Fungal Biology

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Mycorrhizal Fungi in South America

Biodiversity, Conservation, and Sustainable Food Production

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Fungal biology has an integral role to play in the development of the biotechnology and biomedical sectors. It has become a subject of increasing importance as new fungi and their associated biomolecules are identified. The interaction between fungi and their environment is central to many natural processes that occur in the biosphere. The hosts and habitats of these eukaryotic microorganisms are very diverse; fungi are present in every ecosystem on Earth. The fungal kingdom is equally diverse, consisting of seven different known phyla. Yet detailed knowledge is limited to relatively few species. The relationship between fungi and humans has been characterized by the juxtaposed viewpoints of fungi as infectious agents of much dread and their exploitation as highly versatile systems for a range of economically important biotechnological applications. Understanding the biology of different fungi in diverse ecosystems as well as their interactions with living and non-living is essential to underpin effective and innovative technological developments. This series will provide a detailed compendium of methods and information used to investigate different aspects of mycology, including fungal biology and biochemistry, genetics, phylogenetics, genomics, proteomics, molecular enzymology, and biotechnological applications in a manner that reflects the many recent developments of relevance to researchers and scientists investigating the Kingdom Fungi. Rapid screening techniques based on screening specific regions in the DNA of fungi have been used in species comparison and identification, and are now being extended across fungal phyla. The majorities of fungi are multicellular eukaryotic systems and therefore may be excellent model systems by which to answer fundamental biological questions. A greater understanding of the cell biology of these versatile eukaryotes will underpin efforts to engineer certain fungal species to provide novel cell factories for production of proteins for pharmaceutical applications. Renewed interest in all aspects of the biology and biotechnology of fungi may also enable the development of “one pot” microbial cell factories to meet consumer energy needs in the 21st century. To realize this potential and to truly understand the diversity and biology of these eukaryotes, continued development of scientific tools and techniques is essential. As a professional reference, this series will be very helpful to all people who work with fungi and should be useful both to academic institutions and research teams, as well as to teachers, and graduate and postgraduate students with its information on the continuous developments in fungal biology with the publication of each volume.

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Chapter 1
Overview of the Biodiversity, Conservation, and Sustainable Food Production with Mycorrhizal Fungi in South America

Mónica A. Lugo and Marcela C. Pagano

Contents

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1.1 Introduction

It has been proposed that global agriculture will have the challenge to produce doubling food production by 2050 to feed the world (Ray et al. 2013). As a consequence, the use of soils in agronomic ecosystems will increase together with the use of agronomic inputs such as fertilizers and pesticides which would be changed from inorganic to organic formulation taking into account the sustainability of these systems and also the provision of food security (Berrutti et al. 2014; Ray et al. 2013). Thus, soil ecosystems and their health and quality are directly involved in sustainable agronomical practices and recognizing the important role of soil microbial communities (Lehman et al. 2015) and their biodiversity, interactions, dynamic, and functioning (De Vries and Wallenstein 2017). Soil ecosystems and their functioning are under the threat of biodiversity loss by the increase of cultivated areas and agronomic exploitation intensity. Changes in land use alter the structure and functioning of ecosystems where biodiversity plays a vital role in the ecosystem services (ES) provision (Fedele et al. 2018, Millennium Ecosystem Assessment-MA 2005). Further, some agronomic managements have positive or negative effects on soil health and microbial biodiversity (Lehman et al. 2015), affecting also the interaction between different microbial communities including bacteria and fungi (Dodd and Ruiz-Lozano 2012) which are proposed as the key to soil sustainability (De Vries and Wallenstein 2017).

Soils are the support of food production in all terrestrial ecosystems including agronomic ones; they are therefore involved in gas emission and global warming effect. Thus, in agronomic ecosystems, several mitigation practices were proposed to promote the increase of carbon soil stock and the reduction of warming gas emission from soil (Paustian et al. 2016). One of these mitigation strategies proposed was the land-user engagement that requires lesser robust quantification and monitoring technologies than others, and also it is most closely related to farmers and their local knowledge as the stakeholders who will apply the new concepts and local knowledge in their agricultural practices (Paustian et al. 2016). Currently, these mitigation proposals are in agreement with and reinforce the new concept of nature's contributions to people (NCP) that has included a social point of view in the concept of ES popularized by the Millennium Ecosystem Assessment (MA 2005). Thus, NCP are positive or negative contributions of the living nature (biodiversity, ecosystems, ecological and evolutionary processes) to the quality of life of people that take into account the stakeholders' knowledge, especially of local communities, and among them of indigenous people, to define the linkage between humans and nature (Díaz et al. 2018).

In Latin America and the Caribbean, it is estimated that 50% of the people living in rural areas continue to live in poverty and about 30% in extreme poverty. In this region, most of the rural population depends economically on agriculture (crops, animals, forests, fisheries, and aquaculture) and usually works in family units. Therefore, family farming occupies a fundamental role in the economy and rural employment, contributing also to the food and nutritional security of the countries and their territorial development and regional efforts to avoid hunger. However, family farming still faces multiple limitations because the family agriculture together with territorial rural development, food security, nutrition, and eradication of hunger is the priority initiative of FAO in Latin America and the Caribbean, to support countries to achieve eradication of hunger, rural poverty reduction, and sustainable development (FAO http://www.fao.org/family-farming/regions/latinamerica/es/). Furthermore, it is predicted that two thirds of cultivable land may disappear in Africa, a third in Asia, and one fifth in South America by 2025 and that arable land area per inhabitant in the world will be reduced to 0.15 ha in 2050 (Gianinazzi et al. 2010), and diverse ecoregions in South America also were considered as vulnerable by its degree of conservation since 2002 (Olson and Dinerstein 2002).

Plants and soils are increasingly appreciated along the different terrestrial ecosystems as they support several ecosystem services for high-quality human life. Anthropogenic activities introduce fertilizers and pesticides in the fields, modifying the biota in the different ecosystems from South America. Moreover, soil microorganisms are considered a potentially suitable target for studying regional and local effects on diversity, and AMF communities have been modified and are still shaped by anthropogenic factors in the past and now (Pärtel et al. 2017).

Land uses are the most extent of these human interventions on fungal communities including mycorrhizal fungi. Arbuscular mycorrhizal fungi (AMF) are a key soil biota functional group with an important potential to contribute to crop productivity and implement new strategies for sustainable production by the ES they may provide such as the promotion of plant growth by avoiding the use of fertilizers, the increase in crop quality and food security, the increase in plant/soil adhesion, and the structural stability of the soils (Gianinazzi et al. 2010). The different land uses affect the AMF diversity and their function in the community, some AMF species may be used as environmental indicators of different land uses (Oehl et al. 2017), and organic farming has beneficial effects on AMF community (Oehl et al. 2004). Despite the significant relationship between AMF diversity, land uses, and its ES, there are very few studies focused in ES of AMF in Latin America. These topics are detailed in Sects. 1.2, 1.5, 1.6, and 1.7 in this chapter.

Worldwide ectomycorrhizal (EcMF) and saprotrophic fungi richness suffered the land use type effects, and forest ecosystems had greater fungal richness than grassland, pasture, and agricultural lands and markedly greater richness for ectomycorrhizal than saprotrophic mushroom-forming fungi. Ectomycorrhizal fungal richness differed from that of saprotrophs by being positively associated with tree species richness (Andrew et al. 2019). In European oak woodlands with cork oak (*Quercus suber* L.) as dominant tree, EcMF richness and abundance were positively correlated with the regime of silvo-pastoral exploitation and low-mortality *Q. suber*, with extensive agro-silvo-pastoral exploitation under a traditional 9-year rotation cultivation system and recent soil tillage, respectively (Azul et al. 2010). Furthermore, Pérez-Moreno and Martínez-Reyes (2014) showed that among the 1018 species of edible macroscopic fungi, 488 species were ectomycorrhizal symbionts belonging to *Amanita*, *Boletus*, *Cantharellus*, *Cortinarius*, *Hygrophorus*, *Lactarius*, *Leccinum*, *Ramaria*, *Russula*, *Suillus*, and *Tricholoma* genera. Currently, Pérez-Moreno et al.

(2021) have recorded 970 EcMF edible species of mushrooms and truffles. Due to EcMF production of macroscopic esporoma (mushroom or truffle) is related with the presence of tree hosts and its functional and physiological conditions, and taken together the richness and abundance of EcMF basidioma and ascoma production are also influenced by the agroforest management and the edible EcMF productivity in natural and managed forest. Therefore, mushroom and truffle production can also be influenced by changes in land use and forest ecosystem modifications. That is why it is necessary to intensify the study of edible EcMF in these ecosystems to increase their sustainability. In this book these issues in South America are detailed (Chap. 12, Salgado Salomón and Barroetaveña; Chap. 16, Palfner et al.; and Sect. 1.3 in this chapter).

Orchidaceae are the second largest flowering plant family with ca. 26,000–28,000 (30,000) species worldwide, living as epiphytic, lithophytic, and terrestrial and mainly distributed in the tropical areas (Angiosperm Phylogenetic Group; http://www.mobot.org/MOBOT/research/APweb/; The Plant List: http://www.theplantlist.org/1.1/browse/A/Orchidaceae/). In America, Orchidaceae is also the most diverse family, with 12,983 species, followed by Asteraceae and Fabaceae. In South America, epiphytic Orchidaceae is the most diverse family represented in the floras of tropical Andean countries such as Ecuador, Colombia, Perú, Venezuela, and Bolivia and also in Central America and the Guianas and is the second most diverse family in Brazil and Argentina (Ulloa et al. 2017). Orchids are associated forming orchid mycorrhiza (OM) with diverse orchid mycorrhizal fungi (OMF) including Basidiomycota, Ascomycota, Chytridiomycota, Glomeromycota, and Mucoromycota. The most common OMF belongs to Basidiomycota (i.e., *Rhizoctonia* spp., *Tulasnellaceae*, *Ceratobasidiaceae*, *Serendipitaceae*, *Thelephoraceae*) and Ascomycota (i.e., *Tuber*). Surprisingly, the ecological niches range of the OMF is very wide and can fluctuate from saprotrophic up to endophytic in non-orchid plants and/or has mixing nutritional types in some cases among other fungal endophytic groups as dark septate endophytes included melanized hyphal Helotiales (Li et al. 2021 and reference therein). Furthermore, this family is an emblematic group for the protection of endangered plants with 948 species included in the Global Red List of IUNP, and 56.5% of them are reported to be threatened. Moreover, orchids have a great and diverse business prospects such as cut flower, ornamental plants, medicinal compounds, and food (De 2020). In this book, tropical Orchidaceae, OM and OMF, their sustainable culture, and orchid used in human diet are revised (Chap. 8, Alomía and Otero), and terrestrial Argentine orchids and their mycorrhiza conservation are presented in Chap. 9 by Fracchia and Sede (see also Sect. 1.4 in this chapter).

In this chapter we show the occurrence of important plant species from South America with their mycorrhizal associations. Specifically, we focused on the grasses, herbs, vines, and trees with economic importance for the different regions of South America and their association with different types of mycorrhizal fungi (arbuscular, AMF; ecto, EcMF; and orchid, OMF).

1.2 Arbuscular Mycorrhizal Fungi Diversity and Food Production

1.2.1 Grasslands

The vegetation type with prevalence of grasses (*Poaceae*) and other grass-like vegetation, which occurs naturally or is managed (for cutting or cattle-grazing) (Stevens 2018) presents great ecological, economic, and social value but continues to receive limited scientific attention. The microbiota associated to grassland vegetation was also few investigated. Among the microbiota, the arbuscular mycorrhizal fungi represent important components interconnecting soil and plant through the hyphal networks, spores, and secreted substances such as glomalin.

Several reports showed that pasture species were highly mycorrhizal dependent. Moreover, spore density can be low in intensively managed pastures, but fungal richness can be high in semi-natural pastures, compared to native forests, which are usually used as a reference of pristine ecosystem. We compile reports from different sites and countries and analyze them.

A total of 42 AMF species were recovered from seminatural subtropical pastures (Melo et al. 2014). However, only two species were shared between subtropical and tropical pastures, Notably, *Scutellospora calospora* was a common species in both pastures. In general, other species are frequently found preferentially in native forests such as *Acaulospora spinosa*, *Ambispora brasiliensis*, *Dentiscutata heterogama* (Lopes Leal et al. 2013), and *Acaulospora lacunosa* (Melo et al. 2014).

1.2.2 Pasture

1.2.2.1 Intensively Managed Pastures

Under intensively managed pastures in Terceira, Azores, members of Claroideoglomeraceae (a group of AMF with small spores) dominated the soils (Melo et al. 2014). Pastures are obtaining increasing importance worldwide due to the need of sustainable management for increasing or maintaining their productivity. There is much interest to improve grassland resilience, pasture persistence, and productivity under environmental limitations and changes (Porqueddu et al. 2016).

In Chap. 17 García and Chippano (in this book) reviewed the potential and proved the effects of AMF on sustainability and productivity of the pasture in flooding areas in Argentina (see also Sect. 1.2.2.3 in this chapter).

In addition, the economic activities related to extensive livestock production based on the grazing of native vegetation were studied in relation to the high level of desertification processes caused by livestock on the ecosystem extent along Argentine Arid Diagonal (AAD) from the north of Argentina to Patagonian Atlantic coasts. In these environments, AMF (Glomeromycota) have been shown an important ecological role in these poorly fertile environments and are involved in the establishment, assembly, and succession of plant species. In these natural grasslands used for livestock purposes if grazing was moderate, mycorrhizal colonization is beneficial to recover photosynthetic tissue loss and to promote compensatory growth of grass species in arid and semiarid environments; grazing has effects on diversity and functional traits of AMF. Furthermore, mycorrhizal colonization has a fundamental role in grassland sustainable productivity depending onto the management practices and ecophysiological characteristics of plant species of these grasslands. Ambrosino et al. (Chap. 19 in this book) have proposed in AAD grasslands that the use of forage resources must be carried out with caution to conserve the attributes and functional traits of Glomeromycota communities and ecosystem services.

1.2.2.2 Tropical Pastures

Brazil

Pastures grazed by domesticated livestock and products from cows on farms are crucial for milk and cheese production. Permanent tropical pastures of *Brachiaria* sp. are commonly established to maintain pasture lands in Brazil which also contained similar AMF and shared frequent species, such as *Claroideoglomus etunicatum*, *Funneliformis geosporus*, *Scutellospora calospora*, *Acaulospora mellea*, and *Dentiscutata heterogama* (Pagano et al. 2019).

Exclusive AMF species are generally found in different plant communities such as in pastures of Amazonia and Brazil or under forest (Table 1.1). Most of the species include *Acaulosporaceae* (*Acaulospora*) and *Glomeraceae* (*Glomus* and *Rhizophagus*), *Gigaspora*, *Diversispora,* and *Scutellospora*. Species richness of *Acaulosporaceae* was higher in pasture compared to forest, while species of *Glomeraceae* and *Gigasporaceae* were in higher number in forest (Pagano et al. 2019).

Table 1.1 Some grasses and AMF species from Brazil

AMF species	Vegetation type	Reference
Funneliformis geosporus	Grassland	Porqueddu et al. (2016)
Glomus macrocarpum	Forest	Pagano et al. (2019)
Claroideoglomus etunicatum	Forest; pasture	Pagano et al. (2019)
Acaulospora spinosa; *Scu. scutata*	Native forest	Crossay et al. (2020); Porqueddu et al. (2016)
Dentiscutata heterogama	Native forest	Porqueddu et al. (2016)
Glomus sp.1	Native forest	Lopes Leal et al. (2013); Porqueddu et al. (2016)
Claroideoglomus etunicatum	Forest; pasture	Pagano et al. (2019)

Furthermore, Salloum et al. (2022) showed the variability in colonization of arbuscular mycorrhizal fungi and its effect on mycorrhizal dependency of improved for tolerance vs unimproved soybean cultivars in a pot experiment.

Microbial inoculants or biofertilizers in sustainable food production benefit plants by efficiently consuming mineral elements such as nitrogen and phosphorus. Arbuscular mycorrhizal (AM) associations assist several plants to obtain sufficient supplies of phosphorus from rhizospheric soils (Igiehon et al. 2017).

1.2.2.3 Temperate Pastures

Argentina

Grasslands are the sources of many agronomic productions, livestock systems, and environmental issues with positive and recognized impacts on water quality and biodiversity. In this book García and Chippano (see Chap. 17 in this book) discuss the ecological role of AM symbiosis on the functioning of temperate grasslands of Argentina as valuable information to promote better management of forage land sustainably, increasing forage. These authors have been focused in the production and preservation of the beneficial effects of AMF communities in these Argentine Flooding Pampa ecosystems, under effects mainly of grazing and defoliation and P fertilization on mycorrhizal root colonization and AM benefits on forage species of these grasslands. In flooding Pampa, AMF were one of the most common root-associated soil biota, and they have influenced plant productivity. Thus, AMF have shown a high value for the functioning and sustainability of these flooding grasslands; AMF communities also were influenced by soil characteristics, plant species diversity, and climate factors as have been recorded in other temperate grassland ecosystems of South America. Moreover, the uses of these environments for raising livestock to increase the forage resource are agronomic practices which modify not only the plant communities but also the AMF communities and consequently the benefits provided by AMF on grassland productivity. García and Chippano (Chap. 17) also found that phosphorus (P) fertilization was necessary to obtain maximum forage yield in these flooding grasslands despite the fact that this procedure had a detrimental effect on AMF root colonization and mycorrhizal response in several plant species, even if AMF could actively be contributing to plant P uptake.

Chile

Along Chap. 20, Yates et al. (in this book) highlight the ecological relevance of AM symbiosis promoting nutrient and water acquisition, favoring plant defenses to cope with pests or adverse conditions such as drought, salinity, and presence of toxic elements, with multiple ecological meanings and the possible biotechnological applications in Chile. Yates et al. have systematized the scientific information regarding AM and AMF in Chile by macrozones and found also the information gaps to direct future studies in this symbiosis. In total, 53% publications of AM symbiosis in Chile studied anthropogenic ecosystems and how to resolve troubles from mining and agriculture, 42% were focused on functional properties and taxonomy of AMF in natural ecosystems, and natural and anthropic issues together were analyzed in 5% of papers. Notorious information gaps were revealed through analysis by geographical zones, showing the main AM and AMF data availability in the south portion of Chile. In addition, the predominant studies were focused in agroecosystems, mainly monocultures, and also human-impacted environments. These authors concluded that it is crucial and relevant to study AM and AMF in the generation of sustainable agri-food systems adapted to coexist with natural ecosystems and the current climate emergency, to contribute with the restoration of degraded natural ecosystems, particularly in the extreme north and south of Chile.

1.2.3 Vineyards

In South America, vine crops extend over an area of 215,000 ha in Argentina, 207,000 ha in Chile, and 80,000 ha in Brazil (Chap. 18 Aguilera et al. in this book). The vineyards are of great economic importance in Argentina (10.8 million hL) and Chile (10.3 million hL), countries that are mainly world wine-producing (Aguilera et al. 2022). As sustainable wine production is increasing worldwide (Abbona et al. 2007), it was evaluated the sustainability of traditional management in different vineyard systems in Berisso, Buenos Aires province, Argentina. Abbona et al. (2007) compared the conventional management to organic; however, they do not evaluated the mycorrhizal associations in that fields, which would be useful to increase the information about this important economic crop.

Aguilera et al. (Chap. 18 in this book) have studied grapevines (*Vitis vinifera* L.) and viticulture in Chile in relation to water scarcity in the frame of climate change to 2050 and the prediction of drought increase accompanied by increases in temperature, along with different edaphoclimatic Chilean conditions. Surprisingly, they found wine production moving toward the south of Chile, with higher winter and spring precipitations but lower temperatures. Further, in this country viticulture is facing a new constraint such as the production on Andosols, very acidic, P fixing and aluminum (Al) toxicity. These prevailing conditions have been a challenge for wine producers, universities, research centers, and the scientific community and also promoted the joint work of these social actors to understand the ecosystem services that could be improved based on microorganisms like AMF as biostimulants, to involve them into bioformulations based in scientific technological developments to applied new inoculant consortia formed by different growth-promoting microorganisms with special emphasis onto high-tech agriculture acquisition of AMF-based biostimulants produced by research groups.

1.2.4 Agroforestry and Food Production

According to FAO's latest 26th UN Climate Change Conference of the Parties (COP26) and Global Forest Resources Assessment, "agricultural expansion drives almost 90% of global deforestation." Thus, agriculture is the main driver of deforestation in all world regions less Europe. In South America, ca. three quarters of deforestation is due to livestock grazing. This committee of specialists involved in FAO report has emphasized to promote agri-food productivity to provide the new food demands of increasing human population and to cut off deforestation due to the fact that the global deforestation is warning tropical rainforests that are under high pressure from agricultural expansion. Tropical forests still are under threat despite a slowdown in deforestation in South America and Asia; the rainforests in these regions have the highest deforestation rates (FAO, https://www.fao.org/newsroom/detail/cop26-agricultural-expansion-drives-almost-90-percent-of-global-deforestation/en). In these tropical environments, mycorrhizal associations such as AM, ECM, OM and ericoid mycorrhizas (ErM) predominate associated with various plant species, as they can coexist in the same host or differ between them (Smith and Read 2018). In addition, deforestation brings with it soil erosion and increased aridity in the region; these environmental conditions are also accompanied by changes in the mycorrhizal symbioses that will be established and will produce changes in the biodiversity of these modified ecosystems.

Therefore, to know what these mycorrhizal associations are, the plant and fungal symbionts involved, what is the functionality of the symbiosis, and the effects of environmental changes on the partners are essential information for the sustainable use of these forests as sources of food and medicines, carbon reservoir, harmonizers of the water cycle, and for the recreation for the world population.

In Argentina, Dr. Marta Cabello continues the studies of *Ilex paraguariensis*, known as yerba mate, a native species from South America (Velázquez et al. 2020). They also showed the improved AMF colonization in organic trees growing spontaneously in the undergrowth of the forest, compared to the conventional management of the exploited crop.

Mycorrhizal associations in South American tropical environments, including the Amazon region, were analyzed along this topic researcher history (see Chap. 4 Peña Venegas et al.) where 1200 samples were collected during the last decade. Arbuscular mycorrhizal fungal diversity and richness trends across the Colombian Amazon are reported, including their relationship with deforestation activities. AM association with two important Euphorbiaceae plants, such as the manioc (*Manihot esculenta*) and the rubber tree (*Hevea brasiliensis*), is also mentioned, due to their relevance for the local economy. Molecular and phylogenetic results are also very important contributions to the knowledge of the AM symbioses and its functions in this wide area of the Amazon such as the upper Amazon basin.

In the tropical South America, coffee and cacao are among the most widespread and economically important agroforestry crops, traditionally cultivated under a canopy of shade trees that is considered a model of sustainable agriculture prone to favor the biodiversity conservation and forest ecosystem services (Chap. 5 Lovera et al., in this book). Lovera et al. studied AMF in these coffee and cacao crops, showing that AMF have an important role in plant nutrition, nutrient cycling, and the sustainable maintenance of these agroforestry ecosystems. Further, these authors report the increases of plant survival under biotic and abiotic stress conditions by means of their mycorrhizal associations that were a fundamental factor to improve coffee and cacao productivity. AMF species associated with coffee and cacao cultivation in South America were reported by Lovera et al. (Chap. 5); thus, the AMF taxa more commonly associated to coffee crops were *Rhizophagus clarus*, *Gigaspora margarita*, and *Claroideoglomus etunicatum* (to improve the coffee grain yield); *R. clarus*, *C. etunicatum*, and *Diversispora heterogama* (higher resistance to drought stress); and *G. margarita*, *Dentiscutata heterogama*, *Ambispora leptoticha*, *Funneliformis mosseae*, *R. clarus*, *R. fasciculatus*, *R. intraradices*, *R. irregularis*, *R. manihotis*, *C. etunicatum*, *Glomus macrocarpum*, *Acaulospora scrobiculata*, *A. colombiana*, *A. laevis*, and *A. mellea* (increase coffee plant growth); instead, with cacao were recorded the following species: *G. margarita*, *Scutellospora calospora*, *Cetraspora pellucida*, *Ambispora appendicula*, *F. mosseae*, *R. clarus*, *R. irregularis*, *Septoglomus constrictum*, and *A. tuberculata* (positive response on the growth and/or mineral nutrition). Moreover, *A. tuberculata*, *A. scrobiculata*, *A. mellea*, and *C. etunicatum* have been proposed as putative inoculant species due to their positive effects on the growth of coffee and cacao hosts, generalistic habit, and their adaptability to different edaphic and environmental conditions (Lovera et al. Chap. 5). Therefore, coffee and cacao agroforestry management is able to maintain functionally diverse AMF communities with multiple benefits of mycorrhizal association. Nevertheless, the increase of the intensity of agriculture practices reduced significantly AMF diversity to change AMF functionality.

1.3 Ectomycorrhizal Fungi Diversity and Food Production

1.3.1 EcMF in Native and Exotic Forest

Mycorrhizas and their fungal partners are one of the most important and ecologically crucial symbiosis for boreal, temperate, subtropical, and tropical forest, driving plant population biology and community ecology shaping their dispersal and establishment, regulating plant coexistence (Tedersoo et al. 2014, 2020). In South America, among a theoretical framework based on 2–7% of world scientific publications, AM associations have been proposed as prevailing along a wide range of these continental biomes under a biogeographic approach (Brundrett and Tedersoo 2018), with less percentage of other types of mycorrhizas. However, EcM symbiosis, OM, and ErM together with dark septate endophyte presence and importance have been revealed by means and exhausting revision from South America scientific data, but also information gaps have been found (Pagano and Lugo 2019 and reference therein), and new ones are shown in this book (see Chap. 22, Marín et al.).

Dipterocarpaceae is an important tree family that forms EcM symbiosis in the Paleotropics. *Pseudomonotes tropenbosii*, a Neotropical species found in the Colombian Amazonia, was recorded forming EcM (Vasco Palacios and Boekhout, Chap. 3 in this book) with 90 species of EcMF from aboveground/sporocarps (81 spp.) and belowground/root-tip samples (23 spp.). The EcMF community was dominated by the genera *Clavulina* (13 spp.), *Russula* (9 spp.), and *Craterellus*, *Coltricia*, and *Amanita* (6 spp. each). Differences in the diversity and richness of species across sites studied have suggested that environmental differences among sites are important in structuring the EcM fungal communities. The 50% of EcMF species symbionts of *P. tropenbosii* coexisted with other tree species of Fabaceae and Cistaceae that grow in remote Neotropical lowland rainforests. Another surprising result was the high diversity of *Clavulina* (12 spp.); nine of them were previously found associated to Fabaceae-dominant forests in Guyana, showing a broad host diversity and widespread distribution of these EcMF. Moreover, Vasco Palacios and Boekhout (Chap. 3 in this book) suggested that the EcM status of *P. tropenbosii* could indicate the mutualistic relationship of EcMF taxa with a Gondwana ancestor of the Dipterocarpaceae already existed, despite it has been proposed the boreotropical migration or transatlantic dispersal of this EcM tree family.

Forest fragmentation, clear cutting, fires, grazing, and partial conversion to exotic plantations are affecting the temperate forests of southern South America at an increasing rate. It is estimated that about 50% of the native Patagonian forest is degraded by anthropic actions that also impact on mycorrhizal associations and the ways to implement mitigation measures. Salgado Salomón and Barroetaveña (Chap. 12 in this book) have proposed, based on the research of native and exotic Patagonian forest, that the autecology and biodiversity of mycorrhizas in these ecosystems need further and urgent studies. Salgado Salomón and Barroetaveña also highlight the recently reported declines of the relictual *Araucaria araucana* and the endemic *N. dombeyi* and how it could affect other forest species. The effects of fire and grazing on Patagonian forest have been analyzed together with their effects on edible mushroom productivity; furthermore, ectomycorrhizal species suitable for nursery inoculation and good field performance have been studied for exotic *P. ponderosa* planted in Patagonia, and these authors considered also inoculations with expensive edible EcM species such as *L. obliqua* with *Tuber melanosporum* Vittad (the Périgord black truffle) under greenhouse conditions, which allows the possibility of culturing this truffle as a secondary crop during reforestation. In this chapter it is further addressed the trufficulture in Patagonia using different species of oaks (*Quercus robur* L., *Q. ilex* L.) inoculated with *Tuber melanosporum* and *T. aestivum* Vittad in less proportion that have been established in suitable microsites along the region. However, these culture practices involved the introduction of exotic taxa into native forest lands, and more studies are necessary to evaluate its proper implementation.

In the sub-Antarctic temperate forests from Argentina and Chile, *Nothofagus* species are the dominant trees (Chap. 14 Fernández et al. in this book). They are usually colonized by abundant and diverse ectomycorrhizas. Due to the considerable ecological and economic importance of *Nothofagus* and its ectomycorrhizas, Fernández et al. have overviewed field, natural, or managed forest and greenhouse bioassays along different experimental researches used to characterize the ectomycorrhizal symbiosis in different South American *Nothofagus* species since how nursery approaches have been addressed the *Nothofagus* seedlings-EcM relationship during domestication programs, the studies of plant physiological traits, to ecosystem processes involved *Nothofagus*-EcMF symbiosis. These authors highlight some aspects that should be worth considering in the near future such as to consider, in management and sustainable forestry of *Nothofagus* due to the diverse biotic and abiotic factors influencing EcM formation and EcMF diversity, the importance of carrying out field studies together with nursery trials under controlled conditions for providing information to be applied as biotechnological tools on *Nothofagus*-EcMF associations. In this chapter were detected many gaps that need to be studied like the effects of high-magnitude disturbances on sub-Antarctic temperate forests, *Nothofagus*-EcMF symbiosis, and larger geographic and temporal scales to elucidate common ecological and biogeographical patterns and to identify how various factors associated with global change could influence the distribution of EcMF and their host plants to project management plans, mitigation strategies, restoration programs of degraded environments, assistance to forestry species migration, and improvement of sustainable forestry production programs.

Recently, edible ectomycorrhizal fungi that are forming mushrooms (EEMF) were globally reviewed (Pérez-Moreno et al. 2021), resulting in 970 species among Basidiomycota (mushrooms in general sense) and Ascomycota (truffles), and it has been shown their huge values as subsistence source in local and global scale but also as an international food business. These authors showed three main areas in the America where the EEMF are a food resources, and South America is one of them. Although South America has a great diversity of EEMF, the information of uses of edible EcMF and fungi in general as food is scarce or completely missing, probably due to the cultural loss of original people traditions by the presence on the continent of European conquerors who greatly discouraged and destroyed the traditional native cultures.

In this book, Palfner et al. (Chap. 16) have analyzed the EEMF. Thus, they found EEMF have a heterogeneous distribution in South America, occurring mainly associated with native and exotic tree species in the temperate forest ecosystems in southern South America, in the Andean-Patagonian region, along natural and timber monocultures forests, considering also truffle culture progresses. EEMF diversity, their cultural and economic relevance, nutritional properties, cultivation, sustainable management and conservation. In Chile, the conservation status of native EEMF *Boletus loyo* is classified as endangered, and *Cortinarius lebre* has been assessed as vulnerable (MMA 2022) and *Amanita rubescens* as an exotic EEMF which were originally introduced in timber tree monoculture but changed to native hosts. Furthermore, it has been proposed a phytosociological classification of Chilean ecosystems (Pliscoff 2015) following standards of the IUCN (International Union for Conservation of Nature). Moreover, governmental, and scientific institutions, and non-governmental organizations (NGO) have elaborated sustainable harvesting protocols and recommendations (Salazar Vidal 2016; Palma et al. 2021).

Besides, globally biological invasions are an ecological, economic, and human health problem. In plant invasions, belowground biotic interactions such as mycorrhizal associations represent "the hidden side" of this problem that has been overlapped by the most visible components of these symbioses, the hosts, but nevertheless these symbiotic interactions are fundamental and decisive to define and determine the invasion success or failure of their plant partners. Policelli et al. (Chap. 2, in this book) explore some of the ecological aspects of EcMF that settle their invasion success with species of the Pinaceae in southern South America. Policelli et al., meanwhile a "myco-centric framework of the invasion process," address the spatial and temporal zonation of EcMF succession in the non-native plant range. They found that different EcMF species are associated with the different stages of tree invasions and that this is key to understanding the co-invasion process. Along Chap. 2, the authors discuss this problematic issue and propose the way forward, emphasizing also how plant mycorrhizal invasions in South America are an ideal system to explore problems that combine mycorrhizal ecology and invasion biology. Thus, Policelli et al. expose this "hidden side" of plant invasions, exposing and highlighting the relevance of fungal symbionts and symbioses for the study and understanding of biological that may be explained in part by the ecological traits of their specific belowground symbionts.

1.4 Orchid Mycorrhizal Fungi

Recently, the scarce information on mycorrhizas established with orchid and ericaceous hosts and their associated mycorrhizal fungi and in South America has been shown (Pagano and Lugo 2019; Lugo and Menoyo 2019). Regarding to orchid family, along Chap. 8 (in this book), Alomía and Otero have reviewed the orchid mycorrhizas in tropical South America, showing that despite the tropical ecosystems are undoubtedly the cradle of orchid diversity, still few aspects of the biology of this diverse plant group have been explored in the region. Orchidaceae species establish mycorrhizal associations with fungi that supply them with nutrients in the early stages of development to stimulate the germination of their tiny seeds, but also adult photosynthetic orchids could be associated with fungi, and they may change along the host life with different successional fungal symbionts along the time (Li et al. 2021 and reference therein). These fungi are called orchid mycorrhizal fungi (OMF), and their interaction with orchids is called orchid mycorrhiza (OM) (see also Sect. 1.1). Alomía and Otero (op. cit.) found that ca. 50% of research publications are focused on determining the diversity of OMF associated with a few orchid species of interest; the rest of the main topics recorded are studies of phylogeny, morphological and symbiotic seed germination, ultrastructure, and community ecology; however, evolution of OM, mutualistic networks, and metabolism of the interaction are the least explored aspects of this symbiosis. Alomía and Otero propose in conclusion to encourage international scientific collaborations to address the most complex issues still unresolved such as the role of OM in the evolutionary success of tropical orchids. On the other hand, these authors invite researchers to carry out scientific labor that results attractive to the general public and orchid growers (e.g.) and "not only in the academic community to develop the enormous potential of Orchidaceae and OM in the region."

In addition, the symbiotic propagation of South American native orchids and their conservation was addressed in Chap. 9 (Fracchia and Sede, in this book). Fracchia and Sede (Chap. 9) pointed out the life cycle of orchids is unique in the plant kingdom, due to the fact that their seeds germinate only when they are associated in nature with compatible fungi. This first contact and association orchid-fungus is crucial for the development of new seedlings and the population dynamics in orchid's habitats. South America ecosystems harbor more than 30% of the world biodiversity of Orchidaceae, and the greatest species richness occurred along the tropical Andean slopes. Currently, the continuous process of deforestation occurred along South America, even more in tropical forest but also in other South American ecosystems (i.e., arid regions, highlands, dry forest); thus, numerous species are under threat of extinction in the short term. In this context, to study and understand this fungal-orchid association and its propagation is essential to advance in conservation programs by means of the generation of protocols to in situ and ex situ culture and propagation of orchid and fungal symbionts, with the focus on rare or endangered species. In Chap. 9 were also reviewed studies on this symbiosis, Orchidaceae species that have been successfully propagated by symbiotic methods, and the proposal of next research and practical actions that should be taken to promote the endangered orchids and their symbiotic fungi conservation.

1.5 Mycorrhizal Fungi and Land Use Change

Soil is one of the main reservoirs of biodiversity on earth due to its physical, chemical, and microclimatic heterogeneity; in particular, it harbors a great diversity of microbial communities (Guerra et al. 2021). Changes in land uses for crop production, mainly those that involve intense agricultural management, threaten soil diversity, compromising global ecosystem functioning and services (Wagg et al. 2014; Tibbett et al. 2020). Cofré et al. (Chap. 10, in this book) overview the effect of two no-till agricultural practices (crop rotation vs soybean monocropping) on AMF communities of five geographical locations of east-central Argentina. Along these cropping systems, 59 AMF morphospecies were recorded in these 5 geographical locations, and crop rotation- managed soils were the richest in AMF species and density of spores; instead the evenness was the lowest compared to the other cropping systems. Crop rotation management was also related to Glomerales as indicator taxa, such as *Funneliformis mosseae* and *Glomus* sp.4. morphospecies. Otherwise, Acaulosporaceae and Diversisporales were the indicator taxa for monocropping practices. Furthermore, in the studied sites of central Argentina, the soil variables influenced the relative abundance of AMF according to taxonomic categories of family and order. Furthermore, soil physiochemical features like percentage of organic carbon and nitrogen differed between crop rotation and monocropping, positively or negatively depending on the management type. Thus, AMF communities and chemical properties differed among no-till agricultural practices, and crop rotation showed to promote greater richness of AMF morphospecies.

Also in central Argentina, Ontivero and Lugo (Chap. 15, in this book) have studied the AMF diversity but along a more arid ecoregion, the Espinal, that includes three different vegetation areas named Algarrobo, Ñandubay, and Caldén Districts. The Espinal has suffered a significant loss of natural vegetation due to the land use changes, with the expansion of the agricultural frontier and deforestation. Scarce scientific publications from this ecoregion have been recorded. Along the Espinal AMF morphospecies were recorded; the greatest AMF morphospecies richness was registered in the Ñandubay District followed by the Caldén District, and 16 spp. were shared between them; in the Algarrobo District, there were not found publications of AMF morphospecies, but one publication recorded the virtual taxa analyzed by molecular methods (García de León et al. 2018). The AMF communities differed between Ñandubay and Caldén in their species composition; furthermore, agricultural activities seem to have a negative effect on the diversity of AMF species in the Algarrobo District. Therefore, in this region the basic information about AMF diversity is lacking. It is necessary to carry out more research work in this issue, because knowing the biodiversity and structure of the AMF communities could provide useful information to develop management and reforestation plans to solve the drastic loss of biodiversity caused by land use changes.

1.6 Mycorrhizal Fungi in South American Degraded Lands

1.6.1 Potential of Mycorrhizal Fungi for Remediation and Restoration in Degraded Soils

1.6.1.1 Petroleum Hydrocarbon Contamination

Soils and other ecosystems are under serious damage conditions due to petroleum hydrocarbon contamination; that is one of the most common environmental problems causing frequent spill events. Nowadays phytoremediation techniques are applied for the decontamination of soils impacted by hydrocarbon pollution, among them an important place is occupied by the use of plants with their bioremediation capabilities enhanced by their associated mutualistic microorganisms such as AMF and their symbiotic associations, the AM (Cabello 1997). Rosas et al. (Chap. 6 in this book) have studied the impact of a hydrocarbon spill on *Aristida setifolia*, a dominant grass species in the Savanna. Rosas et al. analyzed rhizospheric samples of *A. setifolia* from contaminated vs uncontaminated soils and quantified AMF spore abundance, mycorrhizal colonization, hyphal length, and glomalin production as glomalin-related soil proteins (GRSP). In polluted soils Rosas et al. (Chap. 6) have observed changes in soil physicochemical features such as increases in the electrical conductivity and the nitrogen percentage. Instead, the effects of hydrocarbon pollution on AMF diversity were negative; however, total glomalin (GRSP) amount was higher in contaminated soils, suggesting that the production of GRSP could have been stimulated by pollution as a defense mechanism generated by the presence of hydrocarbons. These authors proposed that the role of glomalin and GRSPs seems to be promising in the protection of mycorrhizal plants against hydrocarbon contamination and that the study of plant-AMF applications to bioremediation should be continued.

1.6.1.2 Heavy Metal (HM) Soil Contamination

A great environmental problem is the heavy metal (HM) soil contamination because the metal's accumulation in the soil is the source from where HM are involved into the food chain and, thus, turning these plants and animals into toxic and harmful organisms that are then consumed by people. There are plant species capable of the hyperaccumulation of HM, the metallophyte; they are useful to remediate contaminated soil. Phytoremediation is "the use of plants and associated microorganisms to remove, contain, inactivate, or degrade contaminants." Among the metallophyte, many plant species are also hosts of AMF and form AM; moreover, these AM-metallophyte function as pioneers at contaminated sites and improve the accumulation of HM by host plants. The improvement of plant tolerance to HM by means of their associated AMF can occur through different mechanisms such as nutrient supply enhancement or water stress decrease, sequestration of HM through binding protein's production (e.g., glomalin), and bioaccumulation in the root cells colonized by AMF structures as well as in the extraradical mycelium. Thus, toxic elements such as Cd, Cu, and Pb among others can travel from soil to roots through AMF hyphae; however, the fungus may also function as a biological barrier against HM transfer from root tissues to the shoot (Molina et al. 2020 and references therein). In this book, Becerra et al. (Chap. 11) provide the information analyzed on metallophytes/hyperaccumulator plants and their mycorrhizal status adapted to HM-contaminated soils in South America, revealing also the scarcity of data on this important topic. Becerra et al. (Chap. 11) showed the mycorrhizal status of metallophytes/hyperaccumulator plant species and focused on the relationship of AMF-metallophytes in soils contaminated with Cu from Chile and soils contaminated with Pb from central Argentina; they also proposed the use of these results for future phytoremediation strategies based on the selection of AMF species with tolerance to HM contamination and their application in HM-contaminated soils.

1.6.1.3 Mycorrhizal Fungi Applied in Restoration

At this time effective ecological restoration of degraded ecosystems and the reduction of gas emissions are possible solutions to mitigate and counteract the negative effects of climate change. It has been pointed out that mycorrhizas are currently widely recognized as fundamental components of plant communities and key modulators of ecosystem functioning (Tedersoo et al. 2014, 2020). Therefore, the mycorrhizal symbioses have to be considered in the context of effective ecological restoration due to the practices applied implying the use of potentially mycorrhizal plants. Among these issues, the experimental evidences predominantly come from the northern hemisphere and showed positive effects on plant biomass and in the plant community richness increased by application of mycorrhizal hosts in restoration field experiments. Silva Flores et al. (Chap. 21, in this book) found scarce restoration experiments from South America and carried them out in few countries (Venezuela, Brazil, Argentina, and Chile); however, most of them showed positive effects of mycorrhizal associations on plant restoration performance. Further, South American field experiments for ecological restoration have used mainly AMF and AM plant species with only one exception for EcM; instead, nor OM neither ErM interactions were applied in restoration assays. Thus, Silva Flores et al. highlighted running experiments with EcM from Brazil and propose to carry forward research in field for ecological restoration that should also consider OM and ErM.

1.7 Mycorrhizal Fungi Diversity, Ecosystem Services, and Huge Information Gaps in South America

In South America are placed the countries with the greatest biodiversity in the world; one of them is Ecuador, where the Amazon region hosted the main biodiverse habitats, settling also the high number of threatened species that also are endemic of the region. Soil microorganisms are considered important drivers of plant biodiversity (van der Heijden et al. 2008); however, little is known about microbial biodiversity associated with the flora of Amazon. Duchicela et al. (Chap. 7, in this book) have conducted the analyses of results of mycorrhiza's research done in Ecuadorian Amazon. They studied the mycorrhizal status and diversity of Ecuatorian Amazon flora from the publishing data. Their results have suggested AMF and AM plant families are dominant in Ecuatorian Amazon ecosystems; that is in agreement with the hypothesis of AMF plant associations which are the best strategy to sustain plant diversity in low P soils as in the Amazon environments. Furthermore, Duchicela et al. highlighted based on the few fungal species described to date that the exploration of the Amazon microbiological diversity is of prime importance and should be addressed. Thus, the Ecuatorian Amazon region has been revealed as a great information gap that urgently needs to be studied on these issues.

In this vast South American continent, also arid and semiarid ecosystems abound from the Andes to the coasts of the Atlantic Ocean, passing through the Patagonian steppe. Along a precipitation gradient, these diverse ecosystems and their mycorrhizal root endophytic associations were revised, and the results summarized by Fontela et al. (Chap. 13, in this book) in Andean and extra-Andean northwest Patagonian subregions, including high-Andean environments, forests, steppes, and meadows. Fontela et al. have concluded that in the Andean region, AM are prevalent in high-Andean plants, native conifers, and other dominant forest trees (except *Nothofagus* spp.) and their understory species. Instead, *Nothofagus* spp. is some of the few ectomycorrhizal Patagonian native species (the *Nothofagus* mycorrhizal symbiosis is detailed studied in Chap. 14 by Fernández et al.); in extra-Andean steppe plants, AM are also abundant; however, non-host plants (NH) were more frequent in the central meadow zones. Also, mycorrhizal colonization decreased from the forests to the meadows, but density of AMF soil spores and infective propagules followed an opposite trend. In general, mixed mycorrhizal colonization was rare, and ErM has a low frequency among other mycorrhizal types and was associated with few plant species, and the fungal symbionts were taxa of *Sebacinales* and other non-identified *Basidiomycota* and *Ascomycota* fungi. Furthermore, the first record for South America of *Rhizoscyphus ericae* was association of EcM with *Gaultheria* host from high Andean in Patagonia. Also *Cadophora finlandia* and *Meliniomyces variabilis* were identified from *Gaultheria* as probable ErM mycobiont and also from *Nothofagus* ectomycorrhizal morphotypes. Thus, Fontela et al. have raised new questions about the capability of *Helotiales* taxa to form both EcM and ErM types. These authors also have recorded dark septate endophytes coexisting with other mycorrhiza types in a wide range of hosts and environments along Patagonia, and they encourage further research to deepen global diversity patterns and the determining factors for these symbiotic associations.

Finally, previous studies in mycorrhizas along South America yielded relevant information on the data gaps for some types of mycorrhizae such as ErM and OM, interactions with dark septate endophytes, and other root fungal endophytes. It was also noted that large areas of important South American biomes remained to be reviewed for their mycorrhizal and endosymbiont symbioses inhabiting the roots, i.e., Patagonia, highland ecosystems, Amazon basin ecosystems, rainforest, dry forest, wetlands, deserts, and extreme arid regions (Pagano and Lugo 2019 and references therein). In this book, several of those information gaps have been filled partially, and new data is revealed throughout the chapters that gave rise to this new book. However, there are still information gaps that must be filled especially in data related to functionality of mycorrhizal associations in South America. Therefore, Marín et al. (Chap. 22, in this book) conducted a meta-analysis of research in South American mycorrhizal biodiversity and ecosystem functions by means of a Web of Science search (1945–2021); they obtained 1528 sampling sites, 80% of them in Brazil, Argentina, and Chile. Among them 63.09% studied mycorrhizal biodiversity, while only 12.70% researches were conducted to know mycorrhizal functioning. Surprisingly 24.21% of the works investigated both mycorrhizal biodiversity and ecosystem functions; this percentage is higher than researches on these issues at global scale research. Marín et al. also concluded that Atacama desert, Patagonian steppe, Cerrado, Chaco, and the Amazonian rainforest biomes need to be studied strongly. Moreover, most mycorrhizal functions (except plant growth and soil aggregation) were understudied, as well as OM and ErM associations, and few specific regions and ecosystems, in general, close to big cities, concentrated the main mycorrhizal research in South America. These authors emphasize the need to carry out more baseline research on plant and fungal taxonomy, mycorrhization, and experiments of response to mycorrhizas to solve the gaps found.

1.8 Conclusions

The excessive use of fertilizers and pesticides is a global threat that affects ecosystems worldwide and particularly in South America. The management of AMF can possibly diminish the utilization of agrochemicals which are dangerous to human populations and to the environment by the introduction of AMF inoculum into the soil of interest. Moreover, possible solutions to the main problems caused by climate change in crops and vineyards will be elucidated especially regarding the symbiosis with mycorrhizas.

In this book, mycorrhizal associations were analyzed and addressed by different points of view of the research applied reported in these topics. The keywords that highlight the information involved in each one of the chapters that engender this book are illustrated in Fig. 1.1. New scientific results have been obtained that fill with relevant information the gaps previously detected by the authors, and new challenges have been found for the investigation of mycorrhizal associations in South America.

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Fig. 1.1 Diagram of chapter’s keywords for mycorrhizal associations in South America

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Chapter 2
Ectomycorrhizal Fungi Invasions in Southern South America

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Contents

2.1 Belowground Invasions: Concepts and Definitions

Today's world is characterized by intense anthropic activity, which leads to the constant movement of organisms that are introduced into areas that are outside their natural range of distribution. Whether intentional or accidental, this movement evades the natural dispersal mechanisms of organisms and the existing biogeographic barriers (Mack et al. 2000; Richardson et al. 2008). Of the group of organisms that are introduced, only a small fraction manage to establish, naturalize, and eventually invade in the regions they are transported to (Blackburn et al. 2011; Jeschke and Pysek 2018). However, the populations that do invade constitute a serious problem at a global scale (Lockwood et al. 2007; Simberloff et al. 2013; Gallien and Carboni 2017). After more than 60 years since the publication of *The Ecology*

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of Invasions by Animals and Plants (Elton 1958), which began the systematic study of biological invasions, the invasion of non-native species is nowadays one of the main causes of global change.

Non-native species invasions have ecological, economic, and socio-cultural consequences. Non-native invasive species can reduce the diversity of native species (Vilà et al. 2011; Pyšek et al. 2012), influence the genetic variation of local populations by hybridization (Vilà et al. 2000), or interfere with pollination networks, dispersal, or other mutualistic associations of the native community (Traveset and Richardson 2006, 2014). Invasive species can also affect biogeochemical cycles (Ehrenfeld 2003; Vilà et al. 2011), disturbance regimes (Simberloff 2011), and ecosystem productivity (Vilà et al. 2011; Simberloff et al. 2013). Some biological invasions can negatively impact the services provided by ecosystems (Ehrenfeld 2010) and cause a negative effect on human health and well-being (Pyšek and Richardson 2010). Biological invasions are, in turn, economically expensive (Pimentel et al. 2005), which is especially problematic for developing countries. Although the estimation of economic costs is complex and can take into account various factors, reports show that in Argentina, for example, invasions cost US$ 6.908 million for the period between 1995 and 2019 (Duboscq-Carra et al. 2021). For developed countries, the estimates rise to 1.7 billion British pounds annually in England (Williams et al. 2010), reaching US$ 120 billion annually in the United States (Pimentel et al. 2005). In addition to the ecological and economic costs, the loss of local diversity and the replacement of native species lead in many cases to the loss of the cultural identity of the invaded sites (Simberloff et al. 2013; Rozzi et al. 2018) and the impact on ecosystem services related to tourism, culture, and recreation (Pejchar and Mooney 2009). Despite these global impacts, and the importance of the ecological mechanisms involved in invasion, one of the main questions of invasion ecology still remains elusive: why are some populations of introduced species successful in their invasion, while others are not (Kolar and Lodge 2001; Richardson et al. 2008)?

The global problem of biological invasions has historically been addressed from an aboveground perspective (Bohlen 2006; Rundel et al. 2014; Wardle and Peltzer 2017). The invasion of animals and plants has received greater attention due to their conspicuity and visible impacts (Brussaard 1997). Less visible organisms, such as those that inhabit the soil, are receiving increasing attention over the last decade as their invasions seem to be as widespread as that of conspicuous organisms (Callaway et al. 2004; Simberloff et al. 2013). Worms, insects, and fungi, together with other soil macro- and microorganisms are transported by humans, most times inadvertently, and establish in new habitats, reproduce, and expand their distribution range (Lockwood et al. 2007; Vellinga et al. 2009; Blackburn et al. 2011). Soil invasive species can shape the aboveground community and impact ecosystem processes (Wardle et al. 2004; Bohlen 2006; van der Putten et al. 2007; Suding et al. 2013; Wardle and Peltzer 2017; Peay 2018). Given their cryptic nature, the difficulties in their study, and the processes involved, belowground invasions are less reported and studied in literature (Reinhart and Callaway 2006; Inderjit and van der Putten 2010).

In this chapter, we will review the current understanding of one of the most widespread and pervasive invasions at a global scale: tree species in the Pinaceae and their co-invasive ectomycorrhizal fungi (EMF). In this first section, we present some concepts and definitions that are relevant to understand the co-invasion process. In the second section, we present a myco-centric framework of the invasion process with a special emphasis on the evidence from southern South America. We address the spatial and temporal zonation of EMF succession in the non-native range and pose new questions about the fungal traits involved in successful invasions. In the third section, we mention other examples of plant-EMF invasions in the region that we think deserve more attention in the future, and we discuss potential implications and future directions. Considering invasion as a process that occurs both above- and belowground, we hope to shed light on the idea that the invasion of some plants might be explained, in part, by the ecological characteristics of their associated symbionts.

2.1.1 Soil Is a Complex Living System

Soil has a set of unique characteristics that make it a system unlike any other found in the biosphere, containing a high diversity of organisms that are susceptible to changes caused by humans. Soil is a highly heterogeneous environment, which has restrictions regarding the availability of nutrients, food resources, and spatial limitations to movement, breathing, and feeding (Wall et al. 2012). The structure of the soil is a conditioning factor for the organisms that inhabit it and is in turn influenced by them (Brussaard 1997). The chemical, physical, and spatial heterogeneity of the soil as a habitat and the adaptations that organisms have developed are probably the cause of its great biotic diversity (Young and Crawford 2004; Wall et al. 2012). Human-induced changes, such as urbanization, agriculture, desertification, and deforestation, or the invasion of non-native species, affect the diversity of organisms that inhabit the soil, as well as its structure and function (Bardgett and van der Putten 2014).

Soil organisms do not have a uniform distribution. For many years, and particularly for microorganisms, soil ecology has been developed under the premise that “everything is everywhere, but the environment selects” (Baas-Becking 1934). In recent decades, evidence has shown that bacteria, protists (Bates et al. 2013), mycorrhizal fungi (Öpik et al. 2006; Tedersoo et al. 2010, 2014), and soil fauna (Wu et al. 2011) have restricted global distributions due to climatic, edaphic, and plant community variations. A clear example of this comes from invasion biology: some non-native plant species manage to escape soil pathogens in their introduced range, while some need their specific soil mutualists to establish and, in some cases, invade in the new range (Callaway et al. 2004; Maron et al. 2015; Dawson and Schrama 2016). The distribution patterns of the species that inhabit the soil are determined by environmental factors, population processes, disturbances, and recolonization events, which operate at different spatial and temporal scales (Bardgett and van der Putten 2014). The spatial scale of these processes can range from millimeters in the rhizosphere or soil pores to hundreds of meters on a regional scale (Ettema and Wardle 2002). The temporal scale includes variations during the same day to those in which decades or millennia are involved (Bardgett and van der Putten 2014). This spatial and temporal heterogeneity of soil influences the growth and structure of the plant communities that grow from it.

Plants influence soil biota, and soil biota differentially affect plant performance (Kulmatiski et al. 2008; Bever et al. 2010; van der Putten et al. 2013). This interaction can be negative or positive for the plant, depending on the balance between the negative effects of soil pathogens, herbivores, and parasites and the positive effects of generally beneficial organisms such as mycorrhizal fungi and nitrogen-fixing bacteria (Klironomos 2002; Suding et al. 2013). In turn, these effects may differ in their intensity between different plant species (Bever et al. 2009) and according to the context in which they occur (Hausmann and Hawkes 2010). Plant-soil interactions play a key role in plant community dynamics, including processes of succession (Kardol et al. 2006), restoration (Eviner and Hawkes 2008; Kardol and Wardle 2010), and invasion (Klironomos 2002; Callaway et al. 2004; Wolfe and Klironomos 2005). In the context of biological invasions, plant-soil interactions could differentially benefit or harm non-native species compared to native ones, facilitating or hindering their invasion, respectively (Kulmatiski et al. 2008; Suding et al. 2013; van der Putten et al. 2013; Gundale et al. 2014; Inderjit and Cahill 2015; Crawford et al. 2019).

2.1.2 Plants Can Co-invade with Soil Mutualists

Within the species that require mutualistic soil symbionts to invade, three main strategies are recognized (Nuñez and Dickie 2014; Nuñez et al. 2016). First, species can establish mutualistic associations with soil organisms that are present both in the range of origin of the invasive species and in the introduced range, called cosmopolitans (Moora et al. 2011). A second strategy involves the formation of novel associations with soil symbionts that are absent in the native range but present in the invaded range. This can occur either when a native symbiont associates with a non-native host species (Tedersoo et al. 2007), or when a native host species associates with an introduced soil mutualistic symbiont (Orlovich and Cairney 2004). The third strategy is co-invasion, which occurs when a mutualist that is present in the native range of an introduced species is also intentionally or accidentally co-introduced with its host (Dickie et al. 2010). The introduction of both members of the mutualistic association can happen simultaneously or not, but both mutualists are new to the invaded ecosystem.

Fig. 2.1 *Pinus contorta* invasion in Coyhaique Alto, Aysén, Chile. The original pine plantation can still be distinguished at the back, with ectomycorrhizal pine trees escaping from the plantation and transforming the landscape – a steppe ecosystem with patches of native ectomycorrhizal *Nothofagus antarctica* – into a monospecific forest. What does the fungal community look like belowground? (Photo credit: N. Policelli)

Co-invasion processes can cause multiple impacts on the native community. One of the main consequences is to enable the invasion of non-native trees. This can result in the conversion of ecosystems previously free of woody species into forests dominated by a single species (Richardson et al. 2000; Nuñez and Dickie 2014; Rundel et al. 2014; Nuñez et al. 2017), as has been reported for vast areas of the southern cone of South America, especially in Chile and Argentina (Fig. 2.1). The co-invading mutualists may, in turn, present new enzymatic mechanisms for the ecosystem such as atmospheric nitrogen fixation (Vitousek et al. 1987) and the acquisition of nutrients that were not previously available. Especially in the case of co-invading ectomycorrhizal fungi, new symbionts are being introduced that have the capacity to produce enzymes to degrade organic matter to access previously occluded nutrients, such as nitrogen (N) (Read and Perez-Moreno 2003), and then transfer those nutrients to their plant hosts. This would be especially impactful in ecosystems with no previously existing ectomycorrhizal fungi, such as the Hawaiian Islands (Hynson et al. 2013). Evidence shows that co-invasion is not a rare phenomenon, being common, for example, for ectomycorrhizae and nitrogen-fixing symbioses (Nuñez and Dickie 2014).

2.1.3 Mycorrhizal Fungi Can Be Strong Determinants of Plant Invasions

Mycorrhizal associations are one of the most widespread symbiotic interactions between plants and diverse groups of soil fungi. These interactions occur between the hyphae of certain fungi, called mycorrhizal fungi, and the subterranean organs of the gametophytes of many bryophytes and pteridophytes, as well as the roots of most seed-bearing plants and the sporophytes of most pteridophytes. Mycorrhizal associations are mainly involved in the absorption of nutrients and water from the soil that the fungus provides to the plant in exchange for carbon compounds (Smith and Read 2008). Evidence shows that mycorrhizae are the main means of absorption of nutrients and water for plants, and were present even in the first land plants that did not have true roots (Strullu-Derrien et al. 2018). Mycorrhizal fungi are members of the vast community of organisms that colonize the rhizosphere, establishing an intimate association with the root. The mycorrhizal condition, unlike symbioses with parasites, is the normal state of most plants under most ecological conditions (Smith and Read 2008). Rather than a friendly relationship between a plant and a fungus, the mycorrhizal symbiotic association can vary throughout a plant's ontogeny on a continuum between parasitism and mutualism (Johnson et al. 1997), depending on both the plant and the fungal partner's needs (Kennedy 2010).

There are several factors that can determine the potential of mycorrhizal fungi to facilitate or hinder the invasion of their hosts (Pringle et al. 2009). For example, the degree of dependence of the plant on its symbiotic fungi can vary from plants that do not require mycorrhizal association (facultative mycorrhizal), to those that are obligate symbionts, which are expected to be constrained during invasion if their symbionts are absent (Pringle et al. 2009; Menzel et al. 2017). However, recent evidence shows that highly invasive plants are more dependent on soil mutualists, counteracting the ideal weed hypothesis (Moyano et al. 2020). The presence of the fungal symbionts in the new habitat and the moment in which they are introduced (Vellinga et al. 2009) are decisive factors in the invasion of their hosts, given that their absence can lead to failed invasions or condition invasion lag phases (Nuñez et al. 2009; Dickie et al. 2017; Sulzbacher et al. 2018). The degree of flexibility that non-native invasive plants exhibit to associate with different species of mycorrhizal fungi, both native and non-native, is also a conditioning factor for invasion (Pringle et al. 2009). Those plants that only associate with highly specific mycorrhizal fungi are expected to be more constrained in their invasion than those that are able to associate with a greater variety of fungal species (Pringle et al. 2009; Menzel et al. 2017). Once both members of the symbiosis are established, non-native fungal symbionts, as well as those of the native community, can influence invasive non-native plants as well as native ones (Pringle et al. 2009). These processes can determine the success of the invasion and the plant community assembly (Kardol et al. 2007) in the invaded range. In the second section of this chapter, we will discuss how not only the mere presence of fungal symbionts but also their identity and ecological traits matter for the invasion to be successful.

2.2 Invasive EMF Co-introduced with Invasive Pinaceae Trees

One of the most studied examples of plant-fungi co-invasions is that between Pinaceae trees and their ectomycorrhizal symbionts. There are no native Pinaceae trees in the Southern Hemisphere (we use “pine trees” to refer to the genus *Pinus* and “Pinaceae trees” to include other genera within the family such as *Pseudotsuga*). Examples of some native conifers from the southern cone of South America are the monkey-puzzle tree (*Araucaria araucana*) or the endemic Alerce (*Fitzroya cupressoides*) in Patagonia. Pinaceae trees have been introduced in South America for productive and ornamental uses, or as an attempt to prevent soil erosion during the early twentieth century. For example, in Isla Victoria, Northern Patagonia, Argentina, Pinaceae species from Europe and North America were introduced as part of a nursery assay to test species’ performance for forestry purposes. Interestingly, the first attempts of pine introductions and establishment failed due to the absence of co-introduced soil (Richardson and Williamst 2015; Nuñez et al. 2017). When they introduced soil, without necessarily knowing about the presence of mycorrhizal symbionts, pine trees – and other species in the Pinaceae – were able to establish. Some of them were able to escape the original plantations and invade the native community (Richardson and Rejmanek 2004; Simberloff et al. 2010; Rundel et al. 2014; Nuñez et al. 2017). One hundred years after those first introductions, the landscape has changed greatly with some pine plantations that have been abandoned and others that are still being managed, but in both cases with severe invasions reported in grasslands and native forests (Fig. 2.1). At a regional scale, there is a growing concern about the spread of invasion, mostly in south and central Argentina and Chile, which require urgent measures to contain and mitigate the invasion (Peña et al. 2008; Simberloff et al. 2010; Nuñez et al. 2017; Franzese et al. 2017; García et al. 2018).

Pine invasions have severe impacts on ecosystem processes in the invaded range. Impacts include changes in water regimes and disturbances such as fire (Simberloff 2011; Simberloff et al. 2013; Cóbar-Carranza et al. 2014), reductions in plant diversity at a local scale (Richardson and Williamst 2015; Franzese and Raffaele 2017), biotic homogenization (García et al. 2018), changes in soil nutrient cycling (Dickie et al. 2011, 2014), and economic losses (Nuñez et al. 2017). In short periods of time, less than 10 years, for example, in many invaded sites in Patagonia, the total transformation of a native ecosystem to a non-native monospecific forest can occur (Fig. 2.1, García et al. 2018). This conversion translates into changes in microclimatic conditions, and the availability of resources leads to a reduction in the richness and abundance of native plants and promotes the modification of soil biota and the alteration of food webs in invaded ecosystems (van der Putten et al. 2007; Simberloff et al. 2010; Richardson and Williamst 2015). To prevent these impacts and predict the invasion, biological attributes of the Pinaceae that affect its invasive potential, such as its high rate of reproduction, its rapid growth, and its ability to disperse over long distances, have been comprehensively studied (Rejmanek and

Richardson 1996). However, the impacts and the possibility of predicting the invasion of pines in relation to their interactions belowground have been less explored.

Pinaceae species require ectomycorrhizal fungi (EMF) in order to thrive (Malloch et al. 1980; Hibbett et al. 2000). Given the obligate nature of this symbiosis, if EMF species are not present in the non-native range, invasion is hampered (Richardson et al. 2000; Nuñez et al. 2009). Evidence shows that Pinaceae species overcome this hurdle by co-invading with their EM fungi (Dickie et al. 2010). Once present in the invaded range, pines and EMF disperse independently (Dickie et al. 2010; Nuñez and Dickie 2014; Horton 2017). Although there is evidence that EMF conditions the invasion of Pinaceae species, little has been investigated about the ecological characteristics of these microorganisms. Aspects such as the identity (e.g., which species of EMF manage to co-invade and what ecological characteristics they possess), their ability to spread, or their ability to establish interactions with other native and non-native species have been scarcely described in the literature. Understanding the mechanisms by which belowground mutualisms influence invasion is a key aspect of the ecology and management of invasive species, as well as the conservation biology of native habitats (Dickie et al. 2016).

2.2.1 A Myco-centric Invasion Framework to Better Understand EMF Success or Failure in the Non-native Range

Not all EMF species are successful in the invaded range. Even from those EMF species that are able to establish a self-sustaining population outside the nursery, only a handful are able to escape the pine plantation and invade. Evidence from South America (Hayward et al. 2015a) and from other regions in the Southern Hemisphere indicates that there is a pattern in the EMF community composition that follows the pine invasion gradient (Fig. 2.2). Here we present here five different groups of EMF species according to the place from which they have been isolated in relation to the pine invasion. This framework combines the spatial and temporal zonation of EMF succession (Peay et al. 2011) in the non-native range, with a unified conceptual invasion model based on what has been described for animals and plant invasions (Blackburn et al. 2011). We hope this framework helps better understand the pine-EMF co-invasion process from a myco-centric point of view and that it opens new questions related to the mechanisms and ecological traits behind these patterns.

2.2.1.1 Non-native EMF That Were Introduced but Never Reported in Pine Plantations

Work by Vellinga et al. (2009) and others has accurately tracked the human-mediated transportation of ectomycorrhizal fungi from mainly Europe and North America to the Southern Hemisphere. Reports come from botanical gardens, isolated

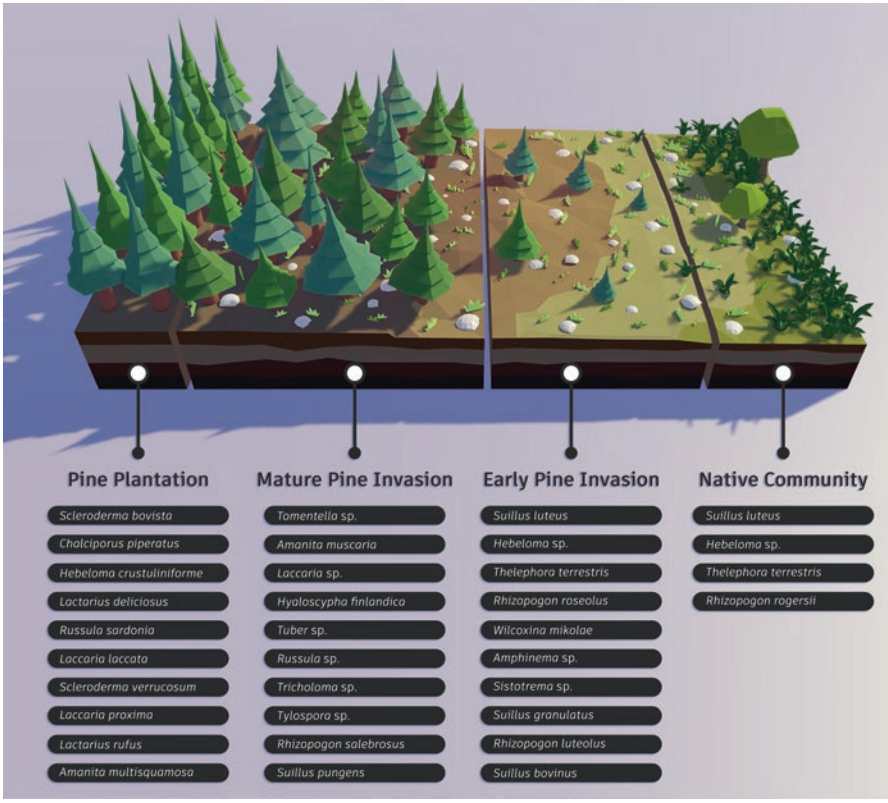

Fig. 2.2 Diagram of four different areas of a Pinaceae invasion and a list of the most reported EMF taxa at each area of global pine invasions. Some EMF species have never been reported outside pine plantations. Other EMF species have been able to invade, but they are always reported to be associated with mature pine invasions in which pine trees are dense. Some taxa, predominantly suilloid fungi – *Suillus* and *Rhizopogon* – are mostly associated with early pine invasions (i.e., the invasion front). A small group of EMF have been reported to be present in the soil of the native community without any Pinaceae hosts nearby. Evidence suggests that each group of EMF may exhibit different ecological traits related to their invasion capacity that could explain the observed spatial pattern. EMF that have been introduced but never reported in pine plantations are not shown in the diagram but included in the proposed invasion framework

observations, herbarium records, and/or plant nurseries. Some records date back from the early 1900s and are based on observations of sporocarps as they predate molecular methods to identify species (Horton and Bruns 2001), but most come from DNA-sequenced fungal material. Interestingly, there is no further published record of many of these introduced species in pine plantations or invasions, even when the number of publications on pine-EMF co-invasions has increased dramatically in the last decade. This suggests that even when these EMF species were able to overcome the geographical and cultivation barriers thanks to humans, they might not have been able to survive or reproduce in the introduced range. Some examples of these EMF species are *Laccaria fraterna, Amanita pantherina, Russula capensis, Clavulina cristata,* and *Lactarius piperatus*. Specifically for South America, examples include *Hebeloma hiemale*, *Inocybe kauffmanii*, *Rhizopogon subolivascens* (Barroetaveña et al. 2005), *Peziza ostracoderma* (Barroetaveña et al. 2010), *Hebeloma sacchariolens*, *Russula consobrina* (Sobestiansky 2005), *Russula albidula*, *Tricholoma equestre*, and *Tricholoma terreum* (Wright and Albertó 2002). Failure to establish in the non-native range can result from a combination of factors related to the EMF species (e.g., low reproductive rate, low responsiveness to colonize roots, high host specificity), the location (e.g., unsuitable soil and/or climate conditions, presence of natural enemies), and stochastic effects which are not constant across repeated introductions (e.g., introduction effort). Hence, this doesn't preclude the possibility that subsequent introductions of the same EMF species in the same sites will be successful.

2.2.1.2 Non-native EMF Never Reported Outside Pine Plantations

When compiling the information of all the reported EMF-pine interactions in plantations in the non-native range, certain EMF species are frequently reported associated with planted pine trees in both managed and unmanaged plantations. However, a subset of those species have never been reported outside plantations (Fig. 2.2). These EMF species have successfully established but haven't been able to spread out of the initial point of introduction yet. Examples of these species are *Chalciporus piperatus*, *Hebeloma crustuliniforme*, and *Lactarius deliciosus.* For South America, examples include *Amphinema byssoides*, *Rhizopogon ellenae*, *Laccaria laccata*, *Russula sardonia*, *Rhizopogon fuscorubens*, and *Scleroderma bovista* (Barroetaveña et al. 2005 and references therein). Even when these species are able to survive in the wild, reproduce, and self-sustain, their dispersion capacity might be an important limitation. Caution should be taken when thinking of these species as non-invasive only because they have never been reported outside pine plantations. Some of these EMF might be under an invasion-lag phase, and when the conditions are suitable (e.g., changes in the soil and/or climate conditions, disturbance, host shift, introduction of a dispersal vector), they might be able to invade. The question of why these species have never been reported outside the plantation is also interesting to understand invasion failures and invasion lags and deserves further exploration in the future.

2.2.1.3 Non-native Invasive EMF Reported Outside Pine Plantations but Always in Mature Pine Invasion

Some EMF species have been able to overcome the dispersal barrier and escape from the initial introduction point (i.e., invade), but have always been reported to be associated with mature invasion sites, where pine trees are dense and older compared to the invasion front (Fig. 2.2). The further from the plantation, the more dissimilar the soil environment is expected to be (Blackburn et al. 2011). Thus, the environmental barrier becomes more relevant at a local scale. Root density could be a key factor at this point that selects for EMF species with different exploration types (Peay et al. 2011). In mature pine invasions, roots are dense due to the proximity of trees. EMF with shorter exploration types will exhibit a more efficient carbon allocation strategy compared to those with longer exploration types and consequently might benefit in mature invasions. Other factors such as soil pH, soil N and other soil nutrient availability, litter quality, surrounding vegetation, and the age of the Pinaceae host might also act as strong filters for these EMF species. Examples of EMF species associated with mature pine invasions are *Amanita muscaria*, *Hyaloscypha finlandica*, *Hyaloscypha bicolor*, *Amanita rubescens*, *Boletus edulis*, *Inocybe curvipes*, *Paxillus involutus*, and *Scleroderma citrinum*, with evidence from South America (Nuñez et al. 2009; Hayward et al. 2015a; Urcelay et al. 2017) and other regions (Dickie et al. 2010; Hynson et al. 2013; Gundale et al. 2016). It is possible that these EMF species are key to sustaining the invasion while pine trees grow older. As the invasion advances, the soil conditions become more favorable for this subset of EMF, and they take over the roots and replace the initial EMF, as they tend to be “more efficient symbionts.”

2.2.1.4 Non-native Invasive EMF Reported in Pine Invasion Fronts

A small group of EMF species, dominated by suilloid fungi (specifically *Suillus* spp. and *Rhizopogon* spp.), predominate at the invasion front and are key at triggering the invasion process (Policelli et al. 2019). These EMF species disperse, survive, and reproduce at sites across multiple habitats far from the invasion source (Fig. 2.2). Apart from being able to disperse further compared to other EMF species, they produce a quantitatively higher number of sporocarps and spores compared to other EMF. Many of these species are also able to generate a resistant spore bank that can last for decades in the soil (Bruns et al. 2009). In turn, those spores are able to germinate faster and colonize the roots of seedlings and young saplings (Policelli et al. 2019). Most of these species are also able to explore greater distances in the soil with their hyphae looking for nutrients and water, as they exhibit a long-distance exploration type. As pine trees are less dense and sparse by several meters in the invasion front, this strategy allows EMF to find new hosts to be colonized. The presence of other EMF species in the invasion front is variable but not less relevant. Other species such as *Thelephora terrestris*, *Wilcoxina mikolae*, *Hebeloma* sp., and *Sistotrema* sp. have also been reported in the invasion front associated with young pine trees (Policelli et al. 2019). In turn, while some suilloid fungi such as *Suillus luteus* and *Rhizopogon roseolus* are within the most invasive EMF, other suilloid fungi have never been reported outside pine plantations or mature invasions, so invasiveness seems to be variable even within the suilloid group.

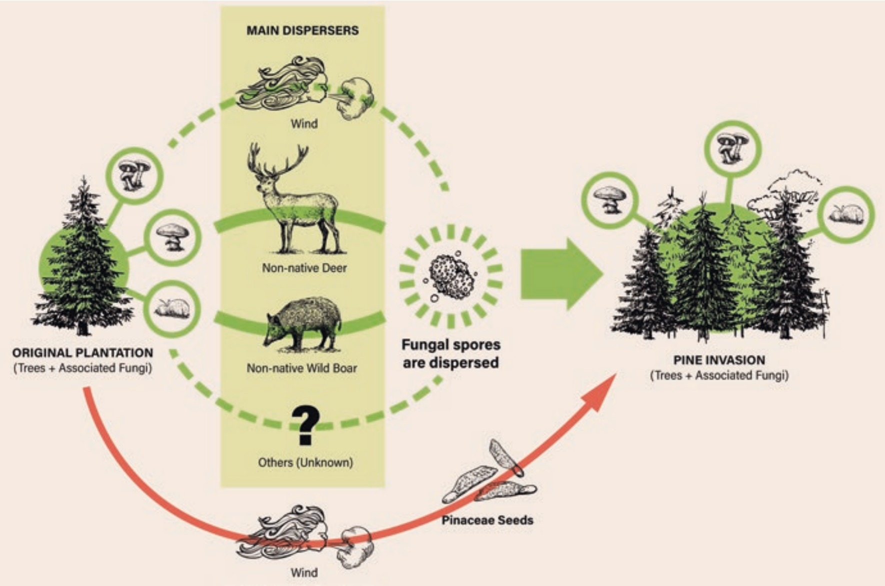

Fig. 2.3 Dispersion is key for successful co-invasion. Both pine trees and EMF disperse independently. While pine trees rely mostly on wind for seed dispersal (red arrow), EMF disperse via wind but also via animal vectors which can be non-native invasive such as deer and wild boars (green lines). However, other dispersal vectors such as birds or humans allow invasive EMF spores to reach sites that are far from the invasion source (the question mark indicates that they have not been described for non-native invasive EMF yet)

2.2.1.5 Non-native Invasive EMF Reported in Native Communities in the Absence of Co-invasive Pine Hosts

Sites dominated by native vegetation, where invasive non-native plants such as pines are not yet observed, may be susceptible to invasion due to the presence of invasive EMF (Policelli et al. 2020a). Evidence from soil samples collected in the field and greenhouse bioassays show that when a Pinaceae host is planted in soil dominated by native vegetation far from other Pinaceae hosts, the plant is still able to be colonized by co-invasive ectomycorrhizal fungi (Policelli et al. 2020a, 2022). A subset of those EMF present in the invasion front, again predominantly suilloid fungi, is able to survive in the absence of compatible hosts (Fig. 2.2). These fungi disperse before their hosts and set up a “waiting for pine trees” scenario, increasing the vulnerability of those sites to further invasion. Most EMF spores disperse predominantly via wind, as the seeds of Pinaceae trees do (Fig. 2.3). At further distances from the invasion source, other vectors such as animals (e.g., wild boars and deer, which are also non-native invasive in South America) are important in the dispersion of invasive EMF, especially for those species with truffle-like sporocarps such as *Rhizopogon* (Nuñez et al. 2013; Soteras et al. 2017). However, recent evidence shows that even in the absence of these mammalian vectors, invasive EMF can reach areas of native vegetation (Policelli et al. 2022). Other dispersal vectors such as birds, or even humans, deserve further exploration (Fig. 2.3). Just as the invasion of plant or animal species generally leads to a biotic homogenization aboveground, the same could be expected to happen in terms of fungal invasion. The effects of this possible homogenization of the native community above- and belowground remain poorly explored.

We are aware of the fact that not all Pinaceae invasions look like the one shown in Fig. 2.1 with a clear invasion front (Hayward et al. 2015b; Policelli et al. 2020a), where pine density correlates with pine age. However, evidence shows that the above presented framework could also be applied to other pine invasion scenarios. Urcelay et al. (2017), for example, found that the EMF community associated with invading pines that are colonizing higher elevations in mountains of central Argentina follows a similar pattern: the higher pines are, the more dominant suilloid fungi become. In a similar study system, Milani et al. (2022) showed that the age of pine trees rather than the degree of spatial isolation between individuals affected the EMF composition. They report a rich EMF community structured by pine age, with more species near older trees and a similar successional pattern in which suilloids are more dominant in younger trees.

In summary, the main point of the above framework is that the identity and the ecological traits of EMF are relevant and act as a determining factor for their invasion. As the previously described patterns occur with pine trees and most biological invasions, it is unclear what the mechanisms behind this pattern of ectomycorrhizal fungi composition are. In other words, why are some EMF able to escape from the plantation and invade, while other EMF have never been reported outside the plantation or even outside nurseries after introduction? Even so, the pine-EMF co-invasion system offers a unique possibility for understanding how the functional traits of soil microbial communities affect the ecosystem in their native and non-native ranges (Hoeksema et al. 2020).

2.3 Unboxing the Black Box: What Is the Way Forward?

Soil ecology has been considered a black box for decades. In relatively few years, a fundamental advance has been made in molecular techniques aimed at analyzing the composition of soil microorganisms (Horton and Bruns 2001; Peay et al. 2008; Branco et al. 2017). However, ecological interactions that are well described for aboveground communities still remain practically unknown in the case of belowground organisms. Processes such as competition between mycorrhizal fungi (Kennedy et al. 2007; Kennedy 2010; Mujic et al. 2016), succession (Peay et al. 2011), or the impact of disturbances on the soil community (Rincón et al. 2014; Glassman et al. 2016) require further study to understand not only the species composition but also the functional role and assembly of the soil biotic community and its impact on the plant community.

Furthermore, the role of plant-fungal co-invasions on impacting soil biogeochemistry is largely unknown, despite the abilities of introduced fungi to alter soil nutrient cycling. For example, invasive EMF may have different metabolic activity and nutrient acquisition capabilities than those of native fungal communities, especially in ecosystems naturally lacking EMF. Suilloid fungi that are dominant on pines in early invasions are relatively nitrophobic and thrive in low-N environments, suggesting that they have a high capacity for obtaining N locked in soil organic matter (Wallander and Nylund 1992; Hatakeyama and Ohmasa 2004). Indeed, *Suillus* has relatively high protease and peptidase activity and polyphenol oxidase/peroxidase activity (Talbot and Treseder 2010; Talbot et al. 2015). Efficient N acquisition traits of invasive EMF may enable the mining of N from invaded soils and conversion into aboveground tree biomass. Hypotheses such as these remain untested, yet are important to understand the full effect of plant-fungal invasions on the biogeochemistry of invaded ecosystems.

Invasion scenarios, such as Pinaceae-EMF co-invasions in South America, are ideal models to investigate the consequences of fungal activity like nutrient acquisition and transfer, interspecies interactions, fungal succession, and community assembly by using manipulative experiments involving a low number of plant and microbial species (Hoeksema et al. 2020). The co-invasion model between EMF and Pinaceae trees represents a possibility to test various questions of the ecology of EMF and their role in plant invasions. The ecological characteristics of suilloid fungi, for example, make them relatively easy to manipulate under controlled conditions, making it possible to inoculate them on to pine seedlings and evaluate both the performance of the EMF and of the seedling under different conditions. A possible question to evaluate in the future could be how these symbiotic organisms interact with the plant under different temperatures, humidities, or soil nutrient contents. Since EMF are essential for the invasion of pines, the change in the intensity of the pine-EMF interaction could be evaluated by simulating climate change conditions, in which the role of EM fungi is expected to be fundamental (Rillig et al. 2002; Compant et al. 2010). In turn, the interaction could be evaluated in relation not only to the plant but also to other soil organisms such as saprobic fungi in different soil and plant nutrient conditions (Fernandez and Kennedy 2016) and how this can affect the invasion. The combination of a model that is easy to manipulate, such as that of pines and suilloid fungi, and the availability of molecular tools opens up a range of future questions aimed at better understanding the functionality of belowground processes and their role in plant invasions.

We know that many fungal – and other microbial – species are involved in the mycorrhizal interaction. However, we know little about the different contributions and impact of each of the fungal species. For example, it could be expected that some EMF species can be more beneficial than others for drought tolerance (Gehring et al. 2017), while others may confer a higher growth to the plant under certain conditions. We know that some groups are different, as in the case of suilloids (Policelli et al. 2019), but there are many important factors associated with the roles of these species that can be fundamental to understanding the invasion systems and probably others. Invasive EMF and invasive pines in South America are a unique scenario to test these questions, due to the reduced number of EMF species – and probably reduced functional redundancy – compared to native ecosystems (Hoeksema et al. 2020).

There is increasing interest in how to integrate EMF ecological knowledge into restoration and management of ecosystems (Policelli et al. 2020b). We think that it is necessary to develop management strategies focused on the interaction between invasive plants and soil mutualists. The ecological characteristics of EMF are important for their management, in particular their dispersal capacity. These characteristics have the potential to determine management priorities when making decisions. Managing an interaction between two invasive organisms could be more effective than managing a single species of interest.

Co-invasion is a generalized mechanism for some invasive ectomycorrhizal plants, such as Pinaceae, but the mechanisms by which non-native invasive species invade in relation to their fungal symbionts can change according to the context in which the invasion happens (Bogar and Kennedy 2013; Moeller et al. 2015). In this sense, the case of pine-EMF co-invasions may constitute an exception, since it occurs mostly in regions that lack phylogenetically related species. If closely related species are present, it may be more probable that non-native invasive species will interact with the native soil biota (Tedersoo et al. 2013). The presence of a native host species related to the non-native invader would function as a facilitating factor for the invasion (Moeller et al. 2015) based on its interaction with native fungal symbionts compatible with both the native and non-native hosts. Using other non-native invasive ectomycorrhizal tree species (e.g., species in the Salicaceae or Myrtaceae), it could be experimentally tested whether the non-native species manages to establish and grow in soil from sites dominated by the native species given the presence of native ectomycorrhizal fungi with which it may associate. In turn, this would allow one to test the generality of the co-invasion process for other woody trees outside the Pinaceae (Bogar et al. 2015; Nuñez et al. 2016).

During the last decades, the black box of soil has been opened (largely through the advent of high throughput DNA sequencing of fungal DNA in soil), but we still do not understand much of what is inside and how it is organized. EMF species invasions provide an exciting opportunity for fertile collaborations within – but not limited to – ecologists, taxonomists, and soil scientists. We are starting to identify the EMF species present across the world's soils and to acknowledge how these species are affected by anthropic activities. However, we still do not understand how they interact with each other and how those interactions affect ecosystem biogeochemistry. Key ecosystem processes in which EMF are involved, such as litter decay and carbon sequestration, are still based on abiotic aspects, with less focus on interactions within the organisms that are responsible for the process itself. Moreover, other soil inhabitants such as protists, bacteria, and viruses are still silently waiting inside the black box of soil. This scenario is stimulating and challenging, especially in regions like South America. Soil microbial communities are not necessarily governed by the same rules as aboveground communities. The conceptual emphasis on the importance of functional diversity, together with the advance of molecular techniques – particularly the advantages of -omic approaches – will hopefully disentangle the mechanisms behind the patterns of invasions. This better understanding of belowground processes involved in plant invasions will hopefully be useful for the conservation, restoration, and sustainable use of soil ecosystems.

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Chapter 3
Pseudomonotes tropenbosii, an Endemic Dipterocarp Tree from a Neotropical *terra-firme* Forest in Colombian Amazonia That Hosts Ectomycorrhizal Fungi

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Contents

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3.1 Introduction

Mycorrhiza, the symbiosis between fungi and plant roots, is the most widespread symbiosis occurring in terrestrial ecosystems (van der Heijden et al. 2015). This association results from the coevolution between plants and fungi and has evolved independently several times in the phyla Ascomycota, Basidiomycota, Glomeromycota, and Zygomycota (Rinaldi et al. 2008; van der Heijden et al. 2015). The most common mycorrhizal interactions are the arbuscular mycorrhizas (AM) and ectomycorrhizas (EcM). EcM fungi play an important role in carbon and nitrogen cycling and influence soil structure, ecosystem stability, and productivity (e.g., McGuire et al. 2010; Courty et al. 2010; Averill et al. 2014; Bâ et al. 2014; Fernández and Kennedy 2015; Corrales et al. 2018, 2022). This group of fungi is often more abundant and diverse in sites with nutrient-poor soils, and this suggested that EcM associations can optimize plant nutrition and might facilitate the dominance of symbiotic plants over others (Torti et al. 2001; Corrales et al. 2016b).

The EcM symbiosis was long thought to be rare or even absent from most of the tropics and only present in Asia and Africa associated with dipterocarp and Fabaceae hosts and in neotropical mountain ecosystems associated with Holarctic plant families, such as Betulaceae, Fagaceae, and Juglandaceae (e.g., Lee 1990; Halling 1996; Mueller 1996; Kennedy et al. 2011; Corrales et al. 2016a, b; Tedersoo and Brundrett 2017). In recent years, the knowledge about the occurrence of EcM associations in the Neotropics has improved, evidencing new plant hosts, new EcM fungal lineages, and insights in biogeography and diversity patterns (e.g., Tedersoo et al. 2010b; Henkel et al. 2012; Roy et al. 2016; Vasco-Palacios et al. 2018, 2019; Corrales et al. 2018). In Neotropical lowland rain forests, EcM plants belong mostly to the families Fabaceae (subfamilies Caesalpinioideae and Faboideae), Nyctaginaceae, Polygonaceae, and Cistaceae (Tedersoo et al. 2010b; Henkel et al. 2012; Moyersoen 2012; Smith et al. 2013; Roy et al. 2016; Corrales et al. 2018; Vasco-Palacios et al. 2018). The EcM fungal species richness, host specificity, and distribution vary among EcM fungal lineages, families of hosts, and habitats (Roy et al. 2016; Corrales et al. 2018; Vasco-Palacios et al. 2018; Corrales et al. 2022). For example, a low diversity of EcM fungi and highly specific host preferences occur with *Coccoloba* (Polygonaceae), *Guapira*, and *Neea* (Nyctaginaceae) species. These ECM hosts occur scattered throughout the forest, integrated among a matrix of AM trees, occur in low densities, and do not form dominant patches (Tedersoo et al. 2010b; Alvarez-Manjarrez et al. 2018). A DNA metabarcoding study showed a similar EcM community composition between soil samples from forests where *Coccoloba* and *Neea* are dispersed among a matrix of arbuscular mycorrhizal trees and forests with the host *Pseudomonotes tropenbosii* (Dipterocarpaceae) (Vasco-Palacios et al. 2019). On the other hand, a high EcM diversity has been found associated with Fabaceae and Cistaceae, plants that occur in high density and form monodominant patches (Singer et al. 1983; Henkel et al. 2012; Moyersoen 2012; Smith et al. 2013; Roy et al. 2016; Vasco-Palacios et al. 2018). Mycobionts associated with these plants have a broader host preference (Henkel et al. 2012; Moyersoen

2012; Smith et al. 2013; Roy et al. 2016; Vasco-Palacios et al. 2018). The EcM associations may contribute to the presence of monodominant tropical forests on sites with nutrient-poor soils, which may be due to the fact that EcM fungi can optimize plant nutrition through different mechanisms (Torti et al. 2001; Henkel et al. 2005; Corrales et al. 2016a, b; ter Steege et al. 2019). However, patterns about monodominance are not consistent even between host plants of the same genus, and those can range from monodominant *Dicymbe corymbosa* (Fabaceae) forests in the Guyana shield to partially dominant stands (25%) of *Dicymbe uaiparuensis* in white-sand forests in western Amazonia (Henkel et al. 2002; Vasco-Palacios et al. 2018).

In the 1990s *Pseudomonotes tropenbosii*, a dipterocarp tree, was discovered in *terra-firme* forests in the Colombian Amazon basin. *Pseudomonotes tropenbosii* is the only Neotropical species of the family Dipterocarpaceae and a monotypic genus in the subfamily Monotoideae (Londoño et al. 1995). This tree occurs in small patches of *terra-firme* forests in the Amazonian region in Colombia, and its discovery emphasized a phytogeographical link of the Colombian Amazonian area with Africa and Madagascar (Londoño et al. 1995; Morton et al. 1999). Dipterocarpaceae is a family of mainly tropical lowland rainforest trees with approximately 16 genera and 695 species (Christenhusz and Byng 2016). The family is widely distributed in Africa and South and Southeast Asia, but only one species occurs in northern South America (Lee 1990; Londoño et al. 1995; Brearley 2012; Bansal et al. 2022). Recently, a boreotropical migration or transatlantic dispersal origin has been proposed for this neotropical species (Bansal et al. 2022). The ectomycorrhizal symbiosis is an ecological feature of at least 61 species of the family, but the ectomycorrhizal fungal diversity of even Asian dipterocarps is still poorly known (Brearley 2012; Diédhiou et al. 2014; Corrales et al. 2022). In a 6-year survey, 296 species of EcM-taxa have been recorded in tropical rainforest of Peninsular Malaysia, and a high number represented undescribed species (Lee et al. 2003). In previous studies in forests with *P. tropenbosii*, at least five putative EcM fungal taxa have been found (Vasco-Palacios et al. 2005; López-Quintero et al. 2012).

Terra-firme forests cover about 80% of the total area of Amazonia and occur in upland and non-flooded areas (ter Steege et al. 2000). These forests occur on acidic sandy to clayey soils that are well drained and have a relatively high cation exchange capacity and a high amount of available phosphorus (Peñuela-Mora 2014; Quesada et al. 2010). Notably, the *terra-firme* forests harbor the world's most diverse tree communities, with few individuals of each tree species present (Duivenvoorden and Duque 2010). In some areas of Colombia Amazonia, *P. tropenbosii* is one of the most important canopy species, representing ca. 17% of the all-canopy individuals (Londoño et al. 1995; Appanah and Turnbull 1998; Parrado-Rosselli 2005). This endemic tree presents a highly restricted distribution with only few populations known from the Colombian territory. These populations grow in small patches of less than 10 ha and are surrounded by *terra-firme* mixed forests or flooding forests (varzeá).

In this study, we explore the diversity of EcM fungi of the endemic dipterocarp species *Pseudomonotes tropenbosii*. The EcM status was confirmed using molecular evidence using the rDNA ITS region employed for fungal identification and the plastid trnL intron for plant identification. We also asked the following questions: (1) Does *P. tropenbosii* harbor a unique EcM fungal species? (2) Does the EcM fungal community exhibit spatial differences? To answer those questions, we performed an exploration of environmental samples from aboveground (sporocarps) and belowground (root tips).

3.2 Materials and Methods

3.2.1 Study Site

The field work was performed in three areas of the Colombian Amazonian region. Two populations were studied in the Middle Caquetá (MC) region in the north of the Amazonia department (Fig. 3.1). In this region, four major forest units have been recognized: floodplain forests, white-sand forests, *terra-firme* forests, and secondary forests (Parrado-Rosselli 2005). The communities of *P. tropenbosii* are present in *terra-firme* forests with a flat to undulating topography with valleys and hills of

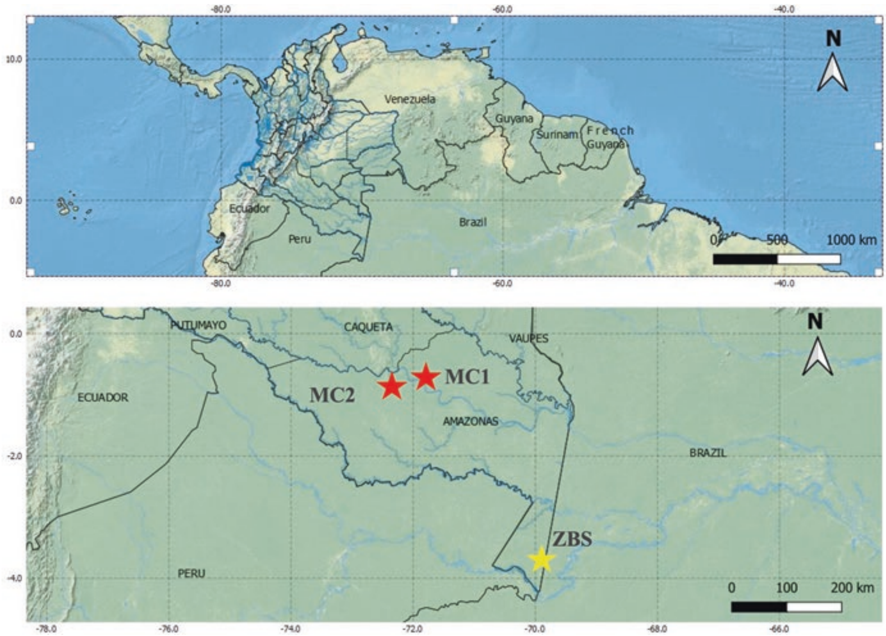

Fig. 3.1 Location of the study sites. The biological station El Zafire (ZBS) in the Southeast Amazon Department in Colombia (yellow start) and the Middle Colombian Amazon plots Peña Roja (MC1) and Puerto Santander (MC2) (red start)

20–40 m height. Soils in this area are well drained with low mineral nutrient content consisting of sands to clays of the Amazonian Upper and Lower Tertiary Sedimentary Plain unit (Parrado-Rosselli 2005). In MC, two sites were visited that are 34 km apart from each other. The first site, called MC1, is about 50 km downstream along the Rio Caquetá near the locality of Peña Roja (00°34′ S, 79°08′ W) in a previously established 1 ha plot of *terra-firme* forests (Fig. 3.6a). The population of *P. tropenbosii* occurred at the top of a hill, and there is no evidence of a broader distribution of the dipterocarp trees beyond this hill. At this site, soils were covered by a deep layer of litter mixed with fine roots. The second site called MC2 is located close to the village of Puerto Santander (00°39′ S, 72°23′ W). Here, the populations grew around the stream of Morelia, and the hills have steeper slopes with smaller and obtuse tops. At this site a thin layer of litter occurs due to the steeper slopes. Both sites are at 200–300 m a.s.l elevation. The vegetation occurring in this landscape unit is characterized by a high species richness. In some areas Mimosaceae, Fabaceae, Lecythidaceae, Arecaceae, and Dipterocarpaceae are partially dominant, with *P. tropenbosii* accounting around 17–19% of canopy trees (Castaño-Arboleda and Betancur 2004; Parrado-Rosselli 2005). The mean annual temperature is 26 °C, and the average annual rainfall is 3060 mm (Duivenvoorden and Lips 1993). Although the region does not have a marked dry season, rainfall decreases between December and February. These localities were visited 1 week per year, between 2010 and 2013. An extra site, called Meta and located close to the Middle Caquetá area, was visited once due to the distance and difficulty of access. This locality was located at 00°45′ S, 71°36′ W and presented a similar climatic condition as MC.

A third population was near the Zafire Biological Station (ZBS) in a previously established 20 ha plot in *terra-firme* forests (coordinates 4°00′ S, 69°53′ W) (Fig. 3.1). The ZBS is located in the south of Colombia and 27 km north of Leticia (Amazon department), close to the border with Brazil, and this location is separated by 420 km from the MC region. The mean monthly rainfall is 277 mm with a drier period from June to September (mean monthly rainfall of 189 mm) and a rainy season from October to May (mean monthly rainfall of 323 mm). The mean temperature is about 26 °C and does not fluctuate significantly during the year. The relative humidity is high with an annual average of 86% (Jiménez et al. 2009; Peñuela-Mora 2014). This area belongs to the Tertiary Sedimentary Plain unit (Proradam 1979; Herrera 1997), which probably originated from the Guiana Shield (Jaramillo et al. 2010). Soils are sandy and are mainly composed of quartz, well drained, and strongly acidic (Jiménez et al. 2009). The terrain is slightly hilly with elevations ranging from 80 to 120 m a.s.l., with a hardpan at a 90–100 cm depth (Quesada et al. 2010). Four major types of forests were present in ZBS, i.e., floodplain forests, white-sand forests, transition forests, and *terra-firme* forests (Jiménez et al. 2009). They all represented primary forests with no evidence of human disturbance, except for hunting. In this area, *P. tropenbosii* trees were only present in a small patch, less than 1 ha, with low abundance of only 20 trees of *P. tropenbosii* occurring in the plot. This plot was sampled four times during the rainy season, between March 2012 and November 2014.

3.2.2 Sporocarp's Sampling

For the sampling of sporocarps, one plot of 0.1 ha was established per site. Sporocarps were collected from each plot with a preference for those that belong to putative EcM taxa. The specimens were photographed in situ. From all specimens, macromorphological characters were described including fresh color according to the *Methuen Handbook of Colour* (Kornerup and Wanscher 1978), macrochemical tests were performed, and spore prints were obtained when possible (Largent et al. 1977; Franco-Molano et al. 2005). A small piece of the sporocarps was preserved in 2% cetyltrimethylammonium bromide buffer (CTAB) for further DNA extraction (Vargas-Estupiñan et al. 2017). The specimens were dried in the field in a sealed container with silica gel. All collections were deposited in the herbarium of the University of Antioquia (HUA).

3.2.3 Root Samples

Soil core samples were randomly collected within a few meters from the stem of 20 trees of *P. tropenbosii* per site. Soil samples were taken from the 0–15 cm upper soil layer. Samples were air-dried, and root tips were examined by light microscopy for the presence of a fungal mantle. Different EcM morphotypes were separately stored in 2× CTAB buffer for further molecular analyses.

3.2.4 Molecular Analyses

DNA from sporocarps preserved in 2% CTAB buffer was extracted using the PrepMan Ultra buffer (Applied Biosystems, Foster City, CA), followed by purification with JETquick general DNA cleanup columns (Genomed; Germany) according to the manufacturer's instructions. The fungal internal transcribed (ITS) region of the ribosomal DNA (rDNA), including the 5.8S rDNA, was PCR-amplified with ITS1F, ITS5, ITS4, and ITS4B primers (Gardes and Bruns 1993; White et al. 1990) following Vasco-Palacios et al. (2014). The D1/D2 domains of the large subunit (LSU) rDNA were also amplified from sporocarps with primers LR0 and LR7 and for sequencing the primers LR0, LR5, and LR7 were used (Vilgalys and Sun 1994). The polymerase chain reaction (PCR) program consisted of 1 cycle of 5 min at 96 °C; 30 cycles of 45 s at 96 °C and 45 s at 52 °C; 1:30 min at 72 °C; and a final extension cycle of 7 min at 72 °C. The amplicons were visualized on a 1% agarose gel stained with GelRed (Biotium Inc., USA). Sanger sequencing was performed using ABI Prism 3700 Genetic analyzer (Applied Biosystems; CA). DNA from mycorrhizal root tips was extracted using the DNEasy plant mini kit (Qiagen, UK) following the manufacturer's recommendations. In cases that the amplification was not successful, PCR amplification and sequencing of the ITS region were repeated using primers ITS 2 and ITS 3 in combination with primers ITS 5 and 4, respectively, which resulted in smaller amplicons (White et al. 1990). To confirm the identity of the plant host, the chloroplast trnL (UAA) intron and trnL-F spacer were amplified and sequenced using the primer combinations trnLc-trnLd and trnLe-trnLf, respectively (Taberlet et al. 2007). All rDNA sequences generated were edited, and a consensus was obtained from forward and reverse sequences with the program SeqMan from the LaserGene package (v8.0, DNAstar).

3.2.5 Sporocarp's Identification

Identification of the sporocarps was based on a combination of a classical morphologically taxonomy and a molecular approached by analysis of the ITS and LSU sequences. Specimens were identified to the species level, and if this was not possible, they were classified as morphospecies. Specimens were assigned to family, genus, and species level based on morphology using keys and descriptions (e.g., Corner 1950, 1970; Bas 1978; Grupe et al. 2016; Pegler and Fiard 1983; Singer et al. 1983; Simmons et al. 2002; Henkel et al. 2011, 2012; Uehling et al. 2012a, b; Wilson et al. 2012). In some cases, we consulted taxonomic specialists, such as Dr. Leif Ryvarden, an expert on *Coltricia* and *Coltriciella*. Major limitations were that sequences from Neotropical EcM fungi are not well represented in the databases. Specimens without sequences, due to our inability to get good sequences, and that could not be named at the species level based on morphology and that were recognized as being a different species of a certain genus were preliminarily treated as distinct morphospecies. The fungal nomenclature was updated using the online version of Index Fungorum (http://www.indexfungorum.org).

3.2.6 Host and Fungal Identification from Root Tips

The consensus sequences obtained were compared using the Basic Local Alignment Search Tool Nucleotide tool (BLASTN) against UNITE database (Unite Community 2017) and the International Sequence Database (NCBI) by using the global search tool, USEARCH7 (Edgar 2010). As Neotropical fungal species are not well represented in the databases, we also used the tool BLASTn against the sequences that we obtained from the sporocarps. The internal transcribed spacer (ITS) sequences were considered to represent the same phylogenetic species, if they differed by <3% across the ITS region (i.e., 97% similarity) and for the genus level if they differed from 3% to 10% (Hughes et al. 2009). The latter taxonomic units were used when the best matches were not informative or when the quality of the DNA from root/tips sequences was too low. Plant host of root tips was identified by comparing sequences with NCBI database using the BLASTn tool (Edgar 2010). Fungal taxonomy and names followed Index Fungorum. All unique sequences were submitted to GenBank (Table 3.1; Suppl. Table 3.1). ITS and trnL sequences from *P. tropenbosii* were generated in this study from plant material collected in the field.

3.2.7 Diversity Analyses

We used the entire set of fungal sporocarp records to compute species rarefaction curves with 1000 permutations and a 95% confidence interval in the Vegan package of R, using the function specaccum (Oksanen et al. 2013; R Core Team 2013). Multivariate permutational analysis of variance with 9999 permutations was implemented in the Adonis routine of the Vegan package (Oksanen et al. 2013) to address the effect of the site on fungal community composition of the aboveground composition. Adonis was calculated on a generated Bray-Curtis distance matrix based on the Hellinger transformation of the abundance of species. Simple Mantel tests were run in Ecodist package of *R* (Goslee and Urban 2007) to determine correlations between sites and fungal community composition. This was based on the abundance of all species per site. Adonis was calculated on a generated Bray-Curtis distance matrix base. The similarity between groups of plots by forest and site were compared by a Mantel test in the Ecodist package of R after 9999 permutations (Goslee and Urban 2007).

3.3 Results

3.3.1 Aboveground Diversity

Seventy-nine morphospecies of EcM fungi were recognized based on sporocarps collected in *terra-firme* forests with the endemic dipterocarp *P. tropenbosii* (Table 3.1; Fig. 3.2b–m). This diversity belonged to 15 lineages of EcM fungi sensu Tedersoo et al. (2010b) and Tedersoo and Smith (2013), namely, /amanita, /boletus, /cantharellus, /clavulina, /coltricia, /cortinarius, /elaphomyces, /entoloma, /inocybe, /pisolithus-scleroderma, /russula-lactarius, /sarcodon, /sebacina, and /tomentella-thelephora, and we include here the lineage /trechispora (Table 3.1). The most species-rich lineages were /boletus and /clavulina (13 species each), /russula-lactarius (12 species), /coltricia (10 species), /amanita (7 species), /cantharellus (6 species), and /cortinarius (5 species) (Fig. 3.3a). These taxa represented 17 families and 29 genera. At the family level, Boletaceae and Clavulinaceae were the most diverse (13 species each), followed by Russulaceae (12 species), Hymenochaetaceae (10 species), Amanitaceae (7 species), Cantharellaceae (6 species), and

Table 3.1 Diversity of EcM species from sporocarps and root tips in *P. tropenbosii* forests

Taxa	Occurrences sporocarps	Occurrences root tips	Site sporocarps[a]	Site root tips	Represent. voucher[b]	Accession number sporocarps and *roots	Similar species, ITS sequences match (>97% similarity) *roots match	Other hosts[c]
Ascomycota								
Elaphomycetaceae								
Pseudotulostoma volvatum O.K. Mill. & T.W. Henkel*	1	1	MC2	MC2x	HUA: 186187	KT724084	*P. volvata* TH8975 JN168735 (97%)*	1, 3
Elaphomyces compleximurus Castellano, T.W. Henkel & S.L. Mill.		1		ZBS			*E. compleximurus* voucher TH8880 JN711441 (97%)	
Basidiomycota								
Amanitaceae								
Amanita campinaranae Bas	2		MC1, MT		HUA: 196961, 196962	KT724057, KT724058		1, 2, 3
Amanita sp.1 sect. Vaginatae	16	1	MC1, MT, ZBS	MC1	HUA: 196966, 196967	KT724059, KT724060, KT757687*	*A.* sp.1 sect. Vaginatae 37JOH KT354675*	2
Amanita sp.2 sect. Vaginatae	14	3	MC1, ZBS	MC1, ZBS, MC2	HUA: 196984, 196985	KT724065, KT724066, KT757691*	*A.* sp.2 sect. Vaginatae 36JOH*	1, 2
Amanita sp.3 sect. Vaginatae	2		ZBS		HUA: 196990, 196991	KT724068, KT724069		
Amanita sp.4 sect. Vaginatae	1		MC1		HUA: 196992			

(continued)

Table 3.1 (continued)

Taxa	Occurrences sporocarps	Occurrences root tips	Site sporocarps[a]	Site root tips	Represent. voucher[b]	Accession number sporocarps and *roots	Similar species, ITS sequences match (>97% similarity) *roots match	Other hosts[c]
Amanita sp.5 sect. Phalloideae	1		ZBS		HUA: 196993	KT724070		
Amanita xerocybe Bas	9	3	MC1, ZBS	MC1, MT	HUA: 186174, 186175	KF937305, KF937306, KT757688*	*A. xerocybe* 1966aAMV KT24071*	1, 2, 3
Bankeraceae								
Sarcodon colombiensis [1]	1	1	ZBS	ZBS	HUA: 186215	KP972654		
Boletaceae								
Aureoboletus sp.1	1		MC1		HUA: 197001			
Aureoboletus sp.2	1		MC1		HUA: 197002			
Austroboletus amazonicus A.M. Vasco-Pal. & C. López-Quint.[1]	8		MC1, ZBS		HUA:186167, 186168, 186169	KF937307, KF937308, KF937309		
Austroboletus festivus (Singer) Wolfe*	4		MC1		HUA: 197009, 197010	KT724085, KT724086	*A. festivus* TH8732 (98%) KT339224	1
Austroboletus sp.3	1		MC1		HUA: 197012			
Boletellus ananas var. *minor* Singer 1983*	1		MC2		HUA: 186186	KT724087		1, 6
Fistulinella campinaranae var. *scrobiculata* Singer	24	2	MC1, MT	MC1	HUA: 197025, 197026	KT757689, KT724090, KT757689*	*F. campinaranae* var. *scrobiculata* 1784AMV 100% KF87835*	5
Singerocomus inundabilis (Singer) T.W. Henkel & Huasbands	1		MC1		HUA: 197035	KT724091	*S. inundabilis* TH10087 98% KT380014	1

Tylopilus pakaraimensis T.W. Henkel		3		MC1, MC2		KT757690*	*T. pakaraimensis* TH8965 97% JN168778	1, 3
Chalciporus sp.1	1		MC1		HUA: 197013	KT724098		
Xerocomus amazonicus Singer*	1		MC1		HUA: 197037, 197036	KT724092, KT724093		1, 3
Xerocomus sp.1	2		MC1		HUA: 197038, 197039			
Xerocomus/*Phylloporus* sp.1	1		MC1		HUA: 197040			
Cantharellaceae								
Craterellus atratoides T.W. Henkel, Aime & A.W. Wilson	4	2	MC2, ZBS	MC1	HUA: 197044, 186192	KT724106, KT724107	*C. atratoides* TH8243 98% KT339209	1, 2
Craterellus atratus (Corner) Watling, Yomyart & Sihan	4	1	MC1, ZBS	MC1	HUA: 186192, 197045	KT724107	*C. atratus* strain TH9203 98–99%	1, 2, 3
Craterellus cinereofimbriatus T.W. Henkel & A.W. Wilson	1	9	MC2	MC1, MC2, ZBS	HUA: 197048	KT354702, KT757706*, KT757707*	*C. cinereofimbriatus* 99% KT354702*	1, 2, 3
Craterellus sp.1	4		MC1, ZBS		HUA: 197049, 197050, 197052			
Craterellus sp.2 negro	1		ZBS		HUA: 197053	KT724108		

(continued)

Table 3.1 (continued)

Taxa	Occurrences sporocarps	Occurrences root tips	Site sporocarps[a]	Site root tips	Represent. voucher[b]	Accession number sporocarps and *roots	Similar species, ITS sequences match (>97% similarity) *roots match	Other hosts[c]
Craterellus strigosus T.W. Henkel, Aime & A.W. Wilson	11	2	MC1, MC2, MT, ZBS	MC1	HUA: 186196, 197057, 197058	KT724109, KT757707*	*C. strigosus* TH9204 type 97% NR120115.	1, 2, 3
Clavulinaceae								
Clavulina amazonensis Corner	18	1	MC1, MC2, MT, ZBS	MC2	HUA: 197076, 197079, 186173	KT724111, KT724112, KT354681, KT757708*	*C. amazonensis* TH9191 99% HQ680356*	1, 2, 3, 4
Clavulina cinereoglebosa Uehling, Aime & T.W. Henkel*	2	1	ZBS	ZBS	HUA: 197086, 197087	KT724113, KT757709*		1, 3
Clavulina cirrhata (Berk.) Corner*	1		MC1		HUA: 197088			3
Clavulina craterelloides Thacker & T.W. Henkel*	10		MC1, MT, ZBS		HUA: 197093, 197094	KT724114		1, 3
Clavulina effusa Uehling, T.W. Henkel & Aime	3		MC1, ZBS		HUA: 197097, 197098, 197099	KT724116		1
Clavulina griseohumicola T.W. Henkel, Meszaros & Aime*	7		MC1, MC2, MT		HUA: 197105, 197106	KT724117		1
Clavulina guyanensis Uehling, T.W. Henkel & Aime*	11		MC1, MC2, MT, ZBS		HUA: 197114, 197115	KT724131		1
Clavulina kunmudlutsa T.W. Henkel & Aime	1		ZBS		HUA: 197118			1, 2, 3

Clavulina nigricans Thacker & T.W. Henkel*	1		ZBS		HUA: 197119	KT724118		1
Clavulina rosiramea Uehling, T.W. Henkel & Aime*	1	1	ZBS	MC1	HUA: 197120	KT724119, KT757710*		1, 3
Clavulina sp.1	2		ZBS		HUA: 197121, 197122, 197123	KT724120		
Clavulina sprucei (Berk.) Corner complex	3		MC1, ZBS		HUA: 197124, 197127			1, 2, 3
Clavulina tepurumenga T.W. Henkel & Aime*	5		MC1, ZBS		HUA: 186178, 197128	KT724121, KF937318		1
Cortinariaceae								
Cortinarius amazonicus Singer & I.J.A. Aguiar		2		MC2		KT757693*, KT757695*		5
Cortinarius sp.1	1		MC1		HUA: 197154			
Cortinarius sp.2	1		ZBS		HUA: 197155			2
Cortinarius sp.3 similar to TH8613	1	1	ZBS	MC2	HUA: 197156, 196902, 196901	KT724155, KT354693, KT354695, KT757694*	*Cortinarius* AMV2240 97% KT354695*	1, 2
Cortinarius sp.4 brown	1		MC1		HUA: 197157			2
Cortinarius sp.7	1		MT		HUA: 197160			
Uncult. *Cortinarius*		10		MC1, MC2		KT757695*, KT757698*, KT757693*	Uncultured *Cortinarius amazonicus* JN168712 98%	1, 3

(continued)

Table 3.1 (continued)

Taxa	Occurrences sporocarps	Occurrences root tips	Site sporocarps[a]	Site root tips	Represent. voucher[b]	Accession number sporocarps and *roots	Similar species, ITS sequences match (>97% similarity) *roots match	Other hosts[c]
Uncult. *Cortinarius*		8		MC1, ZBS		KT757700*, KT757703*	Uncultured *Cortinarius* voucher MCA3928 JN168712 99%	3
Diplocystidiaceae								
Tremellogaster surinamensis E. Fisch	1		ZBS		HUA: 197177	KT724170		1
Entolomataceae								
Entoloma sp.1	2		MC1		HUA: 197161, 197162	KT724166, KT724167		
Hydnaceae								
Sistotrema sp.	1		ZBS		HUA: 197067			
Hymenochaetaceae								
Coltricia barbata Ryvarden & de Meijer*	2		MC1, ZBS		HUA: 186204, 197132	KT724147		6
Coltricia hamata (Romell) Ryvarden	5		MC1, ZBS		HUA: 197135, 197136, 197137, 197138, 197139	KT724142, KT724141, KT354687		2
Coltricia sp.1	1		MC2		HUA: 197143			
Coltricia sp.2	1		MC1		HUA: 197144			
Coltricia sp.4	1		MC1		HUA: 197145			
Coltricia sp. nov.[a]	1		ZBS		HUA: 197146			

Coltriciella dependens (Berk. & M.A. Curtis) Murrill*	1		ZBS		HUA: 197147			3
Coltriciella oblectabilis (Lloyd) Ryvarden	4		MC1, MC2, ZBS		HUA: 197140, 197141, 197142, 197133	KT724137		1, 2, 3
Coltriciella sp. nov.[a]	2		MC1		HUA: 186205, 197134	KT724139		
Coltriciella sp. nov.[a]	7		MC1		HUA: 197153	KT724145		
Inocybaceae								
Inocybe sp.1	10		MC1		HUA: 197168, 197169,	KT724169		
Inocybe sp.2 gris	3		MC1		HUA: 197173, 197174, 197175			
Russulaceae								
Lactifluus annulifer (Singer) Nuytinck		2		MC1		KT757711*	Uncult. *Lactifluus annulifer* JOH71 99% KT354733	1, 2, 3
Lactifluus venezuelanus (Dennis) De Crop		1	MC1	MC1		KT757716*	*Lactarius venezuelanus* G1066 97% KJ786668	2
Lactifluus subgen. *Plinthogalus* sp.5	1		MC1		HUA: 197209	KT724172		
Lactifluus sp.1	1		ZBS		HUA: 197179	KT724171		1
Lactifluus sp.2	2		MC1		468LQ, 474LQ			

(continued)

Table 3.1 (continued)

Taxa	Occurrences sporocarps	Occurrences root tips	Site sporocarps[a]	Site root tips	Represent. voucher[b]	Accession number sporocarps and *roots	Similar species, ITS sequences match (>97% similarity) *roots match	Other hosts[c]
Russula gelatinivelata S.L. Mill., Aime & T.W. Henkel*	11		MC1		HUA: 197183, 186208	KT724176, KT724174,	*Russula* TH7446 98% JN168748	1
Russula puiggarii complex Singer	16	4	MC1, ZBS	MC1, MC2, Meta	HUA: 197196, 197197, 197198	KT724181, KT724182, KT757712*, KT757717*		1, 2, 3, 4, 5
Russula sp. 2	1		MC2		HUA: 197206			
Russula sp.3	1		MC1		HUA: 197207			
Russula sp.4	1		MC1		HUA: 197208			
Russula sp.6 reddish	1		ZBS		HUA: 197205			
Russula sp.7 whitish	1		MC2		HUA: 197180			
Russula sp.8 green	1			MC1	HUA: 197210		*Russula* sp. 8 11JOH 99%	2
Russula sp.9	1		MC1		HUA: 197211			
Uncult. *Lactifluus subiculatus*		4		MC1		KT757713*, KT757711*		1, 3
Uncult. *Russula* sp. nov.3		1		ZBS		KT757714*	*Russula* sp3. AMV2209 99% KT354752	2
Sclerodermataceae								
Scleroderma sp.1	1		MC1		HUA: 197178			
Scleroderma sp.2	1		MT		HUA: 197176			
Sebacinaceae								
Sebacina sp.2		2		MC1		KT757715*, KT354769*, KT757720*		2

Sebacina sp.3	1		ZBS		HUA: 197215			
Tremellodendron sp.1	1		MC1		HUA: 197216	KT724199		
Tremellodendron sp.2	1		MC1		HUA: 197213	KT724200		
Thelephoraceae								
Uncultured *Thelephora* (EcM)	1	2	MC1	MC1, ZBS	HUA: 197212	KT757727*, KT757729*		2, 3
Thelephora sp.1	1		MC1		HUA: 197217, 197218			
Thelephora sp.2 blackish	1		MT, ZBS		HUA: 197219, 197220			
Hydnodontaceae								
Hydnodon thelephorus (Lév.) Banker	14		MC1, MC2, MT, ZBS		HUA: 197067			

Species were identified based on morphology and ITS rDNA sequences

References: All species are assigned to the EcM lineages defined in Tedersoo et al. 2010a and Tedersoo and Smith (2013). Taxa lacking epithets are morphologically distinct species level taxa (morphospecies) as yet unidentified to species; taxa with epithets followed by "ined." have been tentatively determined as new to science but are yet to be formally described

[a] The column sites are the Middle Colombian Amazon region 1 (MC1) and 2 (MC2) and El Zafire (ZBS)

[b] HUA (Corresponds with numbers at Herbario de la Universidad de Antioquia)

[c] Species previously reported from (1) Fabaceae-dominated forest in Guyana (Henkel et al. 2012), (2) WSFs with *Dicymbe uaiparuensis* in Colombia (Vasco-Palacios et al. 2018), and (3) *Pakaraimaea dipterocarpacea* Guyana-Venezuela (Moyersoen 2012; Smith et al. 2013). Novelty: Fifteen species constituted first report to Colombia * and 2 new species recently been described[1]. For the root tips, species-level operational taxonomic units (OTUs) are defined as sequences that are 97% similar across the ITS rDNA sequence region. Taxa defined as genera are 90% similar and family 80%. Taxa labeled with Latin binomials or voucher numbers (HUA) were identified based on ITS matches with sporocarps and with NCBI and UNITE databases. Uncult. is the abbreviation of uncultured.

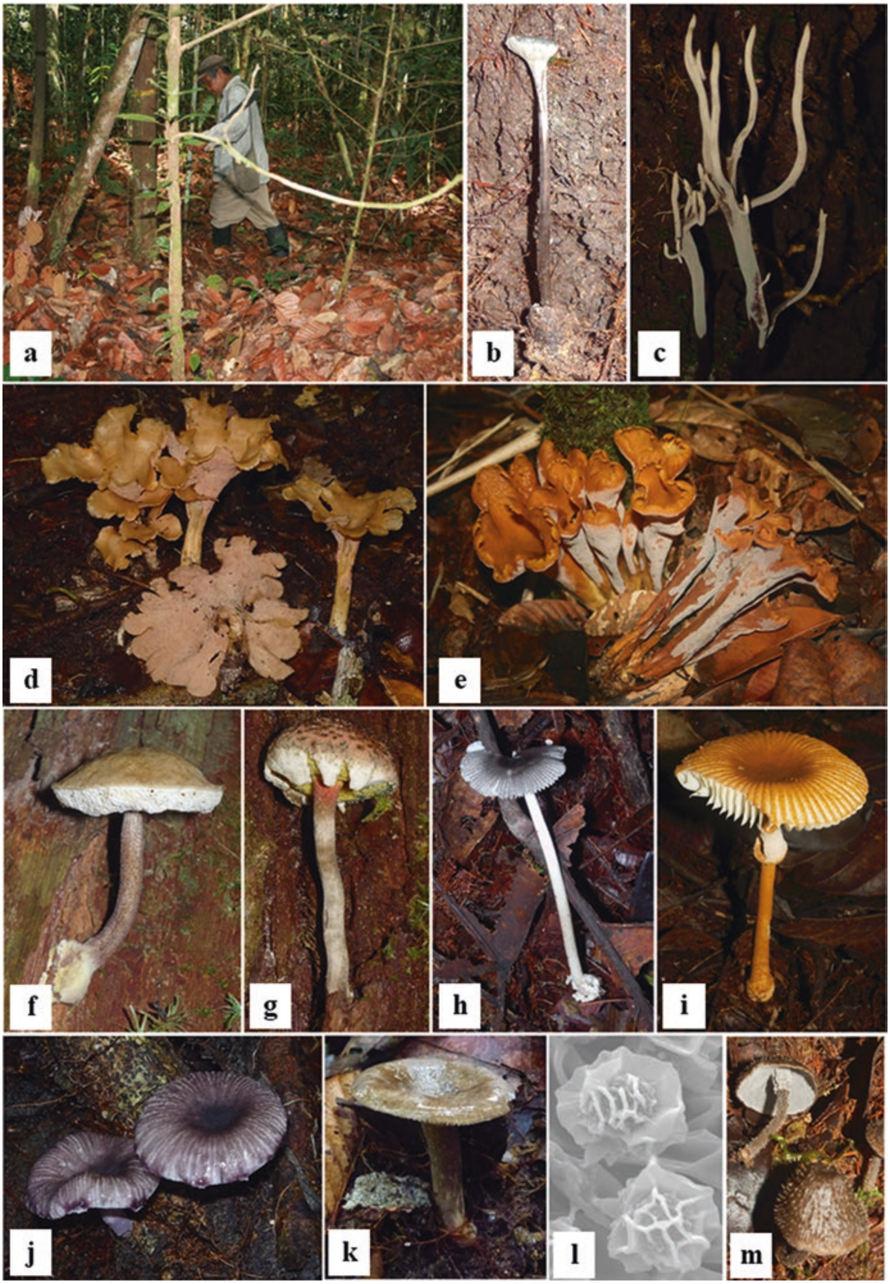

Fig. 3.2 *Terra-firme* forests with *Pseudomonotes tropenbosii* (**a**) and representative ectomycorrhizal fungi collected in the *P. tropenbosii* forest (**b**–**m**). Sporocarps of (**b**) *Pseudotulostoma volvatum*, (**c**) *Clavulina guyanensis*, (**d**) *Hydnodon thelephorus*, (**e**) *Clavulina craterelloides*, (**f**) *Austroboletus festivus*, (**g**) *Boletellus ananas* var. *minor*, (**h**) *Amanita* sp1 secc *vaginata*, (**i**) *Amanita xerocybe*, (**j**) *Russula gelatinivelata*, (**k**) *Lactarius* subgenera *Plinthogalus* sp.; (**l**) basidiospores of *Lactarius* subgenera *Plinthogalus* sp.5 and (**m**) *Craterellus strigosus*. Photo's credits: A. Vasco-Palacios

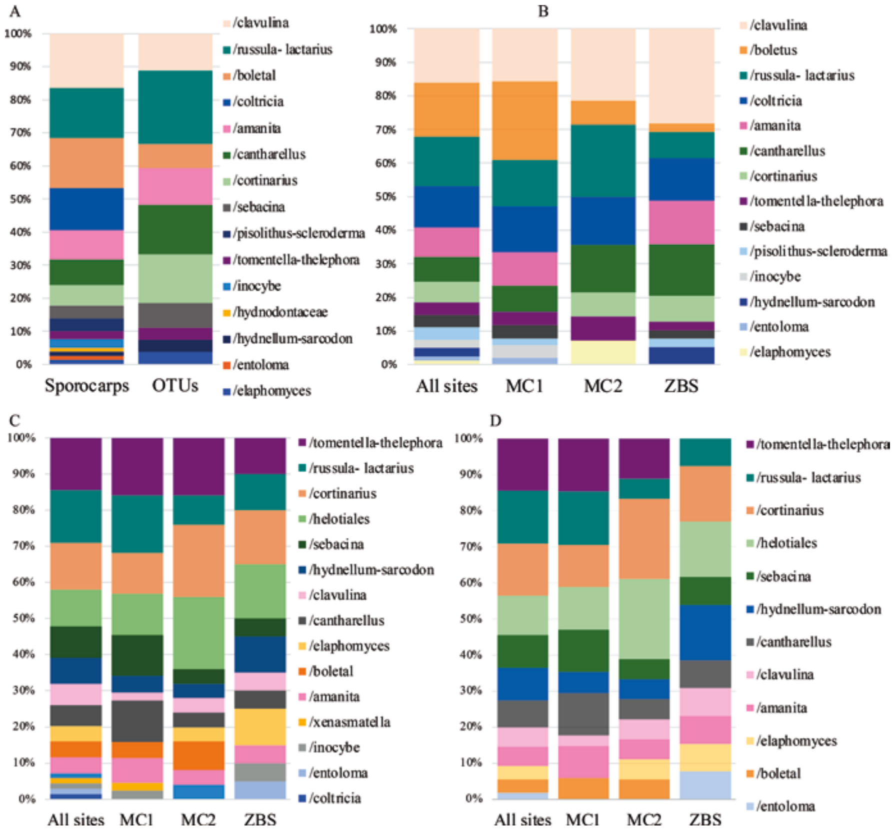

Fig. 3.3 (**a**) Proportion of EcM fungal lineages recovered from sporocarps and from root tips from the *P. tropenbosii* forests. (**b**) Proportion of EcM fungal lineages recovered from sporocarps from the *P. tropenbosii* per site. (**c**) Proportion of EcM fungal lineages recovered from root tips per site (OTUs identified at species and genus level). (**d**) Proportion of EcM fungal lineages recovered from root tips per site (OTUs identified at species level). Sites from the Middle Colombian Amazon region sites MC1 and MC2 and from El Zafire Biological station ZBS. The EcM lineages were defined according to Tedersoo et al. (2010b) and Tedersoo and Smith (2013)

Cortinariaceae (5 species). Additional taxa were found in the Sebacinaceae (3 species); Inocybaceae, Sclerodermataceae, and Thelephoraceae (2 species); Bankeraceae, Diplocystidiaceae, Elaphomycetaceae, Entolomataceae, Hydnaceae, and Hydnodontaceae (represented all by 1 species) (Table 3.1). Fourteen species were new reports for Colombia (Table 3.1), and at least six species were new to science. Two of those have recently been described as *Austroboletus amazonicus* and *Sarcodon colombiensis*, respectively (Vasco-Palacios et al. 2014; Grupe et al. 2016), and the others belonging to genera such as *Russula*, *Coltriciella*, and *Coltricia* are under study.

The presence of species represented by sporocarps varied greatly between sites and visits. In total, 45 species occurred in MC1, 13 species in MC2, and 37 species

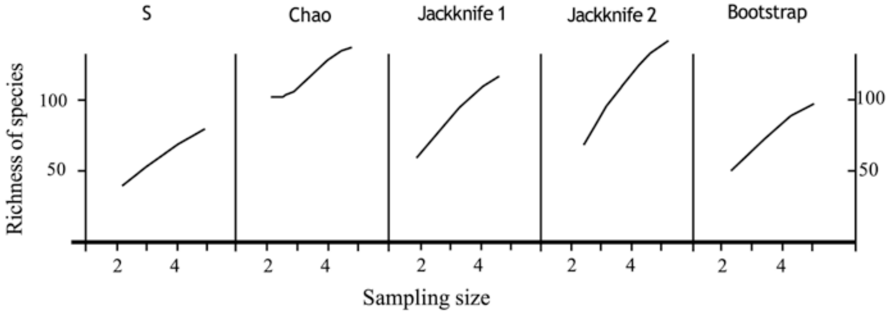

Fig. 3.4 Rarefaction curve with the number of samples (*x*-axis) and number of ectomycorrhizal fungal species recovered from sporocarps (*y*-axis) from Colombian *P. tropenbosii* forests. The curve represents the total number of fungal species with 95% confidence interval, 1000 permutations, and first- and second-order jack-knife, Chao, and Bootstrap estimates of species richness. S is the real observed data

in ZBS (Fig. 3.3b). Nine species, e.g., *Craterellus strigosus* (Fig. 3.2l), *Clavulina amazonensis*, *Clavulina guyanensis*, and two unidentified species of *Amanita* (*Amanita* sp.1 and sp.2), were present in most of the field works and found >10 times. On the other hand, *Fistulinella campinaranae* var. *scrobiculata* and *Russula gelatinivelata* (Figs. 3.2d, e) were also abundant, but only observed in MC1. *Austroboletus amazonicus*, a described species (Vasco-Palacios et al. 2014) from *P. tropenbosii* forests, was collected in MC1 and ZBS.

Although the community of EcM fungi was highly diverse, the species accumulation curve indicated that the EcM fungal diversity was not fully recovered during sampling (Fig. 3.4). The estimated richness suggested that approximately 125–150 species (first-order and second-order jackknife/Chao, respectively) might comprise the total biodiversity of EcM fungi in these *P. tropenbosii* forests (Fig. 3.4). Bootstrap estimates indicated that approximately 100 species are present, which is close to the number of 79 observed species.

3.3.2 Belowground Diversity

The occurrence of ectomycorrhizal fungi on the roots of *P. tropenbosii* corroborated the ectomycorrhizal status of this Neotropical tree. Two hundred eighteen fragments analyzed by DNA sequencing showed fungal sequences (92%), and 177 of those (81%) represented putative EcM fungi. Overall, 26 species were identified (blast match >97%) at species level, 10 genera with 41 sequences at genus level, 11 familias with 51 sequences at family level (Table 3.1 and Suppl. Table 3.1). These EcM fungi belonged to 16 lineages as follows: /amanita, /boletal, /cantharellus, /coltricia, /clavulina, /cortinarius, /entoloma, /elaphomyces, /helotiales, /hydnellum-sarcodon,

/inocybe, /russula-lactarius, /sebacina, /tomentella-thelephora, /xenasmatella, and a /facultative biotrophic saprobe (Fig. 3.3b). Considering only the OTUs that were identified at species and genus level, the most OTUs-rich lineages were /tomentella-telephora, /russula-lactarius, and /cortinarius (8 OTUs each), /helotiales (6 OTUs each), /hydnellum-sarcodon and /sebacina (5 OTUs each), and /cantharellus (4 OTUs) (Table 3.1; Suppl. Table 3.1; Fig. 3.3c). The composition of lineages was similar among MC1, MC2, and ZBS, represented by eight, nine, and ten lineages, respectively (Fig. 3.3d). However, the richness varied between sites with 27 OTUs in MC1, 19 OTUs in MC2, and 14 OTUs in ZBS (Fig. 3.3c, d). The most abundant OTUs were *Craterellus cinereofimbriatus* (8 detections) and unidentified species of *Cortinarius* sp. 866root (10 detections), *Cortinarius* sp. 917root (8 detections), and *Tomentella* sp. 1452root (11 detections), but most of the taxa were detected only twice or once (Table 3.1). Most of the species were not specifically associated with *P. tropenbosii*, e.g., 12 OTUs were also reported previously from roots of *P. dipterocarpacea*, 16 from *Dicymbe* forests in Guyana, and 14 from *Dicymbe uaiparuensis* occurring in white-sand forests in Colombia (Table 3.1). Overall, 15 species were found in both analyses, namely, aboveground as sporocarps and belowground on the root tips of *P. tropenbosii* using DNA (Table 3.1; Fig. 3.5).

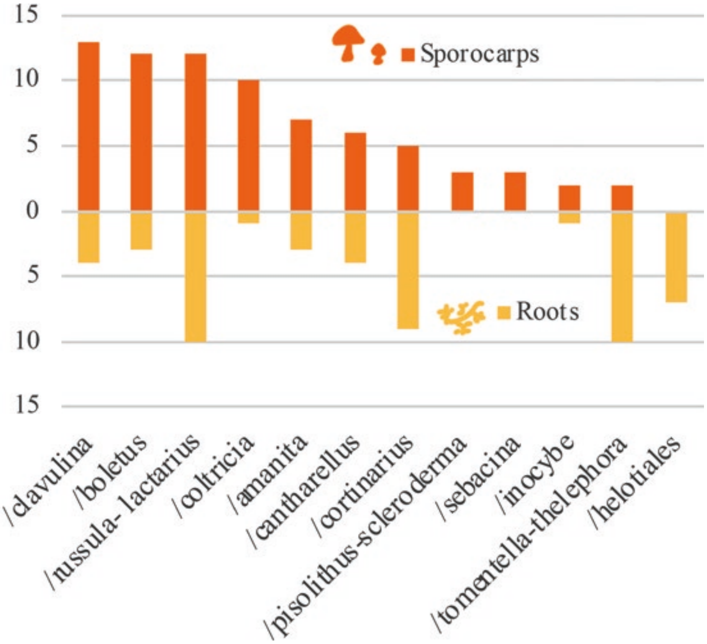

Fig. 3.5 Comparison of abundance between aboveground and belowground diversity. The zero line represents the case where a lineage is equal in belowground (sporocarps) and aboveground (root tips) samples

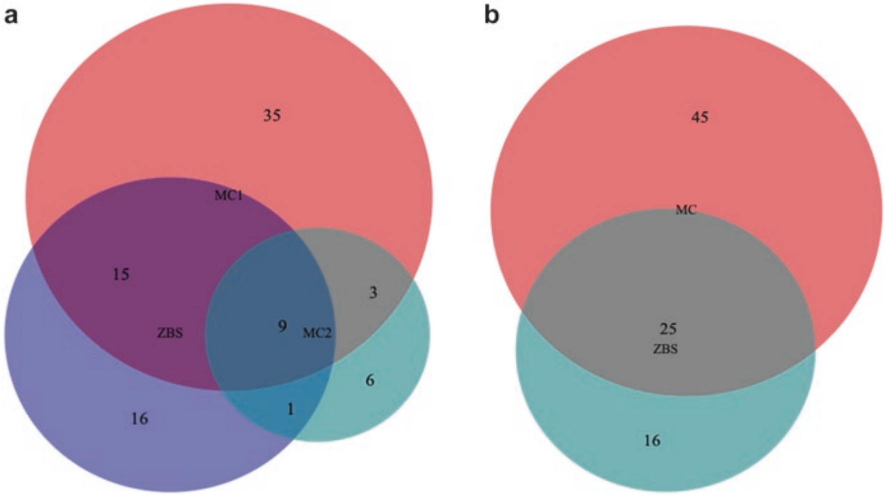

Fig. 3.6 Venn diagrams comparing EcM fungal diversity between Middle Caquetá region sites. (**a**) 1 (MC1) and 2 (MC2) and El Zafire (ZBS), (**b**) between MC sites and ZBS

3.3.3 EcM Fungal Composition

The EcM fungal composition among sites using sporocarp counts revealed significant differences (ADONIS, $P > 0.05$; Mantel $P > 0.05$). Only five species were found in all sites, one species occurred in MC1 and MC2, and 14 species were found in MC1 and ZBS, while no overlap in species was observed between MC2-ZBS (Fig. 3.6a) (ADONIS, $F_{2,3}$ =2.80, $P = 0.001$; MC1-MC2, MC1-ZBS, MC2-ZBS; Mantel $P > 0.001$). In total, 52 species occurred in MC1, 43 species occurred in ZBS, and MC2 showed much less richness with only 13 species. Overall, community composition was most similar between sites MC1 and ZBS that shared 15 species despite the considerable geographical distance between these two forests (Fig. 3.6a). Considering the Middle Colombian Amazon region as a unit, 59 species of EcM fungi were recovered, and 25 species were shared with the geographically separated area ZBS (Fig. 3.6b). The sites MC1 (12 lineages) and ZBS (12 lineages) differed by the lineage /hydnellum-sarcodon, represented by a new species of *Sarcodon*, *S. colombiensis*. In site MC2 only seven lineages were represented. We expected a similar diversity and composition between sites MC2 and MC1, because these two sites are relatively close in distance. MC2, however, presented low EcM diversity and shared only six species with MC1. Thirty-nine species have been previously reported from Fabaceae forests, and twenty-four of those were shared with white-sand forest with *Dicymbe uaiparuensis* in the ZBS area.

3.3.4 Other Taxa Collected Off of the Studied Plots

Twelve species of EcM fungi were recorded from a *P. tropenbosii* forest located at the south of MC1 in a place called Meta, but that was visited only once (Table 3.1). Ten species were shared between the MC and ZBS plots, but *Scleroderma* sp.3 and *Cortinarius* sp.7 were only observed from this site.

3.3.5 Host Plant Identification

The hosts of each of the 98 mycorrhizal roots that corresponded to EcM fungal species were identified as *P. tropenbosii* (83 detections), *Coccoloba* sp. (1 detection), and other plants not reported as EcM hosts (4 detections, e.g., *Brosimun* sp., *Erythroxylum* sp., *Ipomoea* sp., *Protium* sp.). Ten sequences were not informative and could not be used for the identification of the plant host.

3.4 Discussion

This is the first in-depth study on the diversity of EcM fungi associated with the endemic dipterocarp tree *P. tropenbosii* occurring in Colombia Amazonia. Our study confirmed the EcM fungal symbiosis with this Neotropical tree and adds new data on the diversity and distribution of the EcM fungi Neotropical lowland rainforest of the Amazonia region. Here, EcM fungi were common and diverse, both in terms of species and known ectomycorrhizal lineages. A total of 90 species were documented, 79 of the total in the aboveground and 26 species belowground. Only 11 species occurred in both analyses, showing important differences when fungi are studied based on sporocarps or root tips. All of the major EcM records associated with *P. tropenbosii* forests consisted of taxa and lineages that have been reported previously in tropical forests associated with Dipterocarpaceae (i.e., Brearley 2012; Peay et al. 2010; Phosri et al. 2012), Fabaceae (i.e., Henkel et al. 2012; Smith et al. 2013; Vasco-Palacios et al. 2018), and Cistaceae (Moyersoen 2006, 2012; Smith et al. 2013). Members of the /clavulina, /coltricia, /russula-lactarius, and /boletus lineages were particularly abundant in the aboveground counting representing 59% of the species, whereas in the belowground analysis, these were /tomentella-thelephora, /russula-lactarius, and /cortinarius, representing 40% of all OTUs (Fig. 3.5). Discrepancies between EcM fungal diversity estimated from sporocarp surveys versus those based on belowground root tip molecular sampling were observed particularly in the lineages /amanita, /boletales, /clavulina, and /coltricia that were under-represented belowground, whereas /tomentella-thelephora, /helotiales, and /sebacina were over-represented (Fig. 3.5). This bias has been previously reported from temperate and tropical sites (Corrales et al. 2022). In

Fabaceae-dominated forests in Guyana and Colombia, taxa in the /sebacina and /tomentella-thelephora lineages were abundant on root tips, but less frequently recovered from sporocarps (Smith et al. 2011; Vasco-Palacios et al. 2018). Species of *Tomentella* and *Sebacina* have cryptic or atypical sporocarps that may be easily overlooked and poorly recovered as sporocarps during surveys (Smith et al. 2011; Vasco-Palacios et al. 2018). For this reason, it is important to study the fungal diversity both above- and belowground, accompanied by molecular tools, to fully characterize the fungal community.

Considering that our sampling of *P. tropenbosii*-associated fungi remained below of saturation (Fig. 3.3), the cumulative data suggested that the diversity of mycorrhizal fungi associated to *P. tropenbosii* is high. In a previous study of the fungal soil community in PtF forests, 129 OTUS of EcM fungi were detected through 454-pyrosequencing of the ITS2 region (Vasco-Palacios et al. 2019). Incomplete recovery of EcM fungal diversity in our study was corroborated by the notion that 10 OTUs (identified at species level) detected from the roots have not been found as sporocarps and 62 species collected as sporocarps were not detected from the roots of *P. tropenbosii*. These data indicate that more sampling is necessary to fully document the diversity of EcM fungi associated with *P. tropenbosii* in the Colombian Amazon. For example, in Guyana, the species richness associated with host plant legumes in Guyana amounts to more than 170 species over a 13-year sampling period (Henkel et al. 2002, 2011, 2012; Smith et al. 2011). Species richness may also be related to the fact that *P. tropenbosii* does not form monodominant patches or large populations in the Middle Colombian Amazon region. Despite *P. tropenbosii* is one of the most important canopy species, the relative abundance of this tree is not more than 18%, and its populations do not occupy more than a couple of hectares (Appanah and Turnbull 1998; Londoño et al. 1995; Parrado-Rosselli 2005). Henkel et al. (2012) considered that the high α fungal diversity in forests dominated by *Dicymbe corymbosa* in Guyana is because this tree maintains a relative abundance of 60–90% with the presence of high numbers of seedlings and saplings in the Guyana plots, which results in an ample substrate availability of EcM fungi. Considering that *P. tropenbosii* is not monodominant, this implies that less roots are available for EcM fungi to colonize, and therefore more competition is at stake resulting in a lower species richness compared with other tropical ectotrophic ecosystems (Smith et al. 2011; Alvarez Manjarrez et al. 2018).

When doing more studies in Neotropical ecosystems, it is getting clearer that our knowledge on the distribution patterns of fungal species has changed, and ectomycorrhizal fungal species present in the Amazonian region seem to occur associated with a wide range of plant hosts. Based on the analyses we did, species were found to be conspecific with those occurring in other EcM forests dominated by Fabaceae (43% of the species shared) and Cistaceae (14% of the species shared) occurring in tropical lowland rainforests tropical from Colombia, Guyana, and Venezuela (Henkel et al. 2012; Moyersoen 2006, 2012; Smith et al. 2013; Vasco-Palacios et al. 2018). Some examples are *Amanita xerocybe*, *Craterellus atratus*, *Clavulina kunmudlutsa*, *Clavulina sprucei*, and *Coltricia oblectabilis* that were present in the Colombian *P. tropenbosii* forest as well as the other forests (Henkel et al. 2012;

Moyersoen 2006; Smith et al. 2013; Vasco-Palacios et al. 2018). Although a high diversity of EcM fungi was shared between *P. tropenbosii* and Fabaceae forests, this pattern is not homogeneous and seems determined by factors that we do not understand yet. For example, in the ZBS region, only 21 species are shared between *terra-firme* forests with *P. tropenbosii* and white-sand forests with *Dicymbe*. Also, considerable differences between these two forests were detected in a metabarcoding analysis of soils, despite the two populations were only 5 km apart (Vasco-Palacios et al. 2019). Furthermore, the same study revealed a similar EcM fungal community from nearby forests with few individuals of *Coccoloba* and *Neea* occurring scattered within a matrix dominated with plants with AM symbiosis (Vasco-Palacios et al. 2019), suggesting that those are an important host to focus on in future diversity and biogeographic studies.

In addition, the distribution of some fungal species that were previously considered to be restricted to the Guiana Shield region was extended in this study. This is the case of *Pseudotulostoma volvatum*, an ectomycorrhizal ascomycete described from *Dicymbe* forests in Guyana that was collected once (MC2) and detected from root tips (Table 3.1, Fig. 3.2b). Particularly relevant is the case of *Clavulina*, a globally distributed genus. These taxa present a high richness recorded in *Dicymbe*- and *Pakaraimaea*-dominated forests in Guyana. Hence, this locale was proposed as the diversification spot for this lineage (Uehling et al. 2012a, b). In *P. tropenbosii* forests, 13 species collected as sporocarps represented 70% of the total of species reported from Guyana (Henkel et al. 2012). Three species were also detected from roots, namely, *Clavulina amazonensis*, *Clavulina cinereoglebosa*, and *Clavulina rosiramea* (Table 3.1). *C. amazonensis* has been found also in central Brazil, Ecuador, and Venezuela (Corner 1970; Petersen 1988; Tedersoo et al. 2010b; Wartchow 2012; Vasco-Palacios et al. 2018).

Russulaceae has previously been suggested as the most diverse in tropical ecosystems (De Crop et al. 2017). In our study, the family was represented by 14 species, which is a low number of species when compared to observations made in other Neotropical studies. In Guyana, for example, 35 species have been reported from Cistaceae and Fabaceae forests after 2 and 13 years of sampling periods, respectively (Henkel et al. 2012; Smith et al. 2013). In Colombia, 15 species were found after 2 years of collection in white-sand forests with *Dicymbe uaiparuensis* in El Zafire (Vasco-Palacios et al. 2018). Among the species, *Russula gelatinivelata*, a species described from the greater Guiana Shield (Miller et al. 2012), was abundant at both MC sites, but it was not found in the plot at the ZBS site. Despite the low diversity found, eight morphospecies collected in this study could not yet be identified at species level and likely correspond to new species (Table 3.1). So far, only two species of ectomycorrhizal fungi collected in forests with *P. tropenbosii* have been described, namely, *Austroboletus amazonicus* and *Sarcodon colombiensis* (Vasco-Palacios et al. 2014; Grupe et al. 2016). Taxonomic work on some of these potentially new species is ongoing (e.g., *Amanita*, *Coltricia*, *Coltriciella*, and among Russulaceae). An interesting group is this of *Coltricia*-*Coltriciella*, seven species were identified from *P. tropenbosii* forests, and three of those correspond to new taxa. Another genus that should be studied is *Cortinarius*. This is one of the most species-rich genera in Holarctic and Austral regions, and it is rare in tropical areas (Moyersoen 2012; Tedersoo et al. 2010b). However, in this study six morphospecies of *Cortinarius* were identified from sporocarps, which is the same number of species recorded by Henkel and collaborators from *Dicymbe*- and *Pakaraimaea*-dominated forests in Guyana (Smith et al. 2013), and 11 species were recorded by Moyersoen (2006) from Venezuela. Based on ITS sequences only, one species that was obtained as sporocarps and four that have been obtained as OTUs at species level from roots, were shared between *P. tropenbosii* and the Fabaceae and/or *Pakaraimaea* hosts (Table 3.1). Thus, we assume that the diversity of this genus in the Neotropics may be extensive.

The fungal community composition presented significant differences across sites (Fig. 3.3b–d). This is consistent with some other studies in tropical ecosystems (e.g., Peay et al. 2010), where the differences in fungal communities in the tropics may correspond to the patchiness of soil properties (Bahram et al. 2013). Our results suggested that variation in EcM fungal communities may be due to soil composition and landscape structure, features that need to be studied in detail in the future and may drive the species turnover in tropical lowland forests. In general, *P. tropenbosii* populations form relatively isolated stands surrounded by nectotrophic mixed forests. In MC1, the site that presented the highest diversity of EcM fungi, the trees were located at the flatter part of the hills, while in MC2 that presented less diversity, the topography is more irregular, and trees were located at steeper slopes, and for this reason less litter accumulated (Parrado-Rosselli 2005). In ZBS, the density of the trees of *P. tropenbosii* is less than in MC, but the topography is more similar to MC1 and the litter layer around the *P. tropenbosii* stands is considerable, and 43 species of EcM fungi were recovered from this site. The Northern MC area is separated from ZBS by approximately 420 km. No populations of *P. tropenbosii* are known to occur between these two areas, but it does not mean that they do not exist, as the Amazon region in Colombia is vast with large areas that remain to be explored. In addition, EcM fungal species also could form association with other plant hosts (Fabaceae) even with those present in AM-dominant forests such as Nyctaginaceae and Polygonaceae, favoring fungal dispersal between *P. tropenbosii* patches. A long-distance dispersal is also a mechanism that may explain the broad distribution of EcM fungi in the Amazonian region. However, small-scale variations in the soil types and structure, landscape variations, varying abundances of the hosts, and litter quality, among others, also play important roles to enhance the alpha and beta diversity of EcM fungi at small spatial scale, but this also needs to be addressed in further studies.

3.5 Conclusions

Evidence of the EcM status of *P. tropenbosii* was provided in this study, and this may suggest a Gondwana origin of this ectomycorrhizal symbiosis of members of the family Dipterocarpaceae. *Pseudomonotes tropenbosii* presents a high richness of EcM symbionts. Ninety EcM fungal species are reported in this study, even though the trees do not form large canopy forests in dense stands, as occurring in Guyana with Fabaceae and Cistaceae. Discrepancies between EcM fungal diversity estimated from sporocarp surveys versus those based on molecular sampling of belowground root-tips demonstrates the importance to use complementary inventory tools and surveys to fully characterize the fungal communities present. We found differences in the diversity and richness of species across sites, independent of the abundance of *P. tropenbosii* and the proximity of the sites, suggesting that environmental differences among sites are important in structuring the EcM fungal communities. Soil composition has been found to explain the structure of such fungal communities in the amazonian region (Vasco-Palacios et al. 2018), and this factor should be considered in the future to understand how it influences the EcM communities associated to *P. tropenbosii*. Similarities in the EcM fungal community between geographically remote places suggests that long range fungal dispersal and the low host-specificity, are important mechanism for the distribution of these fungi in the region, that is dominated by AM-forests. In this vast region, an enormous number of fungal species form ectomycorrhizae with plants of the Cistaceae, Dipterocarpaceae, and Fabaceae families. Lack of specificity allows fungal species to have a wide distribution even though hosts are present in small populations, often scattered throughout the region. More research is needed on the factors driving the diversity, distribution, and community structure of EcM fungi in neotropical lowland forests, as well as their relationship to plant hosts and their functional roles.

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Chapter 4
Arbuscular Mycorrhizal Fungi in the Colombian Amazon: A Historical Review

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4.1 Introduction

Arbuscular mycorrhizal fungi (AMF) are one of the most studied symbiotic fungal groups with a widespread global distribution (Smith and Read 2008; Davison et al. 2015). Compared to other fungi, AMF diversity estimation tends to be relatively low, which has been argued to be the result of low diversification rates (Öpik et al. 2010; Davison et al. 2015; Perez-Lamarque et al. 2020). Besides, the study of this group is still troublesome due to the difficulty of delimiting its taxonomy, further influencing the quantification of its richness and the construction of a robust phylogeny (Perez-Lamarque et al. 2020; Kolarikova et al. 2021). Despite these issues, around 300 morphologically defined (Öpik and Davison 2016) and more than 1000 molecularly defined taxa (Öpik et al. 2014) have been globally identified.

However, these numbers are not the results of an evenly distributed study of global AMF diversity (Davison et al. 2015), since some regions, such as Europe and North America, have been more intensively studied compared to tropical ones (Öpik et al. 2010; Marin and Bueno 2019). Beyond the sampling bias, it is expected that arbuscular mycorrhizal (AM) fungal communities show biogeographic differences caused by their limitations for autonomous dispersion, their close dependency on host plants, as obligate plant symbionts, and their susceptibility to particular environmental factors such as climate and soil properties (Davison et al. 2015, 2021; Paz et al. 2020). Thus, similarly to other fungi, soil properties, plant diversity, and climate are expected to be the key drivers of AMF variability among different regions (Kivlin et al. 2011; Davison et al. 2021).

The Amazon region is recognized as one of the world's largest and most diverse tropical rainforests, with a current alarming decrease in their conservation status (Amigó 2020). The diversity of AMF in this particular region has been studied in a few locations (Lopes Leal et al. 2009; Stürmer and Siqueira 2011; de Oliveira Freitas et al. 2014; León 2015; Reyes et al. 2018; Peña-Venegas et al. 2019), although this list is not exhaustive, given that unpublished and "grey" literature in the region exists. The Colombian Amazon is one of its most unknown areas within the Amazon region and due to historical, political, and social reasons. Consequently, in 1995 the Sinchi Amazonian Institute of Scientific Research of Colombia started studying AMF and their symbiosis with plants in this region. The Colombian Amazon region covers 483,164 km^2 of the upper Northwestern basin, representing 10% of the whole Amazon basin (Sinchi 2009). In this area, there are different types of soil, going from very weathered and developed soils with low fertility such as Arenosols and Podzols (Ultisols in USDA classification), followed by acidic infertile Ferralsols (Oxisols in USDA classification) and Cambisols (Inceptisols in USDA classification), to the fertile Fluvisols and Anthrosols (WRB 2015).

Given the urgent pressure to preserve the Amazon tropical rainforest, a deeper understanding of their ecosystems is needed. In this book chapter, we present a historical review of the existing information in the study of AMF in the Colombian Amazon. In particular, we review the historical progress of the AM research and present novel studies about AMF diversity in the region, analyzing their biogeographical variation, the effects of deforestation, and the AM association in two plant species with a relevant local economic value. Finally, we identified key knowledge gaps and developed recommended steps in its further exploration, which can ultimately increase our regional functional understanding. Particularly, we want to highlight the emerging challenges regarding data and study compatibility in relation to the fast evolution of molecular analytic techniques for this fungal group in the last decades. This review expects to be a solid base to build future knowledge about this fascinating and relevant group of organisms in the Amazon.

4.1.1 A Historical Approach to Study AMF in the Colombian Amazon Region

4.1.1.1 Morphological Descriptions in the Colombian Amazon

Colombia was one of the first countries in South America to study AMF. Between the 1970s and 1980s, Colombia experienced a boom in the study of AM association with the arrival of North American and European researchers focused on improving the production of key tropical crops (Sieverding and Leihner 1984a, b; Sieverding and Howeler 1985). The first two reports on AMF from the Colombian Amazon date from 1988, when the AMF community associations with the native fruit tree “inchi” (*Caryodendron orinocense*) was determined (Pinto 1988; Pinto and Pedraza 1988). After this, other studies about the description of AMF community comparing natural and anthropic disturbed soils (pastures and traditional agriculture) were done in the region (Pinto 1992; Peña-Venegas 2001; Restrepo 2006). These five documents used classical methodologies to study AMF. The methodologies included the collection of soils in the field, the isolation of spores from these soils (only Restrepo 2006 used trap plants for previous multiplication of AMF propagules) by wet sieving and centrifugation in a sucrose gradient (Sieverding 1991), and the direct description of spores for their taxonomic identification.

These preliminary results indicated for the first time the dominance of the genus *Glomus* in Amazonian soils, now corroborated by other works such as Lopes Leal et al. (2013), Peña-Venegas and Vasco-Palacios (2019), and Stürmer and Siqueira (2011), which report the presence of the sporocarp-forming species. Additionally, these results indicated that the number of AMF spores was higher in places where the natural forest was cut down and replaced by pastures than in undisturbed or moderately disturbed forests or indigenous shifting cultivation plots. High spore numbers in pastures and disturbed ecosystems are consistently reported in Amazon soils (Stürmer and Siqueira 2011; Lopes Leal et al. 2013). One possible explanation for this pattern might be related to the link between AMF sporulation and soil pH;

AMF preferred to sporulate in less acidic soils (Lopes Leal et al. 2013), which corresponds consistently to pastures (5.0–5.5 pH values) rather than to more acid forest soils (3.5–4.0 pH values).

From 1995 to 2004, the Sinchi Institute devoted its efforts to collecting spores from soil samples in the Colombian Amazon and preserving them in slides with lactoglycerine and polyvinyl-lactoglycerine. After the collection, the assignment to already described AMF morphotypes followed, which was done by using prototypical spores with clear and key spore characteristics unique to each morphotype as a proxy to distinguish AMF taxa. However, the AMF spore morphotypes collected included many undetermined morphospores that were not reported in Schenck and Perez (1990), considered the most important reference catalogue of the AMF morphotype description for Colombia. The two key factors that likely hampered the determination of the spore morphotypes to AMF genus and species were (1) the low number of spore samples per putative morphotype and (2) the difficulty of obtaining quality spore samples with the distinguishable morphological features needed for taxonomic determinations. Those difficulties resulted from the samples being collected directly from the soil and, therefore, with different stages of spore development. To reduce those problems, the use of trap (trap culture technique) was recommended, from which it was possible to obtain enough and fresh AMF spores for each particular morphotype (Sieverding 1991). Many (spore) trap cultures using *Phaseolus vulgaris* as a host plant were developed during this time. However, this method was very time-consuming and has been a limitation for most mycorrhizal researchers, particularly the ones who were not entirely devoted to the description of AMF morphotypes. It is important to mention that even if trap culture is a good option to multiplicate and obtain young spores, it has some limitations: not all AMF species sporulate, the biology of each species could include different physiological requirements, and the plant host used could influence AMF sporulation, favoring some morphotypes and limiting others (Jansa et al. 2002; Redecker et al. 2003; Sanders 2004).

In 2005, after the scientific visit to Cuenca's laboratory at the Instituto Venezolano de Investigaciones Científicas (IVIC) in Caracas, Venezuela, many more morphotype identities were reviewed and finally determined. In 2006, as a consequence of this collaborative work, including the revision of more than 400 samples, an illustrated catalogue with the most common AMF spore morphotypes from the Colombian Amazon region was published (Peña-Venegas et al. 2006). This catalogue included 31 AMF spore morphotypes: 20 *Glomus* morphotypes, 5 *Acaulospora* morphotypes, 3 *Scutellospora* morphotypes, and 1 *Archaeospora*, *Entrophospora*, and *Gigaspora* genera morphotype, respectively.

Although these new descriptions were helpful for some morphotypes found in the Colombian Amazon, many unidentified morphotypes remained unknown in the Sinchi Institute database. Most unknown morphotypes were from the *Glomeraceae*, the most common and diverse family in the Amazonian soils. *Glomeraceae* is a difficult taxonomic AMF group due to the reduced spore morphological features to differentiate one species from another. Furthermore, *Glomeraceae* was one of the AMF families that were recently reorganized into 14 genera (Blaszkowski et al. 2021). The AMF diversity estimation was based only on field-collected spores, which were not always in good condition for taxonomic descriptions. As each sampling location was normally visited only once, the AMF diversity described was based on the sporulating species at that specific moment. Additionally, the information about their associated plant species was lacking in these spore descriptions from the samples. In this regard, it seems that the commonly accepted assumption associating the sporulating rhizospheric AMF with the root colonizing AMF may be reconsidered (Saks et al. 2014; Valera-Cervero et al. 2015; Peña-Venegas et al. 2019).

4.1.2 Molecular AMF Descriptions in the Colombian Amazon

In the 1990s, molecular techniques to study AMF became more available and started to appear in the literature. The first works indicated that the taxonomic classification of AMF needed re-evaluation according to the natural phylogenetic organization of Glomeromycota (Schüßler et al. 2001). In the following years, different authors made efforts to harmonize molecular and morphological AMF classification to produce a unique taxonomic key for both spore morphotypes and molecular operational taxonomic units (OTUs) (Sieverding and Oehl 2006; Spain et al. 2006; Walker et al. 2007; Oehl et al. 2008, 2011a, b; Sieverding et al. 2014).

A key step was started in 2004 with the arrival of the student Daniela León to the Sinchi Institute. To define a protocol for Amazon soils and obtaining better results to estimate AMF diversity, the different molecular methodologies published until that time were reviewed and tested. Initially, León tested 14 different protocols, including 3 to isolate AMF DNA from spores and 4 protocols to obtain AMF DNA from roots. We tested eight specific primers to obtain AMF sequences (ARCH1311; ITS4; ITSIF; GLOM5.8R; LETC1670; ACAU1660; GIGA5.8R; and NS5), following Redecker (2000) in five different combinations (León 2006). This work made it possible for the first time to obtain AMF sequences from manioc (*Manihot esculenta*) roots. The results indicated that the AMF genera colonizing manioc roots (*Glomus* and *Gigaspora*) were not always detected from soil samples or/and from the morphological descriptions of spores. Moreover, the opposite was also found in some genera, such as *Acaulospora*, which was present in the soil samples but not in manioc root samples (León 2006). At that time, the inconsistencies between root and soil AMF richness results were commonly attributed to the lack of standardization of the molecular protocols. Nowadays, this result (differing root and soil AMF communities) can even be expected given a putative selective filtering process of plant roots with the soil of AMF communities (Saks et al. 2014; Valera-Cervero et al. 2015).

From 2006 until now, at Sinchi Institute, we have continued using morphological methodologies for the taxonomic classification of AMF spore morphotypes, given that under certain conditions, it is easier and cost-effective to determine spores with particular morphological characteristics. Later, between 2009 and 2010, Öpik and collaborators proposed a new molecular nomenclature for the operational taxonomic units (OTUs) obtained for the Glomeromycota. This new approach to AMF taxonomy is based on the idea that those AMF OTUs can be universally identified in a standard way, assigning stable and comparable labels among studies, similar to the classical taxonomic classification of species. Consequently, a new nomenclature of AMF emerged based on virtual taxa (VT), allowing to identify taxonomically the specific Glomeromycota sequences found (Öpik et al. 2009, 2010). This methodology was then incorporated into the pre-existing traditional methodologies applied at Sinchi Institute, greatly complementing the description of AMF in the region.

4.2 Diversity of AMF of the Colombian Amazon

The global analyses of roots and soil with molecular techniques have provided a comparison of AMF diversities worldwide. These analyses have indicated that tropical forests exhibit a significantly higher AMF taxonomic richness per plant species than temperate forests or habitats under strong anthropogenic influences (Öpik et al. 2006; Toussaint et al. 2020). The reasons for these patterns are aligned with AM symbiosis being one of the oldest plant-fungi mutualist associations (Brundrett and Tedersoo 2018), emerging in the Early Devonian period when Earth's climate changed to a tropical wetland condition (Qie et al. 2019). Indeed, temperate and warm conditions in the tropics are kept stable all year round. These resemble the initial conditions in which AM associations emerged and developed, providing a longer stable evolutionary history and thus allowing the development and maintenance of a higher AMF diversity (Toussaint et al. 2020).

The AMF database of the Sinchi Institute (last update: November 2020) includes 1268 records, among them 855 samples associated with soil-borne spore morphotypes and 413 samples associated with VT; therefore, 70% correspond to information coming from soil samples and 30% from root samples. The AMF records coming from a molecular analysis in soils and roots varied depending on the sequencing method applied (454 pyrosequencing or Illumina sequencing) and on the small subunit rRNA (SSU rRNA) primers used to obtain specific AMF sequences, either NS31 and AML2 (Simon et al. 1992; Lee et al. 2008) or WANDA and AML2 (Lee et al. 2008; Dumbrell et al. 2011). The latest updated database information is summarized in Table 4.1.

We know that the International Nucleotide Sequence Database Collaboration (INSDC) contains a vast amount of AMF sequence data. However, it also contains a high non-negligible proportion of erroneous sequences (Nilsson et al. 2006). These sequences do not occur in other curated databases, where data are included by expert scientists (Kohout et al. 2014). The presence of many erroneous sequences motivated us to use the same sets of primers as other world-leading teams to compare our samples and results with the information published in the Maarj*AM* database. Then, following a common standard protocol, the VT obtained was matched with a sequence similarity of ≥97% against the VT reported in the reference

Table 4.1 Sample characteristics to estimate AMF diversity in the Colombian Amazon

		Samples processed with molecular methodologies				
		Sequencing methodology				
		454		Illumina		
Type of sample	Samples processed from soil-borne spore	NS31/ AML2 primers	NS31/ WANDA primers	NS31/ AML2 primers	NS31/ WANDA primers	Total
Soil samples	855	37	0	19	126	182
Root samples	413	37	0	0	194	231
Total	1268	74	0	19	320	413

Maarj*AM* database or, in a second stage, with a similarity threshold of 90% against sequences published in the INSDC.

Independent of the molecular methodology used to obtain AMF sequences, there is no doubt that those sequences corresponded to previously reported AMF. However, each methodology and pair of primers will offer a different AMF community composition (Dumbrell et al. 2011; Kohout et al. 2014; Lekberg et al. 2018). Thus, comparisons of samples are only possible when the same molecular technique and the same specific pair of primers are used.

When the AMF diversity estimated by the morphology of soil-borne spores is compared to what has been obtained by molecular methodologies, the AMF community composition can also differ (Table 4.2). The soil-borne spore approach has been useful to determine the genus of some morphotypes of the Glomeraceae, which molecular techniques have not discriminated. Also, there are AMF species described by morphological spore features, which did not include molecular determinations. Some of those morphotypes had limited distributions, and it is difficult to find them in cultured collections to determine their sequences. All these have motivated using a combined soil-borne morphological spore approach with a molecular one for a complete determination of the AMF community from soil samples (Oehl et al. 2011a; Öpik and Davison 2016).

It is interesting to observe that independently of the methodological approach used to determine the AMF composition of Amazon soils, the coarse composition of the AMF community at the genus level is similar. According to previous studies, *Glomus* is the most common genus detected molecularly in tropical soils (Öpik et al. 2006), and it is also the most common and diverse genus in Amazon soils.

Although the genus *Glomus* contains the highest number of described species, it is the genus with the smallest proportion of species sequenced for the SSU rRNA gene until now (Öpik et al. 2010). For environments rich in *Glomus* species, it has been suggested (Kohout et al. 2014) the use of a set of internal transcribed spacer (ITS) family-specific primers, such as those developed by Redecker (2000) and Redecker et al. (2003). Also, it has been demonstrated that shorter fragments produced by the use of SSU global fungal primers, such as NS31 and WANDA, with specific primers such as AML2 to target AMF, as used here, exhibited a relatively

Table 4.2 Comparison of the AMF community composition of the Colombian Amazon forest by a morphological soil-borne spore description and by a molecular VT approach

Methodology	Soil-borne spore diversity		VT diversity	
Genus	Undetermined species	Determined species	Undetermined species	Determined species
Acaulospora	19	(7): *A. colombiana*, *denticulata*, *foveata*, *mellea*, *morrowiae*, *rehmii*, *tuberculata*	15	0
Archaeospora	0	0	9	0
Ambispora	0	(1): *A. leptoticha*	0	(2): *A. fennica*, *leptoticha*
Cetraspora	0	(2): *C. pellucida*, *spinosissima*	0	0
Claroideoglomus	0	(1): *C. etunicatum*	3	(1): *C. lamellosum*
Diversispora	0	(1): *D. tortuosa*	3	(1): *D. spurca*
Funneliformis	0	(2): *F. coronatum*, *geosporum*	0	0
Gigaspora	4	(1): *G. albida*	0	(1): *G. decipiens*
Glomus	104	(9): *G. clavisporum*, *glomerulatum*, *magnicaule*, *microaggregatum*, *multicaule*, *pansihalos*, *reticulatum*, *rubiforme*, *sinuosum*	132	(2): *G. coremoides*, *proliferum*
Kuklospora	0	(1): *K. kentinensis*	1	0
Paraglomus	0	0	10	(3): *P. brasilianum*, *laccatum*, *occultum*
Rhizophagus	0	(3): *R. aggregatum*, *intraradices*, *manihotis*	0	(3): *R. clarum*, *intraradices*, *manihotis*
Sclerocarpum	0	(1): *S. amazonicum*	0	0
Scutellospora	3	(7): *S. calospora*, *castanea*, *crenulata*, *spinosa*, *spinosissima*, *striata*, *tepuiensis*	3	(2): *S. castanea*, *heterogama*
Septoglomus	0	(1): *S. constrictum*	0	0
Simiglomus	0	(1): *S. hoi*	0	0
Viscospora	0	(1): *V. viscosa*	0	(1): *V. viscosa*

high-resolution power for the most diverse family (Glomeraceae). However, these primers were weaker in delimiting species from less abundant families such as Claroideoglomeraceae, Diversisporaceae, or Paraglomeraceae (Kohout et al. 2014). In this case, the number of Claroideoglomeraceae, Diversisporaceae, and Paraglomeraceae reported so far is low but consistently found in samples from different sites of the Colombian Amazon. Then, it is possible that not all the species of *Claroideoglomus*, *Diversispora*, and *Paraglomus* in the soil and root samples collected were detected. However, the proportion of detection of these genera is consistent along the different stocks of samples sequenced, indicating that the abundance/proportion of these genera might be correct with respect to the other genera found.

When comparing the AMF inventory obtained from the Colombian Amazon with previous reports of AMF diversity in the region, we found that many AMF taxa are consistently detected and reported (Table 4.3). These AMF species are the target ones to develop inoculants for regional agriculture. This type of species selection is even more recommended when native *Glomus* species dominate the region. Some of them seem to be the most efficient symbionts for important native crops such as the cowpea bean (*Vigna unguiculata*) (Silva et al. 2018).

Table 4.3 Comparison of reported AMF inventories from the Amazon region

AMF species	This chapter (Colombia)	Reyes et al. (2018) (Brazil)	Caproni et al. (2018) (Brazil)	de Oliveira Freitas et al. (2014) (Brazil)	Lopes Leal et al. (2013) (Brazil)	Stürmer and Siqueira (2011) (Brazil)	Lopes Leal et al. (2009) (Brazil)
Acaulospora brasiliensis				X			
Acaulospora bireticulata							X
Acaulospora colombiana	X		X	X	X	X	X
Acaulospora delicata		X			X	X	X
Acaulospora denticulata	X		X				
Acaulospora elegans		X			X	X	
Acaulospora excavata						X	
Acaulospora foveata	X	X	X	X	X	X	X
Acaulospora gedanensis					X	X	
Acaulospora laevis					X	X	
Acaulospora mellea	X	X	X	X		X	X

(continued)

Table 4.3 (continued)

AMF species	This chapter (Colombia)	Reyes et al. (2018) (Brazil)	Caproni et al. (2018) (Brazil)	de Oliveira Freitas et al. (2014) (Brazil)	Lopes Leal et al. (2013) (Brazil)	Stürmer and Siqueira (2011) (Brazil)	Lopes Leal et al. (2009) (Brazil)
Acaulospora morrowiae	X	X	X	X		X	X
Acaulospora paulinae						X	
Acaulospora polonica						X	
Acaulospora reducta		X					
Acaulospora rehmii	X	X	X	X	X	X	X
Acaulospora scrobiculata		X	X	X	X	X	X
Acaulospora spinosa		X	X		X	X	X
Acaulospora tuberculata	X		X		X	X	X
Acaulospora walkeri						X	
Ambispora appendiculata		X		X	X		
Ambispora fennica	X						
Ambispora leptoticha	X		X	X		X	X
Archaeospora trappei				X	X	X	X
Cetraspora pellucida	X				X	X	
Cetraspora spinosissima	X						
Claroideoglomus claroideum			X	X			
Claroideoglomus drummondii				X			
Claroideoglomus etunicatum	X	X	X	X			
Claroideoglomus lamellosum	X						
Claroideoglomus luteum				X			
Dentiscutata scutata		X					

(continued)

Table 4.3 (continued)

AMF species	This chapter (Colombia)	Reyes et al. (2018) (Brazil)	Caproni et al. (2018) (Brazil)	de Oliveira Freitas et al. (2014) (Brazil)	Lopes Leal et al. (2013) (Brazil)	Stürmer and Siqueira (2011) (Brazil)	Lopes Leal et al. (2009) (Brazil)
Diversispora eburnea				X			
Diversispora pustulata				X			
Diversispora spurca	X				X		
Diversispora versiformis				X			
Diversispora tortuosa	X				X	X	
Enthrophospora infrequens					X	X	X
Funneliformis badium				X			
Funneliformis coronatum	X						
Funneliformis geosporum	X	X	X	X		X	
Funneliformis halonatum		X					
Fuscutata heterogama		X	X				
Gigaspora albida	X						
Gigaspora decipiens	X						
Gigaspora gigantea			X				
Gigaspora margarita		X	X				X
Gigaspora rosea				X			
Glomus ambisporum		X				X	
Glomus atrouva				X	X		
Glomus australe				X		X	
Glomus brohultii				X			
Glomus caledonium			X				
Glomus claroideum							X
Glomus clavisporum	X		X			X	
Glomus coremoides	X					X	

(continued)

Table 4.3 (continued)

AMF species	This chapter (Colombia)	Reyes et al. (2018) (Brazil)	Caproni et al. (2018) (Brazil)	de Oliveira Freitas et al. (2014) (Brazil)	Lopes Leal et al. (2013) (Brazil)	Stürmer and Siqueira (2011) (Brazil)	Lopes Leal et al. (2009) (Brazil)
Glomus corymbiforme					X	X	
Glomus diaphanum			X	X			
Glomus etunicatum			X				
Glomus formosarum			X				
Glomus fuegianum				X			
Glomus glomerulatum	X	X	X		X	X	
Glomus heterosporum				X			
Glomus inviernanum			X				
Glomus lacteum					X	X	
Glomus macrocarpum		X	X	X		X	
Glomus magnicaule	X		X			X	
Glomus microaggregatum	X					X	
Glomus microcarpum			X	X			
Glomus minutum				X			
Glomus multicaule	X						
Glomus nanolumen			X			X	
Glomus occultum			X				
Glomus pansihalos	X						
Glomus proliferum	X						
Glomus reticulatum	X		X				
Glomus rubiforme	X			X		X	
Glomus spinosum				X			
Glomus sinuosum	X		X			X	
Glomus taiwanensis				X			

(continued)

Table 4.3 (continued)

AMF species	This chapter (Colombia)	Reyes et al. (2018) (Brazil)	Caproni et al. (2018) (Brazil)	de Oliveira Freitas et al. (2014) (Brazil)	Lopes Leal et al. (2013) (Brazil)	Stürmer and Siqueira (2011) (Brazil)	Lopes Leal et al. (2009) (Brazil)
Glomus tenebrosum				X			
Glomus trimurales						X	
Glomus trufemii		X					
Kuklospora kentinensis	X						
Orbispora pernambucana		X					
Pacispora robigina				X			
Paraglomus brasilianum	X						
Paraglomus laccatum	X						
Paraglomus occultum	X		X				X
Racocetra castanea				X			
Racocetra weresubiae			X				
Rhizophagus aggregatum	X						
Rhizophagus clarum	X		X		X	X	X
Rhizophagus intraradices	X		X		X	X	
Rhizophagus invernianum			X				
Rhizophagus manihotis	X						
Sclerocarpum amazonicum	X						
Scutellospora arenicola				X			
Scutellospora biornata					X	X	
Scutellospora calospora	X		X	X			
Scutellospora castanea	X						
Scutellospora cerradensis				X			

(continued)

Table 4.3 (continued)

AMF species	This chapter (Colombia)	Reyes et al. (2018) (Brazil)	Caproni et al. (2018) (Brazil)	de Oliveira Freitas et al. (2014) (Brazil)	Lopes Leal et al. (2013) (Brazil)	Stürmer and Siqueira (2011) (Brazil)	Lopes Leal et al. (2009) (Brazil)
Scutellospora dipurpurescens				X			
Scutellospora heterogama	X						
Scutellospora persica						X	
Scutellospora scutata					X	X	
Scutellospora spinosa	X						
Scutellospora striata	X						
Scutellospora tepuiensis	X						
Septoglomus constrictum	X						
Simiglomus hoi	X						
Viscospora viscosa	X						

The molecular approaches applied to study AMF have shown remarkable determinations of the Glomeromycota fungal group. However, the molecular techniques have determined differences in the small subunit ribosomal RNA (SSU rRNA) genes of these fungi based on different types of polymerase chain reaction (PCR) sequencing, which has been changing rapidly in the last decade. For example, three different sequencing methods have been used to study Glomeromycota at the molecular level during the last decade: first-generation sequencing or Sanger sequencing; 454; and Illumina next-generation sequencing (Raza and Ahmad 2016).

Vasar et al. (2017) demonstrated that 454 and Illumina Miseq sequencing offered similar AM fungal richness results. This similarity, in principle, might allow us to compare results between these two sequencing methodologies. However, different sets of primers target different AM fungal genomic regions capturing different AM fungal community compositions. Therefore, the results obtained from samples that used the same sequencing methodology but a different set of primers seem to have weak comparability (Lekberg et al. 2018). However the differences in results caused by the use of different sequencing methods or different sets of primers do not seem to meaningfully affect the AM fungal community structure and its responses to the environment (Lekberg et al. 2018). This result suggests a promising avenue for using historical datasets (such as the one built in the Colombian Amazon) to evaluate past, current, and future regional trends.

Taking into consideration the overall conceptual and technical limitations of the results obtained from different molecular methodologies, we want to show the interesting diversity trends of the Colombian Amazon region obtained so far. These trends can be considered the base for future hypotheses, which should be further tested with information collected and processed in a more standardized way.

4.3 Diversity Trends of the AM Fungal Communities Along a Biogeographical Gradient

The Colombian Amazon corresponds to a portion of the upper northwestern Amazon basin. From the northwestern (NW) to the northeastern (NE) region of the Colombian Amazon, a biogeographical gradient is defined by three main factors (Fig. 4.1). From NW to NE, (1) altitude changed from 1000 to 95 m above sea level, (2) the soil texture changed from dominated limed-clayed soils to sandier soils, and (3) natural vegetation changed from the typical tropical rain upland forest with high plant diversity to a shorter forest with lower diversity. In particular, the vegetation

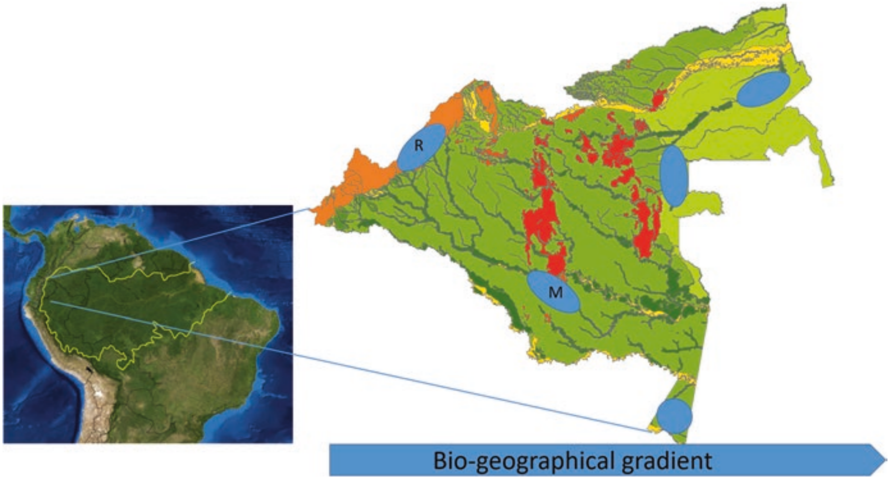

Fig. 4.1 Map of the Colombian Amazon region indicating the origin of soil samples used to study changes in the AMF community composition along a biogeographical gradient. Colors correspond to particular landscapes with different soil types: hills with Cambisols (orange) where rubber trees (R) were also collected, Amazonian planes with Ferralsols and Fluvisols in the alluvial valleys (green) where manioc (M) were also collected, and savannas with Arenosols and Podzols (yellow). Anthrosols are not shown

changes from the tropical rain upland forest, with tall trees (35 m on average) and a closed canopy growing between a dense undergrowth forest (IGAC 1979) and a forest with trees around 10–20 m. tall, growing on a shallow sandy podzolized soil and influenced by the Guiana shield formation. These short forests are also associated with natural savannas of white sands in an almost plain flat landscape.

We selected soil samples from the Sinchi database collected from native Amazon forests in four states located along the biogeographical gradient from the NW (Amazonas and Caquetá states) to the NE (Guainía and Vaupés states) of the Colombian Amazon region. According to the coordinates of the soil samples selected, we filtered those locations in the Colombian Amazonian Herbarium (COAH) database to obtain information on the plant community composition of each studied area. The resulting VT richness and plant species richness were grouped by state. Rarefaction curves were performed previously to AMF information to check whether the number of soil samples selected was enough and representative of the AM fungal community for each state (Fig. 4.2). Given that the rarefaction results indicate similar curves, we proceed to analyze the correspondence between plants and AM fungi.

Shannon and Simpson indexes of AM fungal diversity showed that soils from the NW were more diverse (Shannon index varies between 1.98 and 1.27; Simpson

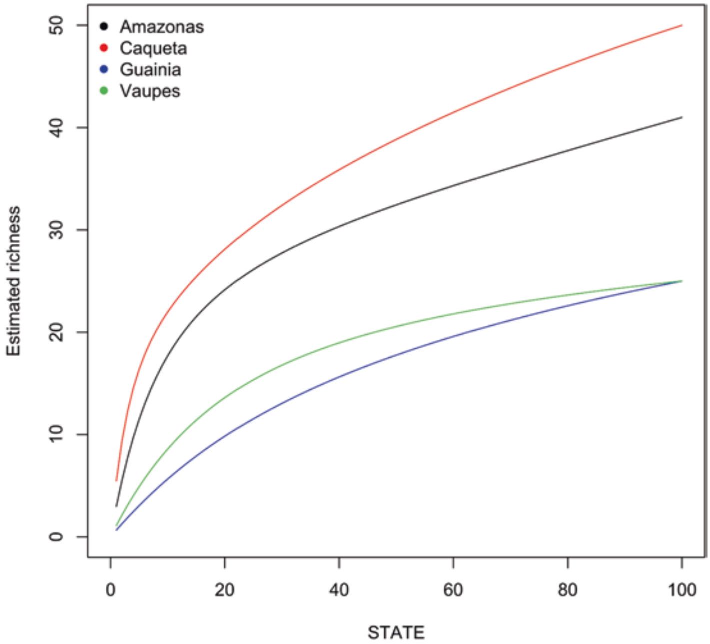

Fig. 4.2 Rarefaction (taxon accumulation) curves of AMF of the northwestern states (Amazonas and Caquetá, in black and red lines, respectively) and northeastern states (Guainía and Vaupés, green and blue lines, respectively) of the Colombian Amazon region

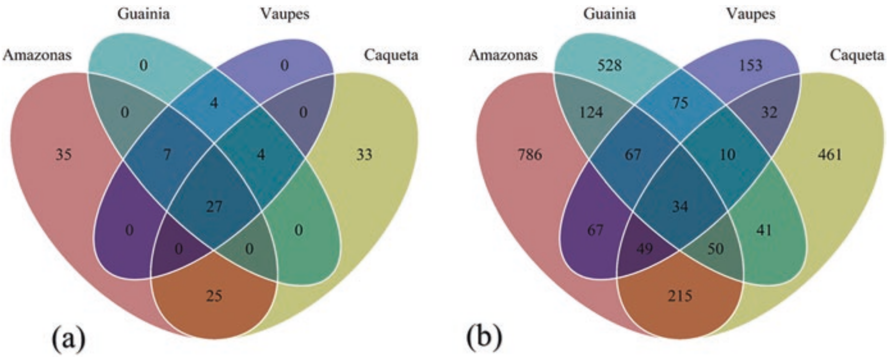

Fig. 4.3 Venn diagrams indicating the number of (**a**) AMF VT and (**b**) plant species shared among northwestern (Amazonas and Caquetá) and northeastern (Guainía and Vaupés) states of the Colombian Amazon region

index, 0.78) than soils from the NE (Shannon index varies between 1.04 and 0.97 and Simpson index between 0.54 and 0.45).

AM fungal and plant communities showed a very contrasted pattern along the biogeographical gradient in the different states (Fig. 4.3). Plant communities differed with more than 51% exclusive plant species in each state, even in soils with a similar composition (Fig. 4.3a). Few plant species (38% of total plant species) and few AM fungal VT (35% of the total VT found) were consistently present along the soil gradient. However, dissimilarities among plant communities and AM fungal communities did not follow similar patterns (Fig. 4.4).

AM fungal communities from well-preserved Amazon ecosystems could vary even if soil or plant communities are similar. On one hand, areas with contrasting plant community compositions shared similar AM fungal communities as occurred between Guainía and Vaupés states. On the other hand, Amazonas and Caquetá states had different plant communities and different AM fungal communities. Additionally, the AM fungal taxa that dominated each studied area (state) were different (Table 4.4). Results indicated that the NW of the Amazon region is dominated by the *Glomus* genus, while in NE, *Acaulospora* became a relevant genus in the AM fungal community composition, as reported by some researchers for Amazon soils of Brazil (Lopes Leal et al. 2013).

These results indicated that the portion of the Amazon region sampled is heterogeneous and that abiotic factors might have a stronger influence on the AM fungal community composition than biotic factors (e.g., plant composition). These results support the idea that dominant AM fungi are context-dependent (Dumbrell et al. 2010), and therefore dominating AM fungal community along the Amazon region is unexpected.

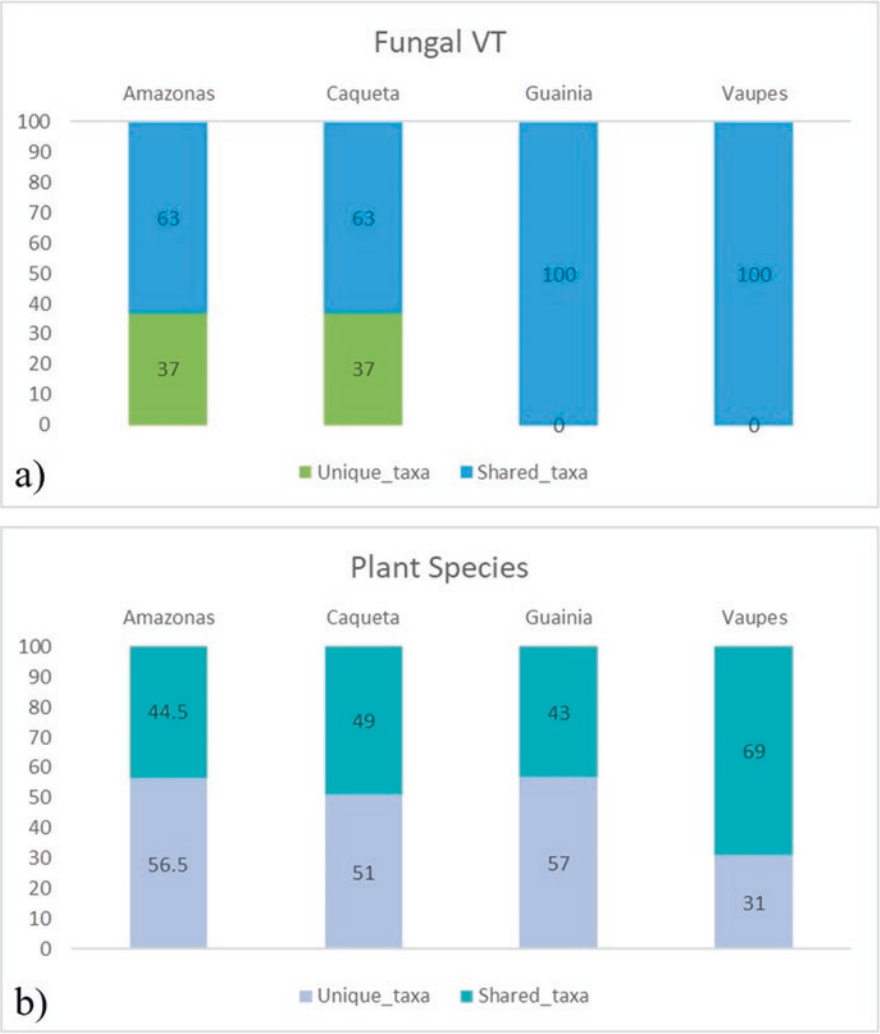

Fig. 4.4 Proportions of AMF (**a**) and plant communities (**b**) among four states of the Colombian Amazon region located along a biogeographical gradient

4.4 Amazon Deforestation and Changes in AM Fungal Communities

The Colombian Amazon region, like most of the tropical forests of the world, is experiencing an increasing rate of deforestation. According to monitoring data of the Colombian Amazon region, between 2016 and 2018, the average annual rate of deforestation was 119.5 hectares, with an average annual rate of transformation of 212 hectares per year (SIAT-AC 2020). Most of these deforested areas were transformed into pastures for cattle production, becoming the main economic activity.

Table 4.4 Arbuscular mycorrhizal fungal taxon with the highest number of sequence reads in the four Colombian Amazon states studied

Amazonas		Caquetá		Guainía		Vaupés	
VT	Reads	VT	Reads	VT	Reads	VT	Reads
Glomus VTMO-G74	1253	*Glomus* VT269	5787	*Acaulospora* VT102	4918	*Claroideoglomus* VTLH-Cl01	2528
Glomus VT126	1132	*Glomus* VT92	4356	*Acaulospora* VT24	1860	*Paraglomus* VT444	1498
Glomus VT183	510	*Glomus* VTGCl-2	2125	*Glomus* VT360	1353	*Acaulospora* VT24	605
Glomus VT80	296	*Glomus* VT292	1688	*Claroideoglomus* VTLH-Cl01	570	*Glomus* VT80	394
Glomus VT89	295	*Glomus* VT79	1174	*Acaulospora* VT231	569	*Glomus* VT70	365

As it was mentioned before, Amazon soils are highly weathered and nutrient-poor. Their fertility depends on the efficient cycling of organic matter coming mainly from forest litter inputs. Once the natural forest is removed, litter inputs disappear, and soil degradation begins. Initial pastures consume the nutrient stocks produced previously by forests. However, after a couple of years, the soils become unfertile and unproductive, often with high aluminum toxicity and severe phosphorus and nitrogen deficiency (Matschullat et al. 2020).

In the case of the Amazon region, most of the native forest is replaced by *Brachiaria decumbens*, an exotic grass species that is highly mycotrophic (Siqueira and Saggin-Junior 2001). The replacement of the natural cover and the cattle grazing activity affect the AM communities and the plant-fungus symbiotic relationship in different ways. AM fungal sporulation increases in pastures, responding to a change in soil pH (less acidic) (Lopes Leal et al. 2013) and new biotic stress (Yang et al. 2020), as native forests with low to moderate grazing levels are transformed into a heavily grazed grassland. Grazing itself directly causes plant stress because of the reduction of photosynthetic biomass, affecting the available nutrient exchange between the host plant and the symbiotic fungus. This is even more intense in host plants with high mycorrhizal dependency (van der Heyde et al. 2017, 2019). However, a reduction of plant stress caused by herbivory can also be mediated by AMF (Frew et al. 2022). Additionally, the composition of AM fungal communities in pastures can differ from native forest soils (Lopes Leal et al. 2013; Barraclough and Olsson 2018), which tend to be mostly dominated by no more than three fungal species (Stürmer and Siqueira 2011). Unfortunately, studies using molecular tools to analyze the changes in AM fungal soil communities caused by the transformation of native forests into pastures in the Amazon region are not yet available.

In addition, we wanted to apply molecular methodologies to evaluate whether the results of previously observed changes might differ according to the methodology used. Our preliminary results indicated that spore abundance was directly related to the abundance of sequences of AM fungi found in the soil, but not with AM fungal richness (Table 4.5), where pastures and native forests were similar.

Table 4.5 AMF abundance, richness, and the five more representative virtual taxa (VT) in pastures and native forest soils in the Colombian Amazon region

	Pasture	Native Amazon forest
Abundance (reads)	2874	107
Richness (No. VT)	18	17
1	*Glomus* VT126	*Glomus* VT359
2	*Glomus* VT60	*Glomus* VT126
3	*Glomus* VT89	*Glomus* VT292
4	*Glomus* VT70	*Paraglomus* VT444
5	*Glomus* VT398	*Glomus* VT79

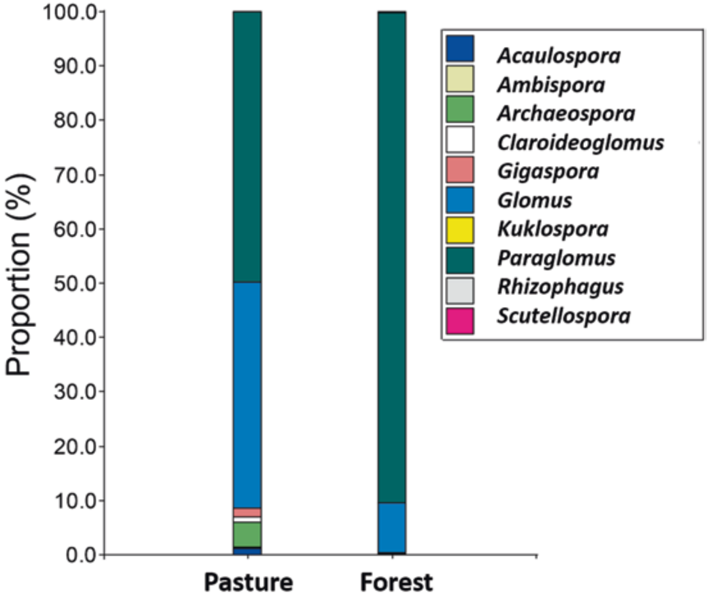

Fig. 4.5 Proportion of genera of arbuscular mycorrhizal fungi in pasture soils and native Amazon forest soils obtained by molecular techniques

However, the AM fungal community composition differed. Both pastures and native forests were composed mainly of *Glomeraceae* VT. However, pasture soils were dominated by *Glomus* species. At the same time, native forest soils also included *Paraglomus* as an important genus in its AM fungal community composition (Fig. 4.5). *Glomus* is generally more resistant to many kinds of disturbance in comparison with other genera (van der Heyde et al. 2017) and is very competitive colonizing roots due to the high number of propagules that produce (Klironomos and Hart 2002), and therefore, *Glomus* is more common in disturbed ecosystems such as those introduced pastures.

The genus *Paraglomus* is commonly abundant in tropical forest ecosystems (García de León et al. 2017; Marinho et al. 2018; Peña-Venegas et al. 2021), which explains why it became an important genus in the AM fungal composition of the Amazon forest.

Interestingly, we found more AMF in pasture soils than in forest soils, in agreement with previous observations where AM fungal richness depended on the level of disturbance and the soil and root matrices (García de León et al. 2017). Higher AMF richness tends to occur in soils of disturbed ecosystems, unlike in natural ecosystems. On the contrary, in conserved ecosystems, AM fungal richness is higher in the roots than in the soils. This can indicate an AMF survival strategy to explore and wait for better conditions and maybe better host plants. In conserved ecosystems, a higher AM fungal richness occurred in the plant roots, suggesting a more stable habitat condition.

Evidencing particular differences in the AM fungal composition between native Amazon forest soils and pasture soils at the *Glomeraceae* level was only possible through molecular tools. In this case, the molecular approach offered a more detailed resolution to discriminate the taxonomy of sequences from AMF with genera sharing spore morphotype similarities than the morphological approach in which this taxonomical resolution is not evident.

4.5 AM Fungal Communities of Two Economically Relevant Euphorbiaceae Native Plants

The Amazon region has provided the world with economically relevant species, including some crops worldwide distributed, such as manioc (*Manihot esculenta*), also known as cassava. Consequently, this region has been recognized as a domestication hotspot, giving additional importance to crops such as "cocoa" (*Theobroma cacao*) and "copoazu" (*Theobroma grandiflorum*); many "chiles" varieties (*Capsicum* sp.); different pineapple varieties (*Ananas comosus*); "avocado" (*Persea americana*); peanut (*Arachis hypogaea*); "cocona" (*Solanum sessiliflorum*); "camu camu" (*Myrciaria dubia*); palm fruits rich in proteins such as peach palm (*Bactris gasipaes*) and asai palm (*Euterpe precatoria*); colorants such as annatto (*Bixa orellana*); stimulant plants such as "coca" leaves (*Erythroxylum coca*) and "guaraná" (*Paullinia cupana*), as well as medicinal plants such as cat's claw (*Uncaria tomentosa*) and "ayahuasca" (*Banisteriopsis caapi*); and significant plant species for industry, such as the rubber tree (*Hevea brasiliensis*) (Schultes 1979; Clement 1999; Simpson and Ogorzaly 2014).

In the last 10 years, the Sinchi Institute has studied the AM association in two important native *Euphorbiaceae* plants: manioc and rubber tree. These studies included molecular approaches to determine the AM fungal species associated with the roots of these species. We took a subset of the AM fungal database to compare the AM fungal communities in the roots of these two plant species from mature plants in productive agrosystems. We included data from four different native manioc landraces (Peña-Venegas et al. 2019) cultivated in shifting agriculture plots on three different soil types. Mycorrhized root samples were obtained at the moment of

Table 4.6 AM fungal community colonizing roots of two *Euphorbiaceae* species

VT	Genus	Species	Percentage of sequence reads			
			Manioc	%	Rubber tree	%
VTX00090	*Rhizophagus*	*manihotis*	16,505	20.76	12,830	10.42
VTX00280	*Glomus*	sp.	12,873	16.19	22,831	18.54
VTX00126	*Glomus*	sp.	8,980	11.29	4,722	3.83
VTX00093	*Glomus*	sp.	6,977	8.77	6,562	5.33
VTX00270	*Glomus*	sp.	6,336	7.97	2,729	2.22
VTX00024	*Acaulospora*	sp.	5,081	6.39		
VTX00082	*Glomus*	sp.	4,877	6.13		
VTX00312	*Glomus*	sp.	2,633	3.31	13,122	10.65
VTX00420	*Glomus*	sp.	2,567	3.23		
VTX00108	*Glomus*	sp.	2,375	2.99	1,987	1.61
VTX00268	*Glomus*	sp.	1,872	2.35	1,210	0.98
VTX00264	*Rhizophagus*	*clarum*	1,248	1.57	1,595	1.29
VTX00418	*Glomus*	sp.	1,175	1.48		
VTX00092	*Glomus*	sp.	1,121	1.41		
VTX00070	*Glomus*	sp.	1,107	1.39		
VTX00089	*Glomus*	sp.	896	1.13	1,516	1.23
VTX00028	*Acaulospora*	sp.	878	1.10		
VTX00269	*Glomus*	sp.	806	1.01		
VTX00248	*Glomus*	sp.	605	0.76		
VTX00227	*Acaulospora*	sp.	602	0.76		
VTX00403	*Glomus*	sp.			11,195	9.09
VTX00076	*Glomus*	sp.			9,305	7.55
VTX00223	*Glomus*	sp.			7,632	6.20
VTX00444	*Paraglomus*	sp.			6,944	5.64
VTX00096	*Glomus*	sp.			4,247	3.45
LH-Gl07	*Glomus*	sp.			3,751	3.05
VTX00099	*Glomus*	*proliferum*			3,553	2.88
VTX00363	*Glomus*	sp.			2,947	2.39
VTX00069	*Glomus*	sp.			2,864	2.33
VTX00364	*Glomus*	sp.			1,631	1.32
Total			79,514	100	123,173	100

manioc harvesting. For the rubber tree, we included data from 19 different rubber clones (nine native clones and ten introduced clones) cultivated in clonal banks in four different soil types (Peña-Venegas et al. 2021). Mycorrhized root samples were obtained from 9-year-old rubber trees. The locations where manioc and rubber tree root samples were collected are far away from each other (see Fig. 4.1). To compare AMF communities in the roots of these two plant species, we selected the twentieth most frequent AMF associated with the roots of each plant species.

Our results showed that the two species shared 50% of the total AM fungal community in their roots (Table 4.6). Four AM fungal virtual taxa were found in the tenth most frequent VT colonizing the roots of the two species, representing 51.55%

of the total AM fungal community for manioc and 43.44% of the total AM fungal community for the rubber tree.

These results indicate that despite soil conditions and biogeographic areas are not similar, the phylogenetic relationship between both crops might have an impact on their symbiotically associated communities. The host specificity for AMF has commonly been considered weak. However, some degree of plant-AMF specificity has been observed at plant species and functional or ecological plant group levels (Helgason et al. 2007; Öpik et al. 2009; Sepp et al. 2019).

Our results, contrary to previous reports, indicate that some plant-fungi specificity might also occur at higher phylogenetic levels (e.g., plant family level), independently of the location where they are cultivated. These results open the door to studying new scenarios where plant-AMF specificity could occur. However, many critical aspects are still unknown, for instance: How does this AM fungal composition respond to a disturbing effect rather than to a plant-fungal interaction? What is the function of the shared vs. exclusive AM fungal community? Which of those (shared vs. exclusive) are more relevant for agriculture in terms of soil health and plant productivity? Future analyses of these aspects will warrant improvements in the way these crops are currently planned and developed.

4.6 Knowledge Gaps and Further Recommendations

The AM research developed in the Colombian Amazon presented in this book chapter indicates that there are still many relevant knowledge gaps to study, in particular, considering the immediate and drastic threats the Amazon basin, as a key global ecosystem, is currently facing (Malhi et al. 2008; Davison et al. 2012), specifically, the alarming rate of deforestation and land use change, expanding the land for intensively managed crops and grazing areas. A basic understanding of the complete composition of the AM fungal communities (Fitter et al. 2004), along with more profound knowledge about their crucial ecosystem multifunctionality (Ryan and Graham 2018), and the resilience of the AM symbiosis to climate change is highly desirable to make decisions about the future of the Amazon basin (Silva-Flores et al. 2021). These decisions should include a substantial increase in research from simple experiments comparing the composition of different land use within a territory to macroecological and biogeographic studies on the diversity and distribution of AMF.

4.7 Conclusions

The study of biological communities, including AMF in environments difficult to access as the Amazon basin, is favored by the presence of permanent research institutions in the region, such as the Sinchi Institute in the Colombian Amazon. After more than 20 years of work on AM fungal communities in the Colombian Amazon, more complete inventories of this fungal group were developed, contributing to the knowledge of the AMF diversity of the region. The results of this research indicate a moderate AMF diversity in the region. However, the functional roles of these AMF seem to be relatively important given their relevance in nutritional functions for natural and valuable cultivated plants. The fast evolution of the methodologies to study AMF, especially those based on molecular techniques, limits the analysis of historical information. More research is needed, but it is the Academy that must soon solve these constraints to advance in the interpretation of the information obtained until now. There had been less evidence of the effects of deforestation and land use changes in the region in communities of microorganisms as the essential elements of the functioning ecosystem, for instance, AM symbiosis and AMF change with deforestation and the transformation of natural forest into agricultural plots or pastures. Although AMF show a good resilience capacity to environmental disturbance, changes in their community composition are not well understood in terms of their ecological functions, the restoration of natural ecosystems, the productivity of native and introduced crops, and their efficiency as mutualistic symbionts. Gaps indicated that the Amazon region would continue to be a fascinating tropical environment to study AMF and the AM symbiosis.

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Chapter 5
AMF Diversity in Coffee and Cacao Agroforestry Systems: Importance for Crop Productivity and Forest Conservation

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Contents

5.1 Introduction

In the face of increasing deforestation of tropical forests, agroforestry represents a promising strategy for forest conservation that reconciles the development of a sustainable agricultural activity with the conservation of biodiversity and the maintenance of the environmental functions of forests. In this type of land management,

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woody species are combined temporal or spatially with crops, creating diversified production systems in the same terrain (Farfán-Valencia 2007). Coffee and cacao are the most widely used agroforestry crops, covering a large part of the world agroforestry surface and together represent the second largest export product of developing countries (De Beenhower et al. 2013). In South America, these products come from the tropical region of the subcontinent, being Brazil, Colombia, Ecuador, and Perú the most important producers (FAO 2021).

In their traditional agroforestry management, these crops are grown under a canopy composed of a high diversity of native shade trees along with introduced timber and fruit trees. This type of agroforestry system preserves diversity levels relatively similar to those of natural forests and maintains many of their environmental functions, which include water conservation, erosion reduction, and carbon sequestration that contribute to climate change mitigation (De Beenhower et al. 2013). Additionally, shade trees buffer climatic extremes and provide large amounts of organic matter, maintaining soil fertility thanks to the action of a diverse community of microorganisms that participate in nutrient cycling and plant nutrition (Schroth and Krauss 2006).

Within this group of microorganisms, arbuscular mycorrhizal fungi (AMF) play an important role in nutrient recirculation, which is crucial for the functioning of these agroecosystems. Mycorrhizal symbiosis is formed by around 80% of vascular plants, increasing water and low-mobile nutrient uptake in soils, particularly phosphorus (P), by means of an extensive mycelial network that explores a greater soil volume than the one reached by plant roots (Cuenca 2015). AMF hyphae have been found profusely colonizing leaf litter (Cuenca et al. 1983), and, although a direct saprophytic activity has not been demonstrated, they may induce a faster decomposition rate through microorganisms present in the hyphosphere (Díaz-Ariza et al. 2020). The hyphal network is able to uptake and transfers to the plant, in very efficient way, the mineralized nutrients reducing their loss by leaching or fixation in the soil. Besides the nutritional benefits, AMF provide increased resistance to pathogens, drought, and heavy metal contamination, improving soil structure through the join actions of the external mycelium and glomalin production (Cuenca 2015).

Both coffee and cacao are highly mycotrophic plants (Cuenca et al. 1990; Cogo et al. 2017; Paguntalan et al. 2020) that depend on mycorrhizal fungi for their development. Also, within the shade trees present in agroforestry systems, woody legumes are highly dependent on mycorrhizas due to their high phosphorous requirement demanded by their association with nitrogen-fixing bacteria (Atangana et al. 2014). In fact, the so-called tripartite symbiosis (legumes, mycorrhizas, and nitrogen-fixing bacteria) is fundamental for the maintenance of these agroecosystems, taking into account that nutrients extracted in each harvest can be compensated by the nutrient input of the nitrogen-rich leaf litter produced by these trees (Cuenca 2015). This delicate equilibrium of biological interactions is unbalanced when agronomic practices are intensified (e.g., reduction of shade trees and increase of agrochemical application) and, although it increases yields in the short term, eventually leads to soil degradation and a progressive loss of soil fertility (Schroth and Krauss 2006). Intensification of agronomic practices significantly reduces AMF diversity and thus the multiple benefits provided by this symbiotic association (Oehl et al. 2010). In this case a great part of the chemical fertilizers are lost by leaching, being a management system highly polluting and inefficient in terms of nutrient use (Wang et al. 2009).

The certification of coffee and cacao as biodiversity-friendly organic products provides better market prices for these crops and represents a successful strategy to promote the reduction of agrochemical use and the conservation of a more diverse tree canopy. The sowing of a higher diversity of woody tree species and the organic conversion of full-sun plantations to shaded ones are practices that are being promoted to recover the productivity and natural fertility of degraded soils (Torres et al. 2008; Vanhove et al. 2016). Despite the growing recognition of the potential of mycorrhizal symbiosis to increase the productivity and sustainability of crops, this issue is generally neglected in management practices. In general, there is a lack of information to select both the appropriate mycorrhizal inoculants to be applied and the adequate agronomical practices for the conservation of native AMF communities. The aim of this chapter is to present the information available regarding i) AMF species associated to coffee and cacao crops in South America; ii) the effect of agroforestry practices on AMF communities; and iii) the influence of AMF diversity on the productivity of these crops.

5.2 AMF Diversity Associated with Coffee and Cacao

The structure of AMF communities is influenced by biotic and abiotic external factors and by intrinsic characteristics of these fungi, such as their dispersal abilities and other life history traits (Chaudhary et al. 2008). In addition to climatic factors such as temperature, light, and humidity, soil properties (pH, texture, organic matter, and nutrients) are abiotic variables that strongly influence the distribution of AMF (Oehl et al. 2010; Herrera-Peraza et al. 2011). Among the biotic factors, the compatibility of host-symbiont interaction influences the mycorrhizal response of host plants and the structure of AMF communities colonizing their roots (Scheublin et al. 2004; Stevens et al. 2020).

Most of the studies describing the AMF community associated with a particular plant are based on the morphological analysis of the spores present in the rhizospheric soil. Spore-based identification has limitations since not all fungi present may be sporulating at the time of sampling and may overestimate the abundance of species with higher sporulation rates (Bever et al. 2001); however, it is still the most widely used method due to its greater accessibility. Molecular methods can detect a greater diversity of AMF and identify which species are colonizing host plant roots, but their application in the South American region is still limited (Cofré et al. 2019).

In this work, the available information about the diversity of AMF associated with coffee and cacao was collected from articles published between 1990 and 2021 obtained through searches in Google Scholar containing the combination of terms "arbuscular mycorrhizal fungi," "cacao or coffee," "*Coffea arabica* or *Theobroma* *cacao*," and "country name" in South America. From the gathered studies, those that included presence records of AMF taxonomically identified to species level were selected (21), which were carried out in Brazil (8), Perú (6), Colombia (4), Venezuela (2), and Ecuador (1). The number of studies for each crop was similar (11–10); however, for coffee, three of the studies conducted in Brazil were review articles, so the information available for this crop in this country is significantly greater. The classification proposed by Redecker et al. (2013) and the list of species at http://www.amf-phylogeny.com/amphylotaxonomy.html published by A. Schüßler (updated November 29, 2021) were used to detect synonyms and unify the nomenclature.

The information obtained about the occurrence of AMF in coffee and cacao agroecosystems in the South American region is summarized in Table 5.1. According to this inventory, there is a high diversity of AMF in these agroecosystems, with a total of 121 species recorded, 104 species in coffee and 67 species in cacao. The greater sampling effort made for coffee could explain the high number of species recorded for this crop compared to cacao, which is similar to that reported for coffee agroecosystem worldwide (>100 species) (Hernández-Acosta et al. 2021). The AMF species found in coffee plantations belonged to eight families, mainly concentrated in Glomeraceae (33%), Acaulosporaceae (28.2%), and Gigasporaceae (20.4%), with the remaining 18.4% in other families. In cacao, nine families were present, being Glomeraceae (37.3%), Acaulosporaceae (26.95%), and Gigasporaceae (10.4%) the best represented families, with 25.4% belonging to other families.

Table 5.1 AMF species present in coffee and cacao plantations according to the review carried out for South America

AMF	Coffee	Cacao
Acaulosporaceae	**29**	**18**
Acaulospora alpina	COL	
Acaulospora bireticulata		BRA
Acaulospora brasiliensis		PER
Acaulospora capsicula	COL	VZL
Acaulospora cavernata	COL	
Acaulospora colombiana	BRA/COL	COL
Acaulospora delicata		VZL
Acaulospora denticulata	BRA/COL	
Acaulospora elegans	COL/PER	
Acaulospora excavata	PER	BRA/VZL
Acaulospora flava	PER	
Acaulospora fragilissima	PER	
Acaulospora foveata[a]	BRA/COL/PER	BRA/COL/ECU/VZL
Acaulospora gedanensis	BRA	
Acaulospora herrerae	BRA/PER	BRA
Acaulospora kentinensis	COL/PER	PER
Acaulospora lacunosa	PER	

(continued)

Table 5.1 (continued)

AMF	Coffee	Cacao
Acaulospora laevis	BRA/COL/PER	
Acaulospora longula	BRA/COL	BRA/PER
Acaulospora mellea[a]	BRA/COL/PER	BRA/COL/PER/VZL
Acaulospora morrowiae	BRA/COL/PER	BRA/VZL
Acaulospora nivalis	BRA	
Acaulospora paulinae	COL	
Acaulospora punctata		PER
Acaulospora rehmii	COL/PER	PER/VZL
Acaulospora rugosa	PER	
Acaulospora scrobiculata[a]	BRA/COL/PER/VZL	BRA/PER/ECU/VZL
Acaulospora sieverdingii	BRA	
Acaulospora spinosa	BRA/COL/PER	PER/VZL
Acaulospora spinosissima	PER	PER
Acaulospora splendida	COL	
Acaulospora tuberculata	BRA/COL/PER	BRA/PER/VZL
Acaulospora verna	BRA	
Incertae Sedis		
Entrophospora infrequens	BRA/COL	
Diversisporaceae	**5**	**4**
Diversispora aurantia	COL	PER
Diversispora pustulata		VZL
Diversispora spurca	PER	COL/PER
Diversispora trimurales	COL	
Otospora bareae	COL	
Sieverdingia tortuosa	BRA	ECU
Pacisporaceae	**0**	**2**
Pacispora chimonobambusae		VZL
Pacispora dominikii		PER
Gigasporaceae	**21**	**7**
Gigaspora albida	BRA	
Gigaspora candida	PER	
Gigaspora decipiens	BRA/VZL	BRA/ECU
Gigaspora gigantea	BRA	BRA/ECU
Gigaspora margarita	BRA/COL/VZL	
Gigaspora rosea	BRA	
Dentiscutata biornata	BRA	
Dentiscutata cerradensis	BRA/COL	
Dentiscutata erythropa	COL	
Dentiscutata heterogama	BRA	PER
Dentiscutata savannicola	COL	
Cetraspora armeniaca	COL	
Cetraspora gilmorei	BRA/COL	VZL
Cetraspora nodosa	COL	
Cetraspora pellucida	BRA	BRA/ECU/PER

(continued)

Table 5.1 (continued)

AMF	Coffee	Cacao
Racocetra alborosea	COL	
Racocetra fulgida		ECU
Racocetra verrucosa	BRA	
Scutellospora arenicola	COL	
Scutellospora calospora	COL	ECU
Scutellospora dipapillosa	BRA	
Scutellospora projecturata	COL	
Claroideoglomeraceae	**3**	**4**
Claroideoglomus claroideum	COL/PER	PER/VZL
Claroideoglomus drummondii		COL
Claroideoglomus etunicatum[a]	BRA/COL/PER	BRA/COL/PER/ECU/VZL
Claroideoglomus luteum		PER
Claroideoglomus walkeri	COL	
Glomeraceae	**34**	**25**
Dominikia aurea	COL	COL
Funneliglomus sanmartinensis	PER	
Funneliformis coronatum	COL	
Funneliformis geosporus	PER	ECU
Funneliformis kerguelensis	COL	
Funneliformis mosseae	BRA/COL	COL/ECU
Funneliformis verruculosus		COL
Glomus ambisporum	COL	VZL
Glomus atrouva		COL
Glomus brohultii	COL/PER/VZL	BRA/PER
Glomus flavisporum	COL	
Glomus fuegianum	BRA	
Glomus glomerolatum	BRA	BRA
Glomus hoi		PER
Glomus macrocarpum	BRA/COL/PER	
Glomus microcarpum	BRA/COL/PER	COL/VZL
Glomus spinuliferum	BRA/COL/PER	BRA
Glomus trufemii	VZL	BRA
Glomus versiforme		PER
Microkamienskia peruviana		PER
Oehlia diaphana	BRA	PER/VZL
Rhizophagus aggregatus	BRA/COL/PER	COL/ECU
Rhizophagus clarus	BRA/COL	COL
Rhizophagus fasciculatus	BRA/COL/PER	COL/PER
Rhizophagus intraradices	BRA/COL	
Rhizophagus invermaium	COL	
Rhizophagus manihotis	BRA/COL	VZLA
Rhizophagus microaggregatum	PER	COL

(continued)

Table 5.1 (continued)

AMF	Coffee	Cacao
Rhizophagus proliferum	COL	
Rhizophagus vesiculiferum	COL	
Sclerocystis clavispora	BRA	
Sclerocystis coremioides	BRA	PER
Sclerocystis liquidambaris	COL	
Sclerocystis rubiformis	COL/PER	PER
Sclerocystis sinuosa[a]	BRA/COL/PER/VZL	BRA/COL/VZL
Sclerocystis taiwanensis	COL	
Septoglomus constrictum	PER	ECU/VZL
Septoglomus deserticola	BRA/COL	
Septoglomus furcatum		BRA
Septoglomus xanthium	COL	
Paraglomeraceae	**4**	**2**
Paraglomus albidum	BRA	
Paraglomus brasilianum	COL	BRA/PER
Paraglomus laccatum	COL	
Paraglomus occultum	BRA/COL	BRA/PER
Ambisporaceae	**3**	**2**
Ambispora appendicula	BRA/COL/PER	BRA/VZL
Ambispora fennica	COL	
Ambispora gerdemannii	BRA	PER
Ambispora leptoticha	BRA	
Archaeosporaceae	**3**	**3**
Archaeospora myriocarpa	BRA	VZL
Archaeospora schenckii	COL	
Archaeospora trappei	BRA/COL	PER
Archaeospora undulata		PER
Total: 121	**104**	**67**

The country of origin of the records reported for each species is indicated (*BRA* Brazil; *COL* Colombia; *ECU* Ecuador; *PER* Perú; *VZL* Venezuela)

[a] Species with the highest frequency of appearance. Data sources: Araujo et al. (2007); Arteaga (2019); Blanco (2006); Bolaños et al. (2000); Carrero (2014); Cogo et al. (2017); Colozzi-Filho and Cardoso (2000); Corazon-Guivin et al. (2021); Cuenca and Meneses (1996); Fernandes et al. (2016); Kramadibrata (2009); Medina (2017); Posada et al. (2016); Prates-Junior et al. (2019); Rojas-Mego et al. (2014); Sandoval-Pineda et al. (2020); Santos et al. (2020); Stürmer and Siqueira (2006); Vallejos-Torres et al. (2022); Vallejos-Torres et al. (2019); Winagraski et al. (2019)

The dominance of the Glomeraceae followed by Acaulosporaceae in both crops is in accordance with that found in natural ecosystems in South America (Cofré et al. 2019) and other regions of the world (Stürmer et al. 2018) and also with the findings on a global scale in coffee agroecosystems by Hernandez-Acosta (2021). The high incidence of the Glomeraceae is associated with its greater capacity for colonization and adaptability to variable conditions (Oehl et al. 2010), also influencing the fact that this family gathers the largest number of species described within the phylum Glomeromycota (Stürmer et al. 2018). Meanwhile, in the Acaulosporaceae, *Acaulospora* was the most species-rich genus (29 for coffee and 18 for cacao), with a high relative frequency in both coffee (Bolaños et al. 2000; Colozzi-Filho and Cardoso 2000; Lozano-Sánchez et al. 2015; Posada et al. 2016; Cogo et al. 2017; Prates-Júnior et al. 2019; Urgiles-Gómez et al. 2020) and cacao (Cuenca and Meneses 1996; Blanco 2006; Carrero 2014; Rojas-Mego et al. 2014; Sandoval-Pineda et al. 2020; Santos et al. 2020). The dominance of *Acaulospora* in coffee has been related to the common presence of this genus in soils with acidic pH, such as the tropical soils in which this crop grows (Hernández-Acosta et al. 2021).

Gigasporaceae was the third most speciose family for both crops (21 for coffee and 7 for cacao), with fewer species reported for cacao. Likewise, at a local scale, Cuenca and Meneses (1996) observed a scarce presence of Gigasporaceae in 16 cacao plantations evaluated in Venezuela, and Blanco (2006) found no species of this family in 20 plantations of this crop in Colombia. Gigasporaceae is usually less abundant in agricultural areas due to its lower tolerance to disturbance and slower colonization rate (Van der Heyde et al. 2017). However, some studies have found a high relative abundance of spores of this family in cacao plantations in Ecuador (Prieto-Benavides et al. 2012), as well as in coffee plantations in the Cerrado area of Brazil with intensive agricultural management (Fernandes et al. 2016), and it is suggested that biogeographic factors could be involved on the observed Gigasporaceae abundance.

Coffee and cacao are crops that naturally grow in understory conditions with differences in altitude range, edaphoclimatic conditions, and forest types with which they are associated (Torres et al. 2008; De Beenhower et al. 2013). Although both crops can coexist in part of their altitudinal range, coffee occurs in the highest parts of the gradient associated with montane cloud forests, while cacao in the lower zones is associated with humid forests such as the Amazon forests from which it is native (Lojka et al. 2017). Despite the different ecological conditions in which these crops can establish, they had in common 50 AMF species, among which *Acaulospora scrobiculata*, *A. mellea*, *A. foveata*, *A. tuberculata*, *Claroideoglomus etunicatum*, and *Sclerocystis sinuosa* showed a high frequency and wide distribution, being found in at least three countries in both crops. These species are frequent in the Neotropics and are highly adaptable to different soil and climatic conditions, being present in a wide variety of biomes (Stürmer and Kemmelmeiner 2021). Other common species shared between both cultures were *Cetraspora pellucida*, *A. morrowiae*, *Rhizophagus aggregatus*, and *R. fasciculatus*. Particularly, *R. aggregatus* was reported by Hernández-Acosta et al. (2021) as the species with the highest presence in the coffee rhizosphere in six Latin American countries.

Some AMF species were found only in coffee (54) or cacao (17) plantations, showing a more restricted distribution (in only one or two countries in the region), which could indicate that they have specific ecological requirements and/or greater affinity for one of these crops. The inventory carried out shows results that could be useful for the selection of AMF species for the preparation of inoculants. Although species with a wide range of distribution and adaptability to different edaphic and environmental conditions are potential candidates, their high colonization and establishment capacity does not necessarily imply a greater effectiveness on the plant. On the other hand, species with crop-specific affinity and/or adapted to particular environmental conditions can also be considered as species of choice for inoculant development. In either case, considering that the effectiveness of mycorrhizal inoculation is a complex multifactorial phenomenon, greenhouse or field tests are essential to assess its effectiveness in the particular crop and ecological condition in which it will be applied.

According to Sturmer and Kemmelmeiner (2021), tropical and subtropical moist broadleaf forests are among the biomes with the highest diversity of Glomeromycota in the Neotropics (186 species). The large proportion of AMF species found associated with coffee and cacao crops in this review (121 species) indicates that these agroforestry systems constitute an important reservoir of AMF diversity. Considering that the South American region is poorly explored in terms of AMF diversity, the presence of these reservoirs becomes even more important. In this regard, Rojas Mego et al. (2014) have pointed out a high proportion of morphotypes present in cacao plantations in the Peruvian Amazon as possible new AMF species and in agreement several recently described species, *Acaulospora flava*, *Funneliglomus sanmartinensis*, and *Microkamienskia peruviana*, have been reported in coffee and cacao agroforestry systems in this region (Arteaga 2019; Corazón-Guivin et al. 2021; Vallejos-Torres et al. 2022).

5.3 Effect of the Agricultural Management on AMF Communities

The capacity of coffee and cacao agroforestry systems to conserve the diversity of AMF communities and the environmental benefits offered by the forest ecosystem varies greatly depending on the cultivation management applied. The classification of agroforestry systems is based on the structural variables of the vegetation (density, richness, and composition of the shade cover) and the agricultural management variables used (fertilization, tillage, use of herbicides and pesticides) (Hernández-Martínez et al. 2009), each of these aspects having an important influence on the AMF communities present.

5.3.1 Shade Cover

In general, the presence of greater tree diversity in agroforestry systems can have a positive effect on AMF diversity and influence the composition of their communities (Bainard et al. 2011). Cuenca and Meneses (1996) found that traditional cacao plantations in Venezuela, with high diversity of shade trees (native and introduced species) and >40 years of cultivation, maintained a diversity of species of AMF similar to that present in undisturbed natural ecosystems. Another study carried out in Venezuela by Carrero (2014) showed that cacao plantations with high shade tree diversity (Fig. 5.1) had significantly higher AMF species richness than the one found in plantations with a forest cover consisting of a single tree species (*Matisia cordata*). It has been suggested that the higher diversity of shade trees promotes a more diverse AMF community due to the greater availability of plant-fungus combinations and increased niche heterogeneity in the ecosystem (Bever et al. 2001).

In coffee plantations maintained as monocultures with full sun exposure (Fig. 5.2), low richness and diversity of AMF are usually found. Although it may be related to the limited diversity of host plants available (Trejo et al. 2011; Fernandes et al. 2016), it is difficult to separate this effect from the high application of agrochemicals that characterizes this type of management. The changes generated in the soil and microclimate due to the absence of forest cover and the greater incidence of light may also be determining factors in the composition of the AMF communities of these agroecosystems (Aldrich-Wolfe et al. 2020).

The identity of shade trees may also influence the arrangement of the AMF present. Muleta et al. (2007) found higher spore density and higher frequency of the genus *Acaulospora* in the rhizosphere of legumes than under the shade of trees of other families. Similarly, Dobo et al. (2018) pointed out that AMF show preferences for specific combinations of tree groups in agroforestry systems based on "tree-enset-coffee." Agroforestry has also been found to promote the distribution of AMF spores at different levels of the soil profile due to the access to deeper layers by the roots of shade trees. The mycorrhizal presence in deep soil layers has important implications on the dynamics of P cycling, making this nutrient more available to plants (Cardoso et al. 2003).

Fig. 5.1 Cacao plantation with shade tree diversity: (**a**) cacao and banana plants in lower strata, (**b**) canopy with diverse shade trees in different strata (Táchira state, Venezuela). Photo's credit: M. Lovera

Fig. 5.2 Coffee unshaded monoculture in deforested agricultural areas (La Guaira State, Venezuela). Photo credit: M. Lovera

5.3.2 Agrochemical Use

In agroforestry systems, the use of agrochemicals tends to increase progressively as the diversity and density of shade trees are reduced, being greater in unshaded monoculture growing in full sunlight. The sustained use of high doses of agrochemicals has a strong effect on abundance and composition of AMF communities leading to a reduction in their diversity (Ma et al. 2020). Particularly, excessive phosphorous fertilization decreases root colonization and sporulation of most AMF (Wang et al. 2009) and may select species less effective in nutrient uptake and plant growth promotion (Johnson and Gibson 2021). In addition, the use of herbicides such as glyphosate reduces both spore germination and mycorrhizal colonization (Druille et al. 2013; Carvalho et al. 2014), and its residual effect hinders the recovery of AMF communities (Wilkes et al. 2020). Furthermore, many of the pesticides and particularly the fungicides applied have an inhibitory effect on the establishment of the mycorrhizal association (Jin et al. 2013; Hage-Ahmed et al. 2019).

The evaluation of AMF diversity of cacao plantations in Venezuela (Cuenca and Meneses 1996) showed that higher concentrations of available P in soils were correlated with less diverse AMF communities. Posada et al. (2016) also found this negative correlation in coffee plantations in Colombia in soils slightly acidic and relatively fertile in comparison with coffee plantations in Mexico (with poor soils strongly acidic). In these last agroecosystems, an intermediate agrochemical management increased AMF richness. The positive effect of low doses of fertilization on physiological activity and AMF diversity present in very poor acidic tropical soils has also been pointed out by Sieverding (1991), indicating that extreme P limitation can affect negatively the functioning of the mycorrhizal association.

On the other hand, Arias et al. (2012) found that coffee plantation with different types of management in Mexico presented similar richness, but different composition in their AMF communities. Management systems that had in common a lack or low level of agrochemical applications presented AMF communities similar to those of the cloud forest taken as reference, despite of having very contrasting vegetation structure. It is suggested that the low level of available P present in these plantations and the reference forest could be the most important selective factor determining the composition of the AMF community. Similarly, Aldrich-Wolfe et al. (2020) found that coffee plantations organically managed with low input did not differ in AMF richness and diversity from conventionally managed ones but did show a change in AMF community composition. *Gigaspora* genus was associated with high radiation intensity and nitrate availability, *Acaulospora* was linked to low nitrate availability, while *Glomus* and *Claroideoglomus* were related with other environmental characteristics of conventionally managed plantations.

In other studies performed in coffee plantations in Brazil, Prates-Junior et al. (2019) showed that agroecologically managed systems harbored higher AMF richness and diversity in comparison with conventionally managed plantations. It is argued that this effect could be associated with the lower application of agrochemicals and decreased competition among AMF species due to the greater heterogeneity and host plant availability of the system. The evaluation by molecular methods of AMF species present showed that the AMF community in agroecological plantations was similar to that of the forest fragment evaluated. This fact was related to the similarity in light availability and host plants between the two environments. Likewise, De Beenhower et al. (2015) in a study conducted in Ethiopia using molecular methods found higher mycorrhizal diversity in coffee plantations established under natural forest in comparison to those with more intensive management systems, and the presence of a group of unique AMF species not shared with the rest of plantations was reported. These results indicate that the maintenance of a high diversity of indigenous shade trees with low application of agrochemicals constitutes a whole desirable management that allows the conservation of a diverse AMF community, similar to that present in the neighboring natural forests.

5.4 Importance of AMF Diversity in the Growth and Productivity of Coffee and Cacao Crops

Agroecosystems that support a higher AMF species richness tended to be more sustainable, due to the functional complementarity between species with different physiological and infective abilities and their synergic effect on plants (Van der Heijden and Scheublin 2007). In farming environments with technical management, the decrease of AMF populations and the disappearance of species are detrimental to the benefits they provide to the crops. In these cases, the functionality of the mycorrhizal association could be recovered by the use of inoculants or the application of agronomic practices that increase AMF populations in the field.

5.4.1 Growth and Plant Nutrition

For coffee and cacao, there are numerous reports about the positive effect of AMF inoculation meanly in nursery stage. There are a variety of responses that seem to depend on the compatibility between the plant-fungus combination and soil fertility, being in general lower the growth response to inoculation when concentration of available P in soil is higher. Within the AMF that have been evaluated individually and have shown a positive response on the growth and/or mineral nutrition of cacao can be cited: *Gigaspora margarita*, *Scutellospora calospora*, *Cetraspora pellucida*, *Ambispora appendicula*, *Funneliformis mosseae*, *Rhizophagus clarus*, *R. irregularis*, *Septoglomus constrictum*, and *Acaulospora tuberculata* (Cuenca et al. 1990; Azizah-Chulan 1991; Oladele 2015; Droh et al. 2016; Padjun et al. 2019; Araujo et al. 2020; Paguntalan et al. 2020; Pongpisutta et al. 2021). On the other hand, AMF species with favorable effects on the growth of coffee plants are *Gigaspora margarita*, *Dentiscutata heterogama*, *Ambispora leptoticha*, *Funneliformis mosseae*, *Rhizophagus clarus*, *R. fasciculatus*, *R. intraradices*, *R. irregularis*, *R. manihotis*, *Claroideoglomus etunicatum*, *Glomus macrocarpum*, *Acaulospora scrobiculata*, *A. colombiana*, *A. laevis*, and *A. mellea* (Sánchez et al. 2000; Bhattacharya and Bagyaraj 2002; Andrade et al. 2009; Cogo et al. 2017; Moreira et al. 2018; Cruz et al. 2020; Hernández-Acosta et al. 2021). It is interesting to note that in the list of AMF with positive effects on growth cited above, there are many AMF species considered as generalists which included several of the most frequently found in the inventory performed (*A. tuberculata*, *A. scrobiculata*, *A. mellea*, and *C. etunicatum*) (Table 5.1). This fact represents an advantage for their use as inoculants, given their adaptability to different edaphic and environmental conditions.

In these agroforestry crops, some studies show that the use of inoculants consisting of a mixture of species (consortia) produces better results than inoculation with a single species (Hernández-Acosta et al. 2018), and in general the best results are achieved by indigenous consortia with high richness of species (Trejo et al. 2011; Droh et al. 2016; Vallejos-Torres et al. 2019). Inoculation with several AMF species applied in consortia can improve P uptake efficiency through the increase in density of external mycelium produced and the complementarity in soil exploration (Van der Heijden and Scheublin 2007). However, according to Hart et al. (2013), the use of consortia could allow the colonization and persistence in the roots of non-efficient AMF species, thus limiting optimal plant growth. Accordingly, a meta-analyses performed by Van Geel et al. (2016) showed that inoculation with individual AMF species was more effective than inoculation with consortia in controlled environments, indicating that these conditions may be beneficial to maintain a high dominance of an AMF effective species previously selected.

AMF inoculation in early stage of plant development is very important considering that substrates used in nurseries are usually sterile or have scarce number of AMF propagules. The use of mycorrhizal inoculants at this stage lets to obtain larger and/or stronger seedlings more resistant to the environmental stress of field transplanting. However, the functioning of the introduced inoculants in the field during the following stages of plant development will depend on their adaptability to edaphic and environmental conditions and their ability to compete with the indigenous AMF present in the site. Thus, it is generally considered advantageous to use inoculants consisting of indigenous AMF species already adapted to field conditions, as compared to the use of commercial AMF inocula (Hart et al. 2017).

Several studies have followed mycorrhizal effect once seedlings are established in the field. Trejo et al. (2011) showed that coffee seedling inoculated with a mix of AMF (from plantations with intermediate technical management) presented greater height values and survival at transplanting than those inoculated with less diverse consortia (from plantations with high technical inputs). The positive effect showed in nursery was sustained during 250 days after sowing. Similarly, Vallejo-Torres et al. (2019) found that inoculation with three different indigenous consortia maintained a positive response on the growth of coffee clones 295 days after planting, with a greater effect of the consortium with the highest diversity in combination with a medium dose of compost.

Regarding the evaluation of long-term crop productivity, Siqueira et al. (1998) measured the grain yield of coffee plants inoculated with three native strains (*Rhizophagus clarus*, *Gigaspora margarita*, and *Claroideoglomus etunicatum*) for 5 years. During the first year, a significant increase in the yield of mycorrhizal plants (>50%) was obtained in the presence of moderate doses of P. This result indicates that in highly leached acidic tropical soils where P is very limited due to its immobilization in aluminum phosphates, a moderate fertilization is necessary to obtain the benefits of the symbiosis. In the following years, the effect of mycorrhizal inoculation was less consistent, generally reducing the observed differences. This decrease in the observed effect was related to colonization in the field by indigenous AMF of control plants (non-inoculated) and to the lower P requirement of mature plants. At the mature stage of crop development, protection against pathogens and other functions related to adaptation to environmental factors are likely to become more relevant than nutritional aspects.

5.4.2 Resistance to Biotic and Abiotic Stress

In addition to nutritional factors, the productivity of coffee and cacao crops can be greatly affected by pathogen attack. It has been found that some of the stress events caused by diseases associated with these pathogens can be alleviated by the presence of AM fungi. For example, root-knot parasitic nematodes (*Meloidogyne exigua*) decrease their pathogenic effect on coffee plants inoculated with AM fungi (Alban et al. 2013; Vallejos-Torres et al. 2020). According to Alban et al.

(2013), the increased plant growth, better phosphorous nutrition, and thickening of the root cuticle observed in mycorrhizal plants are factors that increase resistance to nematode infection. Similarly, Vaast et al. (1998) found that *Pratylenchus coffeae* root lesions were lower in coffee plants with well-established mycorrhizal symbiosis and improved P status. In relation to rust (*Hemileia vastatrix*), a disease with a high negative impact on coffee production worldwide and a lower incidence and severity of this disease has been found in the presence of AMF (Vallejos-Torres et al. 2021). Mycorrhizal colonization also increases resistance to other pathogens such as *Fusarium solani* (Al-Areqi et al. 2015), *Cercospora coffeicola* (Guzmán and Rivillas- and Osorio 2007), *Colletotrichum gloeosporioides* (Colmenárez-Betancourt and Pineda 2006) and *Rhizoctonia solani* (Pereira 1994). In cacao, plants inoculated with AMF exposed to *Phytophthora megakarya* (black pod rot) improved growth and showed a significant reduction in the expression of this disease (Tchameni et al. 2012; Paguntalan et al. 2022), which is one of the main limitations for the productivity of this crop.

Regarding abiotic stress factors, drought is one of the most relevant affecting the survival and growth of plants in the field. AMF facilitate water uptake directly through the mycelial network or indirectly through increased root development and the improvement of soil structure (Cuenca 2015). The benefits of mycorrhizal association under drought stress were evidenced by Moreira et al. (2018) in an assay in which coffee plants inoculated with three AMF strains (*R. clarus*, *C. etunicatum*, and *D. heterogama*) increased their tolerance to moderate water deficit. Likewise, for cacao, the application of selected AMF strains has been proposed as a strategy to increase the drought resistance of this crop (Seutra-Kaba et al. 2021). Finally, the toxicity due to the accumulation of heavy metals (in polluted or metalliferous soil) is an important aspect to consider not only due to its negative effect on crop growth but also because of the limitations associated with its presence in the grain and the risk of entry into the food chain (Arévalo-Gardini et al. 2017). The use of AMF has been proposed as a mitigation mechanism given the ability of some AMF to immobilize these metals in the intraradical hyphae or in the mycorrhizosphere, reducing their concentration in the plant shoot. In particular, the role of mycorrhizae in alleviating cadmium accumulation in cacao has been one of the most studied topics. In this sense, some efforts have been directed to explore the diversity of indigenous AMF in soils with high cadmium concentration in order to select stress-tolerant strains that can be used to mitigate its toxic effect and reduce the presence of this element in the plant shoot (Sandoval-Pineda et al. 2020; Vallejos-Torres et al. 2022).

5.5 Conclusions

Agroforestry systems maintained with low inputs under the shade of native trees represent an excellent strategy for the conservation of biodiversity both above- and belowground, constituting important reservoirs of a wide variety of AMF species with diverse functional traits and different potential benefits. Even though the inventory of AMF species present in coffee and cacao plantations in the South American region is still limited, it provides useful information for understanding the AMF communities associated with these agroforestry systems. In particular, the selection of effective indigenous AMF strains or consortia for early inoculation in the nursery stage is a recommended practice for the management of these crops, being particularly important for the recovery of productivity in plantations where high-input management has caused a significant loss of diversity of the AMF community. Given that coffee and cacao are two crops of great socio-economic importance in South America, the use of AMF in more sustainable management practices is a promising path in the search to balance conservation needs with the economic and food requirements of the population. To achieve this goal, further in-depth research is needed for the effective management of this complex symbiotic interaction.

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Chapter 6
Potential of Arbuscular Mycorrhizas for the Remediation of Soils Impacted with Pollutants

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6.1 Introduction

Soil contaminated by petroleum hydrocarbons is one of the most studied topics for the development of remediation technologies due to the extensive use of crude oil and the frequency of spills that cause serious damage to soils and ecosystems. Phytoremediation is a technique for the rehabilitation of soils impacted by hydrocarbons that has received increasing interest as a passive, aesthetically pleasing and useful procedure for simultaneously attacking a wide variety of pollutants. Its application cost is lower compared to other physical or chemical treatments, in addition to being an environmentally friendly technique. Depending on the geological characteristics of the impacted soil, its physicochemical characteristics, and microbiological activity, pollutants can undergo chemical and/or microbiological transformations that can influence their fate and transport in the environment. In tropical lowland areas, such as Venezuela, phytoremediation is advantageous due to the warm and almost constant temperatures throughout the year, which favor plant growth and the activity of soil microflora, under adequate conditions of humidity and nutrients.

Soil microorganisms play an important role in the bioremediation process and, associated with plants, contribute to the degradation of pollutants. Plants and their associated rhizospheric microbes interact with each other, and in most cases, plant often supplies the microbial population with carbon sources that stimulates the mineralization of organic contaminants by the indigenous microbial populations in the soil environment. Petroleum hydrocarbons present in the contaminated soil are degraded by plant-associated microbes which can involve endophytic and rhizospheric bacteria. The utilization of the synergy between plants and rhizospheric microbes has a great potential to deal with petroleum hydrocarbons in effective soil remediation process.

González-Chávez et al. (2004) sustain that mycorrhizae are used in bioremediation processes, since they intervene effectively in the recovery process of oil-contaminated soils. El-Din Hassan et al. (2014) observed that mycorrhized willows had a better tolerance in oil-contaminated soil than non-mycorrhized ones, suggesting that these plants, when taken to the field, could be potential phytoremediators. Nwoko (2014) evaluated the response of mycorrhized *Phaseolus vulgaris* L. in soils contaminated with different doses of crude oil, finding a 23% hydrocarbon removal rate in a soil with 2% crude oil. Rajtor et al. (2016) showed that in the rhizosphere of mycorrhized plants grown in soils contaminated with hydrocarbon, the number of microbial populations increased with the accumulation of hydrocarbon in the root zone. Their results suggest that the plant-mycorrhiza combination favors the dissipation of crude oil in the soil. Lenoir et al. (2016) observed that mycorrhizal plants reached a dissipation rate of 48% of hydrocarbon in contaminated soil.

Infante et al. (2012) described around 28 plant species in Venezuela associated with arbuscular mycorrhizae present in oil-contaminated soils. They suggest that the symbiosis could favor the establishment of plants in soils contaminated with hydrocarbons. Toro et al. (2017) also showed favorable results of *Brachiaria* *brizantha* inoculated with native mycorrhizae in soils impacted with hydrocarbons, which removed 80% of the total petroleum hydrocarbons (TTP) present in the soil.

Glomalin has been described as a hydrophobic glycoprotein abundantly released from Glomeromycota fungi hyphae in soil, is composed by a persistent carbon fraction, and appears to play an essential role as binding agent of soil particles to form aggregates, improving soil structure. It is also known as glomalin-related soil protein (GRSP) that includes other soil proteins or components than glomalin with similar characteristics (Rillig 2004). It has been proposed that GRSP have a high binding capacity for certain toxic metals (Cu, Cd, and Pb) and mitigate damage of contaminated soils to plants (Vodnik et al. 2008). Chen et al. (2020) found that GRSP reduced significantly soil sorption capacities for phenanthrene. Gałązka et al. (2020) found that mycorrhizal fungi, through GRSP production, significantly increased the efficiency of the remediation process in soils contaminated with crude oil. Our purpose in this work is to evaluate the effect that hydrocarbon contamination has on AM propagules and the production of GRSP in the rhizosphere of *A. setifolia*, dominant plant of a savanna impacted by an oil spill.

6.2 Materials and Methods

6.2.1 Plant Selection: Sampling

The study area is located at 8° 40´09´´N and 62 ° 59´03 W, within the Orinoco River Oil Belt, northeast of Venezuela. In this, the dominance of a single species of grass, *Aristida setifolia* Kunth, typical of the eastern Venezuelan savannas, was observed (Fig. 6.1). Eight samples of the plant and its rhizospheric soil were taken in the area near a hydrocarbon pit, called the contaminated zone (CZ), and three samples from the uncontaminated zone (NCZ) as a control. The samples were taken with a core

Fig. 6.1 General appearance of *Aristida setifolia* Kunth. Source: Royal Botanical Garden. Plants from the world online https://powo.science.kew.org/. November 2021

8 cm in diameter and 20 cm long at a depth between 0 and 20 cm. Once in the laboratory, the samples were refrigerated at 4 °C until processing.

6.2.2 Determination of Saturated, Aromatic Compounds, Resins, and Asphaltenes (SARA) and Total Petroleum Hydrocarbons (% TPH)

This analysis was performed in triplicate on a composite sample from the contaminated and uncontaminated zones. The determinations were based on the hydrocarbon detection method of 3540C (USEPA 1996), according to which the detection of SARA (saturated, aromatic, resins, and asphaltenes) is carried out with the use of IATROSKAN MK 6/6 s and thin layer chromatography coupled by means of a flame ionization detector (FID).

6.2.3 Isolation and Quantification of Spores of Glomeromycota Fungi

It was carried out with the wet sieving and decanting method, according to Gerdemann and Nicolson (1963) and subsequent centrifugation with 80% sucrose (Jenkins 1964), after which the clean spores were placed in a Petri dish with water for observation and quantification.

6.2.4 Quantification of Mycorrhizal Root Length (% LRM) in Roots of A. setifolia

The secondary roots of *A. setifolia* were extracted and washed with tap water. Once cleaned and dried, trypan blue staining was performed, according to Phillips and Hayman (1970). Subsequently, the mycorrhizal root length was quantified according to Giovanetti and Mosse (1980).

6.2.5 Extraction, Staining, and Quantification of Glomeromycota Fungi Mycelium

The mycelium of Glomeromycota fungi in the rhizosphere of *A. setifolia* was extracted using 37% sodium hexametaphosphate as dispersant solution. Then the sample is vacuum filtered using a 0.2 μm nylon filter, according to Boddington et al.

(1999). Once having the mycelium on the filter, the hyphae were stained with trypan blue, as described by Phillips and Hayman (1970). The mycelium measurement was performed using Image Pro Plus software and a LEIKA model MC190HD camera, coupled to the microscope, at 40X magnification. Mycelium measurements were expressed as mycelium length per sample weight (m/g soil).

6.2.6 Determination of Total Glomalin-Related Soil Proteins (T-GRSP)

Determinations were made with the method proposed by Wright and Upadhyaya (1998), which consists of placing 2 g of rhizospheric soil with 8 ml of a 50 mM sodium citrate extracting solution in centrifuge tubes. The mixture is placed in the autoclave for 90 min at a temperature of 120 °C. At the end of this procedure, a reddish-brown solution was obtained, which was stored at 4 °C. This process was repeated until a clear yellow solution was obtained. At the end of the extraction cycle, all the extracts were joined to perform protein quantification by Bradford (1976).

6.2.7 Determination of Easily Extractable Glomalin-Related Soil Proteins (EE-GRSP)

The previous procedure proposed by Wright and Upadhyaya (1998) was carried out, but with a single extraction of 2 g of rhizospheric soil and 8 mL of 8 mM sodium citrate solution. The proteins were then quantified using the method of Bradford (1976).

6.2.8 Quantification of GRSP Present in Sodium Citrate Extracts: Bradford Method

An aliquot of the sodium citrate extracts obtained for T-GRSP and EE-GRSP determinations was taken, and the Bradford (1976) methodology was applied, according to which the amount of proteins present in the extract is determined by reacting with Coomassie blue dye, using bovine serum albumin as a pattern. The results were expressed as mg protein/g of soil.

6.2.9 Statistical Analysis

With the results obtained from each of the analysis, we proceeded to verify whether the data complied with the ANOVA assumptions. Mean comparison tests were performed ($p < 0.05$). When the assumptions of the ANOVA were not fulfilled, the non-parametric statistics test, Kruskal-Wallis, was performed ($p < 0.05$).

6.3 Results

6.3.1 Soil Description

Soils are moderately acidic, with sandy to sandy-loam texture, low availability of N and P, and characteristic of Ultisols present in the Venezuelan savannas (Table 6.1).

Physicochemical comparison between contaminated and non-contaminated areas showed a small increase in the pH of the contaminated soil that corresponds well with the significant increase in electrical conductivity in CZ. Organic carbon is higher in CZ but does not differ statistically from NCZ, while N content is slightly higher in CZ.

6.3.2 Hydrocarbon Analysis Description

The percentage of total petroleum hydrocarbons (% TPH) was determined, as an indirect measure of the percentage of oils and fats, obtaining values of 1.8% of TPH in CZ and 0% of TPH in NCZ. According to the No. 5245 Official Gazette of the Republic of Venezuela, the percentage of fats and oils must be less than 1% in the surface soil. Our results show the presence of hydrocarbons above the values allowed by the legislation. The fractions obtained in the determination of the composition of the hydrocarbon according to the SARA analysis were 15.48 mg/g of saturates, 85.21 mg/g of resins, and 7.311 mg/g of asphaltenes (Fig. 6.2).

Table 6.1 Physicochemical characterization of rhizospheric soil samples from *A. setifolia* growing in hydrocarbon-contaminated (CZ) and non-contaminated zones (NCZ)

Sampling zone	Texture	pH	EC (dS/cm)	% OM	% OC	% *N*	Available *P* (mg/kg)
CZ	Sandy	6.05± 0.60 a	3.68 ± 0.10 a	0.91 ± 0.76 a	0.53 ± 0.40 a	0.05 ± 0.04 a	0.2 ± 0.00 a
NCZ	Sandy-loamy	5.95 ± 0.72 a	1.92 ± 0.06 b	0.54 ± 0.34 a	0.31 ± 0.20 a	0.03 ± 0.01 b	0.2 ± 0.00 a

References: t test for means comparison ($p < 0.05$). Different letters in the same column indicate statistically significant differences. Average values ± standard deviation ($n = 3$)

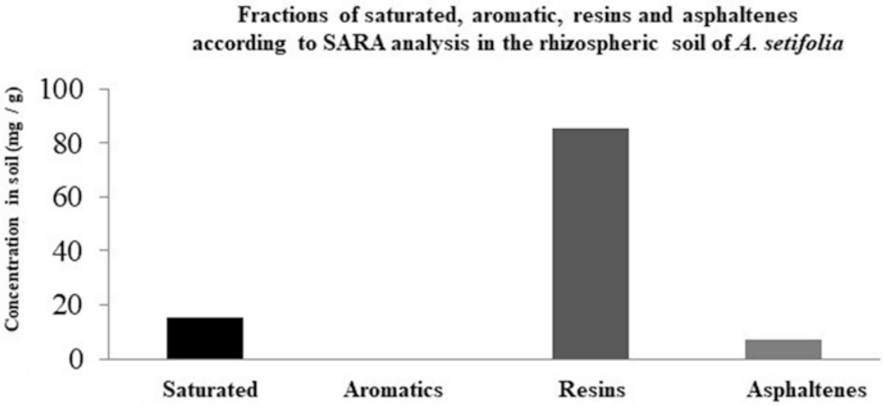

Fig. 6.2 Fractions of saturated, aromatic, resins, and asphaltenes according to SARA analysis in the rhizospheric soil of *A. setifolia*

Table 6.2 Glomeromycota spore number, mycelium length, percentage of mycorrhized root length (% MRL), total (T-GRSP) and easy extractable (EE-GRSP) glomalin-related soil proteins in the rhizospheric soil of *A. setifolia*

Sampling zone	N° Glomeromycota spores/100 g de soil	Hyphal length (m/g de soil)	% MRL	T-GRSP (mg protein/g soil	EE-GRSP (mg protein/g de soil)
CZ	101 ± 19.57 a	31.44 ± 0.20 a	50.60 ± 8.53 a	2.05 ± 1.06 a	1.67 ± 0.68 a
NCZ	168 ± 10.56 b	55.61 ± 0.03 b	85.48 ± 5.93 b	1.48 ± 1.22 b	1.63 ± 0.62 a

Kruskal-Wallis test ($p < 0.05$). Different letters in the same column indicate statistically significant differences. Average values ± standard deviation ($n = 3$)

Aromatics, the most toxic fraction of the hydrocarbons, were not detectable, most likely due to volatilization and natural attenuation of these compounds. If degradation process is occurring naturally, a favorable condition in the recovery of contaminated soils may be happening, since polycyclic aromatic hydrocarbons are highly dangerous for health due to their carcinogenic power. The resin and asphaltene fractions are of low biodegradability and still persist.

6.3.3 *Glomeromycota Spore Number*

The presence of hydrocarbon pollutants had a direct impact on the amount of Glomeromycota spores present in the rhizosphere of *A. setifolia* in CZ (Table 6.2). Verdín et al. (2006) and Debiane et al. (2009) observed the hydrocarbons had a direct impact on AM fungal populations, reducing the amount of spores and mycorrhizal colonization. However, the prevalence of plants present in areas affected by the presence of oil can be mediated by the benefits of symbiotic associations. Infante et al. (2012) found that most of the native species that grew in soils contaminated with hydrocarbons were mycorrhized. The mycorrhizal association could have favored the presence of *A. setifolia* in the soils contaminated with hydrocarbon. Gao et al. (2014) point out that plants with symbiotic associations can tolerate certain concentrations of hydrocarbons, since by means of radical exudates, the biodegradability of the compound is favored. This in turn can increase bacterial populations with the potential to degrade them through enzymatic activities, favoring the use of C and phosphorus and nitrogen. The spore values obtained in NCZ were slightly lower than those reported by López-Gutiérrez et al. (2004) on a *T. plumosus* savanna (189.5 spores/100 g of soil) in the rainy season.

6.3.4 Mycelium Length of Glomeromycota Fungi in the Rhizospheric Soil of **A. setifolia**

In CZ the length of the mycelium is 31.44 m / g of soil, significantly less with respect to the value obtained for NCZ, 55.61 m/g of soil. Borie et al. (2005), Seguel et al. (2008), and Curaqueo et al. (2010) indicate that the density of hyphae decreases in the soil when disturbed by tillage or the presence of contaminants, which would be happening in this case, as the rhizosphere of *A. setifolia* is subjected to oil spill (Table 6.2).

6.3.5 Percentage of Mycorrhizal Root Length (% MRL) of **A. setifolia**

In CZ, mycorrhizal root colonization of *A. setifolia* was 50.61%, significantly lower than that obtained for NCZ, 85.48%. Cabello (1997) found a 30% decrease in the root colonization of *C. dactylon* due to the effect of hydrocarbon contamination. Franco-Ramírez et al. (2007) report that in hydrocarbon-contaminated grasslands, native species *E. polystachya* had 63–77% of mycorrhizal root colonization. This suggests that the infectivity of AM propagules may have been affected in the presence of hydrocarbon, although this response also depends on the type of host plant and the affinity for AMF. The decreases in the number of spores and amount of mycelium in CZ with respect to NCZ suggest that the effect of the hydrocarbon can affect the amount and/or viability of AMF propagules, therefore, its infective capacity (Table 6.2).

6.3.6 *Quantification of T-GRSP and EE-GRSP in Rhizospheric Soil of* A. setifolia

T-GRSP for CZ was 2.05 mg of total protein/g of soil, significantly higher than 1.48 mg of total protein/g of soil in NCZ. Glomalin or GRSP has been observed to have a mitigating effect on organic pollutants on plants. Gao et al. (2017) obtained 1.7 mg protein/g soil in a trial with aromatic hydrocarbons in cultures of *M. sativa*, similar to this work. Furthermore, Thavamani et al. (2012) found that enzyme activities were severely inhibited in soils that were contaminated with both aromatic hydrocarbons and heavy metals. Our results suggest that the increased production of T-GRSP in ZC could be a mechanism by which *A. setifolia* tolerates the adverse effects of the pollutant in the rhizosphere.

Regarding EE-GRSP, the values obtained for ZC were 1.67 mg of protein/g of soil similar to the values detected in ZNC 1.63 mg/g of soil. Lozano (2015) recorded 1.5 mg of protein/g of soil in a plot affected by fire. Due to the presence of gas burners, the sampling area is exposed to high temperatures, which would directly affect the viability of the proteins. Temperature and water availability, among other factors, would affect mycorrhizal propagules and the amount of proteins in the soil, which in turn would affect the EE-GRSP content (Table 6.2).

6.4 Conclusions

Hydrocarbon pollution affected chemical parameters of soil in the contaminated area, a small increase in the pH of the contaminated soil that corresponds with the significant increase in electrical conductivity in CZ. Organic carbon is higher in CZ but does not differ statistically from NCZ. The greater % of total petroleum hydrocarbons (% TPH) determined and the persistence of the hydrocarbon content above the permitted levels indicate that the soil is contaminated. Despite the fact that AM propagules were negatively affected, the higher production of T-GRSP in the rhizosphere of *A. setifolia* would represent a defense mechanism to mitigate the contaminating effect of the hydrocarbon. These results may indicate that more effective protection to the plant occurs due to GRSP production in hydrocarbon-contaminated soils.

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Chapter 7
Diversity of Arbuscular Mycorrhizal Fungi in the Ecuadorian Amazon Region

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7.1 Introduction

As mentioned by Alexander von Humboldt, the Amazon region is a diverse network driven by “active and organic powers.” Given the technology available at Humboldt’s time, the diversity described was mostly limited to what the eye could see: mainly the incredible diversity of “macro-organisms” or the larger life forms such as plants and animals. This only partly tells the story about the biodiversity present in any given ecosystem on the planet. Even with all the technological advances of today, what lies beneath the soil surface remains in part a mystery to science. The diversity, abundance, and ecological importance of smaller life forms such as fungi and other microorganisms are a research field that has yet to be further developed, for instance, in the Ecuadorian Amazon region.

Soil fungi play a central role in understanding macro-biodiversity and how they shape ecological processes fundamental to the Amazon rainforest. To quote Paul Stamets in his book entitled *Mycelium Running*, “What goes on beneath the forest floor is just as interesting - and just as important - as what goes on above it. A vibrant network of nearly microscopic threads is recycling air, soil, and water in a continuous cycle of balance and replenishment. Survival depends not on the fittest but on the collective” (Stamets 2005). Revealing the role mycelial networks play within ecosystem functioning and how they could be linked to large-scale environmental processes which are crucial for plants, animals, and us humans could support efforts to promote the conservation of the Amazon rainforest. Within the fungal kingdom, arbuscular mycorrhizal fungi are known to establish intimate symbiotic relationships with plants and would therefore be an excellent subject to enhance scientific knowledge about the hidden realms of the soil biome as the base of a healthy Amazon rainforest in Ecuador and could even contribute to finding a balance between conservation efforts and sustainable development of the entire Amazon Basin.

Mycorrhizal associations have been explored by different methods over time. Albert Bernard Frank, in 1985, for the first time proposed the concept of the mycorrhizal symbiosis (Antoine et al. 2021). From that point forward, palaeobotanical, morphological, and phylogenetic data have demonstrated that plants and mycorrhizal fungi have co-evolved for more than 400 million years (Brundrett 2009). Four different types of mycorrhizal associations have been described: two major groups are formed by ectomycorrhizae (EcM, mycelia grow outside the root tissues) and endomycorrhizae (with the most common type as arbuscular mycorrhizae or AM), whereas orchid and ericoid mycorrhizae are specialized types which form unique mutualistic relationships with members of the Orchidaceae or Ericaceae, respectively. A common method to determine the type of mycorrhizal fungal colonization involves chemical staining in root tissues, combined with morphological characterization and molecular taxonomy to identify the species level.

Arbuscular mycorrhizae are the most diverse and widespread symbionts of plants. These aseptate filamentous fungi comprise a monophyletic and taxonomically diverse clade within the Glomeromycota (Oehl et al. 2009; Öpik et al. 2014).

As obligate biotrophs, their survival depends on the successful colonization and establishment of the symbiosis with host plant roots by penetrating cell walls and forming intracellular fungal structures, called arbuscules. Here, the fungus receives plant-fixed carbon (C) in exchange for macro- and micronutrients, such as phosphorus (P) and nitrogen (N). AM fungi are known to transfer up to 90% P and 80% N requirements for the plant's overall development (Smith and Read 2010). The bidirectional exchange established between AMF and host plant is considered a costly investment; however, it is through this reciprocal exchange that both partners can meet important physiological needs and increase fitness and survival, particularity in nutrient-deficient soils. AMF symbiosis positively impacts plant productivity (Cofré et al. 2019; Lugo and Pagano 2019) on the one hand through plant dispersal, competition, and coexistence as processes at the community level and on the other hand by soil C sequestration and soil aggregate stabilization at the ecosystem scales (Duchicela et al. 2012; van der Heijden et al. 2015).

Nutrient availability acts as an important selective pressure dictating the specific requirements and circumstances under which plants allow the establishment of AMF symbiosis. In the Amazon rainforest, due to a combination of high temperatures and daily precipitation, soils suffer from leaching which leads to certain nutrient deficiencies. In fact, soils in the Amazon Basin frequently not possess the optimal nutrient balance required to support tropical agriculture. Although high amounts of rainfall and high temperatures create near-optimal environmental conditions to allow high organic matter decomposition rates to return most of the nutrients back to the soil, over time, this process leads to lixiviation. This process leaches nutrients to deeper parts of the soil keeping them out of reach for most plant roots or can inhibit the rate of bioavailability of certain nutrients (Ensley-Field 2016). Leaching due to high rainfall also creates more acidic soils. Along with low nutrient availability, this can result in forests with a low net primary productivity and lower species richness. However, this is not an accurate description of the Amazon rainforest, where high primary productivity in reality leads to high biodiversity. Despite the rapid nutrient cycling in the Amazon rainforest, overall P and N availability is relatively low, which leads to the question: how an ecosystem that features soils with low phosphorus concentrations can harbor the highest rates of plant diversity of the entire planet (Ensley-Field 2016)? Could P as a limiting resource in tropical forest soils provide an increased selective pressure on host plants to engage in AMF symbiosis? Providing an increased host plant phosphorus and nitrogen uptake, AMF could actively play a central role in maintaining and promoting high plant diversity in tropical ecosystems (Bennett et al. 2017; Sheldrake et al. 2017). It is likely that mineralization rates and local leaching processes within Amazonian forest communities will affect the type of mycorrhizal association.

To date, this hypothesis has yet to be explored for the Ecuadorian Amazon region. In this chapter, we review the state-of-art knowledge regarding mycorrhizal explorations in the Ecuadorian Amazon region, and we predict the potential status of mycorrhiza by studying the relative abundance of mycorrhizal plants in the three predominant habitats at the Ecuadorian amazon region, known as Terra Firme, Varzea, and Igapo ecosystems.

7.2 Methods

7.2.1 *Study Site Description*

The Amazon rainforest is the largest natural region in Ecuador, spanning six provinces with a total extension of 73,909 km^2 (Fig. 7.1). This bioregion has elevations below 600 masl, and it is tropical jungle that encounters the highest average rainfall (3349 mm per year) in the country. From the four predominant habitats known as Terra Firme, Varzea, Igapo, and Campinas which have been described for the nine countries that comprise the Amazon Basin, only the first three are abundant in the Amazon region of Ecuador. Each of these habitats is differentiated by plant, animal, fungal, and microbial species abundance and diversity, as well as their particular physical and chemical conditions. Terra Firme forests are habitats that are slightly more elevated, with well-drained soils that contain a higher clay composition. These forests are characterized by vegetation commonly divided by strata as well as small open areas created by falling trees known as tree gaps. Tree diversity in Terra Firme forests is high with approximately 200 to 300 species per hectare. Varzea forests are periodically flooded by white water rivers that originate in the Andean highlands. These rivers carry eroded sediments and therefore contain high amounts of nutrients. When compared to Terra Firme, Varzea forests showcase a distinct soil architecture with higher nutrient availability, a deeper layer of organic matter and less clay (Ensley-Field 2016). Igapo forests are less abundant and have smaller patches, as these forests remain flooded for longer periods of time and have a high concentration of tannins dissolved in water due to vegetation decomposition, giving them the term blackwater ecosystems (Ensley-Field 2016). Riverine forest, riverine island scrub habitats, and palm marshes of *Mauritia flexuosa* are also present in the Ecuadorian Amazon, but with limited geographical extensions. Despite the before-mentioned differences, these three predominant habitat types of the Ecuadorian Amazon rainforest, share high temperature, humidity, and rainfall for most part of the year.

7.2.2 *Mycorrhizal Type and Plant Status*

The relative abundance of mycorrhizal plants served as an estimate for the representation of the symbiosis in the plant community (Gerz et al. 2016). Characteristics of interest include mycorrhizal type (e.g., AM, EcM). Plant mycorrhizal type informed about the role of mycorrhizal symbiosis in nutrient cycling processes (Read and Pérez-Moreno 2003). Moreover, plant mycorrhizal status, defined as whether or not a plant species has the potential to form mycorrhiza (e.g., mycorrhiza and non-mycorrhizal), can serve as a proxy for the extent to which plants rely on mycorrhizal symbiosis (Moora 2014). We analyzed the mycorrhizal status of the Ecuador Amazon flora checklist which we elaborated from published vegetation surveys as published in scientific literature. We determined the plant-mycorrhizal status using

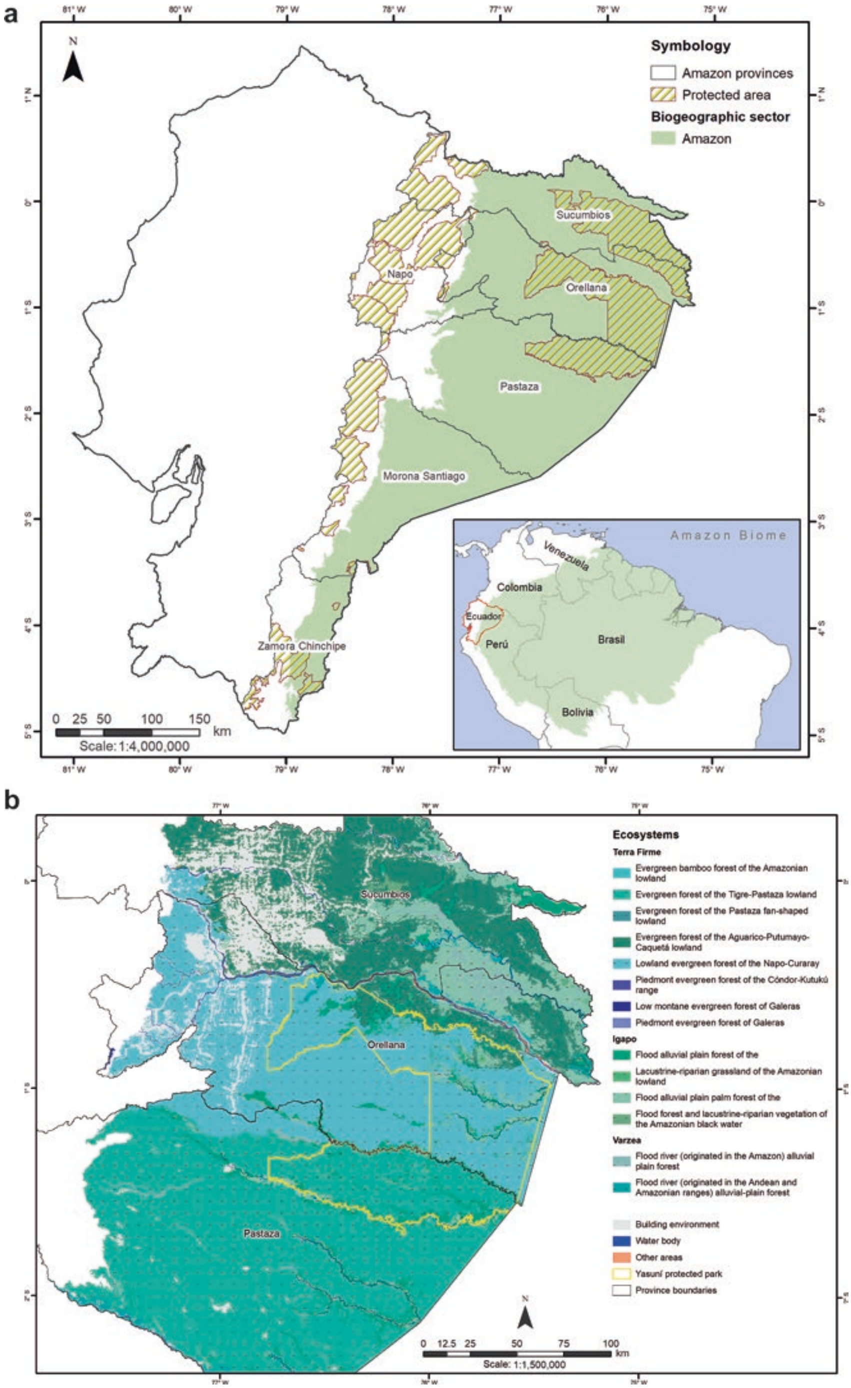

Fig. 7.1 Amazon region in Ecuador. (**a**) Geographical map of the six Ecuadorian provinces with tropical rainforest cover (green areas). Striped, yellow areas indicate protected areas under the National Protected Areas System (SNAP; Ministerio del Ambiente del Ecuador, 2013). (**b**) Location of Yasuní National Park (area highlighted in yellow) and the surrounding Amazon provinces of Sucumbios, Orellana, and Pastaza. The green to blue color gradient indicates the ecosystem cover for Terra Firme, Varzea, and Igapo habitats according to the Ecosystem Classification System of Continental Ecuador. Source: Ministerio del Ambiente del Ecuador (2013)

Brundrett (2009) and Wang and Qui's (2006) checklists of the mycorrhizal status of land plant families. Finally, a chi-square analysis was performed to understand whether mycorrhiza status is directly associated to the plant origin status.

7.2.3 Mycorrhizal Literature Selection Process

We identified publications based on an initial literature search using the Institute for Scientific Information Web of Knowledge (now Clarivate Analytics Web of Science®) and the Google Scholar database, searching the following keywords *mycorrhiz**, *Amazonia**, and *Ecuador**. We narrowed our initial list with selected publications down by filtering for inclusion criteria and scanning for root colonization and mycorrhizal diversity in roots and soil.

7.3 Results

The total list of vascular plants incorporated in our study as presented in this chapter contained 162 plant species of the Ecuadorian Amazon region with information regarding their taxon, origin, and plant functional group. Furthermore, we assessed their mycorrhizal type, and status, for all listed species according to previously published checklists on the mycorrhizal status of land plants families by Brundrett (2009) and Wang and Qui's (2006) (Supplementary information Table S1). This list consisted of 64% native, 6% endemic, and 28% exotic species. Based on information about their plant functional type, 28% of the plant species were attributed to grasses, 2% to legumes, 40% to herbaceous flowering plants or forbs, and the remaining 38% to woody species.

When considering plant type and status, 5% of plant species did not form any type of mycorrhizal associations, 2% of the plant species belonged to families that typically associate with ectomycorrhizal fungi (EcM), 85% of the plants belonged to families that typically associate with arbuscular mycorrhizal fungi (AMF), and 8% could not be found in plant family's records. Regarding specific types of mycorrhizal associations with host plant species, most of the species were characterized to engage in relationships with AMF across plant origin and functional group. Almost 90% of the native and non-native plant families were categorized as mycorrhizal plant status (Fig. 7.2). The chi-square test was not significant, suggesting that these two variables are independent.

Lunt and Hedger (1996) were the first to report mycorrhizal infections observed after root staining methods in an original set of 23 plant species from Terra Firme communities in the Ecuadorian Amazon. As a result, they reported that AMF infections were the dominant mycorrhizal type within plants of these Terra Firme communities. Tedersoo et al. (2010) studied the presence of ectomycorrhizal associations in roots of plants in a 30 ha plot in Yasuni National Park by a combination of direct observations of root tips and fruiting bodies and subsequent DNA sequence

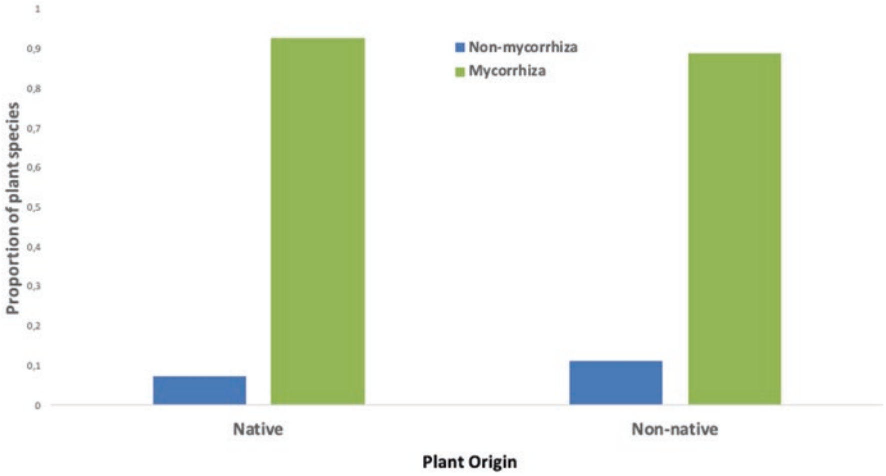

Fig. 7.2 Occurrence of plant mycorrhizal status in native and non-native plants species from the Equadorian Amazon region

analysis. They reported relatively low richness of EcM fungi, contrasting the results of studies from temperate and tropical research sites in the Northern and Southern Hemisphere. The results as previously published in the two beforementioned reports are consistent with the results from our mycorrhizal status analysis presented here.

Moreover, Hickey (2020) observed mycorrhizal colonization of roots of seedlings of the genus *Inga* (Fabaceae) collected in Varzea and Terra Firme forest habitats at the Tiputini Biodiversity Station, located at the north flank of the Tiputini river. This study confirmed AMF colonization in roots of members of the genus *Inga* collected from both habitats.

Regarding molecular identification of AMF, we encountered nine studies which were conducted in the Amazon rainforest of Ecuador within the NCBI and ENA database, which included information of molecular tools to taxonomic analysis of mycorrhizal fungi. Table 7.1 depicts the different methodological experimental designs, sample sizes, and study locations for all nine studies. For example, Haug et al. (2019) analyzed 61 plants from pristine primary and altered secondary forests. To extract DNA of dried mycorrhizae, they used the innuPREP Plant DNA kit and sequenced 18S rDNA amplicons. They used OPTSIL software to define their OTUs, while the taxonomic classification was done using BLAST search against the nucleotide sequence NCBI database. Their results showed a high richness of Glomeromycota in these specific tropical rainforest plots (825 sequences/25%). Garces et al. (2017) conducted a study focused on mycorrhizal diversity and dynamics at La Joya de los Sachas in the province of Orellana, at an oil-polluted site where plant roots were analyzed of the following species: *Monotagma* sp., *Polybotrya* sp., *Geonoma* cf. *deversa*, *Euterpe precatoria*, *Costus scaber*, *Costus pulverulentus*, *Costus lima*, *Carludovica palmata*, and *Polybotrya* sp. DNA was extracted using Fast DNA SPIN Kit for Soil (MPBiomedicals) following the manufacturer's protocol. The complete ITS marker region was amplified using SSUmAf-LSUmAr

Table 7.1 Occurrence of arbuscular mycorrhizal fungi (AMF) species in Ecuadorian Amazon ecosystems determined by molecular DNA sequence analysis

Title of the study	DNA extraction KIT	Primers target	Sequencing platform	Place of Ecuadorian Amazon	Software used to taxonomic classification	Soil analyzed or plant	Results	Reference
Diverging diversity patterns in the *Tulasnella* (Basidiomycota, Tulasnellales) mycobionts of *Aneura pinguis* (Marchantiophyta, Metzgeriales) from Europe and Ecuador	DNeasy Plant Mini Kit (Qiagen, Hilden, Germany)	Universal fungal primers ITS1F/NL4, ITS1F/LR5, and ITS1F/TW14	ABI 3100; Applied Biosystems	Cordillera El Consuelo in South Ecuador, Reserva Biológica San Francisco (RBSF) - Zamora Chinchipe	Blast search using the nucleotide sequence database (NCBI) against tulasnelloid nucLSU sequences	*Aneura pinguis*		Preußing et al. (2010)
Mycorrhizal preferences and fine spatial structure of the epiphytic orchid *Epidendrum rhopalostele*	Plant DNAeasy Mini Kit (Qiagen, Hilden, Germany)	ITS-5.8S rDNA region with the universal primers ITS1 and TW14	Not described	Border of Podocarpus National Park along the Loja-Zamora Road in Zamora-Chinchipe province		*Epidendrum rhopalostele* (Orchidaceae)		Riofrío et al. (2013)

Title of the study	DNA extraction KIT	Primers target	Sequencing platform	Place of Ecuadorian Amazon	Software used to taxonomic classification	Soil analyzed or plant	Results	Reference
Nitrogen and phosphorus additions impact arbuscular mycorrhizal abundance and molecular diversity in a tropical montane forest	PowerSoil DNA Isolation Kit ®	Targeted the variable region of the large subunit rDNA (LSU)	Roche FLX 454 pyrosequencing instrument	Zamora Chinchipe - Podocarpus National Park	MOTHUR, RAxML	Mixed root AMF communities, replicated at three tropical montane forests in southern Ecuador with differing environmental characteristics	64 samples were sequenced, 4 were removed because of low read numbers. 238 OTUs were identified but 113 allowed non-AMF sequences. Finally, 74 AMF OTUs were found: 48 belong to Glomerales order and 26 to Diversisporales order. The proportion of N and P in the different soils did not have any effect on the root length but on the percentage of root colonization specially the increasing N soils. Fertilized soils reduce the percentage of OTU by the high amount of N and P. When P was increasing, the richness of Glomerales decreased; when the N increased, Diversisporales reduced; and when N and P were used together, both orders were reduced. Nevertheless, community composition was not affected by N or P additions	Camenzind et al. (2014)

(continued)

Table 7.1 (continued)

Title of the study	DNA extraction KIT	Primers target	Sequencing platform	Place of Ecuadorian Amazon	Software used to taxonomic classification	Soil analyzed or plant	Results	Reference
Are there keystone mycorrhizal fungi associated with tropical epiphytic orchids?	DNeasy Plant Mini Kit (Qiagen)	(ITS2), the fungal specific primer pair ITS86F and ITS4	Illumina MiSeq® technology by which 300 bp long paired-end reads	Zamora Chinchipe - Podocarpus National Park	UPARSE software, taxonomic assignment using UNITE database	54 plant individuals with evidence of mycorrhizal colonization		Cevallos et al. (2017)
Arbuscular mycorrhizal fungal community composition in *Carludovica palmata*, *Costus scaber*, and *Euterpe precatoria* from weathered oil ponds in the Ecuadorian Amazon	FastDNA® SPIN Kit for Soil (MP Biomedicals, United States)	SSU rRNA gene, the complete ITS region (including the 5.8S rRNA gene) and approx. 800 bp of the LSU rRNA	Sanger sequencing with M13F-M13R primers, Macrogen	Sucumbíos - La Joya de los Sachas	Taxonomic annotations were according to Schübler and Walker	*Carludovica palmata*, *Costus scaber*, *Euterpe precatoria*, and surrounding soil	It identified 32 OTUs that described 4 AMF genera and 7 species, in a proportion of 31% *Glomus*, 25% *Acaulospora*, 22% *Archaeospora*, and 22% *Rhizophagus*. At species level, 31% belonged to *Glomus*, 22% *Archaeospora* sp., 16% *Acaulospora* sp. and *Rhizophagus proliferus*, 6% *Rhizophagus* sp. and *Acaulospora longula*, and 3% *Acaulospora kentinensis*. *E. precatoria* was colonized only by *Rhizophagus* sp. and *R. proliferus*. The alpha diversity calculated by Shannon index showed no difference between AMF species of soil or plant	Garces et al. (2017)

Title of the study	DNA extraction KIT	Primers target	Sequencing platform	Place of Ecuadorian Amazon	Software used to taxonomic classification	Soil analyzed or plant	Results	Reference
Community composition of arbuscular mycorrhizal fungi associated with native plants growing in a petroleum-polluted soil of the Amazon region of Ecuador	FastDNA® SPIN Kit for Soil (MP Biomedicals, United States)	SSUmAf–LSUmAr or SSUmCf-LSUmBr primer pairs	454 pyrosequencing was done by using the 2 XLR GS Junior Sequencing (Nucleomics Core, Leuven Belgium	Charpa field in the province of Sucumbios	QIIME pipeline	Root of 18 different species, detailed at paper	A total of 40 root samples were sequenced; their results showed a total of 17085 raw sequences. In these results, 150 sequences were well defined as AMF representative sequences that were classified as *Acaulospora*, *Archaeospora*, *Rhizophagus*, *Glomus*, *Sclerocystis*, *Dominikia*, and *Kamienskia.* The most frequent genera were *Acaulospora* (73%), with a prevalence of *A. kentinensis*, *A. scrobiculate*, and 4 species that their taxonomic classification at species level was not possible. Analysis of AMF community composition by NMDS analysis showed differences in the three sampling sites	Garces et al. (2019)

(continued)

Table 7.1 (continued)

Title of the study	DNA extraction KIT	Primers target	Sequencing platform	Place of Ecuadorian Amazon	Software used to taxonomic classification	Soil analyzed or plant	Results	Reference
Reforestation sites show similar and nested AMF communities to an adjacent pristine forest in a tropical mountain area of South Ecuador	innuPREP Plant DNA Kit (Analytik Jena; Germany)	18 S rDNA primers NS1/ NS4. primers AML1/ AML2	In-house ABI 3100	Zamora Chinchipe - Podocarpus National Park	Blast search using the nucleotide sequence database (NCBI)	Pristine forest and reforestation forest. Mycorrhizae were sampled by tracing single roots from the stem down to the fine roots	A total of 213 plants were analyzed. For all the sequences obtained, 550 belonged to Glomeromycota sequences. Also, 38 AMF-OTUs were identified with the software OPTSIL. The taxonomic assignment of the OTUs corresponded to Glomerales (58/66%), followed by Diversisporales (18/22%) and Archaeosporales (24/12%). When they compared the richness in pristine forest and reforestation plots, it showed that the richness in both sites is similar, for example, 28/73 OTUs were found on the reforestation plots, and 28/81 OTUs were found in the pristine forest. Something interesting is that 97% of the plants of the study were associated with at least one member of the Glomerales and the 35% with Diversisporales and Archaeosporales	Haugh et al. (2019)

Title of the study	DNA extraction KIT	Primers target	Sequencing platform	Place of Ecuadorian Amazon	Software used to taxonomic classification	Soil analyzed or plant	Results	Reference
Species composition of arbuscular mycorrhizal communities changes with elevation in the Andes of South Ecuador	innuPREP Plant DNA Kit (Analytik Jena, Germany)	NS1/NS4. Primers AML1/AML2	Sequenced by GATC Biotech (Konstanz, Germany)	Bombuscaro area in Parque Nacional *Podocarpus* and Parque Nacional Cajas	OTUs were classified based on sequence similarity using OPTSIL software	646 root samples, directly from the soil without regarding the identity of the plant partner. Collected fine roots from the organic layer or the topsoil (2–10 cm soil depth)	As they analyzed different elevation levels at the site that collected the samples, they demonstrated that the 4 altitudes showed distinct patterns of AMF communities. Also, the most abundant genus at an altitude of 1002/2000 masl was Glomus (OTU16, OTU49, and OTU1), and it is also considered the most diverse genus, with 62% of all OTUs, followed by Acaulospora (17%) and Archaeospora (11%). But the 3000–4000 masl was totally changed, with 37 OTUS. The most abundant were Acaulospora. Samples from ≈4000 masl sites at Cajas National Park ($n = 123$) revealed 32 OTUs (181 sequences); Acaulospora and Glomus were the most dominant AMF	Haugh et al. (2019)

(continued)

Table 7.1 (continued)

Title of the study	DNA extraction KIT	Primers target	Sequencing platform	Place of Ecuadorian Amazon	Software used to taxonomic classification	Soil analyzed or plant	Results	Reference
Moderate phosphorus additions consistently affect community composition of arbuscular mycorrhizal fungi in tropical montane forests in southern Ecuador	PowerSoil DNA isolation kit (MoBio Laboratories Inc., Carlsband, CA, USA)	Glomeromycota specific primer sets developed by Krüger et al. (2012)	Illumina MiSeq platform using 2 9250 paired-end chemistry at the Berlin Center for Genomics in Biodiversity Research (BeGenDiv)	Three sites along the southeastern Andes of Ecuador	USEARCH v.10, MOTHUR	Mixed root AMF communities, replicated at three tropical montane forests in southern Ecuador with differing environmental characteristics	A total of 280 samples were analyzed and produced 12 625 525 reads. A total of 628 OTUs were identified, and 65.6% of the reads belonged to Glomeromycotina. AMF OTU accumulation curves indicate that pre-montane plant communities hosted on average 126 more OTUs than lower and upper montane forests, which relative to each other reached a very similar number of OTUs As a conclusion, the tropical montane forest harbors highly diverse AMF communities. Also, the fertilization effects at the taxonomic composition were related with nutrients and the status of nutrients in each place analyzed	Dueñas et al. (2020)

Data compilation included papers that were published in the last 25 years (1997 to 2021) primers for the first PCR and SSUmCd-LSUmBr primers for a subsequent nested PCR and sequenced using M13F-M13R primers at Macrogen Inc. All sequences were assembled and edited with SeqMan and grouped in OTUs with the OPTSIL program. Alignments were performed with MAFFT, and a maximum likelihood phylogenetic tree was generated using RAxML-HPC2. Taxonomic annotations were labeled according to Schübler and Walker (http://www.amf-phylogeny.com/). Furthermore, diversity was analyzed using the Shannon diversity index using the vegan package in R.

7.4 Discussion

The Ecuadorian Amazon is renowned for its high levels of bird, amphibian, insect, and floral diversity (de Oliveira Freitas et al. 2014). However, little is known about the microbial diversity of Amazon soils. Although plant diversity is incredibly high in the Ecuadorian Amazon, plant endemism is relatively low, and species tend to grow in ample geographical extensions. Thus far, symbiotic plant-microbe interactions and general patterns of occurrence and diversity of mycorrhizal fungi in the Amazon rainforest in Ecuador are largely unknown.

Flora surveys and herbaria records suggest a dominance of flora associated with arbuscular mycorrhizal fungi. This is consistent with the observations of Tedersoo et al. (2010) that reported low ectomycorrhiza richness in the Yasuní region. The dominance of arbuscular mycorrhizal over ectomycorrhizal flora in this region could be explained by the habitat types which are characterized by climatic conditions that favor fast leaf litter and organic matter decomposition with a rapid availability of soil nutrient which could favor arbuscular mycorrhizal associations. Read’s (1991) hypothesis mentions that slow decomposition rates at high latitudes favor mutual relationships with ectomycorrhizal fungi due to their increased capacity to liberate organic nutrients (Peay 2016). Moreover, John et al. (2007) investigated the influence of soil nutrients on niche structure in the Yasuni forest dynamic plot and reported low phosphorus, nitrogen, and pH levels, suggesting the importance of soil resource availability in the assembly of tropical tree communities. This also has implications on spatial heterogeneity in soil resource distributions which could favor specialization at different levels of essential resources available. Thus, soil microbial communities, in particular intimate relationships with beneficial mycorrhizal fungi, could be considered a key mechanism to sustain plant diversity. More explorations are necessary to decipher the underlying mechanisms by which nutrient competition favors dominance of AM associations with the Amazon flora.

The soil nutrient composition and variance influence and promote the dominance of certain AMF species. Previous molecular studies in tropical forests revealed that the order Diversisporales of the Acaulosporaceae is the most abundant group, instead of the order Glomerales as previously thought. Global data have confirmed that Acaulosporaceae prefer mostly acidic soils (Camenzind et al. 2014). Moreover, AM symbiosis occurs across the Ecuadorian Amazon region, from natural pristine ecosystems to anthropogenic agroecosystems. The reported AM taxa are similar to reports in literature from other Amazon regions. For instance, an exploration of AM diversity in Terra Firme biomes in the central part of the Brazilian Amazon identified 41 spore morphotypes, with species belonging to the genera *Glomus* and *Acaulospora* representing 44% of the total taxa (de Oliveira Freitas et al. 2014).

Plants roots in polluted environments were found to be colonized above 50%; nevertheless, it was the first time that AMFs were detected in roots of plants growing in contaminated soils using molecular techniques. Other studies reported more AMF OTUs in roots of plants growing under similar conditions, but these samples were sequenced using a different approach using 18S rDNA as a molecular marker using 454-pyrosequencing and Sanger. It is important to address that many genera could remain undetected when plant roots are stained for classical spore taxonomy using microscopy. The high abundance of spores of genus *Glomus* (31%) revealed the predominance of the Glomeraceae in oil-polluted soils, which was also verified by the high abundance of extraradical hyphae. These results characterized that the genus *Acaulospora* belongs to stress-tolerant AMF. And the genus *Archaeospora* with 22% of dominance was also identified in Amazon region of Brazil and Colombia. Because Glomeraceae, Acaulosporaceae, and Archaeosporaceae belong to AMF families which were previously identified in diverse plants and environments around Ecuador, the authors suggested that human, animal, and wind activities could have shaped their distribution (Garces et al. 2017).

Advances in DNA sequencing has enabled an unprecedented understanding of the diversity of microbial communities (Bahram et al. 2018). Microbes are present in all compartments of the plant such as roots, leaves, flowers, and seeds. The root microbiome refers to the microorganisms, their genomes, and their interactions in and on plant roots (Mendes et al. 2013; Parfrey et al. 2018). The narrow zone directly surrounding plant roots is defined as the rhizosphere, whereas the endosphere refers to the internal plant tissues, and roots are included (Philippot et al. 2013). Soil acts as the microbial reservoir from which plant roots recruit their microorganisms (Schreiter et al. 2014). In addition to the microbial community recruited from the soil, the root microbiome can also harbor members that could have been transmitted via the plant seeds (Lemanceau et al. 2017). Soil type, plant species, and interactions between different microbial populations are considered to be the main factors that drive the assembly of the root microbiome (Lareen et al. 2016). Rhizodeposition, in the form of secreted exudates, polysaccharide mucilage, enzymes, and root border cells into the rhizosphere, is an important substrate and triggers the reaction of both beneficial and pathogenic microorganisms (Lambers et al. 2009). Up to 40% of the total photosynthetic sugars are secreted into the rhizosphere (Hirsch et al. 2013), causing physical-chemical changes and enhanced microbial activity, referred to as the rhizosphere effect (Philippot et al. 2013).

Soil microbial communities are control centers which mediate how trees interact with the soil environment. Microbial networks, like the dense fungal hyphal nets that connect many trees, allow trees to help their nearby offspring with the best possible start in life, while competing with neighboring trees of different species (Aleklett and Body 2021). There is growing evidence that the microbial diversity and abundance present in soils can strongly alter forest ecosystems in temperate regions (Wagg et al. 2014). Nonetheless, soil microbiome information from the Amazon Rainforest is limited, particularly from rainforest ecosystems at the foot of the Ecuadorian Andes. These forests are within the top biodiversity hotspots of the planet, with hundreds of thousands of macro-organisms described, but currently lacking is important knowledge about the microbiodiversity on which the visible world depends.

7.5 Concluding Remarks and Future Perspective

This is a first approximation to understand the ecology of soil mycorrhizal communities in forests of the Ecuadorian Amazonia. The plant mycorrhizas status analysis and the reported diversity of mycorrhiza fungi suggest that arbuscular mycorrhizal plant families and fungi are dominant, which is consistent with the hypothesis of AMF being a strategy to sustain plant diversity in low P environments. Regarding the need of understanding the mechanism that sustains plant diversity, for future direction, in order to deeply address this question, we suggest expanding the exploration with a combination of field and experimental observations that include trade-offs between morphological plant root traits, phosphatase exudation, and microbial dynamics in the rhizosphere.

The reported mycorrhizal diversity in literature from studies of mycorrhizal diversity at the Ecuadorian amazon is similar to other studies in the amazon regions; however it is highlighted the number of undescribed species that need more attention. Regarding the understanding of difference of mycorrhizal association in the three main types of biomes at the Ecuadorian Amazon, future taxonomic identification of AMF should be based on both morphological criteria and molecular analysis. This will contribute to the report of the diversity of mycorrhizas; therefore, research should continue until databases can be completed and the underestimated AMF decreases in this ecosystem. The study of AMF species from natural and transformed ecosystems has a representative potential in applied research on plant establishment, remediation of polluted soils, and conservation.

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7.6 Appendix A

Supplementary Table S1 List of plant species, family, genera, plant origin, and functional group from surveys in the Ecuadorian Amazon*. Mycorrhiza type and status were determined using Brundrett (2009) and Wang and Qui's (2006) checklists of the mycorrhizal status of land plants families

Plant				Mycorrhiza Type				Plant Taxon suborigen					Plant functional groups				
Family	Genus	Species	Common name(s)	Ectomycorrhiza	Endomycorrhiza	Not registered	None	Native	Endemic	Exotic	Cultivated	Other	Grasses	Legumes	Forbs	Woody species	Other
Alismataceae	Saggitaria	latifolia	Patata de Pato		X			X					X				
Alliaceae	Allium	cepa	Cebolla							X	X				X		
Amaranthaceae	Amaranthus	spinosus	Amaranto espinoso					X					X		X		
Amaranthaceae	Amaranthus	viridis	Amaranto verde		X			X					X				
Amaranthaceae	Chenopodium	ambrosioides	Té de Méjico		X					X	X					X	
Anacardiaceae	Astronium	graveolens	Amargoso		X			X								X	
Anacardiaceae	Anacardium	occidentale	Marañón		X					X						X	
Anacardiaceae	Astronium	graveolens	Ron-ron		X			X								X	
Annonaceae	Annona	sp.			X			X	X							X	
Araceae	Anthurium	pentaphyllum			X			X					X				
Araceae	Caladium	bicolor	Alas de Ángel		X			X			X		X				
Araceae	Colocasia	esculenta	Papa china		X					X	X				X		
Araceae	Philodendron	ornatum	Guembé					X									
Araceae	Anthurium	sp.			X			X	X						X		X
Arecaceae	Bactris	gasipaes	Contaduro		X						X		X				
Arecaceae	Euterpe	sp.			X			X								X	

Plant				Mycorrhiza Type				Plant Taxon suborigen					Plant functional groups				
Family	Genus	Species	Common name(s)	Ectomy-corrhiza	Endo-mycor-rhiza	Not registered	None	Native	Ende-mic	Exotic	Culti-vated	Other	Grasses	Legumes	Forbs	Woody species	Other
Asteraceae	Ageratum	conyzoides	Cangay		X					X			X		X		
Asteraceae	Bidens	pilosa	Chipaca, romerillo		X			X							X		
Asteraceae	Hypochaeris	radicata	Hierba del chancho		X					X					X		
Asteraceae	Tagetes	erecta	Tagete							X	X				X		
Balsaminaceae	Impatiens	walleriana	Balsamina		X					X	X		X				
Betulaceae	Alnus	acuminata	Aliso	x				X								X	
Blechnaceae	Blechnum	occidentale	Helecho		X			X									
Bombacaceae	Ochroma	pyramidale	Balsa		X			X			X					X	
Boraginaceae	Cordia	alliodora	Laurel blanco				X	X					X				
Brasicaceae	Nasturium	officinale	Berro de agua				X			X	X				X		
Brasicaceae	Raphanus	sativus	Rábano		X		X			X	X						
Bromeliaceae	Ananas	comosus	Piña		X						X		X				
Burmanniaceae	Burmania	tenella			X	X		X									Saprofita
Burmanniaceae	Thismia	sp.				X		X									Saprofita
Canabaceae	Trema	micrantha	Capulin Cimarrón		X			X							X	X	
Caricaceae	Carica	papaya	Papaya		X			X			X		X				
Caryophyllaceae	Cerastium	arvense	Oreja de ratón		X			X							X		
Caryophyllaceae	Cerastium	fontanum	Merusa		X					X					X		

(continued)

Supplementary Table S1 (continued)

Plant				Mycorrhiza Type				Plant Taxon suborigen					Plant functional groups				
Family	Genus	Species	Common name(s)	Ectomy-corrhiza	Endo-mycor-rhiza	Not registered	None	Native	Ende-mic	Exotic	Culti-vated	Other	Grasses	Legumes	Forbs	Woody species	Other
Clusiaceae	Clusia	minor	Cupeicillo		X			X									Hemiepi-phyte
Clusiaceae	Clusia	sp.			X			X					X			X	X
Combrataceae	Terminalia	catappa	Akom, korina		X					X						X	
Crassulaceae	Costus	scaber	Cañabrava		X			X					X				
Cucurbitaceae	Cucumis	sativa	Pepino		X					X	X						Vine
Cyperaceae	Becquerelia	cymosa			X			X							X		
Cyperaceae	Carex	muricata	Marcie-guilla		X		X			X					X		
Cyperaceae	Cyperus	articulatus			X			X							X		
Cyperaceae	Cyperus	distans	Cebollín		X			X							X		
Cyperaceae	Cyperus	iria	Junca Real		X					X					X		
Cyperaceae	Cyperus	ligularis			X			X					X				
Cyperaceae	Cyperus	luzulae	Zacate de estrella				X	X					X				
Cyperaceae	Cyperus	odoratus	Hierba de Zopilote		X			X					X				
Cyperaceae	Cyperus	pohlii					X	X					X				
Cyperaceae	Cyperus	rotundus	Castañuela		X			X							X		
Cyperaceae	Eleocharis	acutangula			X			X							X		
Cyperaceae	Eleocharis	geniculata			X			X					X				
Cyperaceae	Scleria	latifolia			X			X							X		

Plant				Mycorrhiza Type				Plant Taxon suborigen					Plant functional groups					
Family	Genus	Species	Common name(s)	Ectomy-corrhiza	Endo-mycor-rhiza	Not registered	None	Native	Ende-mic	Exotic	Culti-vated	Other	Grasses	Legumes	Forbs	Woody species	Other	
Cyperaceae	Scleria	melaleuca	Navajuela		X			X					X					
Davalliaceae	Nephrolepis	cordifolia	Helecho serrucho		X			X							X			
Elaeocarpaceae	Sloanea	guianensis	Ablania		X			X								X		
Ericaceae	Gaultheria	erecta	Arrayán			X		X							X			
Ericaceae	Sphyrospermum	buxifolium				X		X					X					
Ericaceae	Sphyrospermum	cordifolium				X		X					X					
Euphorbiaceae	Hyeronyma	alchorneoides	Pilón		X			X								X		
Euphorbiaceae	Hevea	brasiliensis	Arbol de caucho		X					X	X					X		
Euphorbiaceae	Manihot	esculenta	Yuca		X													
Euphorbiaceae	Phyllanthus	urinaria	chamberbitter		X					X			X					
Euphorbiaceae	Acalypha	sp.			X			X	X				X		X	X		
Euphorbiaceae	Drypetes	sp.			X			X					X			X		
Fabaca	Inga	marginata	Guaba		X											X		
Fabaceae	Cajanus	cajan	Gandul		X					X	X			X	X			
Fabaceae	Calopogonium	mucunoides	Calopo		X			X					X	X				
Fabaceae	Calopogonium	caeruleum	Jicama Cimarrona		X			X					X	X				

(continued)

Supplementary Table S1 (continued)

Plant				Mycorrhiza Type				Plant Taxon suborigen					Plant functional groups					
Family	Genus	Species	Common name(s)	Ectomy-corrhiza	Endo-mycor-rhiza	Not registered	None	Native	Ende-mic	Exotic	Culti-vated	Other	Grasses	Legumes	Forbs	Woody species	Other	
Fabaceae	Inga	leiocalycina	Guabo colorado		X											X		
Fabaceae	Erythrina	poeppigiana	Bucare ceibo		X			X								X		
Fabaceae	Trifolium	repens	Trébol blanco		X					X	X				X			
Fabaceae	Vigna	unguiculata	Frejol chino		X					X	X		X					
Fabaceae	Inga	striata	Árbol de guaba		X			X								X		
Fabaceae	Dialium	guianense	Guapaque		X			X								X		
Fabaceae	Inga	sp.			X			X	X							X		
Gentianaceae	Centaurium	erythraea	Centáurea menor		X					X					X			
Gentianaceae	Voyria	aurantiaca				X		X									X	
Heliconaceae	Heliconia	sp.			X			X							X			
Hymenophyla-ceae	Hymeno-phyllum	tunbrigense	Helechillo			X		X							X			
Juncaceae	Juncus	bufonius	Junco de Sapo		X			X					X					
Juncaceae	Juncus	effusus	Junco de esteras		X			X							X			
Lamiaceae	Pruenella	vulgaris	Consuelda menor		X					X					X			
Lamiaceae	Salvia	splendens	Salvia Escarlata		X						X				X			
Lauraceae	Ocotea	sp.			X			X								X		

Plant				Mycorrhiza Type				Plant Taxon suborigen					Plant functional groups					
Family	Genus	Species	Common name(s)	Ectomy-corrhiza	Endo-mycor-rhiza	Not registered	None	Native	Ende-mic	Exotic	Culti-vated	Other	Grasses	Legumes	Forbs	Woody species	Other	
Lauraceae	Ocotea	puberula	Guaica		X			X								X		
Lauraceae	Persea	americana	Aguacate		X						X					X		
Lauraceae	Nectandra	sp.			X			X								X		
Magnoliaceae	Talauma	ovata					X	X								X		
Malvaceae	Ceiba	pentandra	Ceiba		X			X								X		
Malvaceae	Guazuma	ulmifolia	Guacimo		X			X								X		
Malvaceae	Theobroma	cacao	Cacao		X			X			X					X		
Melastomataceae	Clidemia	hirta	Cordoban peludo		X			X							X			
Melastomataceae	Graffenrieda	emarginata		x	X			X								X		
Meliaceae	Cabralea	canjerana	Bateacaspi		X			X								X		
Meliaceae	Cedrela	fissilis	Cedro misionero		X			X								X		
Meliaceae	Guarea	kunthiana	Guamaron		X			X								X		
Meliaceae	Melia	azedarach	Cinamomo		X					X	X					X		
Meliaceae	Trichilia	elegans	Catigua blanca		X			X								X		
Meliaceae	Swietenia	macrophylla	Caoba, palo santo		X			X			X					X		
Meliaceae	Cedrella	sp.			X			X								X		
Moraceae	Brosimum	lactescens	Leche de Vaca		X			X								X		
Moraceae	Artocarpus	altilis	Árbol de pan		X					X	X					X		
Moraceae	Brosimum	alicastrum	Ramón		X			X								X		

(continued)

Supplementary Table S1 (continued)

Plant				Mycorrhiza Type				Plant Taxon suborigen					Plant functional groups					
Family	Genus	Species	Common name(s)	Ectomy-corrhiza	Endo-mycor-rhiza	Not registered	None	Native	Ende-mic	Exotic	Culti-vated	Other	Grasses	Legumes	Forbs	Woody species	Other	
Moraceae	Ficus	sp.			X			X								X	X	
Moraceae	Brosimum	sp.			X			X								X		
Musaceae	Musa	acuminata	Platano malayo o rojo		X													
Myrtaceae	Psidium	guajava	Guayaba		X			X			X				X	X		
Myrtaceae	Eugenia	sp			X			X								X		
Nyctaginaceae	Mirabilis	jalapa	Dondiego de noche				X			X	X		X		X			
Nyctaginaceae	Neea	sp.		x				X							X	X		
Osmundaceae	Osmunda	regalis	Helecho real		X			X							X			
Piperaceae	Piper	sp.			X			X	X				X					
Plantaginaceae	Plantajo	major	Llantén mayor		X					X					X			
Poaceae	Agrostis	gigantea	Vilfa		X					X					X			
Poaceae	Alopecurus	aequalis	Cola de zorra acuática				X	X							X			
Poaceae	Aristida	adscensionis	Escobilla		X			X							X			
Poaceae	Cynodon	dactylon	Grama común		X					X					X			
Poaceae	Digitaria	ciliaris			X			X							X			
Poaceae	Digitaria	violascens	Escobilla blanda		X					X					X			

Plant				Mycorrhiza Type				Plant Taxon suborigen					Plant functional groups				
Family	Genus	Species	Common name(s)	Ectomy-corrhiza	Endo-mycor-rhiza	Not registered	None	Native	Ende-mic	Exotic	Culti-vated	Other	Grasses	Legumes	Forbs	Woody species	Other
Poaceae	Eleusine	indica	Pata de Gallina		X					X			X				
Poaceae	Festuca	rubra	Cañuela común		X	X	X			X					X		
Poaceae	Holcus	lanatus	Saboya		X					X					X		
Poaceae	Homolepis	aturensis	Cansagente		X			X							X		
Poaceae	Leersia	hexandra	Lambedor		X			X							X		
Poaceae	Oryza	sativa	Arroz		X					X	X				X		
Poaceae	Panicum	laxum	Pasto Guínea		X			X					X				
Poaceae	Panicum	maximum								X	X					X	
Poaceae	Paspalum	conjugatum	Pasto amargo		X			X								X	
Poaceae	Poa	annua	Pastito de invierno							X					X		
Poaceae	Rhynchely-trum	repens	Hierba de la lana		X					X	X					X	
Poaceae	Saccharum	officinarum	Caña de azucar		X					X	X		X				
Poaceae	Setaria	glauca	Mijo perla							X					X		
Poaceae	Zea	mays	Maiz		X					X	X		X				
Portulacaceae	Montia	fontana	Borujas				X	X							X		
Portulacaceae	Portulaca	oleraceae	Verdolaga		X			X					X				
Rosaceae	Acaena	ovalifolia	Fruto de Cadillo		X		X	X					X		X		

(continued)

Supplementary Table S1 (continued)

Plant				Mycorrhiza Type				Plant Taxon suborigen					Plant functional groups				
Family	Genus	Species	Common name(s)	Ectomycorrhiza	Endomycorrhiza	Not registered	None	Native	Endemic	Exotic	Cultivated	Other	Grasses	Legumes	Forbs	Woody species	Other
Rosaceae	Fragaria	vesca	Fresera		X					X	X				X		
Rubiaceae	Psychotria	sp.			X			X	X						X	X	
Rubiaceae	Coffea	arabica	Cafeto		X					X	X				X	X	
Rubiaceae	Randia	sp.			X			X					X			X	
Rubiaceae	Palicourea	sp.			X			X	X				X			X	
Rutaceae	Zanthoxylum	sp.			X			X							X	X	
Sapindaceae	Allophylus	sp.			X			X	X							X	
Schizaeceae	Anemia	phyllitidis	Fraxinifolia		X												
Secrophulariaceae	Veronica	peregrina	Verónica america		X			X							X		
Secrophulariaceae	Veronica	persica	Azulete pérsico		X					X					X		
Secrophulariaceae	Veronica	serpyllifolia	Ontineta		X			X					X				
Silacacea	Casearia	sp.			X			X					X			X	
Silacacea	Xylosma	sp.			X			X								X	
Similaceae	Smilax	sp.			X			X									X
Solanaceae	Cestrum	sp.			X			X	X				X			X	
Solanceae	Solanum	dulcamara	Dulcamara		X			X					X		X		
Solanceae	Solanum	tuberosum	Papa		X						X		X				
Thelypteridaceae	Macrothelypteris	torresiana	Penquita		X												
Thelypteridaceae	Thelypteris	interrumpta						X							X		
Thismiaceae	Tiputini	foetida				X			X				X				

Plant				Mycorrhiza Type				Plant Taxon suborigen					Plant functional groups					
Family	Genus	Species	Common name(s)	Ectomy-corrhiza	Endo-mycor-rhiza	Not registered	None	Native	Ende-mic	Exotic	Culti-vated	Other	Grasses	Legumes	Forbs	Woody species	Other	
Urticaceae	Urtica	urens	Ortiga menor		X					X					X			
Urticaceae	Urera	sp.			X			X					X			X	X	
Urticaceae	Boehmeria	sp.			X			X					X		X	X		
Verbenaceae	Aegiphila	sellowiana	Taman-queira		X			X							X	X		
Verbenaceae	Lantana	camara	Lantana		X					X	X		X		X			
Verbenaceae	Tectona	grandis	Teca		X					X	X					X		
Zingiberaceae	Renealmia	sp.			X			X							X			
Total		162		3	137	9	12	105	10	45	35	0	46	3	65	62	10	
Ratio				2	85	5	8	64	6	28			28	2	40	38	6	

*Source:

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Chapter 8
Orchid Mycorrhizas in South America: Tropical and Subtropical Ecosystems

Yasmín A. Alomía and J. Tupac Otero

Contents

8.1 Introduction

Orchids have fascinated enthusiasts and naturalists since at least the end of the eighteenth century (Cullen 1992). Certainly, Darwin's work (1862) inspired by highly specialized floral adaptations for attracting, deceiving, and manipulating insects to promote allogamy (Dressler 1981) was one of the most interesting and stimulating approaches for future generations of researchers. In addition, with about 30, 000 species and a worldwide distribution, Orchidaceae is the second most diverse family among angiosperms (Bánki et al. 2021). Its amazing floral shapes as well as the intricate relationships with both pollinators and mycorrhizal fungi make this plant group one of the most bizarre throughout the plant kingdom.

Among mycorrhizal associations (Peterson et al. 2004; Smith and Read 2010), orchid mycorrhiza (OM) is a special type that only occurs within Orchidaceae. Since orchids form minute seeds (like dust) with a reduced endosperm (Fig. 8.1) in

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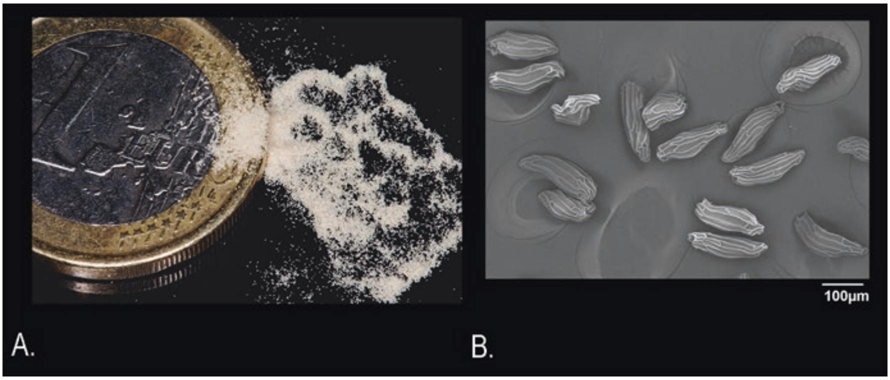

Fig. 8.1 Seeds of *Dichaea andina* orchid. This genus has one of the smallest sizes of seeds among Orchidaceae: (**a**) seeds like dust on one Euro coin, (**b**) view through scanning electron microscope (SEM). (Photo credit: Y. A. Alomía)

Fig. 8.2 Cross section of orchid root with pelotons (brown hyphal coils) formed by fungi into the parenchyma cells of cortex; view through of light microscope (200X). Photo credit: Y. A. Alomía

natural conditions, they need a fungal partner that provides the organic source to support the germination and seedling establishment (Bernard 1904; Rasmussen 1995; Brundrett 2017). This symbiosis is recognized for the formation of complex hyphal coils called pelotons within the root cortex tissue (Peterson et al. 2004; Zettler and Corey 2018) (Fig. 8.2).

In early stages of development, the plants are entirely dependent on the supply of nutrients and carbon by the orchid mycorrhizal fungi (OMF). In later stages, most species become green plants (i.e., photosynthetic orchids), and some species can obtain additional carbon supplies from fungi that remain until the adult stage. This mode of nutrition in which the plant gains carbon simultaneously from two sources, its own photosynthesis and the fungal supply, is called partial mycoheterotrophy

(Leake 1994; Gebauer 2018) (some authors use also the term mixotrophy; see Selosse et al. (2016)). In a broad sense, all orchid species are mycoheterotrophic in some state of the life cycle. Those species without chlorophyll as adults (i.e., non-photosynthetic orchids), which entirely rely on fungi throughout their lives for mineral and carbon nutrition, are called fully mycoheterotrophic orchids (Gebauer 2018).

Photosynthetic orchids are generally associated with fungi of the *Rhizoctonia* complex. This group consists of a phylogenetic heterogeneous assemblage that includes saprophytic, parasite, and endophyte species. Orchids also form mycorrhizas with fungi mainly included in the genera *Ceratobasidium*, *Tulasnella*, *Sebacina*, and *Serendipita* (Suárez et al. 2008; Taylor and McCormick 2008; Kottke and Suárez 2009; Weiß et al. 2016; Fritsche et al. 2021). On the other hand, partially mycoheterotrophic orchids, as well as fully mycoheterotrophic orchids, are associated with ectomycorrhizal fungi (*Russula*, *Thelephora*, *Tomentella*) of other nearby plants or with saprotrophic fungi (*Resinicium*, *Gymnopus*, and *Mycena*) (Martos et al. 2009; Zettler and Corey 2018).

Bernard (1904) reported the first record of mycorrhizal associations in orchids with *Rhizoctonia*-like fungi and proposed the first techniques for the cultivation of these fungi from sections of infected roots to promote seed germination. Morphologically, members of *Rhizoctonia* group share some common traits: hyphae without clamp connections branched at right angles, constriction of the hyphal branch, a septum close to the bifurcation site, and production of chains of swollen monilioid cells (Fig. 8.3). Detailed studies such as nuclear condition, septal ultrastructure, enzymatic activity, and the formation of anastomosis groups (Suárez et al. 2006, Suryantini et al. 2015, Thakur et al. 2018, Sathiyadash et al. 2020, Ghirardo et al. 2020, Nandeesha et al. 2021) can provide more taxonomic confidence. In addition, advances in molecular techniques have facilitated the identification of OMF mainly through sequencing of the internal transcribed spacer (ITS) region of ribosomal DNA (Kristiansen et al. 2001; Taylor and McCormick 2008; Kottke and Suárez 2009; Yokoya et al. 2015; Fritsche et al. 2021).

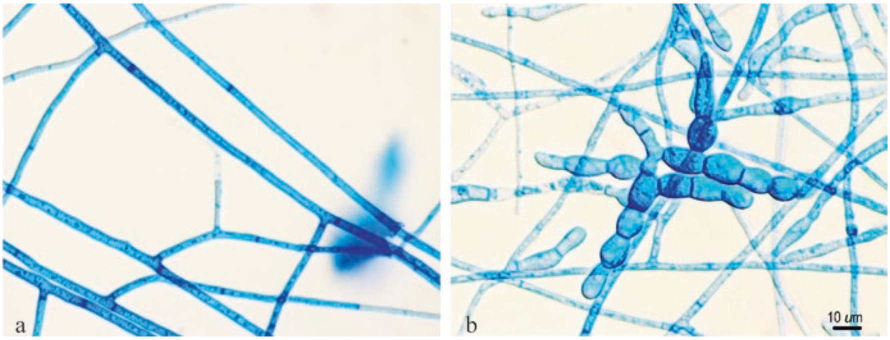

Fig. 8.3 Typical *Rhizoctonia* mycelium (*Ceratobasidium* sp.) stained with lacto-phenol blue; (**a**) branching pattern at 90, septum and constriction close to the bifurcation, (**b**) monilioid cells. Photo credit: Y. A. Alomía

An extensive literature has been published about the structure of orchid mycorrhiza, mechanisms of attraction, infection, nutrient exchange, phylogeny, and isolation techniques (Peterson et al. 2004; Smith and Read 2010; Dearnaley et al. 2012, 2016; Selosse et al. 2016; Swarts and Dixon 2017; Zettler and Corey 2018). Most of the knowledge on this topic comes from studies conducted in temperate ecosystems with terrestrial orchids (Kristiansen et al. 200; Gebauer and Meyer 2003; Bonnardeaux et al. 2007; Roche et al. 2010; Waterman et al. 2011; Jacquemyn et al. 2014, 2015; Těšitelová et al. 2015). In this chapter, a synopsis of the relevant literature on the interactions between OMF and their host plants in subtropical and tropical regions from South America is presented. After that, some perspectives and potential collaborations will be discussed.

8.2 Studies on Orchid Mycorrhizae in South America

A great majority of studies on OM in South America are academic documents named as "gray" literature and deposited in the repositories of university libraries. Others are published in local scientific journals written in native languages (e.g., Spanish, Portuguese). We have compiled the studies conducted in Brazil, Colombia, and Ecuador (Table 8.1), where it was possible to identify the taxon or taxa of the orchids studied, the methods for the identification of mycorrhizal fungi, and their taxonomic determination. The names of the orchid and fungal species were preserved as reported. The contribution of OMF of other countries in the tropical region of South America (Venezuela, Guyana, Surinam, French Guyana, Perú, Bolivia) has been limited or is not available to consult.

Pioneering studies on OM in tropical zones of South America were presented in the early twenty-first century by Díaz et al. (2000) in Colombia, when they were exploring the mycorrhizal associations in several orchid species. This study used morphological traits of mycelium to identify the fungal partner as *Rhizoctonia* for all species, although the main aim of the research was to identify what type of plant secondary metabolites was producing in the tissues where the endophytes were found. Around the same time, in Brazil, the first studies on orchid mycorrhizae were beginning in the laboratory of Professor Maria Catarina Megumi Kasuya of the Federal University of Viçosa. This time, it included, in addition to morphological characterizations, genetic information (ITS sequencing and RAPDs) (Pereira 2001). The interest in this subject in the country then expands, resulting in several researches throughout the next decade (Table 8.1). Around 2002, in Colombia, mycorrhizal interactions began to be explored in economically important species such as *Vanilla planifolia* (Ordóñez et al. 2012), and, under the guidance of the second author of this chapter from the National University of Colombia, an important field of research in the country was opened, resulting in multiple studies (Table 8.1). Although Colombia and Brazil were the pioneer countries, Ecuador has established itself as the leading country in research on OMF in the region. Many of the studies carried out in this country are rigorous investigations that have been published in scientific journals (unlike Brazil and Colombia, where many reports are part of gray

Table 8.1 Studies on fungal associations with orchids in tropical South America: a timeline

Taxon	Fungal origin	Identification method	Reported OMF	Study focus	Reference	Country
Elleanthus, *Epidendrum*, *Liparis*, *Maxillaria*, *Odontoglossum*, *Oncidium*, *Pleurothallis*, *Sobralia*, *Stelis*	Fungal cultures	Morphological	*Rhizoctonia* sp.	Diversity, plant secondary metabolites	Díaz et al. (2000)	Colombia
Epidendrum rigidum, Isochilus linearis, Maxillaria marginata, Oncidium flexuosum, *Oncidium varicosum*, *Oeceoclades maculata*, *Polystachya concreta*	Fungal cultures, pelotons	Morphological, enzymatic assays, molecular (RAPD, RFLP-ITS)	*Epulorhiza* sp., *Epulorhiza repens*, *Epulorhiza epiphytica*, *Ceratorhiza* sp.	Diversity, morphological studies	Pereira (2001), Pereira et al. (2005a)	Brazil
Isochillus linearis, *Polystachya concreta*, *Gomesa crispa*, *Campylocentrum* sp., *Bifrenaria tyrianthina*, *Oncidium gracile*, *Epidendrum secundum*, *Pleurothallis limae*	Fungal cultures	Morphological, polyphenol-oxidases activity, molecular (nrDNA ITS/Sanger sequencing)	*Epulorhiza* sp., *Ceratorhiza* sp.	Diversity, phylogeny	Nogueira (2004)	Brazil
Bulbophyllum weddelii, *Epidendrum dendrobioides*, *Maxillaria acicularis*, *Oncidium gracile*, *Pleurothallis teres*, *Prosthechea vespa*, *Sophronitis milleri*, *Sarcoglottis* sp.	Fungal cultures	Morphological	*Epulorhiza* sp., *Ceratorhiza* sp. *Rhizoctonia*	Diversity, morphological studies	Nogueira et al. (2005)	Brazil
Oncidium flexuosum	Fungal cultures	Molecular (RAPD, RFLP-ITS)	*Epulorhiza repens*, *Epulorhiza epiphytica*, *Ceratorhiza*, *Rhizoctonia* sp.	Symbiotic seed germination	Pereira et al. (2005b)	Brazil

(continued)

Table 8.1 (continued)

Taxon	Fungal origin	Identification method	Reported OMF	Study focus	Reference	Country
Gomesa crispa, *Campylocentrum organense*, *Bulbophyllum* sp.	Fungal cultures	Morphological	*Ceratorhiza*, *Rhizoctonia*	Diversity, ultrastructural analyses	Pereira et al. (2005c)	Brazil
Stelis hallii, *S. superbiens*, *S. concinna*, *Pleurothallis lilijae*	Root tissue with pelotons, fungal cultures	Morphological, molecular (nucLSU and nrDNA ITS/Sanger sequencing)	*Tulasnella*	Diversity Phylogeny, ultrastructural analyses	Suárez et al. (2006)	Ecuador
Masdevallia coccinea	Fungal cultures	Morphological	Binucleate *Rhizoctonia*	Morphological studies	Ordoñez (2006)	Colombia
Cyrtopodium vernum	Fungal cultures	Morphological	*Rhizoctonia*	Diversity, symbiotic seed germination	Gonçalves et al. (2008)	Brazil
Several species of Pleurothallidinae	Root tissue with pelotons	Molecular (nucLSU/ Sanger sequencing)	Tulasnellales, Sebacinales	Diversity, Community ecology, Phylogeny	Kottke et al. (2008b)	Ecuador
Stelis hallii, *S. superbiens*, *S. concinna*, *Pleurothallis lilijae*	Root tissue with pelotons	Morphological, Molecular (nucLSU and nrDNA ITS/Sanger sequencing)	*Opadorhiza*, *Epulorhiza*	Diversity, Phylogeny, Ultrastructural analyses,	Suárez et al. (2008)	Ecuador
Epidendrum secundum	Fungal cultures	Morphological, Molecular (RAPD, ITS-RFLP, nrDNA ITS/Sanger sequencing)	*Tulasnella/Epulorhiza*, *Sebacina/Opadorhiza*	Morphological studies, Diversity	Pereira (2009)	Brazil
Epidendrum secundum	Fungal cultures	Morphological	*Epulorhiza* spp.	Morphological studies, Diversity	Pereira et al. (2009)	Brazil

Bletia, *Stelis*, *Pleurothallis*, *Octomeria*, *Maxillaria*, *Pseudolaelia*, *Epidendrum*, *Artorima*, *Elleanthus*, *Sobralia*, *Prosthechea*	Root tissue with pelotons	Morphological, Molecular (nrDNA ITS/Sanger sequencing)	Atractiellomycetes (Pucciniomycotina)	Diversity, Phylogeny, Ultrastructural analyses,	Kottke et al. (2010)	Ecuador
Epidendrum secundum	Fungal cultures	Morphological	*Epulorhiza* spp.	Symbiotic seed germination	Pereira et al. (2011)	Brazil
Hadrolaelia jongheana, *Hoffmannseggella cinnabarina*, *Hoffmannseggella caulescens*	Root tissue with pelotons	Molecular (nrDNA ITS/Sanger sequencing)	*Tulasnella*, *Sebacina*	Diversity, Phylogeny	Oliveira (2012)	Brazil
Oeceoclades maculata	Fungal cultures	Morphological	*Rhizoctonia*	Morphological studies, Symbiotic seed germination	Pessoa et al. (2012)	Brazil
Coppensia doniana	Fungal cultures	Morphological, molecular (nrDNA ITS/Sanger sequencing)	*Ceratobasidium*	Diversity, phylogeny, morphological studies, symbiotic seed germination	Valadares et al. (2012)	Brazil
Epidendrum rhopalostele	Root tissue with pelotons	Molecular (nrDNA ITS/Sanger sequencing)	*Tulasnella* (clades A and B)	Diversity, phylogeny	Riofrío et al. (2013)	Ecuador
Notylia sp., *Habenaria* sp., *Epidendrum melinanthum*, *Trizeuxis falcata*, *Maxillaria* sp., *Cranichis* sp., *Dichaea* sp.	Fungal cultures	Molecular (nrDNA ITS/Sanger sequencing)	*Ceratobasidium*, *Thanatephorus*	Diversity, phylogeny	Mosquera et al. (2013)	Colombia
Epidendrum hemiscleria	Pelotons	Molecular (nrDNA ITS/Sanger sequencing)	*Tulasnella violea*	Diversity	Cueva (2014)	Ecuador

(continued)

Table 8.1 (continued)

Taxon	Fungal origin	Identification method	Reported OMF	Study focus	Reference	Country
Vanilla calyculata, *V. odorata*, *V. rivasii*	Fungal cultures, root tissue with pelotons	Molecular (nrDNA ITS/Sanger sequencing)	*Tulasnella* *Ceratobasidium*	Diversity, symbiotic seed germination	Alomia (2014), Alomia et al. (2017)	Colombia
Epidendrum marsupiale, *Odontoglossum pardinum*	Root tissue with pelotons	Molecular (nrDNA ITS/Sanger sequencing)	*Tulasnella*, *Ceratobasidium*, Sebacinales	Diversity	Guzmán and Moreno (2014)	Ecuador
Epidendrum secundum, *Acianthera limae*, *Polystachya concreta*	Fungal cultures	Molecular (nrDNA ITS/Sanger sequencing)	*Epulorhiza repens*, *E. epiphytica*	Diversity	Nogueira et al. (2014)	Brazil
Cyrtopodium paludicolum, *Cyrtopodium saintlegerianum*	Fungal cultures	Molecular (nrDNA ITS/Sanger sequencing)	*Rhizoctonia*, *Tulasnella*	Diversity, symbiotic seed germination	Carvalho (2015)	Brazil
Ionopsis utricularioides, *Psygmorchis pusilla*	Fungal cultures	Molecular (nrDNA ITS/Sanger sequencing)	*Ceratobasidium*	Diversity	Valadares et al. (2015)	Colombia
Cyrtochilum flexuosum, *Cyrtochilum myanthum*, *Maxillaria calantha*	Root tissue with pelotons	Molecular (ITS2/Illumina sequencing)	Serendipitaceae, Ceratobasidiaceae, Tulasnellaceae	Diversity, community ecology	Cevallos et al. (2016)	Ecuador
Teagueia spp.	Root tissue with pelotons	Molecular (nrDNA ITS/Sanger sequencing)	*Tulasnella*, Atractiellales (Pucciniomycotina)	Diversity, phylogeny, evolutive implications	Suárez et al. (2016)	Ecuador
Maxillaria spp.	Fungal cultures	Morphological	*Tulasnella*, *Ceratobasidium*	Diversity	Rodriguez & Lora (2016)	Colombia
Stanhopea tricornis	Fungal cultures	Morphological	*Rhizoctonia*	Diversity	Córdoba-Díaz et al. (2015)	Colombia

Prescottia sp., *Oeceoclades maculata*, *Arundina bambusifolia*, *Vanda* sp., *Cattleya tigrina*, *Cattleya walkeriana*	Pelotons	Morphological	*Epulorhiza*, *Ceratorhiza*, *Moniliposis*, *Rhizoctonia*	Diversity, symbiotic seed germination	Minamiguchi (2017)	Brazil
Cyrtochilum flexuosum, *Cyrtochilum myanthum*, *Cyrtochilum pardinum*, *Epidendrum marsupial*, *Maxillaria calantha*	Root tissue with pelotons	Molecular (ITS2/ Illumina sequencing)	*Agaricales*, *Cantharellales*, *Thelephorales*, *Hymenochaetales*, *Sebacinales*, *Atractiellales*	Diversity, community ecology	Cevallos et al. (2018)	Ecuador
52 orchid species: *Stelis* (18 spp.), *Pleurothallis* (13 spp.), *Maxillaria* (9 spp.), *Epidendrum* (5 spp.), *Elleanthus* (3 spp.), *Artorima* (1 sp.), *Prosthechea* (1sp.), *Sobralia* (1sp.), *Oncidium* (1 sp.)	Root tissue with pelotons	Molecular (nrDNA ITS/Sanger sequencing)	*Tulasnellaceae*, *Serendipitaceae*	Diversity, mutualistic networks	Herrera et al. (2018)	Ecuador
Cyrtochilum myanthum, *Stelis superbiens*	Fungal cultures	Molecular (nrDNA ITS/Sanger sequencing)	*Tulasnellaceae*, *Ceratobasidiaceae*	Diversity	Novotná et al. (2018)	Ecuador
Zygopetalum mackayi, *Z. pedicellatum*	Fungal cultures	Molecular (nrDNA ITS/Sanger sequencing)	*Tulasnella*	Diversity	Regina et al. (2019)	Brazil
Oncidium luteopurpureum, *Oncidium lehmannii*	Fungal cultures	Molecular (nrDNA ITS/Sanger sequencing)	*Ceratobasidium*	Diversity	Henao-Mejía et al. (2020)	Colombia

(continued)

Table 8.1 (continued)

Taxon	Fungal origin	Identification method	Reported OMF	Study focus	Reference	Country
Cattleya jongheana	Fungal cultures	Morphological, Molecular (nrDNA ITS/Sanger sequencing)	*Tulasnella*, *Serendipita*	Morphological studies, diversity, phylogeny	Freitas (2021)	Brazil
Dichaea andina	Fungal cultures	Molecular (nrDNA ITS/Sanger sequencing)	*Ceratobasidium*	Diversity	Alomia et al. (2022)	Colombia

References: *mtLSU* mitochondrial large subunit ribosomal gene; *nucLSU* nuclear large subunit ribosomal gene; *nrDNA ITS* nuclear ribosomal internal transcribed spacer gene; *ITS-RFLP* restriction fragment length polymorphism of the fungal nuclear internal transcribed spacer region; *RAPD* random amplified polymorphic DNA. In the "study focus," we refer as morphological studies to those where the macroscopic characteristics of the mycelium are described and an optical microscope is used for the microscopic traits. In contrast, ultrastructure analyses are more detailed studies of the anatomy of fungi on the characteristics of hyphae and other mycelial organs, using transmission electron microscopy literature). Professor Juan Pablo Suárez from Universidad Técnica Particular de Loja (Ecuador) as the leader in this field with the academic collaboration of Professor Ingrid Kottke (University Tübingen, Germany) has proposed a more evolutionary and ecological perspective on this type of association, including detailed ultrastructural analyses by transmission electron microscopy and community ecology approaches (Kottke et al. 2008a; Kottke and Suárez 2009; Kottke et al. 2013; Suárez and Kottke 2016).

The initial studies identified the fungi associated with orchids from the isolation of the mycelium that was morphologically characterized (Díaz et al. 2000; Pereira 2001). This approach required a great knowledge of fungi or the consultation to experts and did not provide very precise determinations due to the lack of reliable taxonomic characters to reach the species level, which would only be possible by obtaining the teleomorph or sexual phase of the isolated strain. In the region, unlike the temperate zones, obtaining teleomorphs was not a method addressed among the scientific community. With advances in molecular biology, the first genetic identifications were made with the random amplified polymorphic DNA (RAPD) and restriction fragment length polymorphism (RFLP) methods (Pereira 2001, Pereira et al. 2005a). Later, with the new findings on Sanger sequencing and the primers designed to capture the genetic information of the groups of mycorrhizal fungi frequently found in orchids (Kristiansen et al. 2001, Taylor and McCormick 2008), many researchers bypassed the time-consuming phase of isolation of mycelium in vitro to obtain the genetic information from root tissues infected with orchid mycorrhiza, verifying the presence of pelotons (Suárez et al. 2006; Kottke et al. 2008b; Suárez et al. 2008; Kottke et al. 2010; Oliveira 2012; Valadares et al. 2012; Alomía et al. 2017). The most studied genes are those of the nuclear ribosomal internal transcribed spacer (nrDNA ITS) gene and to a lesser extent those of the mitochondrial large subunit ribosomal (mtLSU) and nuclear large subunit ribosomal (nucLSU) regions. Sanger sequencing is the most widespread approach and is still used today for studies in which the strains are not required to be used in germination experiments or to be conserved in mycelium banks for later purposes. Although recent next-generation sequencing (NGS) techniques with Illumina technology offer more complex and robust information on fungi associated with orchids (Cevallos et al. 2016, 2018), this approach is not the most used due to the costs involved, which are not necessarily within the common financial source availability of researchers from developing countries in the region.

8.3 Research Interests

Some studies explored more than one topic in relation to the fungus-orchid associations. However, most studies (46%) have been focused on determining the diversity of OMF associated with few orchid species of interest. Phylogeny, morphological, and symbiotic seed germination studies are other of the main topics addressed (16%, 12%, and 11%, respectively). Ultrastructure and community ecology

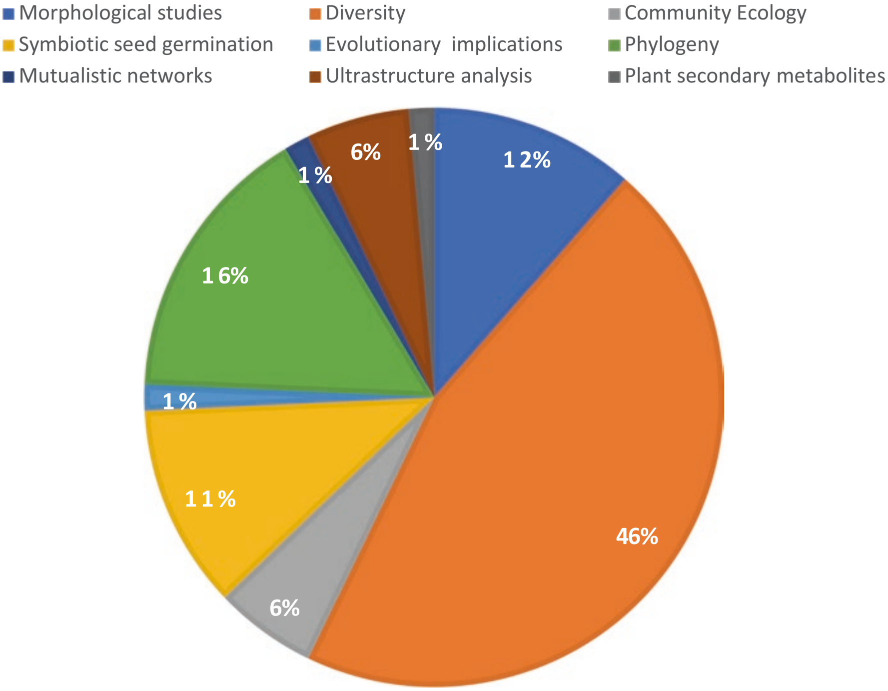

Fig. 8.4 Main studied topics in the OMF-orchid interaction from tropical and subtropical ecosystems in South America

researches are hardly representative (6%), while evolutionary implications, mutualistic networks, and metabolic aspects are the least explored topics (1%) (Fig. 8.4). We did not find studies aimed at understanding physiological topics of the OMF-orchid interaction.

These data indicate that basic questions are still being asked in the region as most of the studies describe the diversity of fungi associated with orchids. Few studies ask analytical questions that lead to a better understanding of the interactions between orchids and fungi. This may in part because a low resource availability limits the development of complex issues, such as those related to physiological or biochemical effects on each partner. Since the biodiversity of orchids in tropical South America is so extensive, the knowledge of OMF diversity is far from complete.

8.4 Challenges and Perspectives

Surprisingly, in tropical regions of South America where the Orchidaceae is especially diverse (Meisel et al. 2015; Kirby 2016), studies on orchid mycorrhiza symbiosis are underrepresented. However, in the last 20 years, the interest in endophytic orchid fungi, both mycorrhizal and non-mycorrhizal, has increased in the region (Herrera et al. 2010; Hernández and Alomía 2020).

Future directions for OMF include: (i) The developing of a broader understanding of OMF. Although most studies focus on fungal diversity, they do not understand the evolutionary implications of the orchids and OMF relationship. We encourage collaborations with the international scientific community to continue investigating complex questions that allow us to understand the role of mycorrhizae in the evolutionary success of tropical orchids. (ii) The developing of new fungal culture techniques to be able to use OMF in bioassays since many OMFs are difficult to grow under in vitro conditions. (iii) To publish for a wider audience. We found that most of the studies are in the “grey” literature. For this, it is necessary to generate innovative research that sparks interest in the academic community. (iv) The application of the information produced on OMF for orchid conservation, orchid propagation, and plant protection (Otero et al. 2013). Orchid conservation programs rarely use OMF technologies to propagate endangered orchids. Similarly, orchid growers do not use OMF for propagation. To develop the enormous potential of the region in the cultivation of orchids, we invite companies interested in the production of orchids such as “Ecuagenera” in Ecuador, “Lima Orquídeas” in Perú, and “Colombo Orquídeas,” “Orquídeas del Valle,” “Orquídeas Eva,” and “Libia Orquídeas” in Colombia, to be linked with the academy so that through research projects, added value will be generated in their business. More specifically, the use of mycorrhizae in the cultivation of orchids can contribute to enhancing production and reducing costs in sexual propagation (seeds) represented by asymbiotic media. Furthermore, OMF could also reduce the development time for flower production, generating significant financial gain for orchid sellers.

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Chapter 9
Symbiotic Propagation of South American Orchids

Sebastián Fracchia and Silvana M. Sede

Contents

9.1 Introduction

All orchid species depend on interactions with specific fungi for completion of their life cycle, particularly during early developmental stages (Rasmussen and Rasmussen 2009). In nature, seed germination occurs only when compatible fungi colonize internal seed tissues inducing the germination process and protocorm development (Dearnaley 2007). On the other hand, the establishment of strictly orchid mycorrhizae occurs a posteriori when suitable mycobionts associate with true roots of orchid seedlings, being often the same fungal strain involved in both events (Arditti et al. 1990; Favre-Godal et al. 2020). Thus, orchid mycobionts are expected to be important drivers for orchid species, not only for seedling establishment and development but also for niche occupancy, plant nutrition and recruitment, and stress mitigation among other ecological variables (McCormick and Jacquemyn 2014). Fungal mycobionts as drivers of orchid species distribution

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are still a matter of discussion, with contrasting positions by different authors (Li et al. 2021).

Most of the taxa identified as fungal mycorrhizal or germination inducers belong to a polyphyletic group of Rhizoctonia-like basidiomycota fungi from the Ceratobasidiaceae, Sebacinaceae, Serendipitaceae, and Tulasnellaceae (Dearnaley et al. 2012). However, other taxa were also identified belonging to the Theleophoraceae, Corticiaceae, and Polyporaceae, although to a much lesser extent (Jacquemyn et al. 2017). When considering orchid habits, terrestrial species are commonly associated with a wider range of basidiomycota fungi than the epiphytic and litophytic species, where isolates of the widespread Tulasnellaceae prevail (Qin et al. 2020). A few ascomycota fungi were also reported as orchid mycobionts and, in specific cases, as mycorrhizal fungi and promoters of seed germination in terrestrial orchids (Hou and Guo 2009; Fracchia et al. 2014a). Most orchid mycobionts are free-living saprophytes, and their distribution is assumed to be independent of their partner plants (Jacquemyn et al. 2017).

Although new insights into the mycorrhizal symbiosis functioning have been published recently (Ghirardo et al. 2020; Zhang et al. 2020), there are still several issues at the ecosystem level that need further research to understand the symbiosis and advance in orchid conservation programs. The specificity and proper identity of mycorrhizal fungi, their role in the distribution of orchid species at different scales, and the temporal variability of the mutual association are still open questions (Li et al. 2021).

9.2 Orchids in South America

The Orchidaceae comprises about 28,000 described species, which represent circa 8% of all vascular plants (Givnish et al. 2016). To date, over 200 new species are described every year, mainly in tropical regions (Chase et al. 2015). The species are distributed from the tropics to high latitudes with cold climates and from sea level to elevations greater than 4500 meters. Most of the species, around two thirds, are epiphytes that are restricted to tropical and subtropical habitats, nearly 5% are lithophytic species also restricted to the tropics, and around 25% are strictly terrestrial that can grow in tropical but also temperate and cold habitats (Gaskett et al. 2018).

South America concentrates approximately one third of the world's biodiversity of orchids, being Colombia the country with the largest number of species in the world with more than 4000 species that were described (Dolce et al. 2020). Brazil has also a high biodiversity with approximately 205 genera and 2650 species, of which about 1800 are endemic (Freitas et al. 2020). Some American genera are amazingly diverse, as is the case of the genus *Epidendrum*, with more than 1500 known species, most of them inhabiting in the tropical rainforest of South America (Pinheiro and Cozzolino 2013).

As in other regions, species richness is correlated with tropical and subtropical ecosystems, meanwhile only 23 terrestrial species inhabit the southern Patagonia in the far south of the Americas, including habitats in Cabo de Hornos islands and the Malvinas archipelago (Schinini et al. 2008; Herrera et al. 2019). Epiphytic species are restricted to latitudes below 34° S approximately, being *Gomesa bifolia* probably the most austral epiphytic orchid species on the planet (Cellini et al. 2009). On the other hand, there are entire regions where only terrestrial species grow, as in Patagonia and high Andean ecosystems above 4000 meters. Particularly, in Chile, all 72 described species belong to terrestrial habits, being almost half endemic of this country (Novoa et al. 2015).

The distribution of orchid species in South America is extremely variable. For example, *Sacoila lanceolata* has a wide distribution on a panamerican level; meanwhile, several epiphytic species are restricted to very small areas as inselberg species in central Brazil (Pinheiro et al. 2014). Another species, *Gavilea insularis,* grows only in the Alejandro Selkirk island in the Juan Fernández archipelago, with an estimated population of less than 300 individuals (Danton 1998). Although first South American orchid species were originally described more than 250 years ago by Linnaeus, new species are periodically described in the region (Pansarin and Miranda 2016; Kolanowska and Szlachetko 2020; Pérez-Escobar et al. 2021). As many species are endemic to very narrow areas, it is expected that new taxa are still waiting to be described, mainly in the tropical Andean mountains and the Amazon rainforest but also in the Gran Chaco, the Yungas, and remote Andean valleys.

This great diversity of orchid species is not exempt, like all living organisms, from the extreme anthropogenic pressure that is constantly increasing in all earth ecosystems. Loss of natural habitat and climate change are the main risk factors for South American orchids (Dolce et al. 2020). The uncontrolled expansion of farmland areas has drastically deteriorated natural ecosystems in the region by direct deforestation and subsequent area fragmentation (Siqueira-Gay et al. 2020). This is notable in biomes as the Atlantic Forest and Amazonian rainforest in Brazil, the Humid Chaco in Argentina and Paraguay, the Yungas in Argentina and Bolivia, and the Cloud Forest on the slopes of the Andes of Perú, Ecuador, Colombia, and Venezuela (Fajardo et al. 2017; Li et al. 2018). Several recognized hotspots of orchid diversity are located in these degraded biomes, so an increasing number of endangered or extinct species is expected in the times to come (Crain and Tremblay 2014). Although there is no record of verified orchid species extinctions in South American countries, this is probably due to the lack of extensive sampling and updated records. The International Union for the Conservation of Nature (IUCN) evaluate species' risk of extinction on the basis of rigorous criteria and the best available scientific information (Zizka et al. 2021).

There are still huge gaps in the basic knowledge of South American orchid species, mainly referred to species taxonomy and phylogeny, species delimitation and phenotypes, distribution range, population size, pollinators, and mycorrhizal interactions among others (Fracchia et al. 2016; Dolce et al. 2020). Fortunately, several new surveys related to conservation strategies have been published on the last years, which compile species richness and conservation status of specific orchid taxa in protected areas, as national parks and reserves (Antolin Barberena et al. 2018; Martel et al. 2021; Alba-Patiño et al. 2021). On the other hand, biotechnological approaches for in vitro propagation of endangered species as well as cryopreservation and germplasm conservation were also addressed worldwide, opening new possibilities for the conservation of South American orchid species (Merrit et al. 2014; Seaton et al. 2015; Dolce et al. 2020).

9.3 Orchid Symbiosis From a Conservationist Perspective

As mentioned above, anthropogenic pressure is taking the natural biomes of South America to a critical stage. In this context, due to the increasing deforestation rates in orchid hotspot areas (i.e., tropical mountain rainforest) but also emerging climatic change effects, it is expected that a large number of orchid species will gradually be considered at extinction risk (Barman and Devadas 2013; Freitas et al. 2020). For this reason, the functioning of orchid mycorrhizal symbiosis acquires great relevance, since this association plays a pivotal role in orchids' life cycle, such as seed germination, plant growth, and stress mitigation processes. When considering species conservation projects, both in situ and ex situ approaches, as assisted migration, symbiotic propagation, and reintroduction programs, knowledge and proper management of the symbiosis is essential to achieve some degree of success (Keel et al. 2011).

Despite the great diversity and wide distribution of orchids in South America, only in the last 10 years, different research groups have published consistent studies on the mycorrhizal symbiosis of native orchids. Prior to these works, a few citations refer to mycorrhizal status of some native species, as well as isolation of fungal strains and morphological characterizations (Godoy et al. 1994; Pereira et al. 2005a, b).

Aiming at symbiotic propagation, fungal isolation strategies and culture methods can be determinant to recover effective mycobionts (Herrera et al. 2019). In most surveys in the region, strains were isolated from thin slides of surface-sterilized orchid roots or by picking directly the pelotons from root parenchymal cells (Fracchia et al. 2014a). Other methodologies, as the seed baiting technique (Brundrett et al. 2003), were also explored for native orchids (Fracchia et al. 2016; Freitas et al. 2020). In this case, protocorms are obtained by incubating orchid seeds in soils inside a plastic mesh contained in diapo slides or other devices. This method allows us to remove the slides from soil and pick the protocorms to isolate directly the strains that promoted germination. An interesting adaptation of this method was employed in epiphytic species in an Ecuatorian reserve with the aim to detect early colonizing fungi (Cevallos et al. 2018). A plastic cylinder with orchid seedlings inside, previously obtained in in vitro culture, was placed in selected tree branches; in this way, the mycorrhizal fungal community composition was assessed by genomic sequencing. The isolation of fungi from more precise strategies is fundamental, since it allows isolation of those strains that promote germination, or are internally associated with the orchid roots forming pelotons.

To date, a total of 21 South American orchid species were symbiotically propagated in agarized media, 14 of them being of terrestrial habit and 7 epiphytes (Table 9.1). Only in some of these works did they achieve symbiotic propagation until adult plants, or even seedlings with green leaves. Most surveys focused on fungal interactions related to seed germination and early protocorm development.

Table 9.1 Symbiotic propagation of South American orchid species: fungal isolates that promoted symbiotic germination/protocorm development

Country	Orchid species	Habit	Fungal isolates[a]	GeneBank accession	References
Argentina	*Gavilea australis*	Terrestrial	*Thanatephorus cucumeris* *Ceratobasidiaceae* *Tulasnella calospora* ***Ceratobasidiaceae*** ***Ceratobasidium albasitensis*** *Ceratobasidium* sp.	KF151201 KJ713697 KJ713701 **KJ713698** **KJ713699** KJ713700	Fracchia et al. (2014b)
	Aa achalensis	Terrestrial	***Gaeumannomyces cylindrosporus*** ***Gaeumannomyces cylindrosporus*** ***Pezizaceae*** ***Thanatephorus cucumeris*** ***Thanatephorus cucumeris***	**KF151198** **KF151199** **KF151200** **KF151201** **KF151202**	Fracchia et al. (2014a)
	Chloraea riojana	Terrestrial	*Thanatephorus cucumeris* *Thanatephorus cucumeris* ***Rhizoctonia solani***	KF151201 KF151202 **KX267766**	Fracchia et al. (2016)
Brazil	*Cyrtopodium paludicolum*	Terrestrial	***Tulasnellaceae*** *Tulasnella*	**KP973894** –	Carvalho et al. (2017)
	Cyrtopodium paludicolum	Terrestrial	*Epulorhiza* sp.	–	Guimarães et al. (2013)
	Cyrtopodium glutiniferum	Terrestrial	*Epulorhiza epiphytica* (two isolates) *Epulorhiza repens* *Epulorhiza* sp. (two isolates)	– – –	Pereira et al. (2015)
	Cyrtopodium saintlegerianum	Epiphyte	*Waitea circinata*	–	Sousa et al. (2019)
	Epidendrum secundum	Terrestrial	***Epulorhiza sp.*** (16 isolates)	–	Pereira et al. (2011)
	Gomesa flexuosa	Epiphyte	*Epulorhiza epiphytica* (two isolates) ***Ceratorhiza* sp.** *Epulorhiza repens*	– – –	Pereira et al. (2005b)

(continued)

Table 9.1 (continued)

Country	Orchid species	Habit	Fungal isolatesa	GeneBank accession	References
Chile	*Chloraea chrysantha*	Terrestrial	*Tulasnella* sp. ***Tulasnella* sp.** *Tulasnellaceae* *Tulasnellaceae*	KP278149 **KP278151** KP278153 KP278156	Herrera et al. (2017)
	Chloraea gavilu	Terrestrial	***Tulasnella* sp.** ***Tulasnellaceae*** *Tulasnella* sp. *Tulasnellaceae*	**KP278149** **KP278150** KP278151 KP278156	Herrera et al. (2017)
	Chloraea bletioides	Terrestrial	***Tulasnellaceae*** *Tulasnellaceae* *Tulasnellaceae*	**KP278153** KP278154 KP278156	Herrera et al. (2017)
	Bipinnula fimbriata	Terrestrial	*Tulasnellaceae* ***Tulasnellaceae*** *Tulasnellaceae* *Tulasnellaceae* *Tulasnellaceae*	KP278147 **KP278148** KP278150 KP278153 KP278156	Herrera et al. (2017)
	Chloraea crispa	Terrestrial	***Ceratobasidium* sp.** *Ceratobasidium* sp. ***Tulasnellaceae*** *Tulasnellaceae*	**KP278168** KP278169 **KP278154** KP278156	Herrera et al. (2017)
	Chloraea longipetala	Terrestrial	*Ceratobasidium* sp. ***Ceratobasidium* sp.** ***Thanetophorus* sp.** ***Tulasnellaceae*** *Tulasnellaceae* ***Tulasnellaceae*** *Tulasnellaceae*	KP278168 **KP278169** **KP278146** **KP278147** KP278154 **KP278155** KP278156	Herrera et al. (2017)
	Chloraea grandiflora	Terrestrial	*Tulasnella* sp. ***Tulasnellaceae*** *Tulasnellaceae* *Tulasnellaceae* *Tulasnellaceae*	KP278149 **KP278152** KP278154 KP278155 KP278156	Herrera et al. (2017)
	Bipinnula fimbriata	Terrestrial	***Ceratobasidium* sp.** (two isolates) ***Tulasnella calospora*** ***Ceratobasidium* sp.** ***Ceratobasidium* sp.** ***Tulasnella calospora*** ***Ceratobasidium* sp.** ***Thanatephorus cucumeris***	DQ102417[c] AY373298[c] DQ102413[c] EF154356[c] EF393622[c] EU668908[c] AY387570[c]	Steinfort et al. (2010)
	Chloraea gavilu[b]	Terrestrial	*Ceratobasidium* sp. (two isolates)	–	Pereira et al. (2021)

(continued)

Table 9.1 (continued)

Country	Orchid species	Habit	Fungal isolatesa	GeneBank accession	References
Colombia	*Vanilla rivasii*	Epiphyte	***Epulorhiza* sp.** *Ceratobasidium* sp. (two isolates) *Tulasnella* sp. (two isolates) ***Epulorhiza sp*.** (two isolates)	**AJ313448**[c] HM623619[c] EF127682[c] **GU166424**[c]	Alomía Aguirre, (2014)
	Vanilla columbiana	Epiphyte	*Ceratobasidium* sp.	HM623619[c]	Alomía Aguirre, (2014)
	Comparettia falcata	Epiphyte	*Rhizoctonia solani* *Ceratobasidium* sp. (two isolates)	– –	Karol Chávez et al. (2015)
Ecuador	*Epidendrum secundum*	Terrestrial	*Ceratobasidium* sp. *Sebacina vermifera*	– –	Durán-López et al. (2019)
	Trichoceros antennifer	Terrestrial	***Rhizoctonia* sp.**	–	Ordoñez et al. (2016)

References:

[a] selected isolates that significantly promoted seed germination/protocorm development; names in bold were isolated from the orchid species under study;

[b] asymbiotic seedlings inoculation

[c] close relative GenBank accession numbers

Currently, only three species were symbiotically propagated in Argentina, all endemic and of terrestrial habit (Fracchia et al. 2014a, b, 2016). Fungal isolates of *Tulasnella*, *Tanathephorus, Rhizoctonia*, and *Ceratobasidium* genera were able to induce seed germination and protocorm development until seedling stage, in the terrestrial species *Aa achalensis* (Fig. 9.1)*, Gavilea australis,* and *Chloraea riojana* (Fig. 9.2). Moreover, three melanized ascomycete fungi, isolated from *Aa achalensis,* two isolates of *Gaeumannomyces cylindrosporus*, and one isolate of Pezizaceae, induced seed germination in the same species.

In Chile, several species, most of them of the terrestrial genus *Chloraea*, were successfully propagated in symbiotic in vitro assays. The germination ability of several isolates of *Tulasnella, Ceratobasidium*, and *Thanatephorus*, isolated from the roots of seven orchid species, including six *Chloraea* species and *Bipinnula fimbriata* was evaluated by Herrera et al. (2017). Seed germination and protocorm development until green leaves and diverse fungal strains were observed in all species, denoting that at least in the study species of the genus *Chloraea*, low specificity is the norm.

On the other hand, Claro et al. (2020) found that fungi isolated from the roots of two rare Chilean species of the genus *Bipinnula* (*B. appinula* and *B. volckmanni*) were unable to induce seed germination, suggesting that seeds may require additional treatment for successful germination, or fungal partners isolated from adult

Fig. 9.1 *Aa achalensis.* From left to right: plant with mature inflorescence with tiny white flowers growing in Chaco Serrano Forest, Córdoba province, Argentina; seedlings obtained by symbiotic propagation in an in vitro culture assay with a *Tulasnella* isolate; seedlings with two leaves ready for transplant to solid substrates. Photo's credit: S. Fracchia

Fig. 9.2 *Chloraea riojana.* From left to right: individual plant growing in the mountain slopes of Velasco range in La Rioja province, Argentina; symbiotic propagation in an in vitro culture assay with a *Rhizoctonia solani* strain that promoted seed germination and protocorm development, forming melanized coilings and pelotons inside the protocorms. Photo's credit: S. Fracchia

plants may not be the same that support the germination of seeds. This last hypothesis was raised by other authors (Li et al. 2021) and could be the case of several South American species. It would be an interesting challenge to detect different fungi that promote different stages in the development of some species of orchids.

The Brazilian epiphytic species *Oncidium flexuosum* was symbiotically propagated by Pereira et al. (2005a). From a total of ten isolates that effectively promoted seed germination and further protocorm growth until seedlings with green leaves, they found only one fungal strain (*Ceratorhiza* sp.), in a trial of 50 days of dual incubation. Another interesting Brazilian species, *Cyrtopodium glutiniferum*, with ornamental and medicinal value, was propagated in an in vitro assay with an *Epulorhiza* isolate (Guimarães et al. 2013). In this work, the authors also evaluated different substrates for the acclimatization of orchid seedlings, propagating efficiently the species with the tested mycobiont, with corncob and pine bark as substrates. Recent studies on the diversity of 50 *Tulasnella* isolates obtained from pelotons of four rare and endangered orchids from a hotspot in Brazil showed an interesting diversity of fungal species with the detection of four new undescribed *Tulasnella* taxa (Freitas et al. 2020). This kind of survey is very interesting, since species of the genus *Tulasnella* have been reported as mycorrhizal fungi of numerous endangered and rare species of neotropical orchids (Almeida et al. 2007).

In Colombia and Ecuador, countries with the highest diversity of species worldwide, five species have been symbiotically propagated, including two species of the genus *Vanilla* (Alomía Aguirre 2014). Although most of the works on symbiotic germination and propagation are aimed at rare or endangered species, only a few explore species that have commercial value as medicinal, ornamental, or edible species.

Many neotropical orchids were propagated asymbiotically in agarized media, including mainly ornamental species that are commonly traded globally (Dolce et al. 2020). A recent work by Pereira et al. (2021) proposes an interesting alternative of symbiotic culture a posteriori, inoculating seedlings obtained in vitro asymbiotic culture. They observed greater vegetative development in inoculated plants, a methodology that could allow greater efficiency in seedling acclimatization and reintroduction programs of endangered species. Failure of symbiotic germination is relatively common in many species of orchids. Noneffective fungal isolates, pH, suitable nutrient media, temperature, light photoperiod, or other factors could be involved in the process.

In a recent work, Herrera et al. (2020) explored endophytic bacteria associated to mycorrhizal roots of *Chloraea* and *Gavilea* orchid species. They found interesting strains with plant growth-promoting attributes such as phosphate solubilization and the production of indole acetic acid (IAA), suggesting that bacterial isolates could be managed as plant growth-promoting rhizobacteria (PGPR) for orchid species. An interesting line of research derived from these results could be the study of the interaction of mycobionts and bacteria in both processes: germination and protocorm development.

9.4 Conclusion

Huge challenges lie ahead for South American orchids. The indiscriminate felling of native forests, added to the increasing climate change effects, predicts a gloomy outlook for the great diversity of orchids in the subcontinent. However, current research on this particular symbiosis on most countries of the region, focused mainly on the conservation of species by symbiotic propagation, is a first contribution to understanding this association and protecting rare and endangered species. There are still many aspects that need urgently to be improved to move forward collaboratively and more quickly. An important factor is the proper identification of fungi, as well as their conservation in registered germplasm banks. In many of the published researches, there is no record that the strains are deposited in fungal banks. It is known that fungal strains that promoted seed germination maintained this effect more than 20 years after its storage in the laboratory. New approaches for in situ conservation are actually considered, taking into account in addition to the specificity of the different species of orchids, the distribution patterns of the mycobionts in the ecosystem. Contrasting hypotheses still coexist about the role of mycobionts on the distribution of orchid species at different scales. Recent studies in Brazil, Ecuador, and Chile give a sample of the variability of fungi associated with native species, including the description of new *Tulasnella* species. More exhaustive sampling, combining with genomic analyses, will be essential to consider protected areas and orchid hotspots in South America.

Finally, an interesting unexplored issue in orchid culture is the incorporation of other plant bioinoculants, as dark septate endophytes, that is, *Trichoderma* isolates, or plant growth-promoting rhizobacteria. Synergistic effects have been observed in the co-inoculation of selected bacteria and other mycorrhizal fungi in several crop species. Improvement in plant fitness and survival rates could be achieved, increasing the chances of success in reintroduction programs.

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Chapter 10

Arbuscular Mycorrhizal Fungi in Agroecosystems of East-Central Argentina: Two Agricultural Practices Effects on Taxonomic Groups

Noelia Cofré, Nicolás Marro, Gabriel Grilli, and Florencia Soteras

Contents

10.1 Introduction

Modern agricultural intensification is one of the major threats to global biodiversity of the Anthropocene (Boivin and Crowther 2021). The associated activities with agriculture, such as chemical applications, tillage, monoculture practices, among others, are considered the most important drivers of global changes through their effects on the biodiversity and ecosystem functions around the world (IPCC 2019). In agroecosystems, some practices like no-tillage (NT) systems, characterized by a reduction in the intensity and/or number of tillage operations (Kabir 2005), have evidenced an increased crop diversity, improved soil fertility and water use, and a decreased soil erosion and chemical input, compared to conventional tillage (Hamel

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1996). In addition, NT practices are more beneficial in terms of the sustainability of the agricultural system by improving crop yield (Zhao et al. 2017).

The Pampean phytogeographic region, comprising an area of 50 million ha at East-Central of Argentina (Hall et al. 1992), represents the main agricultural land of the country. In Argentina, NT practices have increased in the last four decades as one of the models of sustainable agriculture (Lal 2004; Viglizzo et al. 2011). These systems include row crop and reduced soil disturbance practices such as tillage operations or crop residue harvest that are instead implemented under conventional production systems (D́iaz-Zorita et al. 2002; Albertengo et al. 2013). Currently, NT systems occupy a large surface of the cultivated area of Argentina, reaching around 20 million ha (Albertengo et al. 2013) with two crop strategies usually being applied: monocropping and crop rotation (Derpsch et al. 2010). The main crops of the area are maize (*Zea may*s L.), soybean (*Glycine max* (L.) Merrill), and wheat (*Triticum aestivum* L.) that are usually grown in rotation with winter annual forage crops such as oat (*Avena sativa* L.) and triticale (*Triticum aestivum* L x *Secale cereale* L.) and pastures such as alfalfa (*Medicago sativa* L.) and fescue (*Festuca arundinacea* L.) for livestock rearing (Viglizzo et al. 2003). Although NT practices are less imperious for the soil ecosystem than conventional ones (D́iaz-Zorita et al. 2002), these strategies have also been affecting the biodiversity of plants, animals, and microorganisms worldwide (Altieri 1999; Finckh and Wolfe 2006). Therefore, NT agricultural practices must be evaluated including other components, such as crop rotation in the context of the history of land use and the physical and chemical characteristics of each area or region (Derpsch et al. 2014). Among their effects on soil ecosystems, one of the best documented are the changes on soil biota and particularly on the communities of arbuscular mycorrhizal fungi (Oehl et al. 2003, 2010; Cofré et al. 2017; Marro et al. 2020).

Arbuscular mycorrhizal fungi (AMF) are one of the most important functional groups of soil biodiversity because they contribute greatly to crop productivity (van der Heijden et al. 2008). AMF can have beneficial effects on plant performance and soil quality, which are essential for agroecosystem functioning (Jeffries et al. 2003; Barrios 2007). However, the benefits of AMF abundance and diversity on crop yields have been overstated in the last decades (Ryan and Graham 2018). For these reasons, the comprehensive evaluation of soil quality in the context of agroecosystems should include the study of AMF communities. In this chapter, we attempt to give an up-to-date overview of AMF communities in agroecosystems of Argentina. In the first section, we provide a characterization of AMF and their current taxonomic classification considering their functioning. The following sections are focused on our research of the variation of AMF community under different agricultural practices of agroecosystems of Argentina.

10.2 General Characteristics of Arbuscular Mycorrhizal Fungi and Taxonomic Groups

Arbuscular mycorrhizal fungi establish symbiosis with more than 80% of land plants including the cultivated ones (Smith and Read 2008). In the soil, extra-radical hyphae help to acquire soil nutrients (mainly phosphorus and nitrogen), while the intra-radical hyphae provide these nutrients to the plant, across the arbuscular structure, in exchange for carbon in form of hexoses and lipids from the plant (Smith and Read 2008; Lanfranco et al. 2018). The association with AMF promotes plant growth and development and also helps them to face biotic stress, such as pathogen or parasite attack (e.g., Azcón-Aguilar and Barea 1997; Marro et al. 2018), or abiotic stresses, such as drought, salinity, nutrient deficiency, or heavy metal toxicity (e.g., Augé 2001; Porcel et al. 2012; Malicka et al. 2021).

It is well known that the benefits of AMF vary among species and taxonomic groups (e.g., van der Heijden et al. 1998; Klironomos et al. 2004; Jansa et al. 2008). The particular benefits that each AMF species or taxonomic group provides to plants are linked to their fungal traits, such as the reproduction strategy (allocation of extra- and intra-radical biomass) and growth rate (Maherali and Klironomos 2007; Chagnon et al. 2013). AMF fungal traits differ among the three main taxonomic families of Glomeromycotina (i.e., Gigasporaceae, Glomeraceae, and Acaulosporaceae) (Powell et al. 2009; Koch et al. 2017). Members of Gigasporaceae produce extensive extra-radical mycelia and sporulate late in the growing season. Meanwhile, Glomeraceae colonizes mainly intra-radically and sporulates early, and Acaulosporaceae produces low biomass both inside and outside the roots (Hart et al. 2001; Hart and Reader 2002). In the context of Grime's framework, Gigasporaceae would be considered as "competitors," Glomeraceae "ruderals," and Acaulosporaceae "stress tolerant" (Chagnon et al. 2013). Considering the functional differences among AMF families and following Oehl et al.'s (2011) classification, we hereafter will refer to four AMF taxonomic groups: Glomerales, Diversisporales, Gigasporales, and basal lineages (comprised by Archaesporales and Paraglomerales).

The AMF taxonomic groups differ in their effect on host performance under both regular and stressful conditions, with members of Diversisporales being the most beneficial to plants under regular conditions and Gigasporales to plants facing biotic stress (Marro et al. 2022). In the context of agriculture, AMF community changes due to different land uses might be relevant not only in terms of biodiversity and functioning changes of the soil ecosystems but also for crop productivity. However, the effect of agricultural practices on AMF taxonomic groups has seldom been studied (Verbruggen et al. 2010; Jansa et al. 2014). Therefore, in this chapter, we compared the composition of the main AMF taxonomic groups under different land uses (monoculture vs. crop rotation) in five different crop fields in East-Central Argentina.

10.3 AMF Communities Under Different Agricultural Practices

Since 2009, we have been studying the AMF communities of the agricultural ecosystems of Argentina (Cofré 2014). We used the morphological traits and ontogeny of spores for the AMF identification; therefore, we refer to AMF species as "morphospecies" (Robinson-Boyer et al. 2009). In this section, we compiled our data and analyzed the influence of agricultural practices on AMF morphospecies in agroecosystems. For these analyses, we considered the soil samples collected in five geographical locations: Manfredi, Bengolea, Monte Buey (Córdoba Province), Pergamino (Buenos Aires Province), and Viale (Entre Ríos Province) in East-Central Argentina (Fig. 10.1). Two no-till agricultural practices were compared: crop rotation (CR) and soybean monocropping (MC). After cropping, three to ten soil core samples were collected per treatment in each geographical location, and the spores were extracted by wet sieving and decanting (see Cofré et al. 2017 for more details). Species were identified using current species classifications (www.mycobank.org). In addition, in order to evaluate the effect of soil characteristics on AMF composition, the main chemical soil properties were determined by standard methods. For each soil sample, we determined soil acidity (pH), percentage of organic carbon (% OC), percentage of nitrogen (% N), and available phosphorus (ppm of P).

The plots under both NT systems (i.e., CR and MC) were selected at four different locations: Bengolea (33° 01' 31" S, 63° 37' 53" W, 231 m.a.s.l.), Monte Buey (32° 58' 14" S, 62° 27' 06" W, 930 m.a.s.l), Pergamino (33° 56' 36" S, 60°33' 57" W, 1000 m.a.s.l.), and Viale (31° 52' 59" S, 59° 40' 07" W, 1156 m.a.s.l.). In each plot, three samples (as repetitions) of the top 10 cm of soil were collected, each one was a composite of 20 randomly selected cores (10-cm-diameter metal corer) collected within an area of 5 m^2 (see Cofré et al. 2017, 2020 for more details). Meanwhile, at the location of Manfredi (31.5° S, 63.5° W, 292 m.a.s.l.), the soil samples were taken from one long-term trial initiated in 1995, at the Instituto Nacional de Tecnología Agropecuaria (INTA). From the experimental trial, we also selected plots with both treatments (CR and MC). In each plot, we collected five soil samples (replicates) of the top 10 cm of soil, separated by at least 25 m from each other (see Marro et al. 2020 for more details).

To test for differences of AMF morphospecies between cropping systems, we compared the morphospecies richness, spores density, Pielou's evenness, and Shannon–Weaver diversity index (Magurran and McGill 2011) obtained with the function *diversityresult* as implemented in BiodiversityR package (Kindt and Core 2005). To determine the difference of these indices between CR and MC, we fitted generalized linear mixed models in the lme4 package (Bates et al. 2015), with cropping system (two levels: CR and MC) as fixed factor and location as a random term. AMF richness was fitted with a Poisson error structure and spore's density, Shannon diversity index, and evenness with a Gamma error structure.

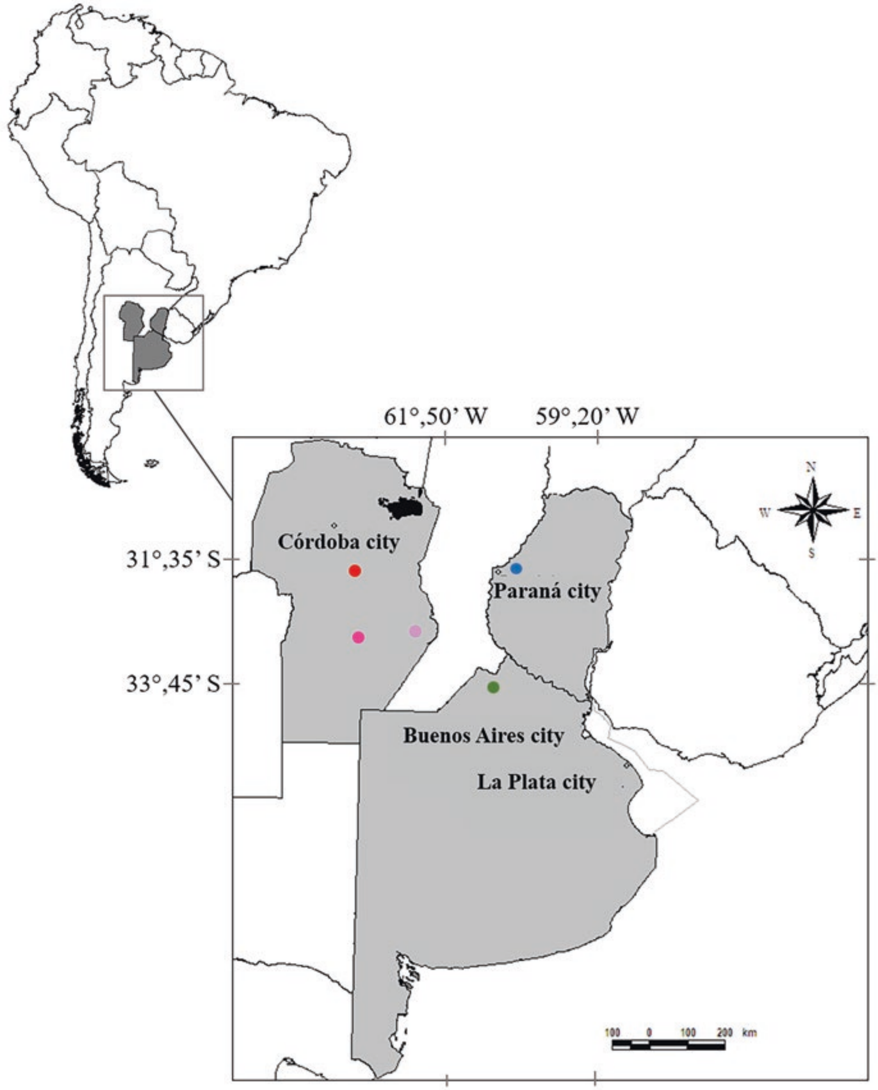

Fig. 10.1 Map showing the sites in each geographical locations included in this study:
(●) Manfredi
(●) Bengolea
(●) Monte Buey
(●) Pergamino
(●) Viale
in East-Central Argentina

In order to describe the AMF community, we calculated the indicator values of each AMF morphospecies, AMF families, and AMF taxonomic groups for each agricultural practice using the *indval* function of the labdsv package (Roberts 2019). We considered nine AMF families (i.e., Acaulosporaceae, Ambisporaceae, Archaeosporaceae, Claroideoglomeraceae, Entrophosporaceae, Gigasporaceae, Glomeraceae, Pacisporaceae, and Paraglomeraceae) and four AMF taxonomic groups (i.e., Glomerales, Diversisporales, Gigasporales, and basal lineages [Archaeosporales and Paraglomerales]).

The effect of each agricultural practice on AMF families and taxonomic groups was tested with permutational multivariate analysis of variance (perMANOVA) using the function *adonis* in the vegan package (Oksanen et al. 2020), and this allows to fit a linear model to partition the variation of community distance matrices among NT systems. Differences in the relative abundance of each AMF family and taxonomic group were calculated using the Bray-Curtis dissimilarity index. We graphically visualized the ordination of the AMF community in two dimensional spaces using nonmetric multidimensional scaling (NMDS) employing *metaMDS* and *ordiplot* functions in the vegan package. Finally, we projected the polygons of agricultural practices and the vectors of soil variables (soil acidity, % organic carbon, % nitrogen, and ppm of phosphorus) in the NMDS. The significance of each vector was assessed with the *envfit* function of the vegan package after 999 permutations. To determine whether the significant effects could be attributed to multivariate dispersion rather than multivariate location (i.e., changes of abundance in AMF community), we calculated the beta diversity within and between groups with the *betadisper* function of vegan. Finally, to evaluate the relationship of the relative abundance of each AMF family and taxonomic group and soil variables, we performed Pearson pair-wise correlations. All the analyses were performed with R (R Core Team 2021).

10.3.1 AMF Morphospecies in Both No-Till Agricultural Practices

A total of 59 morphospecies, comprising 18 genera and nine families, was found in the studied area. Only 15 morphospecies were identified at the genus level (Table 10.1 and Fig. 10.2). There were two AMF morphospecies indicative of CR system: *Funneliformis mosseae* and *Glomus* sp. 4 (indicator value = 0.338, *P* = 0.025; indicator value = 0.229, *P* = 0.022, respectively). Meanwhile, MC showed nonsignificant indicator morphospecies. The species *F. mosseae* and the genera *Glomus* sp. are globally widespread and considered to have ruderal strategies associated, such as fast sporulating AMF successful in disturbed systems (Sýkorová et al. 2007; Oehl et al. 2010); thus, it draws attention that was indicative of CR but not for MC. In particular, *F. mosseae* is a generalist species widely used for experimental studies due to its easy culturing, but it shows a low to moderate effect on the promotion of plant growth, nutrient uptake, as well as protection facing biotic and abiotic stress comparing with other taxa (Marro et al. 2022). Probably,

Table 10.1 List of the arbuscular mycorrhizal fungal morphospecies found in both no-till agricultural practices (crop rotation-CR- and monocropping -MC-) in the five geographical locations in East-Central Argentina

	Manfredi		Bengolea		Monte Buey		Pergamino		Viale	
AMF morphospecies	CR	MC	CR	MC	CR	MC	CR	MC	CR	MC
Basal lineages										
Archaeosporales										
Ambisporaceae										
Ambispora leptoticha	X			X	X		X			
Archaeosporaceae										
Archaeospora trappei	X			X	X	X	X	X	X	X
Paraglomerales										
Paraglomeraceae										
Paraglomus bolivianum				X				X		
Diversisporales										
Acaulosporaceae										
Acaulospora alpina	X			X			X	X		X
A. bireticulata			X	X	X	X	X	X	X	X
A. cavernata	X									
A. delicata	X									
A. excavata			X		X	X			X	X
A. kentinensis	X	X								
A. mellea					X				X	X
A. rehmii	X	X	X	X		X	X	X		
A. scrobiculata	X	X	X	X	X	X	X	X		
A. spinosa	X			X			X	X	X	X
Acaulospora sp. 1	X	X								
Acaulospora sp. 2	X									
Pacisporaceae										
Pacispora sp. 1							X			
Gigasporales										
Gigasporaceae										
Cetraspora pellucida					X					
Dentiscutata heterogama				X						
Gigaspora decipiens							X		X	
G. gigantea				X			X		X	X
G. margarita	X		X	X	X	X	X	X	X	X
G. rosea			X	X	X	X	X	X		
Gigaspora sp.1	X									
Racocetra fulgida			X		X					
R. persica	X									
Scutellospora biornata			X					X		X
S. calospora	X		X	X			X	X	X	
S. dipapillosa				X						
S. rubra			X	X				X		
Scutellospora sp. 1				X				X		

(continued)

Table 10.1 (continued)

	Manfredi		Bengolea		Monte Buey		Pergamino		Viale	
AMF morphospecies	CR	MC	CR	MC	CR	MC	CR	MC	CR	MC
Scutellospora sp. 2	X									
Scutellospora sp. 3	X									
Glomerales										
Claroideoglomeraceae										
Claroideoglomus claroideum			X	X	X	X	X	X	X	X
C. etunicatum	X	X	X	X	X	X	X	X	X	X
C. lamellosum	X									
C. luteum					X				X	
Entrophosporaceae										
Entrophospora infrequens	X	X	X	X				X	X	
Glomeraceae										
Funneliformis coronatus					X		X	X		
F. geosporum	X	X	X	X	X	X	X	X	X	X
F. mosseae	X		X		X	X	X	X	X	X
Glomus brohultii	X		X	X	X	X	X	X	X	X
G. fuegianum	X	X						X	X	X
G. melanosporum	X	X								X
Glomus sp. 1					X	X				
Glomus sp. 2					X					
Glomus sp. 3			X							
Glomus sp. 4	X	X								
Glomus sp. 5	X									
Glomus sp. 6	X									
Glomus sp. 7	X									
Glomus sp. 8	X	X								
Rhizoglomus microaggregatum				X			X	X	X	X
R. clarum	X			X			X	X	X	X
R. fasciculatum				X			X			
R. intraradices			X	X	X	X	X	X	X	X
Sclerocystis sinuosa	X				X					
Septoglomus constrictum	X	X	X		X	X		X		
Sieverdingia tortuosa	X									
Simiglomus hoi	X									

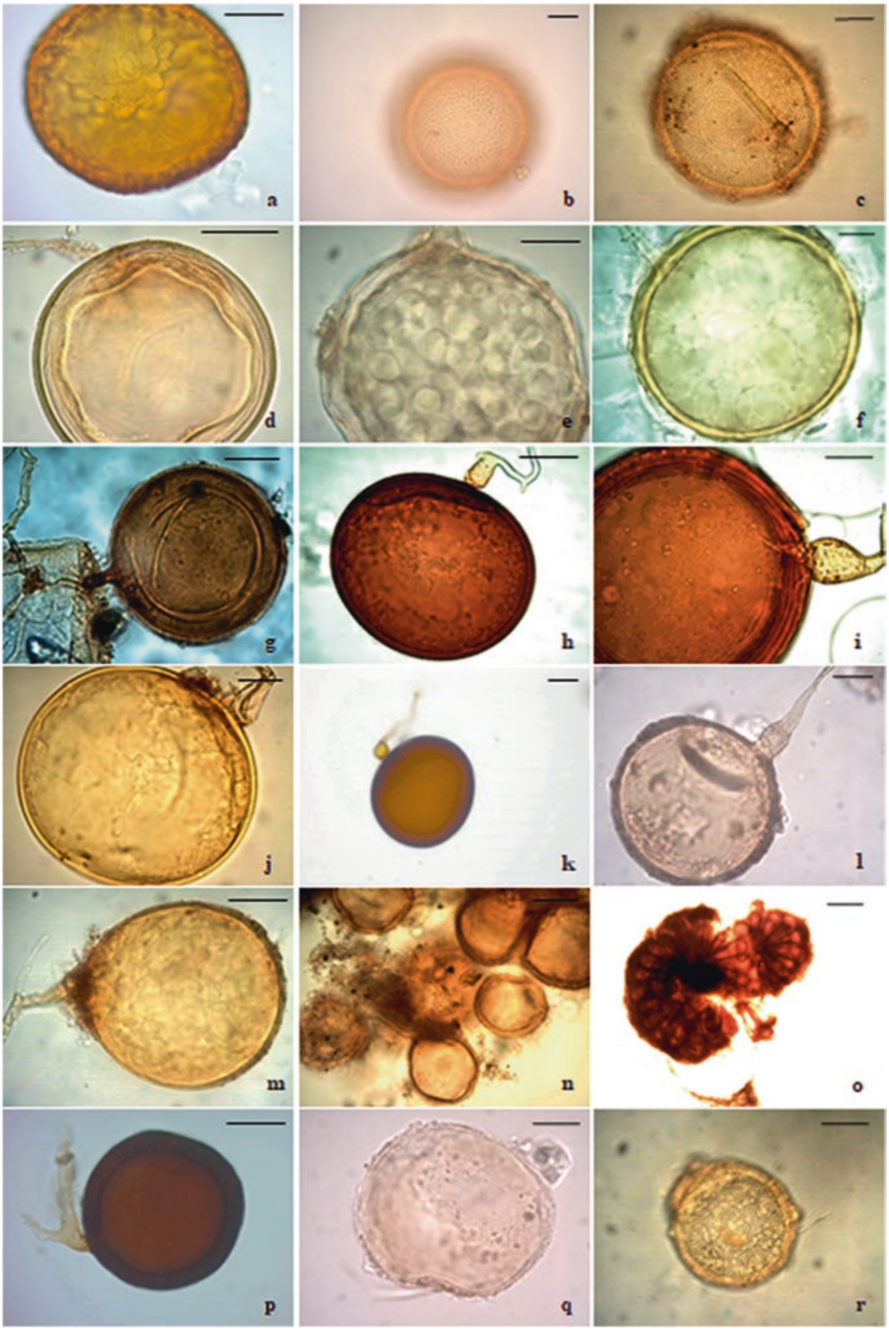

Fig. 10.2 Some of the arbuscular mycorrhizal fungi morphospecies identified in the agroecosystems of East-Central Argentina: (**a**) *Acaulospora bireticulata*, (**b**) *A. rehmii*, (**c**) *A. scrobiculata,* (**d**) *Ambispora leptoticha*, (**e**) *Archaeospora trappei*, *(***f**) *Claroideoglomus claroideum,* (**g**) *Entrophospora infrequens,* (**h**) *Dentiscutata heterogama,* (**i**) *Gigaspora margarita,* (**j**) *Scutellospora calospora,* (**k**) *S. rubra,* (**l**) *Funneliformis coronatus,* (**m**) *F. mosseae,* (**n**) *Rhizoglomus microaggregatum,* (**o**) *Sclerocystis sinuosa,* (**p**) *Septoglomus constrictum,* (**q**) *Pacispora* sp., (**r**) *Paraglomus bolivianum.* Scale*:*50 µm. Photo's credit: N. Cofré

the change of host plant identity over time during CR is promoting *F. mosseae* sporulation, which might explain its higher occurrence in CR than in MC practice.

Richness of AMF morphospecies (CR: 7.57 ± 2.61 and MC: 6.06 ± 2.32) and spore's density (CR: 82.87 ± 86.74 and MC: 31.77 ± 21.61) were significantly higher in CR than in MC (richness: $z = 2.37$, $P = 0.02$; density: $z = 2.65$, $P = 0.008$). However, evenness was significantly higher in MC than in CR (0.71 ± 0.16 and 0.80 ± 0.11, respectively; $z = 2.71$, $P < 0.001$). Therefore, despite soils of CR harbor more AMF morphospecies and spores than MC soils, the community of MC was more evenly distributed. However, Shannon diversity index was similar between both agricultural practices ($z = 1.53$, $P = 0.126$). In line with our results, MC under no-tillage reduced the bacterial diversity (Figuerola et al. 2015) and phosphorus-solubilizing bacteria abundance (Agaras et al. 2014) in previous studies. In addition, we have previously evidenced, for soybean, that CR soil promotes higher seed quality and, to a lesser degree, higher yield than MC soil (Marro et al. 2020). Therefore, beyond particular AMF species, the community composition of AMF together with the other soil microorganisms and chemical characteristics of CR soil might be improving its quality and thus crop yield differentially than MC soil does.

Acaulospora bireticulata, *A. scrobiculata*, *Archaeospora trappei*, *Claroideoglomus claroideum*, *C. etunicatum*, *Gigaspora margarita*, *Funneliformis geosporus*, *F. mosseae*, *Glomus brohultii*, and *Rhizoglomus intraradices* occurred in both agricultural practices, in at least four of the five geographical locations studied (Table 10.1). Other morphospecies were more restricted; some of them to crop rotation and some to monocropping. Twenty-one morphospecies were exclusively recorded in CR management (*A. cavernata*, *A. delicata*, *Acaulospora* sp.2, *Claroideoglomus lamellosum*, *C. luteum*, *Cetraspora pellucida*, *Gigaspora decipiens*, *Gigaspora* sp. 1, *Racocetra fulgida*, *R. persica*, *Scutellospora* sp. 2, *Scutellospora* sp. 3, *Simiglomus hoi*, *Glomus* sp. 2, *Glomus* sp. 3, *Glomus* sp. 5, *Glomus* sp. 6, *Glomus* sp. 7, *Sclerocystis sinuosa*, *Sieverdingia tortuosa*, *Pacispora* sp. 1). On the other hand, *Dentiscutata heterogama*, *Scutellospora dipapillosa*, *Scutellospora* sp.1, and *Paraglomus bolivianum* were only registered in MC. Furthermore, Paraglomeraceae, with only one morphospecies (*P. bolivianum*), was exclusive to MC (Table 10.1).

Leaving the soil organic matter during agricultural management, such as in NT practices, not only helps prevent alteration of soil ecosystems but also improves carbon stocks and reduces CO_2 emissions from the soil during agricultural production (Kladivko 2001; Carrera et al. 2007; Wang et al. 2020). Here, we evidenced that the NT systems of East-Central Argentina harbor higher diversity of AMF compared to other agroecosystems under conventional tillage (de Pontes et al. 2017). Moreover, the AMF diversity found in this study under NT systems is similar to that found in some natural ecosystems at Central Argentina (Cofré et al. 2017, Longo et al. 2016, Soteras et al. 2015; Grilli et al. 2012). In particular, the richer AMF community in the CR system than in MC might be caused by an alteration in soil chemical properties that facilitates the diversification of AMF assemblage (Magurno et al. 2014). In addition, CR increases the quantity and quality of residues in soil that positively influence the soil biota associated with AMF and promotes soil fertility (Tiemann et al. 2015).

10.3.2 AMF Families and Taxonomic Groups Change with Agricultural Practices

The families that showed the highest number of morphospecies in East-Central Argentina were Glomeraceae (22), Gigasporaceae (16), and Acaulosporaceae (12), followed by the other families displayed in Table 10.1. Claroideoglomeraceae was represented by few morphospecies, but it was one of the dominant in both NT systems (Fig. 10.3a, b). Regarding the dominance of these families, for CR practice, Glomeraceae represented the 45% of total morphospecies number, Claroideoglomeraceae 30%, and Acaulosporaceae 16% (Fig. 10.3a). In MC, Acaulosporaceae was predominant with 35% of the total morphospecies number, followed by Glomeraceae (31%) and Claroideoglomeraceae (24%) (Fig. 10.3b).

In both agricultural practices the taxonomic groups followed the same pattern of variation, Glomerales was the dominant taxonomic group with 76% and 56% for CR and MC, respectively, followed by Diversisporales (16% and 34%), Gigasporales (6% and 7%), and Archaeosporales (2% and 3%) (Fig. 10.3c, d).

Relative abundance of AMF families (perMANOVA: pseudoF = 4.359, $P = 0.007$) and AMF taxonomic groups (perMANOVA: pseudoF = 8.488, $P = 0.003$) was significantly influenced by agricultural practice (Fig. 10.4). Dispersion within and between groups was homogeneous (families: $F = 0.148$, $P = 0.701$; orders: $F = 2.199$, $P = 0.14$); thus, differences could be attributed to the agricultural practice

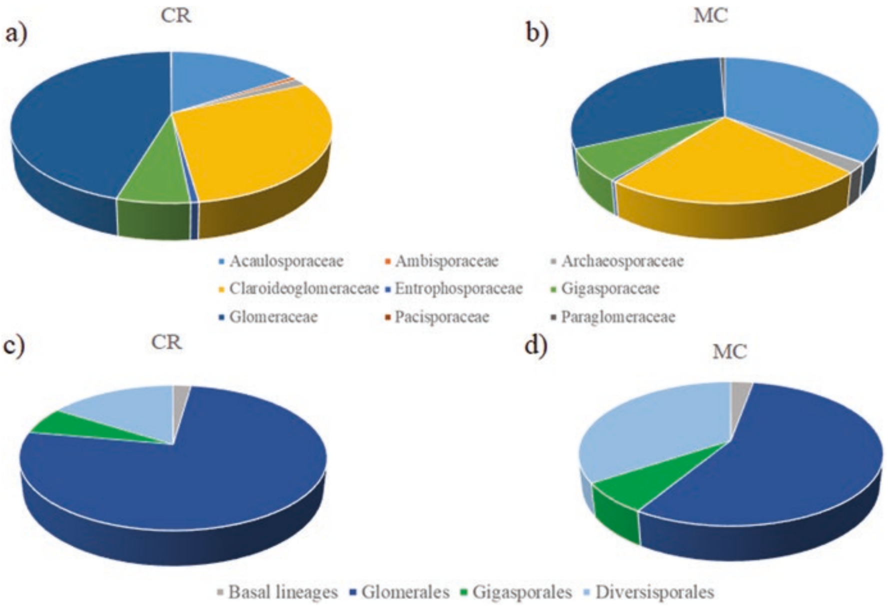

Fig. 10.3 Arbuscular mycorrhizal fungal families (**a** and **b**) and orders (**c** and **d**) distribution in both no-till agricultural practices (crop rotation-CR- and monocropping -MC-) of East-Central Argentina

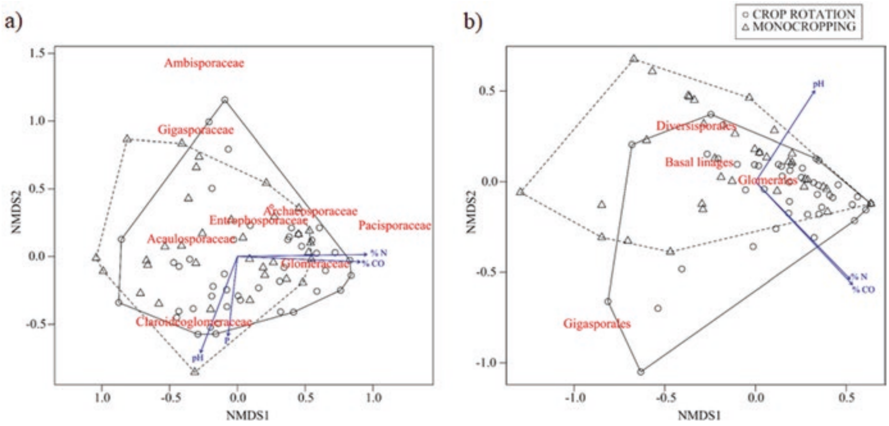

Fig. 10.4 Two-dimensional nonmetric multidimensional scaling (NMDS) plot of variation in arbuscular mycorrhizal fungal taxonomic groups among both no-till agricultural practices (crop rotation and monocropping). AMF were grouped in (**a**) families (stress = 0.133) and (**b**) orders (stress = 0.078). Significant ($P < 0.05$) soil variables are fitted as vectors (pH: soil acidity, % OC: percentage of organic carbon, % N: percentage of nitrogen, and P: ppm of phosphorus)

and not the multivariate dispersion. Soil variables significantly influenced the relative abundance of AMF families and taxonomic groups (Fig. 10.4). Increasing percentages of organic carbon and nitrogen were positively associated with CR and negatively with MC. The NT systems modify the soil chemical properties that shape AMF communities in agroecosystems, such as pH (Davison et al. 2021), nitrogen (Egerton-Warburton and Allen 2000), and phosphorus (Smith and Read 2008). AMF spore diversity being affected by nitrogen has been observed when this nutrient is the most deficient instead of phosphorus (Jefwa et al. 2006). An increase of organic carbon in CR systems suggests a higher level of carbon sequestration due to a higher density of AMF hyphae in CR than in MC (Rillig et al. 2001).

Acaulosporaceae was a significant indicator family for MC (indicator value = 0.621, $P = 0.003$). The relative abundance of this family was positively correlated with an increasing soil acidity ($t = 2.647$, $P = 0.009$) and a decreasing percentage of organic carbon and nitrogen ($t = -3.536$, $P = 0.001$; $t = -3.533$, $P = 0.001$, respectively; Fig. 10.4a). Acaulosporaceae members tend to have very delicate and diffuse hyphae, which may be less “costly” in terms of carbon allocation to its host (Hart and Reader 2002), and generally occur in acidic soils (Veresoglou et al. 2013), which might explain the predominance of this family in MC soils.

Glomerales was a significant indicator order for CR and Diversisporales for MC (indicator value = 0.558, $P = 0.002$; indicator value = 0.619, $P = 0.001$, respectively). Glomerales and Diversisporales were positively related to soil acidity ($t = 2.958$; $P = 0.004$; $t = 2.617$; $P = 0.011$, respectively). In addition, Diversisporales was negatively associated with the percentage of organic carbon ($t = -3.527$, $P = 0.001$) and nitrogen ($t = -3.533$; $P = 0.001$).

10.4 Conclusion

The conversion of natural areas into zones with agricultural crops is one of the main causes of global change (IPCC 2019); thus, the implementation of agricultural practices less aggressive to nature as an alternative to the conventional ones is outstanding. In this context, agroecosystems which comprise NT agricultural practices, have been encouraged as a sustainable agriculture (Albertengo et al. 2013; Calegari et al. 2020; Chen et al. 2020; Cordeau 2022). However, the different NT systems vary in their impact on soil ecosystems as we evidenced in this chapter for the AMF communities between soybean MC and soybean-maize CR systems. We observed that NT practices implemented in East-Central Argentina have differentially affected the AMF community and chemical properties of soil ecosystems. MC harbored a less rich AMF community with more representatives of Acaulosporaceae than CR systems, and CR were associated with an increasing % CO and % N.

The agroecosystems presented in this chapter, which do not exceed 1000 km, showed 17% of the Glomeromycotan taxa identified worldwide (~340). This finding supports the ubiquity and low endemisms presented by this Phylum around the globe (Öpik et al. 2016). These morphospecies are distributed in nine of the 14 AMF families globally recognized (Oehl et al. 2011), with representatives in the four AMF taxonomic groups. It is worth noting that agroecosystems under NT in Argentina hold up a highly diverse AMF community in the region studied here, which in addition belongs to the Caatinga Chaco diagonal, where the greatest AMF richness was observed (Cofré et al. 2019). Therefore, future agricultural practices in these soils should take particular caution with the conservation of AMF communities. The higher AMF richness of CR soils and their higher positive effect on soybean productivity (Marro et al. 2020) than MC soils highlights the importance of carrying out less imperious agricultural practices in Argentina.

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Chapter 11
Metal Soil Contamination, Metallophytes, and Arbuscular Mycorrhizal Fungi From South America

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11.1 Introduction

Soils are the most valuable ecosystem in the world (Pepper et al. 2009) because they provide ecosystem services needed for life on the earth (FAO and ITPS 2015). However, soil, water, and air pollution represent severe environmental problems that can affect quality and human health (FAO and UNEP 2021).

Soil contamination has frequently been used as a synonym for soil pollution. Soil pollution is defined as the presence of a chemical or substance (heavy metals, pesticides, etc.) out of place with a higher than normal concentration with adverse effects on nontargeted organisms (FAO and ITPS 2015). Human activities release pollutants to the environment (Swartjes 2011) affecting various species and ecosystems on the planet.

Heavy metals (HM) are a group of metals and metalloids with a relatively high atomic mass (>4.5 g/cm^3) such as Pb, Cd, Cu, Hg, Sn, and Zn that cause toxicity problems. These elements naturally occur at low concentrations in soils, and many are essential for plants, animals, and humans. High HM concentrations may cause phytotoxicity, which accumulate in tissues and living organisms. The main anthropogenic sources of HM are industrial areas, mine tailings, disposal of metal wastes, leaded gasoline and paints, fertilizers, pesticides, coal combustion residues, etc. (Alloway 2013). Among the HM, Zn, Ni, Co, and Cu are more toxic to plants; meanwhile, As, Cd, Pb, Cr, and Hg are more toxic to higher animals (McBride 1994).

Remediating polluted soils requires advanced technologies. Researchers and remediation companies are investing in technologies that enable environmentally and sustainable remediation (Duarte et al. 2018). A promising biological method proposed for cleanup of contaminated environments is phytoremediation (Sessitsch et al. 2013; Thijs et al. 2017). Phytoremediation uses plants and their associated microorganisms to remove, reduce, transform, mineralize, degrade, volatilize, or stabilize contaminants through their metabolic activities (Kelley et al. 2000; Miretzky et al. 2004; Cherian and Oliveira 2006; Cho et al. 2008). Depending on the type of pollutant, different strategies for phytoremediation are recognized: phytoextraction, phytodegradation, rhizofiltration phytostabilization, and phytovolatilization (Ashraf et al. 2019). Briefly, (1) phytoextraction is the uptake of HM by the harvestable parts of plant, (2) phytodegradation is the decomposition of pollutants by plants and microbes, (3) rhizofiltration is the absorption of metals from polluted waters, (4) phytostabilization produces a decreased mobility and immobilization of pollutants in soil by plant roots and microbes, and (5) phytovolatilization is the volatilization of pollutant into the atmosphere by plant roots (Chaudhry et al. 1998; Khan et al. 2000). A wide diversity of plant species has been identified, due to their great capacity to accumulate HM, which are called *metal hyperaccumulating plants*.

In phytoremediation, soil microorganisms must include the mycorrhizal fungi in soil restoration programs (Haselwandter and Bowen 1996). Arbuscular mycorrhizal (AM) associations enhance soil phytoremediation processes since they improve plant growth-promoting, pollutant-degrading, and/or detoxification capacities (Chen et al. 2018; Begum et al. 2019). Arbuscular mycorrhizal fungi (AMF) are ubiquitous in soil being present on the roots of plants growing on HM-contaminated soils playing an important role in metal accumulation and tolerance (Gaur and Adholeya 2004; Khade and Adholeya 2009; Miransari 2011). When polluted soil is treated with AM-colonized plants, an important point to be considered is the selection of appropriate AMF species since they are most efficient and have the ability to survive under metal stress conditions being more efficient that other AMF species (Miransari 2011; Meier et al. 2012b).

The objective of this chapter is to show a list of metallophyte/hyperaccumulator plant species registered for South America with their mycorrhizal status. Specifically, we focused on the relationship between AMF symbiosis with metallophytes in soils contaminated with Cu from Chile and soils contaminated with Pb from Central of Argentina.

11.2 Heavy Metal Soil Contamination, Metallophyte/Hyperaccumulator Plants and Arbuscular Mycorrhizal Fungi

All soils contain HM due to the mineral composition of the lithosphere. HM can be derived from natural and anthropogenic sources. From natural sources, the HM concentration depends on the type of rock and environmental conditions. For instance, the olivine mineral from igneous rocks contributes with Mn, Co, Ni, Cu, and Zn to the soils. However, in sedimentary rocks, the minerals can release Cr, Mn, Co, Ni, Cu, Zn, Cd, Sn, Pb, and Hg (Nagajyoti et al. 2010). Volcanoes, another natural source of metals, can emit gases and particles with Al, Zn, Mn, Pb, Ni, Cu, and Hg.

HM can also be derived from anthropogenic sources. In urban and industrial areas, main factors that contribute to the increases in HM content are the disturbances caused by rapid urbanization, the emission of gases, and deposition of particles on the soil (Lyanguzova 2017; Navarrete et al. 2017). HMs are not considered as a pollutant if they persist in low concentrations. The term "*heavy metal pollution*" refers to HM levels that are higher than the normal concentration (Alloway and Ayres 1997).

Soil contamination by HM is one of the most serious ecological problems all over the world. The use of plants for the decontamination of a polluted environment is named phytoremediation and was described in the mid-1990s (Sandermann 1994; Schnoor et al. 1995; Cunningham and Ow 1996). Plants used for phytoremediation must be capable of growing in soils with a high metal concentration. These plants have the ability to accumulate very high concentrations of metal or metalloid elements in their shoots or roots (Marchiol et al. 2004). They can survive, grow, and reproduce on natural metalliferous soils as well as on sites polluted with HM as a result of anthropogenic activities. Plants that grow in metalliferous soils are also known as *metallophytes* (Baker 1987; Rascio and Navari-Izzo 2011). *Metallophytes* that can accumulate high concentrations of metals in their shoots, translocate to the shoot, and accumulate in organs, especially leaves, are called *hyperaccumulators* (Baker et al. 2000; McGrath and Zhao 2003). Hyperaccumulator plants can grow and reproduce on natural metalliferous soils such as calamine soils (enriched in Zn and Pb) and serpentine soils (derived from Fe- and Mg-rich ultramafic rocks, also enriched in Ni, Cr, and Co) (Vassilev et al. 2004). Most hyperaccumulators are endemic to metalliferous soils, namely, "*strict/obligate metallophytes*,"; meanwhile "*facultative metallophytes*" can live also on non-metalliferous soils, but they are prevalent on metal-enriched habitats (Assunção et al. 2003).

Metallophyte species have evolved efficient metal-uptake mechanisms and effective metal sequestration in internal tissues (Fernández and Henríquez 1991; Ernst et al. 1992; Briat and Lebrun 1999). When plants accumulate metals, these metals can be ingested by animals, thus creating the potential for toxic effects at higher trophic levels. The hyperaccumulation of HM depends on the plant species, soil condition (pH, organic matter content, cation exchange capacity, etc.), and type of HM (Xian and Shokohifard 1989; Otte et al. 1993; Barman et al. 2001; Espinoza-Quinones et al. 2005).

The number of identified hyperaccumulator plants has increased in the last years. In 2015, over 450 HM-hyperaccumulating species were found in 45 angiosperm families (Krzciuk and Gałuszka 2014). In July 2017, the global database contains 721 hyperaccumulator species (523 nickel, 53 copper, 42 cobalt, 1 chromium, 42 manganese, 20 zinc, 2 rare earth elements, 41 selenium, 2 thallium, 7 cadmium, 5 arsenic, and 8 lead) with some species showing hyperaccumulation of more than one element (Reeves et al. 2017, 2021).

Soil microorganisms, such as AMF, play an important role in protecting plants against HM. AMF are cosmopolitan, occurring even in areas with high concentrations of HM (Klauberg-Filho et al. 2005; Stürmer and Siqueira 2006). They are found in almost all families of herbaceous and arboreal species of interest for environmental recovery. AMF has great potential for assisting HM hyperaccumulators in the remediation of contaminated soils (Miransari 2011; Meier et al. 2012b). The combined use of plants with AMF has advantages over the use of hyperaccumulator plants proposed as one of the most promising green remediation techniques (Yang et al. 2016). Hyperaccumulating plants include families such as Brassicaceae, Plumbaginaceae, Juncaceae, Caryophyllaceae, Juncaginaceae, and Amaranthaceae and some members of the Fabaceae, which are usually non-AM species, although there are several reports of the presence of AMF associated to hyperaccumulating plants (Chandra et al. 2018).

The countries with the greatest numbers of published hyperaccumulator plant species are Cuba (Reeves et al. 1999), New Caledonia (Jaffré et al. 2013; Gei et al. 2020), Turkey (Reeves and Adıgüzel 2008), and Brazil (Reeves et al. 2007). Latin America is the least studied continent, with few metallophyte (metal tolerant and/or hyperaccumulator plants) species reported (Ginocchio and Baker 2004). Making focus in South America, in Table 11.1, we show a list of naturally metallophyte/hyperaccumulator plants growing in metalliferous soils and their

Table 11.1 List of the metallophytes, hyperaccumulator, and metal indicator plant species from South America

Heavy metal tolerance	Geographic distribution	Plant species and metal type (Mt, H, Mi)	Mycorrhizal Status / References ([a])
Ni	Brazil	*Adiantum* sp. H	Nd
		Chromolaena sp. nov H	Nd
		Chromolaena nmeyeri H	Nd
		Porophyllum aff. angustissimum H	Nd
		Cnidoscolus cf. *bahianus* H	Nd
		Vellozia sp. H-facultative	Nd
		Turnera trigona H	Nd
		Turnera melochioides H	Nd
		Turnera aff. *oblongifolia* H	Nd
		Piriqueta sidifolia H	Nd
		Cuphea aff.*erectifolia* H	Nd
		Mitracarpus cf. *parvulus* H-facultative	Nd
		Richardia grandiflora H	Nd
		Lophostachys villosa H-facultative	Nd
		Justicia lanstyakii H-facultative	Nd
		Ruellia geminiflora H	Nd
		Phaphia sarcofila H-obligate	Nd
		Heliotropium salicoides H-facultative	Nd
		Croton campestris H	Nd
		Cipura aff. *xanthomelas* H	Nd
		Camptosema aff. *ellipticum* H	Nd
		Esterhazya sp. H	Nd
		Lippia aff. *geminata* H	Nd
		Lippia aff. *lupulina* H	Nd
		Lippia aff. *gracilis* H	Nd
		Stevia parviflora H	Nd
		Sida linifolia H	Nd
Pb		*Vetiveria zizanioides* Mt	P (1)
		Sida cordifolia Mt	P (1)
		Pteris vittata Mt	P (1)
		Pteridium aquilinum Mt	P (1)
		Ricinus communis Mt	P (1)
		Asteraceae sp. Mt	P (1)
Zn, Cd		*Gomphrena claussenii* H	P (2)

(continued)

Table 11.1 (continued)

Heavy metal tolerance	Geographic distribution	Plant species and metal type (Mt, H, Mi)	Mycorrhizal Status / References (^{a})
As		*Anadenanthera peregrina* Mt	P (3)
		Brachiaria sp. Mt	P (4)
		Turneraceae Mt	P (4)
Cu	Chile	*Dactylium* sp. Mt	Nd
		Nolana divaricata Mt	Nd
		Mulinum spinosum Mt	Nd
		Erigeron berterianum Mt	Nd
		Cenchrus echinatus Mt	Nd
		Mimulus luteus var. *variegatus* Mt	Nd
		Oenothera picensis Mt	P (5)
		Imperata cylindrica Mt	P (6)
		Baccharis linearis Mt	P(7)
As	Peru	*Bidens cinapiifolia* H	Nd
		Paspalum racemosum H	Nd
		Paspalum tuberosum H	Nd
Cu		*Bidens cinapiifolia* Mt?	Nd
Se	Venezuela	*Lecythis ollaria* H	Nd
Ni		*Waltheria americana* H	Nd
		Oyedea sp. H	Nd
		Croton sp. H	Nd
		Lepidaploa remotiflora Mt?	Nd
		Borreria verticillata Mt?	Nd
		Wedelia calycina Mt?	Nd
Zn	Argentina	*Prosopis alba* Mi	Nd
		Prosopis nigra Mi	Nd
Cu, Zn, Ni, Sr, Li, Cd, Bi		*Prosopis alba* Mi	Nd
Cu, Zn, Ni, Sr, Li, Cd, Bi		*Larrea divaricata* Mi	Nd
Cr		*Ricinus communis* Mt	P (8)
		Conium maculatum Mt	P (8)
Cr, Cu, Pb, Zn		*Eleocharis montana* Mi-facultative	NP (9)
		Cyperus eragrostis Mi-facultative	NP (9)
Cr, Pb		*Hydrocotyle bonariensis* Mi-facultative	P (9, 10)
As, Cd, Cr, Cu, Hg, Fe, Ni, Pb, Zn, Mn		*Juncus pallescens* Mi	P (10)
Pb		*Bidens pilosa* H	P(11)
		Tagetes minuta H	P(12)

(continued)

Table 11.1 (continued)

Heavy metal tolerance	Geographic distribution	Plant species and metal type (Mt, H, Mi)	Mycorrhizal Status / References ([a])
		Sorghum halepense Mt	P(13)
		Jarava plumosa Mt	P (14)
		Solanum argentinum Mt	P(14)
		Zinnia peruviana Mt	P(14)
Crude oil		*Cynodon dactylon* Mt	P (15)
Zn	Ecuador	*Baccharis amdatensis* Mt	Nd
		Rumex crispus Mt	Nd
		Pennisetum clandestinum Mt	Nd
Pb		*Chenopodium ambrosioides* Mt?	Nd
		Pennisetum clandestinum Mt?	Nd
As		*Holcus lanatus* Mt?	Nd
		Pennisetum clandestinum Mt	Nd
Au, As, Sb	Bolivia	*Baccharis incarum* Mi	Nd
		Fabiana densa Mi	Nd
		Festuca orthophylla H	Nd

The list is based on Ginocchio and Baker (2004), Global database (http://hyperaccumulators.smi.uq.edu.au/collection) and local references. Plant species type (Mt: Metal tolerance, H: hyperaccumulator, Mi: metal indicator). Obligate metallophytes: species restricted to metalliferous soils; facultative metallophytes: accumulate HM on metalliferous soils but occur commonly on non-metalliferous soils. Heavy metal tolerance: Ni: nickel, Cd: cadmium, Zn: zinc, Pb: lead, As: arsenic, Se: selenium, Bi: bismuth, Sr: strontium, Cr: chromium, Cu: copper, Li: lithium, Fe: iron, Hg: mercury, Mn: manganese, Au: gold, Sb: antimony. Geographic distribution, arbuscular mycorrhizal status (P: present, NP: not present, Nd: not determined)

[a] References: (1) Schneider et al. (2016); (2) Carvalho et al. (2019); (3) Gomes (2011); (4) Schneider et al. (2013); (5) Meier et al. (2011); (6) Meier et al. (2012c); (7) Menares et al. (2017); (8) Gil-Cardeza et al. (2014); (9) Mendoza et al. (2015); (10) Colombo et al. (2019); (11) Becerra et al. (2017a); (12) Becerra et al. (2017b); (13) Becerra et al. (2016); (14) Menoyo et al. (2021); (15) Cabello (1997)

geographical areas. This table is based on Ginocchio and Baker's (2004) work, completed with the Global Hyperaccumulator Database (http://hyperaccumulators.smi.uq.edu.au/collection) with data from Brazil and local references. The presence and diversity of metallophytes were discriminated as Mt: tolerant to metalliferous soils or accumulating HM in roots, Mi: metal indicator plants present in the soils, and H: hyperaccumulator plants that accumulate HM in roots and shoots. Besides, we have included their mycorrhizal status based on local literature. As we can see, the information is limited and deserves wider investigation (Ginocchio and Baker 2004).

11.3 Arbuscular Mycorrhizal Fungi Symbiosis with Metallophytes in Contaminated Soil From Chile and Central Argentina

In Chile, mining is the main economic activity, highlighting the production of Cu, being Chile the main producer worldwide (Cornejo et al. 2017b). Under this scenario, it is common to find places that have been significantly affected by the deposition and accumulation of mining wastes, especially in the north and center Chile, being recognized several hundred sites as mining tailings that have not received an adequate closure (MMA 2011), with a high probability of producing serious contamination issues in the near future. The strong mining activity associated with the extraction of Cu has produced large amounts of toxic wastes with high concentrations of this element (Cornejo et al. 2008; Ávila et al. 2010; Castañón-Silva et al. 2013).

In Central Argentina, lead (Pb)-polluted soil was recorded in the proximity of a former battery recycling factory, which operated for two decades (1984–2005) and finally closed due to a lack of emission control and inadequate waste disposal that caused a severe accumulation of Pb over an extensive zone (Salazar and Pignata 2014). Pb is one of the most widespread metal contaminants in soil, and Pb decontamination is crucial for the maintenance of environmental health and ecological restoration (Moosavi and Seghatoleslami 2013).

Heavy metal contamination changes the abundance and diversity of the AMF population in the soil (Del Val et al. 1999). Nonetheless, studies on the occurrence and diversity of AMF in South America soils contaminated with HMs are scarce. In this section of the chapter, we focus on the relationship between AMF symbiosis in Chile contaminated with Cu and Central of Argentina contaminated with Pb.

11.3.1 Chile

In Chile, the Puchuncaví Valley (Valparaíso Region - Central Chile, Fig. 11.1a) is highlighted as one of the main studied areas where the impact of mining activities is strong. In this area coexist various thermoelectric installations, oil refineries, and the Ventanas Cu smelter. For more than five decades, the multiple industrial activities have generated an enormous impact on soils, represented by the loss of high amounts of organic matter and the presence of an extreme erosion (Cornejo et al. 2008). Additionally, the Puchuncaví Valley shows a very scarce plant cover currently represented by a few (pseudo) metallophyte species capable of tolerating and growing under high levels of metals present (Fig. 11.1b-d) and the acidity produced by the acid rain (González et al. 2008). In spite of the above, the impacts on microbial communities and their functioning under these conditions have been poorly studied (Aponte et al. 2020), which is even more evident in other areas of the country with the presence of metal-contaminated sites, such as Northern Chile.

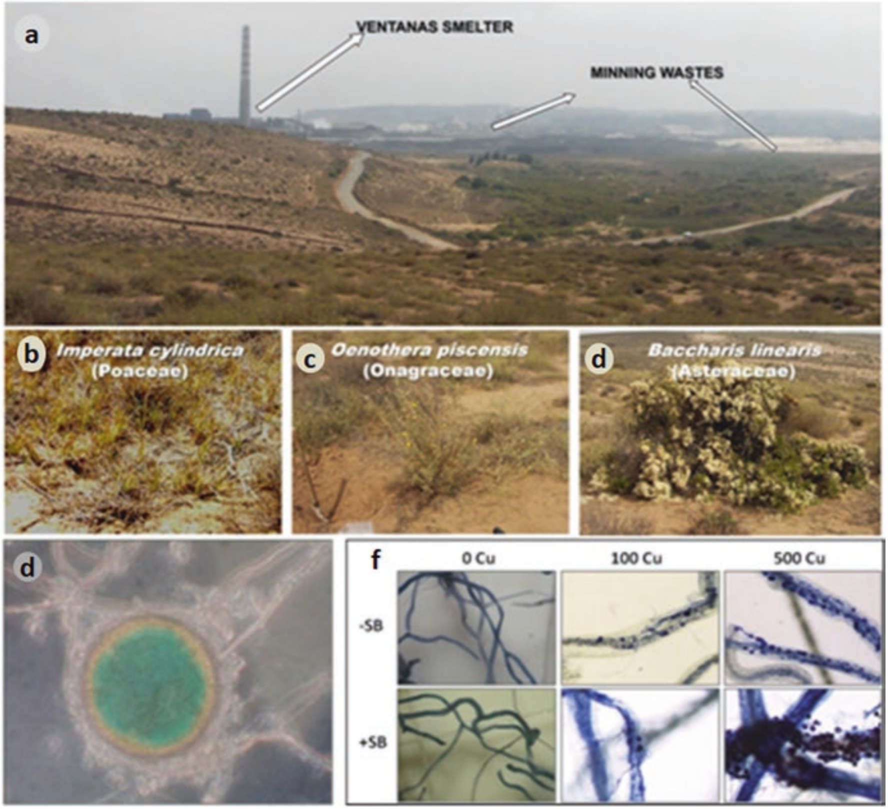

Fig. 11.1 Some representative images regarding the study of AMF in metal contaminated soils in Chile. (**a**) General view of the Puchuncaví Valley (central Chile) where it is easily distinguishable by the strong effect on the plant cover and erosion by the deposition of mining wastes. In this area, only few plant species are present. Among them, (**b**) *Imperata cylindrica,* (**c**) *Oenothera picensis*, and (**d**) *Baccharis linearis* have demonstrated a high mycorrhizal dependence and the capability to be used in phytoremediation programs. (**e**) View of resistance spores of the AM fungus *Claroideoglomus claroideum* showing accumulation of Cu salts. (**f**) Arbuscular mycorrhizal colonization in *Oenothera piscensis* roots at different Cu levels in interaction with (+SB) and without (-SB) sugar beet (SB) wastes as amendments. Photo credit: P. Cornejo

Although Cu is an essential micronutrient for the plant and soil microorganisms' growth, its presence in excessive concentrations can be highly toxic (Cuillel 2009; Vidal et al. 2020, 2021). Numerous studies have shown the mechanisms of AMF to cope with the toxicity produced by Cu in plants. The compartmentalization of excessive amounts of Cu in the cytoplasm of AMF spores is a mechanism that allows the survival of other AMF spores allowing the functionality and the colonization of roots of host plants in the Cu-contaminated environment (Cornejo et al. 2013) Another mechanism corresponds to the generation and accumulation of glomalin in the soil. The glomalin is a glycoprotein of fungal origin that has the ability to sequester various HM, such as Cu and Zn, also contributing to the stabilization of highly contaminated soils, such as those described in the Puchuncaví Valley (Cornejo et al. 2008, 2017a, b).

In Central Chile, some populations of plants naturally generate tolerance mechanisms against the presence of metals in contaminated soils, such as *Oenothera picensis* and *Imperata cylindrica* (Meier et al. 2012b, Fig. 11.1b, c). In a study regarding the effects of inoculation with AMF in Cu-contaminated soils, the efficiency of a consortium including AMF adapted to Cu (obtained from soils at the Puchuncaví valley) was compared with the fungus *Claroideoglomus claroideum* (obtained from agricultural soils in the southern Chile and presumably not Cu tolerant, Fig. 11.1e) in plants of *O. picensis*, *I. cylindrica*, and *Helianthus annuus.* Results showed that *C. claroideum* produced better behavior in terms of plant establishment and growth, suggesting its capability to be used in phytostabilization programs of Cu-contaminated soils (Meier et al. 2012b). Microorganisms isolated from contaminated soils are considered to have better tolerance levels compared to other organisms isolated from non-limiting conditions. In this case, *C. claroideum*, and especially the production of high levels of glomalin, seems to be responsible for this increased tolerance. In the same way, the use of *O. picensis* together with the AM fungus *C. claroideum* resulted in an optimal alternative to perform the Cu phytostabilization in contaminated soils from central Chile (Cornejo et al. 2017a).

In soils contaminated with Cu, the AMF favored the colonization and propagation of *Baccharis linearis* (Fig. 11.1d). In detail, root colonization improved the soil water retention capacity allowing the development of exploratory plant tissues. However, high levels of Zn in the tailings decreased the AMF colonization (Menares et al. 2017), which also highlights the need to seek solutions that include the multi-contaminated condition of these environments, where several toxic elements may be present at the same time.

Another alternative to promote the plant growth in Cu-contaminated environments is the use of organic amendments to increase the efficiency of phytostabilization programs (Medina et al. 2015). In this sense, the addition of agro-industrial organic wastes has been carried out, such as sugar beet wastes, together with the inoculation of AMF adapted to high Cu levels. The interaction of both technologies allowed an increased establishment of *O. picensis* seedlings under high soil Cu concentrations, mainly due to the increase of AM root colonization (Meier et al. 2011, 2015, Fig. 11.1f). Recently, the incorporation of compost also improved the response of *O. picensis* plants inoculated with *C. claroideum* directly growing in Cu mining tailings. The results showed a decreased Cu bioavailability and also increased the production of photosynthetic pigments, which generated notable increases in plant growth (Pérez et al. 2021). Another study reported that the inoculation of *O. picensis* plants with AMF in conjunction with the addition of biochar strongly contributed to decrease the concentrations of bioavailable Cu in soil. This generates a decrease in the Cu concentrations in parallel with an improvement of the rhizosphere environment, which increased the density and diversity of soil microorganisms as well as the plant growth, suggesting the use of this amendment in the sustainably phytoremediation of metal-contaminated soils (Meier et al. 2017a, b).

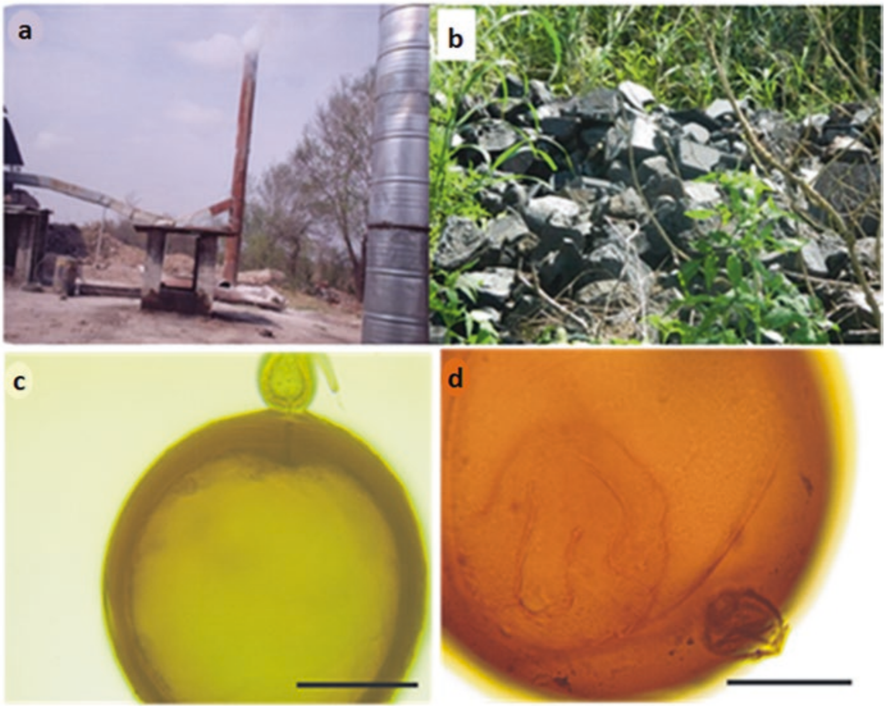

Fig. 11.2 The study site in Bouwer city (Córdoba, Central Argentina). (**a**) The smelter factory, (**b**) slag of batteries, (**c**) *Gigaspora decipiens* Scale: 0.5 mm, (**d**) *Denticustata biornata* Scale: 0.5 mm. Photo's credits: J. Salazar, E. Menoyo, and A. G. Becerra

11.3.2 Central Argentina

In Córdoba province, central Argentina, Bouwer city is located. In this locality, a former battery operated between 1984 and 2005. The smelter worked inappropriately, without emission control and an inadequate waste disposal (Fig. 11.2a, b). Salazar and Pignata (2014) reported the presence of diverse HM: Zn, Co, Cu, Ni, Mn, Fe, and Pb around the smelter. The battery recycling factory was closed in 2005, and the affected area has not received any treatment for its ecological restoration. In the place, high levels of Pb contamination exceed the limits for industrial land use; the Argentinean legislation (Law 24051-1991) contemplates 1000 mg kg^{-1}.

The AMF community (morphological and molecularly) was described in the rhizosphere of native (*Bidens pilosa*, *Tagetes minuta*) and exotic metallophyte herbs (*Sorghum halepense*) on different levels of Pb around the smelter. A total of 24 morphotypes of AMF including Acaulosporaceae, Claroideoglomeraceae, Gigasporaceae, Glomeraceae, Paraglomeraceae, and *Entrophospora*, Incertae sedis, were determined in the rhizospheric soil. By molecular approach, a total of 115 virtual taxa (VT) of AMF were detected. VT richness was negatively correlated with soil Pb concentration (Faggioli et al. 2019). According to the soil Pb level, the AMF taxonomic groups varied being Paraglomeraceae and Glomeraceae the predominant families stated present in a control site and in Pb-contaminated soil. Acaulosporaceae, Ambisporaceae, Archaeosporaceae, Claroideoglomeraceae, Diversisporaceae, and Gigasporaceae were less abundant. However, a noteworthy increase of Gigasporaceae was found in the soils with higher Pb content.

Differential taxonomic responses to Pb concentration suggest that some AMF species are less affected by these stressed conditions. Probably, these species have developed any survival strategy in sites with HM. The immobilization of HM in different fungal structures that allows alleviating their phytotoxicity may be one of the prevailing mechanisms. This was recorded for Al (Aguilera et al. 2011), Cu (Cornejo et al. 2013), and Cr (Gil-Cardeza et al. 2014). Thus, the capability of the AMF community present in Pb-polluted soils is to accumulate this HM in spores. The HM accumulation is dependent on the combination AMF-plant species (Salazar et al. 2018). Considering AMF spore community, high Pb concentrations were registered in these structures of resistance. Analyzing AMF species, in the site with more Pb content (16186 μg g^{-1}), the most abundant spores belong to the Gigasporaceae: *Gigaspora decipiens* and *Denticustata biornata* (Fig. 11.2c, d). Both species accumulated Pb in spores (Salazar et al. 2018).

Surrounding the smelter, there is an agricultural area with *Sorghum bicolor* crops. Blanco et al. (2016) analyzed the relationship between Pb accumulation and the toxicological human risk of this crop. Since *S. bicolor* accumulated Pb in the roots, these crops could have a phytostabilization role in soils with high Pb level. Regarding the rhizospheric microorganisms, the relative abundance of AMF families varied along sites with different Pb content (Fig. 11.3). Glomeraceae was present in all study sites. Notably, in the sites with high Pb (1104–145 mg kg^{-1}), the

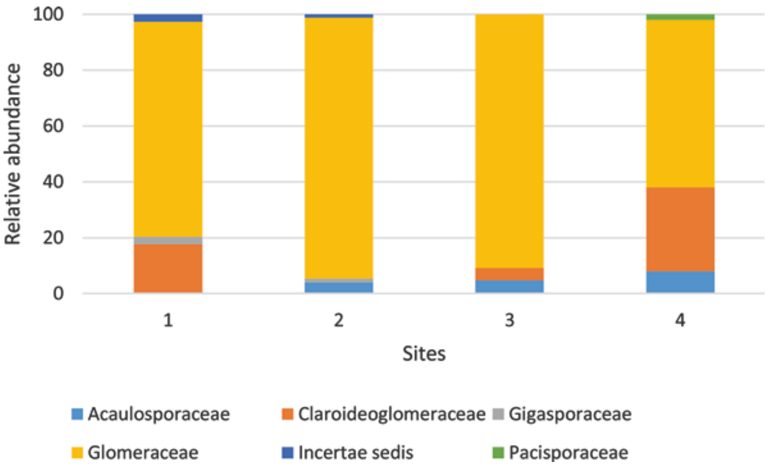

Fig. 11.3 Relative abundance of AMF families present in the *Sorghum bicolor* rhizosphere sampled in four sampling sites from an agricultural area in Bouwer. Sites 1 and 2 closer to the smelter factory. Sites 3 and 4 far away from the smelter factory

proportion of Glomeraceae was higher, between 77% and 92% of the spores belonged to this taxonomic group. Another noticeable result was the variation in the relative abundance of Gigasporaceae. Although this family was present at low proportion, it was only registered in the most contaminated sites (868–1104 mg kg^{-1}). In contrast, Acaulosporaceae increased the frequency as the Pb content of the soils decreased. Thus, there are AMF species resistant, sensitive species, and indicator species of soils contaminated with Pb.

The environmental phytoremediation practices should not exclude the study of the combination between plant species and their native fungal symbionts. In these combinations, the most tolerant associations to HM should be detected representing a biological source of great potential for use in soil phytoremediation strategies.

11.4 Conclusion

Soil pollution is a global threat that affects every ecosystem and region worldwide. To remediate or minimize soil pollution, it should have considered the phytoremediation process. In order to restore contaminated soils, it is necessary to identify and characterize metallophyte/hyperaccumulator plants, including their AM status. Here, we summarized the studies carried out in South America providing information about the arbuscular mycorrhizal status of metallophyte/hyperaccumulator plants. Knowing the AM status of these plants is a promising field and needs to be addressed in phytoremediation processes. As evident in this chapter, the current knowledge regarding AM status of metallophyte/hyperaccumulator flora is still incipient. Regarding the relationship between AMF symbiosis in soils from Chile contaminated with Cu, it is noticeable that the fungus *Claroideoglomus claroideum* is an optimal alternative to perform the Cu phytostabilization in contaminated soils from central Chile. Besides, the use of organic amendments in Cu-contaminated environments is an important alternative to promote plant growth and to increase the efficiency of phytostabilization programs. In Central Argentina, the AMF community showed a high tolerance to Pb-polluted soils and accumulated this pollutant inside spores. Besides, Paraglomeraceae and Glomeraceae are the predominant families present in Pb-contaminated soil. A noteworthy increase of Gigasporaceae was found in the soils with higher Pb content. Consequently, further research is necessary on the interaction of HM-AMF-metallophytes especially regarding the phytoremediation with mycorrhizas.

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Chapter 12
Native and Exotic Woodland from Patagonian Andes: Anthropic Impacts and Mycorrhizas

María Eugenia Salgado Salomón and Carolina Barroetaveña

Contents

12.1 Introduction

12.1.1 Mycorrhizas in Patagonian Woodlands

The Patagonian Andes of Argentina harbor more than 4.2 million hectares of mycorrhizal native tree species, comprising Nothofagaceae Kuprian., Araucariaceae Henkel & W. Hochst., and Cupressaceae Rich. ex Bartl. (Rojas et al. 2011), as the most oustanding families. They coexist with more than 120.000 ha of implanted

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forests, including *Pinus ponderosa* Dougl. ex Laws., *P. radiata* D. Don, *Pinus contorta* var. *latifolia* (Engelmann) Critchfield and *P. contorta* var. *murrayana* (Balfour) Engelmann, *Pseudotsuga menziesii* (Mirb.) Franco, *Salix* L spp., and *Populus* L. spp. (Peri et al. 2016; MinAgrI 2017). Furthermore, around 2,225,000 ha of grasslands in the Patagonian ecotone are suitable for fast-growing exotic conifer plantations (MinAgrI 2017).

Mycorrhizas are one of the most important and ecologically crucial symbiosis for boreal, temperate, subtropical, and tropical forests, driving plant population biology and community ecology by affecting their dispersal and establishment, regulating plant coexistence (Tedersoo et al. 2020). On the other hand, Soudzilovskaia et al. (2019) analyzed the relation between the dominance of mycorrhizal types and soil carbon (C) stocks, showing that across large geographical scales, higher cover of ectomycorrhizal (EM) vegetation is broadly associated with greater soil C stocks in both topsoil and subsoil; their analysis also suggests that EM biomass has declined in all continents, primarily due to a replacement of natural forests by agricultural lands, so restoration of native vegetation, especially in abandoned agricultural and barren land, may help alleviate anthropogenic soil C losses and ameliorate increases in atmospheric greenhouse gasses.

Native forests and afforestation from Argentinian Patagonia present different types of obligate mycorrhizal associations, needed for the establishment and growth of these tree species (Carrillo et al. 1992; Horton et al. 1999). Nothofagaceae forests are distributed between 33° and 56° S and comprise six species forming extensive monospecific and also mixed forests, all of them EM symbionts (Singer and Morello 1960; Carrillo et al. 1992). Cupressaceae species comprise the iconic *Araucaria araucana* (Mol.) K. Koch, which grows only in Neuquén province; the relictual, giant, and longest living species *Fitzroya cupressoides* (Mol.) Johnst., *Austrocedrus chilensis* (D. Don) Pic. Serm. & Bizzarri, and *Pilgerodendron uviferum* (D. Don) Florin are all obligate arbuscular mycorrhizal (AM) hosts (Fontenla et al. 1998). All nontarget or understory species in Nothofagacea and Cupressaceae forest are AM or ericoid hosts, with the exception of the non-mycorrhizal *Lomatia hirsuta* Diels ex J. F. Macbr. (Fontenla et al. 1998; Bruzone et al. 2015). Regarding afforested species, all *Pinus* spp. are obligated EM, while *P. menziesii* and Salicaceae Mirb. are able to form dual mycorrhizas (i.e., simultaneously associate with AM and EM fungi) (Horton et al. 1999; Gehring et al. 2006; Salgado Salomón et al. 2013a, 2018).

12.1.2 Anthropic Impacts on Forests

Forest fragmentation, clear-cutting, fires, grazing, and partial conversion to exotic plantations are affecting the temperate forests of Southern South America (Fig. 12.1) at an increasing rate (Mohr-Bell et al. 2019). It is estimated that about 50% of the native Patagonian forest is degraded by these mentioned anthropic actions (Peri and Ormaechea 2013; Mohr-Bell et al. 2019). Disturbances alter biological diversity, which includes mycorrhizal associations (Sapsford et al. 2021). Regarding

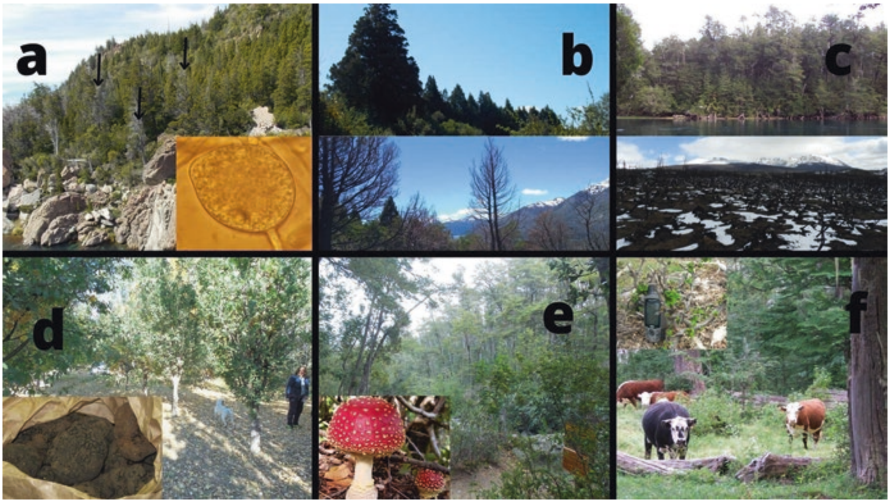

Fig. 12.1 (**a**) "Mal del Ciprés" caused by *P. austrocedri*, detail: asexual zoosporangium (Ph. ML Vélez), (**b**) burnt and unburnt *A. chilensis* forest, (**c**) burnt and unburnt *Nothofagus* forest, (**d**) Trufficulture, oaks inoculated with *T. melanosporum*, detail: black truffles, (**e**) *N. dombeyi* forest invaded by *A. muscaria*, detail: *A. muscaria* basidiome, (**f**) Silvopastoral system, *N. pumilio* forest affected by cattle, detail: browsed seedling. Photo's credits: J. O. Bava

Nothofagaceae forests, the most distributed species *N. pumilio* (Poepp. & Endl.) Krasser and *N. antarctica* (G. Forst.) Oerst. suffer herbivory and impacts of fire (Fig. 12.1c), the former by both cervids and cattle (Fig. 12.1f) (Quinteros et al. 2017) with an increased frequency of forest fires during the last decades (Mundo et al. 2017) that are preventing their regeneration. *Nothofagus antarctica* forests show approximately 90% of its stands affected to silvopastoral systems, often associated with burning to promote grass production (Peri and Ormaechea 2013), sustaining sheep and cattle raising, and providing some wood products including poles, firewood, and timber for rural construction purposes (Peri and Ormaechea 2013). On the other hand, *Austrocedrus chilensis*, the native conifer with wider distribution, exhibit large degraded areas as it has been widely exploited for its wood quality (Aparicio et al. 2009), for cattle grazing (Quinteros et al. 2017), and also because of the large mortality originated by the accidental introduction and spread of the exotic *Phytophthora austrocedri* Gresl. & E.M. Hansen (Fig. 12.1a) (Greslebin and Hansen 2010; Vélez et al. 2014).

Even Patagonian forests have been reported as fire adapted, 95% of forest fires in Patagonia have anthropic causes, either intentional or through negligence (SAyDS 2012). The frequency of fire occurrence and level of intensity have increased during the last decade; between 2001 and 2009, 8.837 ha have been burned, between 2009 and 2013, 11.920 ha, and between 2013 and 2017, 43.583 ha, showing an accelerated intensification (Moreno et al. 2018; Mohr-Bell et al. 2019). Years of severe drought with the unprecedented combination with warm summers have been creating conditions favorable to widespread, more frequent, and severe fires than in the past several centuries (Moreno et al. 2018). Although ecosystems experience a constant process of adaptation, the effects of Climate Changes caused by anthropogenic activities suppress the natural ability of ecosystems to adapt and be resilient, affecting the geographical location, species diversity, and dynamic balance of ecosystems, diminishing their ability to grant benefits to society (Córdova and Blanco 2009). Projections made by the Intergovernmental Panel on Climate Change (IPCC 2021) warn that during the 20 th century, tree mortality and forest decay will increase driven by the interaction of pathogens spread, forest fires, biological invasions and drought, risking ecosystem's C storage, biodiversity conservation, soil stability, wood production and aesthetic values (IPCC 2021; Robinson et al. 2020).

The course of native forest invasion with exotic introduced trees planted in Patagonia also raise concerns about their effects on native trees and their associated mycorrhizal biota. Some studies have alerted that the presence of invasive plants can decrease, sometimes drastically, the mycorrhizal colonization of native plants, causing the absence of new native seedlings (Inderjit et al. 2006; Mummey and Rillig 2006; Zhang et al. 2007; Rudgers and Orr 2009), maladaptation processes (Kranabetter et al. 2015), and/or changes on the mycorrhizal community diversity (Dickie and Johntson 2008; Dunk et al. 2012; Policelli et al. 2019).

12.2 Arson Forest Fire and Mycorrhizas

Fire alters soil properties and reduces the microbial activity, causing deep changes on nutrient availability and directly impacting the vegetation recovery (McMullan-Fisher et al. 2011). Particularly, EM fungi are affected by fire through the increase of soil temperature, the combustion of the organic layer that removes litter and woody debris, the deposition of ashes, the soil surface runoff (Cairney and Bastias 2007), and the detrimental effects on fungal mutualistic partners, affecting mycelia and fruit bodies occurrence needed for spore dispersal (Dahlberg 2002). Additionally, fire causes the loss of vegetation and thus the lack of colonizable roots, disrupting the continuity of the mycorrhizal interaction and affecting the life cycle of the EM fungi (Rincón et al. 2014). When tree species are obligatory mycorrhizal, post-fire-regenerated seedlings are highly dependent on the availability of active EM fungal propagules in soil (i.e., mycelium, spores) to establish the symbiotic partnership that will help them to cope with the adverse environmental conditions imposed by fire (Dahlberg et al. 2001; Buscardo et al. 2011).

Some studies carried out in Patagonia after forest fires show that impact on mycorrhizal community depends on fire intensity, site features, and in the time elapsed since the disturbance (Palfner et al. 2008; Longo et al. 2011; Talarico et al. 2017, Chávez et al. 2020), in agreement with what has been previously reported in forests from other parts of the world (Cairney and Bastias 2007). In Nothofagaceae sites recently burned, it was found that EM colonization was lower compared with no burned sites, but the difference disappears after 6–10 years (Palfner et al. 2008; Longo et al. 2011). However, Longo et al. (2011) found no significant differences in

EM species diversity, while Palfner et al. (2008) found that post-fire conditions clearly favor *Descolea antarctica* Singer as an early EM colonizer of *Nothofagus* Blume seedlings. Regarding the direct effects of fire on AM fungi, results have been contradictory, reporting either negative (e.g., Valariño and Arine 1991; Allsopp and Stock 1994), neutral (e.g., Treseder et al. 2004; Docherty et al. 2012), or positive effects on spore abundance (Eom et al. 1999; Moreira et al. 2006). A study carried out in burned *Austrocedrus chilensis* forest in Patagonia (Fig. 12.1b) showed that AM spores were significantly more abundant under moderate fire severity compared with unburned or high severity and that these differences disappear after 5 years (Talarico et al. 2017; Salgado Salomón et al. unpublished). However, seedlings grown on soils recently burned showed low rate of AM colonization, regardless of fire severity, showing that spore abundance did not correlate with infectivity; non-burned soils showed 40% higher AM colonization than moderate and severe burned soils (Salgado Salomón et al. unpublished).

Thinking in restoration strategies, the analysis of fire intensity and the evaluation of surviving trees and viable soil inocula seem necessary in order to define seedlings inoculation strategies, accounting for tree species diversity present in the area, along with suitable EM species (Barroetaveña et al. 2019) or AM fungi in the case of Cupressaceous and other tree and shrub species (Cofré et al. 2019).

12.3 Tree Species Introduction and Mycorrhizas

The introduction of exotic conifers with productive purposes, with partial shrublands/forest conversion to exotic plantations, became a source of controversy (Gyenge et al. 2010), as tree introductions lead to their EM-associated species introduction. This has been widely shown for exotic EM species spread in Patagonia (Barroetaveña et al. 2005, 2006, 2007, 2010; Salgado Salomón et al. 2011, 2013b, 2018; Hayward et al. 2015; Policelli et al. 2019) as well as in other Nothofagaceae ecosystems from New Zealand (Dickie et al. 2010; Moeller et al. 2015).

The ectomycorrhizal species richness associated with *P. ponderosa* and *P. menziesii* in Patagonian plantations (Argentina) is very reduced compared with their native forests; 18 taxa from *P. ponderosa plantations versus 157 from native forests and 15 taxa from P. menziesii plantation versus 514 from native forest were reported; among them, 28% of the former were recorded from native P. ponderosa forests and 60% of the latter recorded from native P. menziesii forests (Barroetaveña et al.* 2007). Considering that some of these EM species have cosmopolitan distributions, such as *Hebeloma mesophaeum* (Pers.) Quél. and *Wilcoxina* Chin S. Yang & Korf spp., and have been reported from both conifer plantations and Nothofagaceae forests (Salgado Salomón et al. 2018; Barroetaveña et al. 2019), along with the recent register of *Amanita muscaria* (L.) Lam. fruiting in pure *N. dombeyi* (Mirb.) Oerst. stand (Giles et al. 2020), there is increasing concern about the process of "*switching*" host and maladaptation facilitated by shared EM (Fig. 12.1e).

12.3.1 *Mycorrhizal Maladaptation*

The positive fitness of plants with mycorrhizal fungi leads to local co-adaption, feedback expected to occur through coevolutionary time. In contrast, when plant and fungal relative fitness are negatively correlated, this negative feedback will lead to maladaptation (Bever 1999; Klironomos 2003), defined as a relative decline in the associated tree species due to altered mycorrhizal communities occurring in native settings (Kranabetter et al. 2015). This dynamic can be similar to that observed in host-enemy coevolution, where rapidly evolving enemies often exert more negative effects on hosts (Hoeksema and Forde 2008). Several authors have described negative effects on seedling fitness and establishment, drought tolerance, N uptake, along with pathogens outbreak due mycorrhizal maladaptation process (McCarthy-Neumann and Ibañez 2013; Bagchi et al. 2014; Kranabetter et al. 2015; Rúa et al. 2016; Gehring et al. 2017). The presence of invasive plants can also decrease, sometimes drastically, the mycorrhizal colonization of native plants, causing the absence of new native seedlings in invaded sites (Mummey and Rillig 2006; Zhang et al. 2007). The decreased or null Nothofagaceae seedling recruitment detected in invaded native forests with *P. menziesii* in Patagonia (Salgado Salomón et al. 2013a) was further analyzed with a soil bioassay in an invasion matrix. That study showed that seedling grown in soil from invaded stand presented significantly lower shoot dry weight and height, collar diameter, root-specific length index, and less survival rate (40%) that were always associated with lower EM colonization rates compared with those grown in soils from non-invaded stands (Salgado Salomón et al. 2018). Even though the mechanism involved in this drastic decline in mycorrhizal colonization is not clear, some authors have hypothesized it can be due to modified site's biochemical features, allelopathic effects, shifts in mycorrhizal communities, and/or inhibition processes (Inderjit et al. 2006; Rudgers and Orr 2009).

In the Climate Change context, forest species migrations are expected and determined either by population expansion into new habitat range or population retreat from historical habitat range (IPCC 2021). Thus, EM maladaptation raises concern about survival and productivity of forest species experiencing rapid shifts in site features, with the potential risk of a mismatch in host genetics with native mycorrhizas that results in maladaptation for the associated tree species (Winder et al. 2021).

12.3.2 *Switching Host*

Ectomycorrhizal fungi have been considered mainly host-specific (Borowicz and Juliano 1991; Newton and Haigh 1998), because of the fungi and plant host coevolution. Host specificity refers to a relationship in which a fungus derives its nutrition from a live host plant during some phase of its life cycle and is restricted to a particular host or group of related species but does not occur on other unrelated plants in the same habitat (Zhou and Hyde 2001). However, there are contradictory observations concerning this statement; some researchers have found no host specificity in some conifers (Horton and Bruns 1998), or species in symbiosis with a comparatively wide range of hosts (Bobbu 2016).

Some EM fungi have been observed "*switching*" associated tree species from exotic conifers to *Nothofagus* forest in Patagonia. *Hebeloma hiemale* and *Wilcoxina* sp., common mycorrhizal partners for *P. menziesii* (Barroetaveña et al. 2007) and not previously reported from Nothofagaceae forest, were found associated with *N. antarctica* seedlings (Salgado Salomón et al. 2018). On the other hand, a native Pyronemataceae EM species was found associated with invasive *P. menziesii* seedlings (Salgado Salomón et al. 2018). Also, forest ranger reports from Los Alerces and Lago Puelo National Parks (Chubut, Argentina) indicate *Amanita muscaria*, a species present in Patagonia and central Argentina associated with Pinaceae (Daniele et al. 2005; Barroetaveña 2006), is fruiting in pure, non-invaded *N. dombeyi* stands, indicating it should be forming EM with this species, the only ectotrophic one (Giles et al. 2020). *Amanita muscaria* occurs naturally in the Northern Hemisphere as an EM associated with pine, spruce, fir, cedar, chestnut, and birch. A similar process has been reported with this EM species in Nothofagaceae forests from New Zealand, Tasmania, and Australia (Robinson 2010; Dunk et al. 2012). Even though more exhaustive studies are needed, the high density of *A. muscaria* sporocarp production implies a high level of resource capture, impacting on critical endangered EM native species from those Nothofagaceae forest (Dickie and Johnston 2008), process that would also need to be elucidated in Patagonia.

12.4 Introduced Plant Pathogens and Mycorrhizas

Numerous studies have proposed that mycorrhizal fungi (either AM or EM) have the ability to protect host plants from attack by radical pathogens such as *Phytophthora* de Bari, *Fusarium* Link, *Pythium* Ness, *Gaeumannomyces* Arx & D.L. Olivier among other diseases (Alejo-Iturvide et al. 2008; Castellanos-Morales et al. 2011; Elmer and Pignatello 2011; Durán et al. 2018; Chu et al. 2019, 2021; Ravnskov et al. 2020; Nakashima et al. 2021). However, this protective role is not entirely clear and would be conditioned by the species combinations, the mycorrhizal colonization rates, and the environmental conditions (Vierheilig et al. 2008; Khaosaad et al. 2007; Veresoglou and Rillig 2012). No experiences of EM pathogen biocontrol have been yet published to our knowledge, regarding Patagonian forests or nursery seedlings.

The native *A. chilensis* forest currently under a process of severe decline due to the widespread and injurious pathogen *Phytophthora austrocedri* Gresl. and E. M. Hansen (Greslebin and Hansen 2010) shows an interesting interaction model with its AM colonization. *Phytophthora austrocedri* was identified as an introduced, although not previously reported, soil pathogen (Vélez et al. 2014, 2020). In a preliminary work carried out with *A. chilensis* seedlings from affected stands, it was found that affected seedlings had a significantly higher AM colonization than healthy ones (Salgado Salomón et al. 2014, Salgado Salomón unpublished). Furthermore, a wider ecological survey of seedlings from affected stands indicates that in *P. austrocedri* presence, *A. chilensis* could induce changes in AM colonization, promoting higher abundance of arbuscules and coils; on the other hand, healthy seedlings showed lower rates of colonization and higher abundance of vesicles (Salgado Salomón unpublished). As the infection with *P. austrocedri* decreases *A. chilensis* hydraulic conductivity (Vélez et al. 2012, Troncoso and Greslebin 2018), the increased AM colonization could be due to a plant attempt to supply this deficiency. It should be remarked that nursery studies with the other native Cupressaceae *Fitzroya cupressoides* and *Pilgerodendron uviferum* also showed high indexes of tissue affectation and mortality caused by this pathogen (Taccari et al. 2019), warning of the extreme vulnerability of these endangered tree species. More studies are required to validate and understand the disease complex ecology of the lethal *P. austrocedri* and the possible protective effects of programmed AM inoculation, considering that AM taxa diversity is still unknown for Patagonia.

There is clear experimental evidence that AM and EM fungi can act as biocontrol agents in a range of systems. However, this may go unnoticed in the environment when improved plant growth is the only measure of efficacy available; direct competition or inhibition, biochemical changes associated with plant defense mechanisms or induced resistance, and development of an antagonistic microbiota may all be taking place. The current application of molecular analytical techniques promises that features driving biocontrol activities in mycorrhizas will begin to be understood (Chu et al. 2019, 2021; Ravnskov et al. 2020).

12.5 Fertilization, Nutrition and Mycorrhizas

Studies on mature forests of NW Argentinean Patagonia indicated that N is the main growth-limiting nutrient in most dominant tree species, while P limitation is uncommon, despite the soil's volcanic origin, which are characterized by low atmospheric N deposition and high plant N conservation (Pérez et al. 2003; Diehl et al. 2008; Godoy et al. 2009). Although P availability in Andisols is low due to high P retention, no evidence of P limitation was found in nine out of the ten dominant tree species in mature forests of Argentinian Patagonia, a fact that was attributed to high mycorrhizal infection (Diehl et al. 2003, 2008). As threshold values for foliar N/P had been suggested as a useful predictor of nutrient limitation in terrestrial ecosystems, Agüero et al. (2014) conducted a greenhouse trial with seedlings of *Lophozonia alpina* (Poepp. & Endl.) Heenan & Smissen and *L. obliqua* (Mirb.) Heenan & Smissen, using soil characteristic of each species. Seedlings were fertilized with three levels of N (100, 200, and 400 mg kg^{-1} soil) with or without the concurrent application of a single P dose (60 mg kg^{-1} soil) during their second growing season. They showed that N primarily limited both species: the addition of N resulted in higher shoot and root masses, an increased number of nodes, taller stems, and greater basal and root diameters, while P did not show any effect. N/P ratios in green leaves and N and P resorption proficiencies indicate that with increased N, P availability can become a secondary limiting nutrient for *L. alpina*. This was accompanied by the maintenance of EM infection and mass allocation to roots in this species. Anyway, a trend toward decreased ectomycorrhizal infection with increased nutrient addition was observed; *L. obliqua* colonization decreased significantly from 67% in the control to 47–57% in four of the six fertilized treatments (the lowest value corresponding to 400 N+P), whereas in *L. alpina*, a significant decrease, from 61% to 51%, was found only in one treatment (400 N) relative to the control. *Lophozonia alpina* seems to be more sensitive to P limitation than *L. obliqua* since it has half the concentration of foliar P and occupies soils with lower P availability (Satti et al. 2007). Adaptation to the low P availability of volcanic soils through high EM infection and mass allocation to roots might have contributed to their null or marginal responses to P addition. The association of EM infection with P acquisition by tree species has been highlighted in several previous studies (Plassard and Dell 2010).

Pinus ponderosa seedlings produced in greenhouses with high levels of fertilization, without EM inoculum applications, showed low or null EM infection at the end of nursery stage but had satisfactory performance in plantation sites during the first years. In order to elucidate this paradox, Salgado Salomon et al. (2009) studied the EM status of greenhouse *P. ponderosa* seedlings produced under these high fertilization regimes and inert substrate. They showed that seedlings had null or very low levels of mycorrhization and low morphotype's richness at the moment of plantation, but showed very high mycorrhizal percentages after 6 months maintained under greenhouse conditions without fertilization and after 24 months in field conditions. Massone (2020) studied the effect of fertilization on *A. chilensis* AM status through a greenhouse bioassay with inert substrate controlling P availability and without AM inoculum management. He found that 1 ppm P concentration produced a higher AM colonization rate than 10 or 100 ppm, although it was never null, and that those differences disappeared after a year in pots or under field conditions, without fertilization. In both cases, the analyzed nurseries fortuitously incorporated mycorrhizal inoculum that rapidly colonized seedlings, during the fertilization or immediately after it was interrupted. These facts demonstrated again that the hypothesis that nursery seedlings can establish and grow without mycorrhizal symbioses is false.

High doses of NPK fertilization are currently used to fasten tree growth and reduce production time in Patagonian nurseries (Massone 2020), although extensive evidence accounts for the inhibitor effect of this practice on seedling mycorrhization (Salgado Salomón et al. 2009). Many other studies are needed to define the adequate doses, type, and application regimes of fertilizer to be used in seedling of most tree species produced in Patagonia, in order to establish fertilization regimes that adjust conditions for both prolific mycorrhization and reasonable production cycles in financial terms (Óskarsson and Halldórsson 2008).

12.6 Mycorrhizal Inoculations

Tree inoculation with mycorrhizal fungi during nursery cultivation increases plant growth and improves subsequent performance under natural conditions when they are outplanted to the field (Rincón et al. 2005, 2007; Oliveira et al. 2010). Restoration efforts with *Nothofagus* spp. in partially or completely degraded forests converted to grasslands can be limited by a lack of remaining EM inoculum. However, the degree of mycorrhizal inoculum limitation and how far mycorrhizal inoculum spreads to degraded or lost *Nothofagus* areas need further analysis. A lack of mycorrhizal infection may slow tree establishment, particularly in combination with other environmental filters such as competition, but cannot be taken as an absolute barrier to tree expansion (Dickie 2007). Facilitation of seedling mycorrhizal infection across plant species has been demonstrated in some other systems such *Arctostaphylos* Adans.–*Pseudotsuga* Carrière (Horton et al. 1999), *Helianthemum* Miller–*Quercus* L. (Dickie et al. 2004), *Salix* L.–*Betula* L.–*Larix* Mill. (Nara 2006), *Arbutus* L.–*Quercus* L. (Richard et al. 2009). Moreover, a widespread native genus of Myrtaceae trees in New Zealand are able to form symbioses with both AM and EM fungi (Weijtmans et al. 2007), which permit them to establish into AM grasslands and accumulate ectomycorrhizal fungal inoculum that thereby facilitate Nothofagaceae establishment (Dickie et al. 2012). In Patagonia, no native dual mycorrhizal shrubs or trees are known that can fulfill a cross-species facilitation role for Nothofagaceae forests. Nursery seedling inoculation with suitable and adapted EM species seems the only strategy to plan successful restoration actions.

In this frame, it is necessary to determine fungal species that can increase tree tolerance to adverse conditions and are suitable to be utilized as mycorrhizal inoculants. Little is known regarding how the mycorrhizal symbiosis varies according to Nothofagaceae species growth stages and to the altered site conditions of degraded lands to be reforested in Patagonia. Many EM species are specific or dominant in specific plant growth stages, so it is important to know which one are present and common during the seedling stage to select candidate species for nursery inoculations (Barroetaveña et al. 2019). Several EM taxa have been reported from nurseries and naturally established Nothofagaceae seedlings as *Descolea* Singer (Palfner et al. 2008; Valenzuela et al. 2008; Álvarez et al. 2009; Fernández et al. 2015; Salgado Salomón et al. 2018), *Hebeloma* (Fr.) P. Kumm. (Fernández et al. 2015; Salgado Salomón et al. 2018), *Sebacina* Tul. & C. Tul., *Inocybe* (Fr.) Fr., *Laccaria* Berk. & Broome, *Genea* Vittad. (Fernández et al. 2015; Marín et al. 2018), *Tomentella* Pers. ex Pat. (Kuhar et al. 2016; Fernández et al. 2015; Salgado Salomón et al. 2017, 2018), Clavulinaceae Donk (Fernández et al. 2015), Thelephorales Corner ex Oberw. (Fernández et al. 2015), and Pyronemataceae Corda (Fernández et al. 2013; Salgado Salomón et al. 2018). It is important to remark that non-artificially inoculated *L. alpina* nursery seedlings have been reported colonized by naturally established EM after 1 year (Fernández et al. 2013), although the abundance and diversity of ectomycorrhizal fungi found in those seedlings compared with naturally established *L. alpina* specimens were different (Fernández et al.

2015), so inoculation programs should not rely on this fortuitous colonization when offering EM-inoculated seedling, at least not before checking EM tip identities.

The endemic genus *Descolea* has been widely studied (Kuhar et al. 2017). Palfner et al. (2008) demonstrated that *D. antarctica*, with previous reports of successful mycelial inoculations by Valenzuela et al. (2008), was the most abundant fungal symbiont on post-fire seedlings of *Lophozonia alpina*, suggesting it behaves as an *early-stage* symbiont. Available data also suggest that species of *Descolea* may be among the most saprotrophically active EM fungi (and so easy to grow in axenic cultures) due to highly adaptable enzymes (phosphatases, amylases, cellulases) that are active over a wide pH and temperature range (Álvarez et al. 2004; Valenzuela et al. 2008). Alberdi et al. (2007) found that the inoculation of *N. dombeyi* seedlings with *D. antarctica* or *Pisolithus tinctorius* (Mont.) E. Fisch. favored a better functionality maintenance of the photosynthetic apparatus under drought compared with non-EM seedlings, and those plants colonized by *D. antarctica* were less physiologically stressed than seedlings inoculated with *P. tinctorius*. Moreover, since the sequestrate *D. brunnea* (E. Horak) Kuhar, Nouhra & M.E. Sm. may constitute an important source of spore inoculum (Kuhar et al. 2017), while other species are easily cultivable, the genus came up as a promising greenhouse inoculant to produce EM Nothofagaceae seedlings for reforestation. Other species such as *Laccaria laccata* (Scop.) Cooke has been reported as a growth promoter for *N. pumilio* seedlings (Marín et al. 2018).

Inoculation with spores has been proposed because they are easy to apply, do not require expensive infrastructure nor qualified personnel for their production, are very abundant in several fungal species, are low volume and easy to handle, and tolerate long storage periods (Barroetaveña et al. 2019; Bassani et al. 2013). Anyway, to broaden the perspective of EM species best suited to inoculate nursery plants, especially for those with low frequency of fruiting, small amounts of spores or corticioid habit, culture obtention, and mycelium inoculum production are required to further test mycorrhizal infection and field performance. Vegetative inoculum has been reported as more convenient for certain species, but its production is difficult and expensive to carry out (Brundrett et al. 2005).

Ectomycorrhizal species suitable for nursery inoculation and good field performance have been studied for *P. ponderosa* planted in Patagonia (Barroetaveña et al. 2012, 2016). Considering inoculations with highly prized edible EM species, Pérez et al. (2007) showed that it is possible to infect *L. obliqua* with the Périgord black truffle (*Tuber melanosporum* Vittad.) under greenhouse conditions, which allows the possibility of cultivating this truffle as a secondary crop during reforestation. Trufficulture is a young productive activity in Patagonia; different species of oaks (*Quercus robur* L., *Q. ilex* L.) inoculated with *Tuber melanosporum* and *T. aestivum* Vittad. in less proportion have been established in suitable microsites along the region (Fig. 12.1d). Nevertheless, as it would imply the introduction of an exotic taxa to native forest lands, further studies are required to evaluate its behavior and productivity.

12.7 Conclusion

Forest fires and changing climatic conditions determined by the accelerated effects of Climate Change, along with the movement and introduction of plants, fungi, and other associated microorganisms determine new, challenging scenarios that include plant diseases and invasion, with their associated phenomena such as maladaptation and biodiversity loss. Sustainably managed forests serve as C storages, requires efforts to develop mycorrhizal technologies that could ensure the bioprotective role of this symbiosis. The role of mycorrhizas' increasing plant tolerance to biotic and abiotic stresses can help decrease plant extinction risks and provide time for plant dispersal and adaptation. In this scenario, the autecology and biodiversity of mycorrhizas in Patagonian forests need further and urgent studies, not only considering recently reported declines of the relictual *Araucaria araucana* and the endemic *N. dombeyi* but also in light of new future outbreaks that could affect other species. Restoration efforts that will be growing in perspective of Climate Change will demand the inoculation of selected, well-suited seedlings, in order to permit seedling survival in degraded areas, which are exposed to drastic environmental situations.

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Chapter 13
Current Knowledge on Mycorrhizal Symbiosis and Endophytes in Northwest Patagonia, Argentina

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13.1 Introduction

13.1.1 Northwest Patagonian Region

Patagonia is a geographical region that encompasses the southern end of South America, from 39 °S to 55 °S. It is characterized by longitudinal and latitudinal precipitation gradients (Paruelo et al. 1998). Annual precipitation decreases from about 4000 mm at the west in the north, to 100 mm at the east in the south of the hemisphere, imposing different soil and vegetation patterns and notably affecting ecosystem composition and functions (Cabrera 1976; Soriano et al. 1983). In northwest Patagonia, Argentina, there are two main geomorphological subregions: Andean and Extra-Andean. The first one occupies a narrow north-south strip of land of 63,000 km^2 along the slopes of the Andes and includes High-Andean ecosystems as well as temperate Andean forests. The Extra-Andean subregions are characterized by irregular plateaus, low hills, and riverine depressions and include steppes and meadows. Patagonia is considered a hotspot region of biological diversity; in this context, mycorrhizal fungi and other soil- or plant-associated microorganisms are not exceptions (Nouhra et al. 2012; Fernández et al. 2015; Bruzone et al. 2016; Duo Saito et al. 2018; Carron et al. 2020; Mestre et al. 2011, 2014; Mestre and Fontenla 2021). Major advances have been made in the last decades regarding the behavior, distribution, and function of the mycorrhizal symbioses in addition to soil microorganisms in Patagonia. Each of these contributions was focused on different subregions, ecosystems, or environments and included different taxonomical or ecological aspects of diverse microbial groups (e.g., fungi, bacteria). A broader view of these achievements was missing so far. This chapter summarizes the current knowledge on mycorrhizal associations in High-Andean ecosystems, Andean forests, steppes, and meadows of northwestern Patagonia. In addition to published information, here, we include important data (presented in-depth) from less accessible reports, such as theses and presentations in scientific meetings; mainly, regional bibliographies were cited with the same objective. The relationships between mycorrhizal behavior and altitudinal-precipitation gradients were highlighted in this report.

13.1.2 Microorganisms Associated with Plant-Soil Systems

Mycorrhiza play important roles in biological communities and are the key sources of energy for many fungi. They benefit plants by enhancing nutrient uptake, inducing resistance to stressful conditions, and may become essential in nutrient-deficient habitats (Gardes and Dahlberg 1996; Read 1999). Mycorrhiza favor inter- and intraspecific nutrient transfer among plants, through a strongly interconnected common mycorrhizal network (Chen et al. 2018). There are four main types of mycorrhiza: arbuscular mycorrhiza (AM), ectomycorrhiza (EcM), ericoid mycorrhiza (ErM), and orchid mycorrhiza (OrM). The EcM and AM symbioses are the most abundant and frequent types, while ErM and OrM are less frequent. AM is the most broadly distributed type of mycorrhiza and is formed by all kinds of plants (herbs, shrubs, trees, pteridophytes, and mosses). EcM normally colonize woody plants and are broadly important in forests worldwide. ErM are limited to some members of Ericaceae and Diapensiaceae, whereas OrM are associated to one of the most diversified plant families, Orchidaceae. Non-host (NH = non-mycorrhizal) plants have secondarily lost their mycorrhiza-forming capacity permanently, or intermittently (= facultative mycorrhiza), depending on the environmental context (e.g., type of habitat, co-occurring plants, soil fertility, or pollution). Many NH plants are habitat specialists, growing under particular soil or climatic conditions and disturbed sites, or nutritional plant specialists, such as carnivores, parasites, and cluster-rooted species. The mycorrhizal type or the capacity to form mycorrhiza is usually uniform for each plant genera or family. Nevertheless, some plants exhibit variable levels of mycorrhizal colonization or even behave as facultative or NH species in different environmental conditions. Other plant species have mixed or dual infections, or change their main mycorrhizal type during their life cycle or depending on different environmental conditions. See these general mycorrhizal knowledge in Brundrett and Tedersoo (2018), Tedersoo et al. (2020), and bibliography cited within them. The interaction of host and NH plants is also important as it has been observed that the latter produce substances that may inhibit AM colonization of host plants. This inhibitory effect, which depends on plant species, age, and size of NH and host plants, decreases the AM infectivity of soils possibly by acting on soil fungal structures. The inhibitory effect of NH plants is of fungistatic, transient, reversible, and nonsystemic nature and depends on volatile substances produced by their roots (Fontenla et al. 1999).

In addition to mycorrhizal fungi, other biotrophic microorganisms, including endophytic fungi and bacteria, often colonize the roots (Chaia et al. 2006; Bruzone et al. 2016; Gentile 2016; Arancibia et al. 2018; Bella 2019; Carron 2021). These plant-microorganism interactions can result in beneficial symbioses, seemingly neutral endophytic cohabitation, and also in harmful relationships (Spanu and Panstruga 2017). For example, dark septate endophytes (DSE) conform a heterogeneous and widely distributed group of fungi, which can be found in plant roots (Diehl and Fontenla 2010; Fernández et al. 2008, 2010; Salgado Salomon et al. 2013; Bruzone 2009, especially common in polar and alpine habitats (Fernando and Currah 1996; Lugo et al. 2003, 2008; Newsham 2011; Urcelay et al. 2011). Some evidence suggested mutualistic associations between DSE and plants, but neutral or even negative effects have also been reported (Mandyam and Jumpponen 2005; Jumpponen 2001; Newsham 2011). Mycorrhizal fungi might co-occur with DSE and with N_2-fixing microorganisms such as the actinobacteria *Frankia* in Patagonia (Chaia et al. 2006, see also Rhamnaceae records in Table 13.1). All these topics will be addressed in the following text, with especial emphasis on the information available for northwest Patagonia.

Table 13.1 Intraradical fungi of High-Andean plants and comparisons with other subregions of northwest Patagonia

Plant taxonomy	Plant habit[a]	Occurrence and colonization of of High-Andean plants			Mycorrhizal occurrence of Andean forest and Extra-Andean steppe plants[b]			
Families and species		Type[c]	AM %[d]	DSE[e]	Ecosystem	Family	Genera	Species
Apiaceae					Steppe	AM		
Azorella andina	PH	AM	91	+				
Pozoa volcanica	PH	AM	69	+				
Asclepiadaceae					Forest	AM		
Diplolepis nummulariifolia	PH	AM	65; 60[f]	++	Forest			
Asteraceae					Forest and steppe	AM+NH		
Baccharis magellanica	SH	AM	65; 79	+++	Forest and Steppe		AM	
Chaetanthera villosa	PH	NH	0	+				
Chiliotrichum diffusum	PH	AM	59; 61	ND				
Erigeron andicola	PH	AM	98	+++				
Erigeron leptopetalus	PH	AM	96	+++				
Nassauvia pulcherrima	PH	NH	0	+	Steppe		AM+NH	
Nassauvia pygmaea	PH	NH	0	+++				
Nassauvia revoluta	SH	NH	0	++	Steppe			
Senecio crithmoides	SH	AM	30; 61	+++	Forest and steppe		AM	
Senecio hieracium	PH	AM	86	+++				
Senecio peteroanus	SH	AM	14; 44	++				
Senecio portalesianus	SH	AM	9	++++				
Berberidaceae					Forest and steppe	AM+NH		
Berberis empetrifolia	SH	AM	49; 59	+++	Forest and steppe		AM+NH	
Brassicaceae					Steppe	NH		
Cardamine sp.	PH	NH	0	++				
Onuris graminifolia	PH	NH	0	+				
Calceolariaceae					Forest and steppe	AM		
Calceolaria biflora	PH	AM	67	++	Forest and steppe		AM	AM
Calyceraceae					Steppe	AM+NH		

(continued)

Table 13.1 (continued)

Plant taxonomy	Plant habit[a]	Occurrence and colonization of of High-Andean plants			Mycorrhizal occurrence of Andean forest and Extra-Andean steppe plants[b]			
Families and species		Type[c]	AM %[d]	DSE[e]	Ecosystem	Family	Genera	Species
Gamocarpha selliana	PH	AM	92	+++				
Moschopsis caleofuensis	PH	AM	79	+++				
Caryophyllaceae					Steppe	NH		
Cerastium arvense	PH	NH	0	++,+++	Steppe		NH	NH
Colobanthus lycopodioides	SH	NH	0	+				
Empetraceae					Forest and steppe	ErM+AM		
Empetrum rubrum	SH	ErM	ND	ND	Forest		ErM	ErM
Ericaceae			**ND**	**ND**	Forest and steppe	ErM		
Gaultheria pumila	SH	ErM	ND	P	Steppe		ErM	
Gaultheria caespitosa	SH	ErM	ND	P				
Loasaceae					Steppe	AM		
Pinnasa nana	PH	NH	0	+++				
Oxalidaceae					Forest	AM		
Oxalis adenophylla	PH	AM	52; 66	+	Forest	AM		
Oxalis erythrorhiza	SH	AM	47	+				
Plumbaginaceae					Steppe	AM		
Armeria maritima	PH	AM	10;12,50	+++	Steppe		AM	AM
Poaceae					Forest and stepee	AM+NH		
Poa obvallata	PH	AM	25	+++	Forest and steppe		AM+NH	
Koeleria barbinodis	PH	AM	41	+++				
Ranunculaceae					Steppe	AM		
Anemone multifida	PH	AM	93	++	Steppe		AM	AM
Callianthemoides semiverticillata	PH	AM	70	++				
Rhamnaceae[g]					Forest and steppe	AM		
Ochetophila nana	SH	AM	78	++	Forest and steppe		AM	
Rosaceae					Forest and steppe	AM		

(continued)

Table 13.1 (continued)

Plant taxonomy	Plant habit[a]	Occurrence and colonization of of High-Andean plants			Mycorrhizal occurrence of Andean forest and Extra-Andean steppe plants[b]			
Families and species		Type[c]	AM %[d]	DSE[e]	Ecosystem	Family	Genera	Species
Acaena macrocephala	PH	AM	61	+	Forest and steppe		AM	
Acaena pinnatifida	PH	AM	98	+	Forest and steppe			AM
Rubiaceae					Forest and steppe	AM		
Galium richardianum	PH	AM	68	+	Steppe		AM	AM
Oreopolus glacialis	PH	AM	65,73	++	Steppe		AM	AM
Schoepfiaceae								
Quinchamalium chilense	PH	AM	1,2	ND				
Valerianaceae					Forest and steppe	AM		
Valeriana carnosa	PH	NH	0	+++	Forest and steppe		AM	AM
Valeriana macrorhiza	PH	AM	80	+++				
Violaceae					Forest and steppe	AM		
Viola columnaris	PH	AM	77	+++	Forest and steppe	AM		
Viola maculata	PH	AM	67;79; 92	+++	Forest and steppe			AM
Viola sacculus	PH	AM	9	+				

References:

[a] Habits: *PH* perennial herbs, *SH* subshrubs

[b] Bibliography information about mycorrhizal symbioses in forest and Patagonian steppe at different taxonomic plant levels (Fontenla et al. 1998; Fontenla 2000)

[c] Type: mycorrhizal types, *AM* arbuscular mycorrhiza, *ErM* ericoid mycorrhiza, *NH* non-host/non-mycorrhizal plants. *AM %* percentage of AM colonization

[d] AM %: Percentage of arbuscular mycorrhizal colonization

[e] *DSE* dark septate endophytes; occurrence in a range: + = 1–25%, ++ = 26–50%, +++ = 51–75%, ++++ = 76–100%; *P* presence of ErM. *ND* not determined. Empty cells are due to lacking data

[f] Data from different sampling sites

[g] Rhamnaceae: Plants with AM and *Frankia* symbiosis. Fontenla, Fernández, Ezcurra *pers.com.* (2007); Bruzone (2009)

13.2 High-Andean Ecosystems: Andean Subregion

13.2.1 Mycorrhiza and DSE Status

High-mountain ecosystems impose unfavorable conditions for plant establishment and development. AM have been reported to be more common in ecosystems located at low altitudes than in European Alpine ecosystems, where the abundance of AM plant species and AM colonization tend to decrease, often in favor of DSE or NH plants (Read and Haselwandter 1981; Dhillion 1994; Körner 1999; Ruotsalainen et al. 2004). Some studies conducted in high-mountain environments along the Andes arrived at similar results (Lugo et al. 2008; Schmidt et al. 2008; Urcelay et al. 2011). However, this is not the case for all high-mountain environments, since the prevalence of AM has been also recorded in elsewhere in the Andes, and South Alps of Japan (Kagawa et al. 2006; Silvani et al. 2017). In Patagonia, High-Andean ecosystems occupy extensive areas above the forests, and their altitudinal range decreases from north to south (2000–500 m a.s.l.). They are characterized by low temperatures, snow accumulation, low relative humidity, high radiation levels, strong winds, and low partial pressures of oxygen (Ferreyra et al. 1998, 2006). The relief of Patagonian High-Andean ecosystems includes both gentle and steep slopes and rocky or sandy soils, which are usually loose and unstable. These scree environments also include islands of High-Andean wetlands and steppes. Patagonian High-Andean ecosystems harbor more than 300 plant species (Ferreyra et al. 1998), and the mycorrhizal status of many of them was evaluated by Fontenla, Fernández, and Ezcurra within the Nahuel Huapi National Park (*pers.com.*). This study was carried out in Mt. Catedral and Mt. Challhuaco, which have different precipitation regimes (1500–1000 mm annually), and included several High-Andean environments such as screes, steppes, and meadows. In total, 46 plant species and 21 families were studied, and it was observed that AM plant species prevailed (70%) over NH (24%) and ErM (6%) plant species. Mixed mycorrhizal colonization or other mycorrhizal types were not registered, and colonization values varied widely between different plant species (15–96%, Table 13.1). These results contrast with those previously mentioned for other high-mountain ecosystems of the world, where AM are not so frequent and NH prevails. It is possible that the interaction between altitude and latitude affects the relevance of the AM symbioses in these High-Andean ecosystems, but more studies are needed to validate this idea.

Most of the families studied in these Patagonian High-Andean ecosystems included species with the same mycorrhizal type (64% of AM families). Although the Calyceraceae and Poaceae species analyzed for these ecosystems had AM, both mycorrhizal or NH species were observed for these families in other Patagonian areas (Table 13.1). The Asteraceae, Berberidaceae, and Valerianaceae families (13%) included both AM or NH species, as observed in previous studies for Patagonia. *Pinnasa nana* is a NH species belonging to the Loasaceae family; these family also presented AM species in other Patagonian regions. NH species were found in 9% of the families, some of which are considered typical non-mycorrhizal families (Brassicaceae and Caryophyllaceae). The Empetraceae and Ericaceae families had ErM, in accordance with previous works. Interestingly, all plant species were also colonized by DSE (Table 13.1), thus demonstrating that the co-occurrence of both fungal types is more the rule than an exception in High-Andean ecosystems.

13.2.2 Mycorrhiza in Plant Species Growing in High-Andean Environments and in the Patagonian Steppe: Andean and Extra-Andean Subregions

High-Andean ecosystems significantly differ from the Patagonian steppe, mainly in terms of snowfalls and rainfalls. However, these distinctive subregions also have some environmental features in common, such as low water availability, high radiation, and wind. Mycorrhizal and DSE colonization, in addition to the occurrence of AM fungi (AMF) spores in soils, was studied for High-Andean to steppe environments (Bruzone 2009; Fontenla and Fernández *pers.com.*; Velázquez et al. 2016). Four environments around 2006 to 830 m a.s.l. were selected from west to east, following a decreasing precipitation (mm annually) gradient: Mt. Tronador (3500), Mt. Catedral (1550), and Mt. Challhuaco (1000) in the High-Andean ecosystems and the Extra-Andean steppe (830 annual mm). *Armeria maritima*, *Baccharis magellanica*, and *Quinchamalium chilense* were sampled in these four environments. Another six plant species were not found all along the gradient so that they were sampled in three sites, according to the following two groups: (a) Western sites (High-Andean environments): *Nassauvia revoluta*, *Senecio crithmoides*, and *Chiliotrichum diffusum*; (b) Eastern sites (Mt. Catedral, Mt. Challhuaco, and Patagonian steppe): *Berberis empetrifolia*, *Oreopolus glacialis*, and *Viola maculata.* As observed in previous studies (see Sect. 13.2.1 and Table 13.1), the majority of the analyzed specimens (85%) had AM; *Nassauvia revoluta* was the only non-mycorrhizal species. When considering together the nine AM species, similar levels of AM colonization were observed for all species and environments (Fig. 13.1a). However, in the case of the species that were present in the four sampling sites, a significant difference between Mt. Tronador and the Patagonian steppe was noticed (Fig. 13.1b). *Armeria maritima* and *Q. chilense* presented a facultative AM behavior with values ranging between 0–65% and 0–39%, respectively. For both species, AM colonization tended to increase from High-Andean environments (including NH specimens and the lower colonization values) to the Extra-Andean steppe (where the highest colonization values were registered). In contrast, the opposite tendency was registered for *O. glacialis*; its colonization values ranged from 3% in the steppe to 88% in high-mountain ecosystems.

The comparison of the symbiotic behavior between High-Andean and Patagonian Steppe environments indicates that the percentage of colonization might vary significantly between extremes of the environmental gradient. In addition, non-mycorrhizal specimens were more frequent in High-Andean environments than in the Patagonian Steppe.

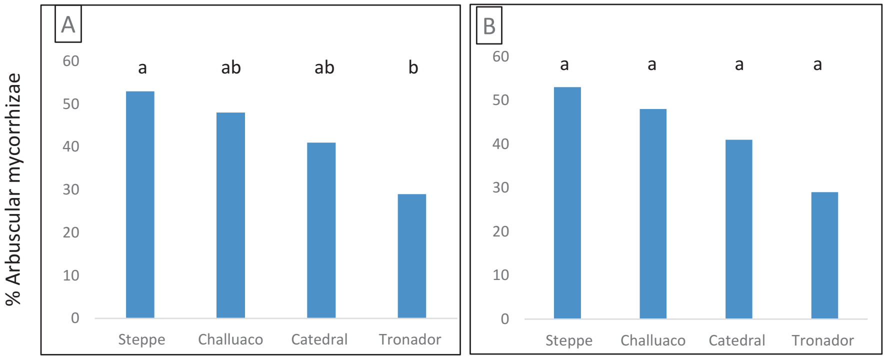

Fig. 13.1 Mycorrhizal colonization of the plant species studied along an altitudinal and precipitation gradient including three High-Andean ecosystems and a Patagonian steppe: (**a**) All species in each sampling site were considered (nine spp.; six of them were found in three out of the four sampling sites); (**b**) only the species present simultaneously in all sampling sites were considered (*A. maritima*, *B. magellanica,* and *Q. chilense*). Data from Bruzzone (2009). Different letters in each graphic indicate statistically significant differences

DSE were present in all plant species and in most of the individuals (83%) that were analyzed. In general, AM colonization was more frequent, in terms of number of individuals, than DSE and more frequent in shrubs than in herbs. Interestingly, AM and DSE colonization was positively correlated, which contradicts what has been described for high-mountain ecosystems in other parts of the world, such as those mentioned before. According to these results, both types of intraradical fungi widely co-occur in the root systems of most of the plant species studied in High-Andean and Steppe ecosystems, supporting the idea that they play a strategic role for plants inhabiting Patagonian ecosystems.

Several studies on high-mountain ecosystems have reported the diversity of soil AMF species (Lugo et al. 2008; Velázquez et al. 2008; Palenzuela et al. 2014). Bruzone (2009) and Velázquez et al. (2016) provided the first records of AMF spores richness and diversity in Patagonian High-Andean ecosystems. A total of six families and 27 species of the Glomeromycota phylum were identified in the same environments and sampled plants cited above (Mt. Tronador, Mt. Catedral, Mt. Challhuaco, and Steppe). Acaulosporaceae was the dominant family in contrast to other high-mountain environments, which were dominated by Glomeraceae (Gai et al. 2009; Carvalho et al. 2012). *Acaulospora* and its species (12) represented the most abundant taxa, with a broad distribution in all four environments. *Acaulospora delicata*, *A. dilate, A. laevis*, and *A. scrobiculata* were the most abundant species. Glomeraceae (with the exception of *Glomus* sp.1), Gigasporaceae, and Pacisporaceae were represented by species with rare or common abundance and variable distribution. The species of Claroideoglomeraceae and Ambisporaceae presented variable abundances; neither of them was absent in the Extra-Andean steppe. The total number of AMF soil spores in the sampled environments ranged from 27 to 92 spores/g and was different only between the two intermediate precipitation environments (Mt. Catedral and Mt. Challhuaco). AM spore's richness was similar among all environments and comparable to other high-mountain areas (Lugo et al. 2008; Carvalho et al. 2012; Gai et al. 2009). Altitude and precipitation were not correlated either with the number of AMF spores or with species richness, suggesting that variations among AMF communities were mostly site dependent, whereas environmental conditions determine AMF local diversity such as mentioned by Velázquez et al. (2008, 2010).

13.3 Patagonian Andean Forests: Andean Subregion

13.3.1 Mycorrhiza and Dominant Tree Species

Andean forests in Argentina extend over 2000 km latitudinal, with a maximum longitudinal width of approximately 100 km. In pure and mixed mesophilic forests, some of the major native tree species are EcM hosts: *Nothofagus antarctica, N. pumilio, N. dombeyi, N. obliqua*, and *N. nervosa* (angiosperms). Seedlings and adults of all species of *Nothofagus* have only EcM (Flores et al. 1997; Diehl et al. 2003, 2008; Tedersoo et al. 2010; Fernández et al. 2013, 2015; Nouhra et al. 2013, 2019). Most of other tree species are AM hosts: *Araucaria araucana* and *Austrocedrus chilensis* (gymnosperms) and *Schinus patagonica* and *Maytenus boaria* (angiosperms). Only one tree species is NH: *Lomatia hirsuta* (angiosperm) (Fontenla et al. 1998, 2001; Diehl et al. 2003, 2008). In the wetter western forests of this Argentinian subregion (referred to as temperate rainforests in Argentina) and in Chile, the diversity of tree species is far higher and includes the following AM host species, among others: *Lepidothamnus fonkii, Fitzroya cupressoides*, *Pilgerodendron uviferum*, *Podocarpus nubigenus*, and *Saxegothaea conspicua* (gymnosperms) and *Laurelia sempervirens*, *Dasyphyllum diacanthoides, Drimys winteri*, and *Luma apiculata* (angiosperms). *Gevuina avellana* and *Embothrium coccineum* are NH species (Carrillo et al. 1992; Godoy et al. 1994; Fontenla *pers.com.*). In northwestern Patagonia, the only EcM host plants seem to be the native Nothofagaceae species and the exotic trees of Pinaceae, Salicaceae, and *Eucalyptus*. Unlike *Nothofagus*, these exotic species may also associate with AM, especially at early developmental stages. Adults of *Populus* and *Salix* trees may also present mixed AM-EcM colonization (Wang and Qiu 2006; Teste et al. 2019). For example, in Patagonia, *Populus* (Salicaceae) adult trees showed EcM colonization (Mestre and Fontenla *pers.com.*), while one-year-old *Populus* rooting cuttings showed mainly AM colonization and the incipient presence of EcM (Mestre et al. 2017; Mestre and Fontenla *per.com.*). *Salix humboldtiana* (a native Salicaceae) also develops mixed AM-EcM colonization in the central area of Argentina (Becerra et al. 2009; Burni 2011; Lugo et al. 2012), but there are no records about its mycorrhizal behavior in Patagonia.

The maximum estimation of EcM plant distribution in the world includes 335 genera and 8500 species; EcM plants constitute one of the main components of forest ecosystems in Boreal temperate and Mediterranean climate zones (Nouhra et al. 2013; Brundrett and Tedersoo 2018). In temperate Andean forests, the EcM type is far less common in terms of the number of genera and species involved (only five species of *Nothofagus* in NW Patagonia). Nonetheless, Argentinian *Nothofagus* spp. are ecological highly relevant due to the broad distribution of *Nothofagus* forests in Andean forests. All dominant native conifers of Patagonia develop AM, in contrast to northern hemisphere conifers (Kernaghan and Harper 2001). In *A. araucana*, two root morphology classes were observed (long-thin vs. short-globular roots), and both classes had similar AM colonization (Diehl and Fontenla 2010). It is interesting to mention the co-occurrence of DSE and mycorrhiza in roots of different *Nothofagus* species, *A. chilensis*, *M. boaria*, and *L. hirsuta*.

The relation between mycorrhizal colonization, soil fertility, and plant nutrient content was studied for ten native tree species in NW Patagonia (Diehl et al. 2003, 2008). The EcM colonization of the five *Nothofagus* species ranged between 73% and 79%. In *A. araucana, A. chilensis, F. cupressoides*, and *M. boaria*, AM colonization ranged between 80% and 90%. *Nothofagus pumilio* and *N. obliqua* forests develop on the most fertile soils, whereas AM native conifers are more associated with the lower values of soil nutrient requirements and N/P ratio (Diehl et al. 2008). According to these studies, the lack of plant P limitation in these species is probably related to the prevalence of EcM and AM in plant roots. The only species apparently limited by P would be *L. hirsuta*, which has cluster roots and was the only NH species included in these works (Diehl et al. 2003, 2008). To explore the vertical distribution of mycorrhizal colonization up to 0.8 m in soil depth, another study was carried out on roots of the same tree species and locations mentioned above. The persistence of EcM or AM colonization and similar colonization values regardless of root depth were demonstrated. These results could be related to the functional importance of mycorrhizal colonization for water access (Fontenla and Diehl *pers.com.*).

13.3.2 Mycorrhiza and DSE of Understory Vegetation

Spermatophytes (Seed Plants). Most of the plants in Patagonian forest understories are low trees, shrubs, and herbs. Several studies in northern Patagonia surveyed the understory of forests dominated by *A. chilensis* (AM forest, 77 spp.), *Nothofagus* (EcM forest, 69 spp.), and *L. hirsuta* (NH forest, 20 spp.). In each of these environments, most of the species formed AM (79, 83, and 85%, respectively), or were NH species (11, 9, and 15%, respectively). Low values of ErM and OrM plant species (6 and 7%, respectively) were recorded under canopy trees of AM forests and EcM forests (Fontenla et al. 1998; Fontenla *pers. com.*). This means that, regardless of the mycorrhizal behavior of the dominant tree species, the understory of all forests had high abundance and distribution of AM plant species. Thus, EcM forests presented a mixed mycorrhizal state that possibly upgrades the benefits of both types of symbiosis. The relative importance of each mycorrhizal type, in terms of EcM-dominant tree versus AM understory; their influences on nutrient, water, and energy cycles, and the way they interact are unknown.

Pteridophytes. These plants are common in the deeply shaded temperate forests of NW Patagonia, especially in the rainforests (Cabrera 1976). Several authors have registered the occurrence of AM in sporophytes and gametophytes of pteridophytes worldwide (Muthukumar and Udaiyan 2000). This symbiosis has been described also for some fossil pteridophytes, which means that it has persisted for more than 400 million years. In Patagonia, the mycorrhizal status of several pteridophyte species was first studied by Fernández et al. (2008, 2010). They analyzed ten families and 12 genera in a temperate rainforest: 50% of these families comprised only mycorrhizal species (Blechnaceae, Dryopteridaceae, Gleicheniaceae, Dicksoniaceae, and Pteridaceae), 20% included facultative mycorrhizal species (Equisetaceae and Lycopodiaceae), and 30% NH species (Aspleniaceae, Polypodiaceae, and Hymenophyllaceae). Arbuscular mycorrhiza were found in 48% of 21 species of pteridophytes considered in these studies. The mean colonization values per species ranged from 54 to 96%. The AM species percentage (48%) is lower than those recorded in other parts of the world (Muthukumar and Udaiyan 2000) and also lower than the percentage recorded for spermatophytes in Patagonian High-Andean and Andean forests (see Sects. 13.2 and 13.3). This may be related to the fact that 52% of the sampled pteridophytes were mostly epiphytic or NH (11 species). An interesting observation concerning pteridophytes, in the rainforests studied here, is that quite often, AM fungi penetrated into root hairs and then spread along the root cortex. These root hair penetrations (common in *Rhizobiales,* a symbiont bacteria) were not frequently observed in Patagonian Angiosperms or Gymnosperms (Fontenla *pers.com.*). Dark septate endophytes were also recorded in the roots of all pteridophyte species that were analyzed, even in those that lacked AM, supporting the idea that DSE are very ubiquitous in plants of Patagonian forests and can co-occur with AMF (Fernández et al. 2008, 2010).

Bryophytes. The relationship between AMF and Anthocerotophyta has been studied previously, and it is known that mycorrhizal colonization may differ in occurrence and abundance depending on genera and environmental conditions. The first record of AM on anthocerophytes from Patagonia has been registered for *Phaeoceros laevis* and *Nothoceros fuegiensis*, which live in *Nothofagus* forests of five Patagonian provinces of Argentina (Cottet and Messuti 2000, 2017).

13.4 Ericoid Mycorrhiza and Related Root Endophytes Along the Andean Subregion: Study of High-Andean and Andean Forests and a Comparison Between Their Native Plant Species Versus Exotic Plant Species from Productive Farm

Ericoid mycorrhiza (ErM) is one of the least studied mycorrhizal types. In general terms, ErM have a low frequency in most ecosystems but a worldwide distribution (Wang and Qiu 2006). This symbiosis implicates a relatively low number of Ascomycota fungi, some uncultivable Sebacinales (Agaricomycetes, Basidiomycota), and other no-sebacinoid Basidiomycota fungi (Vohník and Albrechtová 2011; Vohník et al. 2012, 2016 and references therein). Some of the main fungal species forming European ErM mycorrhiza are *Rhizoscyphus ericae* and *Oidiodendron maius* (Helotiales, Ascomycota) (Gorzelak et al. 2012). *Rhizoscyphus ericae, Meliniomyces* spp., and *Cadophora finlandica* belong to the so-called *R. ericae* aggregate (REA) and have been recently grouped in the *Hyaloscypha* genera (Fehrer et al. 2019). The REA is a widespread group of root-associated fungi that may behave as saprobe root endophytes or ErM, or as EcM fungi (Fehrer et al. 2019), and in general could be isolated by cultivation. While *R. ericae* seems to be restricted to the ErM lifestyle, normally in Ericaceae plants; *M. variabilis, M. bicolor*, and *C. finlandica* form ErM with Ericaceae (Ohtaka and Narisawa 2008) but may also colonize roots of NH and EcM hosts and form fruit bodies (Piercey et al. 2002; Vohník et al. 2013). Most of the information on ErM fungi has been obtained by microscopy and culture-independent techniques (Selosse et al. 2007); information from fungal isolation and re-synthesis assays is quite limited. Until recently, ErM from the southern hemisphere had been mostly described in plants from Australia (McLean and Lawrie 1996; McLean et al. 1999), New Zealand, and South Africa (Bizabani et al. 2016). In Argentina, one of the first reports of ErM was published by Urcelay (2002), who studied the co-occurrence of ErM, AM, and DSE in *Gaultheria poeppigii* (Ericaceae, subfamily Vaccinoideae) from the central part of Argentina.

In Patagonia, the first studies of ErM were performed on blueberry cultivars, located near native Patagonic Andean forest (*Vaccinium* spp., exotic in this region) and in four native *Gaultheria* species from native forests and High-Andean ecosystems (Bruzone 2009; Bruzone et al. 2015, 2016; Fontenla and Fernández *per. com.*). Eleven varieties of *V. corymbosum* and *V. ashei* from different farms located near Lago Puelo (Chubut province, Argentina) were investigated. These studies used different experimental approaches such as microscopic observation, ErM fungi and endophytic culture and isolation, and molecular identification. High and variable ErM colonization was observed in all *Gaultheria* and *Vaccinium* species, regardless of host species/variety or sampling environment/site. Three different ErM colonization patterns of rhizodermal cells were microscopically observed: ErM fungi from REA aggregate, Sebaciales, and hyphal loops possessing clamp connections typical for basidiomycetes (not Sebacinales). DSE were also typically observed in all the analyzed species and varieties.

Cultural techniques resulted in the isolation of fungi from *Vaccinium* spp. cultivated in commercial farms (160) and from *Gaultheria* spp. growing in Andean forests (257) or in High-Andean environments (234). Regardless of genera/species/environment, a prevalence of cultural Ascomycetes (more than 90%) over Basidiomycetes was observed. However, different compositions of fungal communities were detected between *Vaccinium* and *Gaultheria* species. In the case of *Vaccinium* spp., typical European ErM fungi (*O. maius* and *Rhizoscyphus* sp.) and other REA fungi (*C. finlandia* and *M.* cf. *varibilis*) were isolated, all of them included in the Ascomycota. Basidiomycota isolates corresponded to only 2% of the total number of cultures, and they belonged to non-mycorrhizal species. The ascomycetous DSE *Leptodontidium orchidicola* and *Phialocephala* cf. *fortinii* were also identified. In native Andean forests, typical European ErM-forming fungi, such as *Rhizoscyphus* or *Oidiodendron,* were not identified in *G. poeppigii* and *G. mucronata*. In this case, the isolated ascomycetous fungi mainly corresponded to the Helotiales, with *Phialocephala* (possible DSE) as the most frequent genera, and the non-mycorrhizal fungi *Pochonia* and *Tetracladium*. To elucidate the apparent contradiction between the observed high ErM colonization and the low proportion of typical ErM fungi through cultivation, the amplification of total root DNA using specific ErM primers was performed. By this means, the presence of the fungi belonging to Sebacinaceae was confirmed in all samples, whereas REA were detected in very few samples (Bruzone et al. 2015). When *G. pumila* and *G. caespitosa* from a High-Andean community were studied, 27% of the identified fungi belonged to *Rhizoscyphus* spp.; this was the first citation of these typical European ErM fungi in Patagonian environments and also in South America.

In summary, the ErM in Ericaceae plants from NW Patagonia present high incidence of *Rhizoscyphus* spp. in High-Andean ecosystems and high incidence of *O. maius* and some REA in *Vaccinium* spp. Sebacinales, an ErM fungi difficult to cultivate, co-occurred in all plants of all ecosystems and were the principal in *Gaultheria spp.* in forests. According to microscopical analyses, there were several structures corresponding to no-sebacinoid Basidiomycota fungi, which could not be isolated. Consequently, these Basidiomycota fungi, possibly forming ErM, may had been underestimated. Further studies are necessary as they may be more relevant than expected for ericoid roots in our region. From all these results, the following question arises: are environmental conditions determining different symbiotic patterns, or are they affecting only by the presence of REA fungi belonging to *Hyaloscypha* genera following a new classification (Fehrer et al. 2019).

13.5 Fungi Associated with Ectomycorrhizosphere

Soils are complex ecosystems in which several regions can be distinguished: bulk soil, generally referred to as the region further away from roots; rhizosphere, surrounding roots and under the direct influence of root activity; and ectomycorrhizosphere, which is under the direct influence of EcM symbioses. Each of these soil regions may harbor distinct microbial communities. The dominant EcM morphotypes and associated ectomycorrhizospheric fungi were studied in four Patagonian *N. antarctica* and *N. pumilio* forests (Andean subregion) located in the Nahuel Huapi National Park (Mestre and Fontenla 2021). These last authors focused their work on the molecular identification of the mycobionts of each ectomorph type (root apices modified by the colonization of each EcM fungi); and most of the sequences were assigned to EcM fungi within eight lineages: /aleurina, /amanita, /cenococcum, /cortinarius, /descolea, /genea-humaria, /inocybe, and /tomentella-thelephora, which had previously been recorded in the region (Nouhra et al. 2013;

Fernández et al. 2015). Less than 25% of the sequences corresponded to non-ectomycorrhizal lineages mainly reported as endophytic fungi. Within the Helotiales order (Ascomycota), the genera *Leptodontideum* and *Phialocephala,* and the species *Cadophora finlandia* and *Meliniomyces variabilis* (Fehrer et al. 2019) were identified. *Leptodontideum* and *Phialocephala* usually form DSE and were also identified in *Gaultheria* spp. roots (Bruzone 2017) and in soil samples from other Patagonian *Nothofagus* forests (Duo Saito et al. 2018; Carron et al. 2020). It has been described that *C. finlandia* and *M. variabilis,* also Helotiales of the RAE group, may form ErM in Ericaceae plants and EcM in European conifers (Vohník et al. 2013). This raises the question of whether some of the Helotiales recorded in Andean forests of Patagonia could form EcM associations with *Nothofagus* trees and ErM with the understory shrubs (*Gaultheria* spp.), or if their presence in *Nothofagus* ectomycorrhizosphere corresponds to an endophytic or rhizospheric saprobiotic role.

13.6 Mycorrhiza and DSE in the Steppe: Extra-Andean Subregion

The Extra-Andean Steppe (Steppe sensu *lato*) of Patagonia is currently affected by desertification. It is ecologically described as a semiarid steppe with less than 500 annual mm of precipitation. In northern Patagonia, this subregion includes the Patagonian steppe (steppe sensu stricto) and meadows. Meadows appear in undulating foothills and lowlands and are graminoid prairies dominated by Juncaceae, Cyperaceae, and Calyceraceae, where desertification processes normally reach low levels; they are characterized by temporary water excess, poor soil aeration, and the permanent accumulation of plant debris.

13.6.1 Mycorrhizal Status

Information regarding mycorrhizal behavior in plants from the Extra-Andean steppe is scarce. The mycorrhizal status of several plant species was studied in the Extra-Andean steppe by Fontenla et al. (2001). This study included 230 plant species from high- to low-shrub steppe ecosystems (sensu stricto) and meadows located at an altitude from 1300 to 900 m a.s.l., where mean annual precipitations vary between 1000 mm and 267 (see references in Fontenla et al. 2001). One pteridophyte, two gymnosperms, and 155 angiosperms were identified and classified into 85 families. As found for the other Patagonian ecosystems, species that belong to the same family had the same mycorrhizal type or were NH. Exceptions to this common behavior were found in species from the typical AM Asteraceae and NH Cyperaceae families. The mycorrhizal status of the Extra-Andean steppe subregion showed a predominance of AM species (72%), resembling the temperate and semiarid grassland of North American, European, and Russian steppes (Fontenla et al. 2001 and references therein). All NH species in this subregion reached 28% of the total number of plant species and were less representative in the steppe sensu stricto (24%) than in the meadows (30%). Besides, it was observed that the Extra-Andean steppe had a low number of plant species usually forming ErM (0.6%) and the absence of EcM, OrM, and dual mycorrhizal colonization. Arbuscular mycorrhiza prevailed in the steppe (sensu stricto) among all plant species (76%, native and exotic), native species (62%), and among the most representative plant species (in terms of cover and abundance). In the meadows, AM were also prevalent in terms of number of species (70%), but not in terms of species abundance or dominance. The NH species *Juncus balticus* was, by far, the most abundant plant species in the meadows, both in cover and density; NH species of Caryophyllaceae, Cyperaceae, Brassicaceae, and Juncaceae were also relatively abundant in these environments. Despite the predominance of the AM status previously mentioned for meadows, the abundance and dominance of NH plants in the center of the meadows defined an important influence in these environments.

13.6.2 Mycorrhizal Behavior of Plants in Patagonian Steppe (Sensu Stricto) and Meadow Zones and Comparisons with Andean Forests

Fontenla (2000) and Fontenla et al. (2001) carried out a geographically extensive study, which included 14 sites with different environmental characteristics along a 1300 mm precipitation range from the Extra-Andean steppe to the Andean forest subregion. In this study, meadows, high-shrub steppes, low-shrub steppes, ecotone environments, and Andean forests were considered. Meadows were categorized as “very good,” “good,” “regular,” and “poor” according to different ecological aspects defined by vegetation abundance and soil cover, physicochemical soil characteristics, soil appearance in terms of water availability (very humid to very dry), and occurrence of desertification factors (see description in Table 13.2). In each meadow category, plant communities vary from the center, with higher water availability, toward the periphery, characterized by much drier conditions. This humidity gradient determined different ecological zones of the meadows: center, periphery, and border. All the studied environments were analyzed regarding plant species composition and their mycorrhizal category: mycorrhizal (M) and non-mycorrhizal/nonhost (NH), category of patches with very low or nil vegetation cover (WV), numbers of AM spores, and infecting propagules of AM in the soil. As mentioned in the preceding Sect. 13.6.1 most mycorrhizal plant species had AM. Thus, the mycorrhizal category was focused only in this mycorrhizal type (AM) and in NH species, and considered the abundance of the most representative species with each mycorrhizal behavior (AM and NH, see data from Sect. 13.6.1). The AM infective

Table 13.2 Characteristics of vegetation, soil, and mycorrhizal behavior of different environments of the Extra-Andean subregion and Andean forests

Environments/ zones #	Vegetation and soil description		Mycorrhizal behavior		
Sites (precipitation) #	Characterization	Dominant and abundant plants	Dominant Category[a]	AM IP[b] (MPN/ soil g)	AM spores (N°/ soil g)
Meadows[c, d, e] Pilcaniyeu, 267 mm					
1[e]*-Very good meadow*	Non-disturbed conditions				
Center	Graminoid prairie, flooded soil, C[e#]> 75	Juncaceae, Cyperaceae, Caryophyllaceae; low *Azorella trifurcata*	NH	1600	510
Periphery	Tussock grass prairie, humid soil, C: 25–50	Juncaceae, Cyperaceae, *Festuca pallescens*; low Poaceae	NH +M	350	324
Border	Transition with steppe; dry soil, C: 25–50	*Festuca pallescens*, *Acaena magellanica*, *Senecio* spp., *Pappostipa* sp.; very low Cyperaceae and Juncaceae	M	35	506
2-Good meadow	Some disturbed conditions				
Center	Graminoid-low shrubs prairie, humid soil, C > 75	Juncaceae, Cyperaceae; low *Senecio neaei*, *S. bracteolatus*	NH + M	900	323
Border	Transition with steppe, dry soil, C:25–50	*Pappostipa* spp., low *Senecio* spp., very low Juncaceae	M	30	183
3-Regular meadow	Moderately disturbed. Slight salinity				
Center	Graminoid prairie; flooded soil and moving water, C > 75	Juncaceae, Cyperaceae	NH	1800	119
Border with steppe	Transition with steppe, dry soil, C: 25–50	*Festuca pallescens*, *Acaena magellanica*, *Senecio* spp., *Pappostipa* spp., very low Cyperaceae and Juncaceae	M	150	298
4-Poor meadow	Highly disturbed. Intensive salinity				
Center	Dry and crumbly soil, salt presence, C: 1-5 scarce vegetation	Low abundance: *Juncus balticus*, *Nitrophila australis*, *Arenaria* sp.	WV	95	9
Periphery	Tussock grass prairie with shrubs and herbs, dry soil, C: 5–15	*Senecio* spp., *Pappostipa* spp., very low Cyperaceae	M	170	138

(continued)

Table 13.2 (continued)

Environments/ zones #	Vegetation and soil description		Mycorrhizal behavior		
Sites (precipitation) #	Characterization	Dominant and abundant plants	Dominant Category[a]	AM IP[b] (MPN/ soil g)	AM spores (N°/ soil g)
Border with steppe	Transition with steppe, dry soil, C: 25–50.	*Senecio* spp., *Pappostipa* spp.	M	17	149
Low-shrub steppe					
Pilcaniyeu, 276 mm	Plateaus and low hills (wind deflation); dry conditions, herbs and shrub, dry soil, C: 25–50	*Azorella prolifera*, *Poa ligularis*, *Pappostipa speciosa* var. *speciosa*, [*Festuca argentina*]	M	175	55
High-shrub steppe					
Bariloche district, near airport 700 mm	plateaus and low hills, with shrub and herbs, dry soil, C: 50–75	*Anarthrophyllum rigidum*, *Berberis microphylla*, *Discaria articulata*, *Azorella prolifera*, *Poa ligularis*, *Pappostipa speciosa*	M	900	437
Ecotone between steppe and forest					
Pampa de Huenuleo 1000 mm	Low hills with shrubs and herbs, some disperse trees, C: 50–75	*Discaria articulata, Poa ligularis, Pappostipa* spp., *Maytenus chubutensis*, *Azorella prolifera*, *Senecio bracteolatus*, *Acaena splendens*, [*A. chilensis*]	M	700	456
Andean forest					
Llao-Llao county forest, 1600 mm	Hill with tree, shrub, and herbaceous strata; C: >75	*A. chilensis*, *N. dombeyi*, *Lomatia hirsuta*, *Schinus patagonica*	M	350	19

References:

[a] Dominant mycorrhizal category of each environment following dominant and abundant plant species: *M* mycorrhizal, *NH* non-host/non-mycorrhizal; *WV* very sparse or nill vegetation

[b] *AM IP:* AM infective propagules

[c] Meadow categories (very good, good, regular, and poor meadow) and ecological zones into each one (center, periphery and border)

[d] Soil parameters: *OM* organic matter (%)

[e] C: soil coverture (%)

[f] Chemical soil characteristic of: Meadows range from center to border: very good: pH = 8-8, OM = 8-3, CIC = 14-8, [Na + K] = 5-4; Good: pH = 9-7, OM = 2-1, CIC = 9-8, [Na + K] = 7-1; Regular: pH = 10-8, OM = 3-1, CIC = 11-5, [Na + K] = 19-2; Poor: pH = 10-8, OM = 2-1, CIC = 14-5, [Na + K] = 29-1. Low-shrub steppe: pH = 6, OM = 1, CIC = 8, [Na + K] = 1; high-shrub steppe: pH = 7, OM = 2, CIC = 9, [Na + K] = 1; ecotone steppe forest: pH = 6, OM = 1, CIC = 13, [Na + K] = <1; Andean forest: pH = 5.6, OM = 0.7, CIC = 33, [Na + K] = <1

#See bibliography in Fontenla (2000) and Fontenla et al. (2001) for precipitation, cover and other descriptions of the environments propagules (IP) were evaluated by an experimental assay of most probable number (MPN) by means of a modified method using trap host plants and soil from each of the 14 sites as a source of inoculum to analyze the potential AM infective capacity (Fontenla 2000; Fontenla et al. 2001). The NMP measure this natural AM infective capacity that was conferred by spores, hyphae from soil, and from host-colonized roots. Soil humidity was categorized as flooded, humid, or dry; soil features included moderate and intensive salinity; vegetation cover varied from less than 5% to more than 75%. Meadow peripheries and borders and the surrounding low-shrub steppe had a similar and low plant cover (25–50%). Intermediate plant covers (50–75%) were observed in a high-shrub steppe and ecotone between the steppe and the forest, and the highest cover (75–100%) was observed in the centers of non-disturbed meadows and in Andean forest. Little disturbed or non-disturbed meadow centers were in general dominated principally by *J. balticus* and other NH Juncaceae and Cyperaceae species. Other NH species and AM plant species were followed depending on their ecological conditions. In meadows with severe desertification conditions, plants were absent or very scarce (WV). Plants in the peripheries of all meadows were either NH+M or only M. All meadow borders, the surrounding steppe, and the forest presented the M category because they were dominated by AM species (Table 13.2). In summary, environmental characteristics together with desertification factors influence the Patagonian mycorrhizal behavior.

In general, the mycorrhizal category (M) prevailed in most communities (Table 13.2), with the exception of meadows' centers. The numbers of spores and infective propagules of AM, in general, decreased from the center of the meadows to the dry low-shrub steppe and the Andean forest. Soil inocula in this study presented predominantly AM structures, which varied with environmental characteristics; forests (characterized by high plant density, stratified vegetation, and higher precipitation) had high numbers of infective propagules but lower numbers of spores, suggesting the predominance of hyphae and infected root pieces in soil. Steppes and meadow borders (which have low plant density, low precipitation, and dry soil conditions) contained high numbers of spores and infective capacity (comparable between them, the spores and the infective capacity value) suggesting a prevalence of spores as infection propagules over hyphae strategies. It is possible to assume that borders of meadows, together with the steppes and forests, behave like AM inocula-generating communities. The center of meadows (non-disturbed) had the higher numbers of spores and infective capacity. The centers of almost all meadows, in contrast, seem to be repository areas for AM inocula, independently of the abundance of NH plants, or the negative effect of non-host substances on AM soil inocula (as we mentioned in the introduction, Sect. 13.1.2).

13.7 Conclusion

All around NW Patagonia, AM is the predominant mycorrhizal type in terms of number and abundance of spermatophyte species in most of the studied environments, with the exception of meadows' centers. This predominance is higher than that found in pteridophytes, but studies on the latter group need to be diversified. In the case of Andean forests, in spite of the high predominance of AM (including understory species), the dominant tree species have principally two types of mycorrhiza. *Nothofagus* forests are the only native ecosystems of Patagonia that are dominated by EcM trees, possibly upgrading the benefits of AM and EcM. Native conifer forests (in contrast with north hemisphere forests) associate with AM, which are present not only in the dominant trees but also in most of the understory vegetation. In general, NH species are in low proportions in terms of number and abundance of species. Pteridophytes seem to be disproportionately represented among NH species, maybe due to the high proportion of epiphytic ferns. The occurrence of other types of mycorrhiza is low or nil, and dual mycorrhizal infection is not observed in native species along the study area. DSE are represented in a high proportion of all plant species of NW Patagonia, co-occurring with mycorrhizal or NH species, including High-Andean plants.

At ecosystem level, mycorrhizal symbioses were more frequent in Andean forests than in the Extra-Andean steppe and in High-Andean environments, which are comparable in this regard. At environmental level, the mycorrhizal status varies following this tendency: EcM-forest > AM-forest > NH-forest > Andean steppe (sensu stricto) > Andean meadows = High-Andean environment. The mycorrhizal status and the number of AM spores and AM infective propagules followed opposite tendencies.

Spore numbers and infective capacity decrease from the center of the meadows to the dry low-shrub steppe and the Andean mixed forest (*A. chilensis* and *N. dombeyi).* In NW Patagonian environments, the prevalence of certain types of AM structures in soil inocula depends on environmental characteristics such as main vegetation type, land geomorphology, and water availability. High infective capacities are provided principally by AM hyphae from soil and root pieces in the steppe and mainly by spores in the center of meadows. It is remarkable that the latter environments, where NH species abound, maintain high infective capacities through the accumulation of resistance structures.

Ericoid mycorrhiza was determined in shrub species belonging to a single genus, *Gaultheria*, which is distributed in High-Andean environments and EcM *Nothofagus* forests. The same type of mycorrhiza is associated with blueberries from a commercial farm located into an Andean forest. The identified ErM fungi were variable between these environments; colonization by REA, Sebacinales, and other Basidiomycota ErM fungi was evidenced. The presence of *Rhizoscyphus ericae* is the first report in South America.

A significant number of endophytes were also found without a clear ecological role; the possibility that some species of fungi, such as *Cadophora finlandia* and

Meliniomyces variabilis, could be shared between ErM and EcM emerges as a possibility.

To conclude, this chapter summarizes current knowledge on mycorrhizal associations in northwest Patagonia and proposes general assumptions and hypotheses. It is clear that there are diverse and complex factors that contribute to mycorrhizal relationships in this large area. Further studies need to be pursued in order to deepen the knowledge on these communities; this will ultimately lead to a better understanding of global diversity patterns and their determinants in view of the climate changes that are having worldwide effects.

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Chapter 14
Mycorrhizas in *Nothofagus* From South America: What Do We Know From Nursery and Field Experiences?

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14.1 Introduction

In the southern cone of South America, Subantarctic Temperate Forests develop on both sides of the Andes Mountains, in Chile and Argentina. These ecosystems are the southernmost forests on Earth and have been relatively undisturbed by man, which makes them unique and extremely valuable for diverse research purposes (Marchelli et al. 2021). They cover a narrow but long latitudinal strip from 35° S (Maule River, Chile) to 55° S (in the Southern extreme of Chile and Argentina) and are considered a biogeographic island due to their geographic discontinuity from the other forests of South America. The climate of these forests is cold temperate, with an average temperature ranging from 9.5 °C in the North to 5.4 °C in the South. Precipitation depends primarily on the humidity of the Pacific Ocean and forms an abrupt decreasing gradient, fluctuating from more than 4000 mm in the West to 700 mm in the East over less than 50 km (Armesto et al. 1995). Altitude gradients are also characteristic of this region, particularly at Northern latitudes where the Andes reach higher heights (Fig. 14.1a). Together with temperature and precipitation, elevation gradients also impose strong adaptive challenges for plants because of the marked changes in environmental conditions over relatively short distances, such as low temperatures, frosts and snow, high radiation, shorter growing season, and changes in soil characteristics (Marchelli et al. 2021). Disturbances of different origins (natural and anthropogenic) and at different scales have also influenced, and still do, the structure and dynamics of Subantarctic Temperate Forests. Some of the main influencing factors are those of geological origin, being volcanic eruptions relatively frequent high-magnitude disturbances in this region (Fig. 14.1c, j). Fire is another disturbance severely affecting these forests and probably the most important since the European colonization (González et al. 2014). At present, a large proportion of these forests is under protection (e.g., National Parks, natural reserves), but this was not always the case, and some regions have a long history of unsustainable exploitation and use, mainly because of grazing, overexploitation, and the establishment of extensive plantations of fast-growing exotic species, such as pines. Therefore, these forests are characterized by a high heterogeneity imposed by prominent environmental gradients, which influences species distribution and determines the occurrence of different forest formations (Marchelli et al. 2021, Fig. 14.1).

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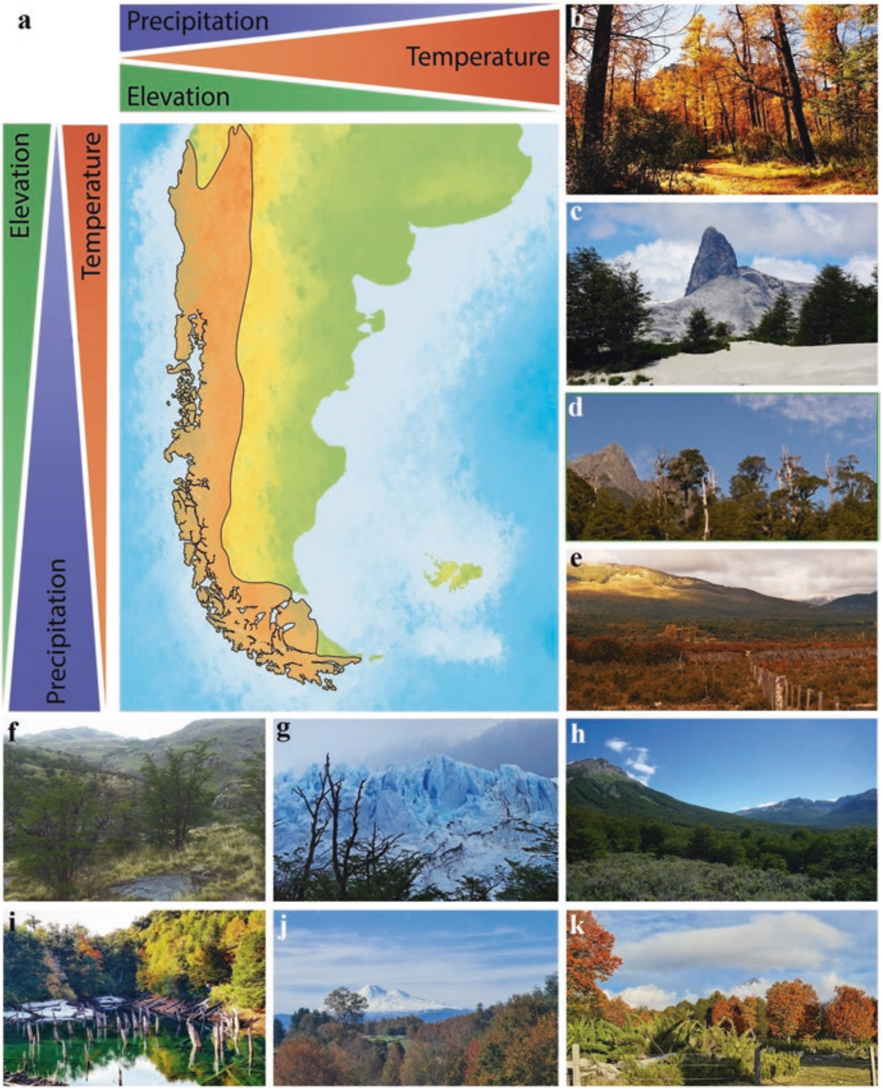

Fig. 14.1 Environmental gradient characteristic of the Subantarctic Temperate Forests in Southern South America (**a**) and some typical *Nothofagus* forests from Argentina (**b–h**) and Chile (**j–k**). (**b**) Yuco, Neuquén; (**c**) Paso Cardenal Samoré, Neuquén; (**d**) Puerto Blest, Río Negro; (**e**) El Foyel, Río Negro; (**f**) El Chaltén, Santa Cruz; (**g**) Calafate, Santa Cruz; (**h**) Ushuaia, Tierra del Fuego; (**i**) Laguna arcoiris, Conguillio; (**j**) Llaima volcano and (**k**) Curacautín, Araucanía region. Photo's credits: Fernández N: (**b–e**, **g**); Knoblochová T: (**f**, **h**); and Cornejo P: (**i–k**)

Subantarctic Temperate Forests are mainly composed of monotypic or mixed stands of two or three dominant tree species, with an understory of bamboo and/or several accompanying shrubs and herbs. In the tree layer, there is a predominance of angiosperms, accompanied by some long-lived gymnosperms, like *Austrocedrus* *chilensis*, *Araucaria araucana*, *Fitzroya cupressoides*, and *Saxegothaea conspicua* (Armesto et al. 1995; Marchelli et al. 2021). Among the angiosperms, *Nothofagus* is by far the dominant genus, conforming about 80% of these forests (Fig. 14.1). This genus has a disjoint distribution with more than 40 species worldwide, occurring in New Zealand, Australia, New Guinea, New Caledonia, and South America (Gondwanan origin). In South America, ten species and three recognized hybrids can be found. Six of these species are present in both Argentina and Chile (*N. alpina*, *N. antarctica*, *N. betuloides*, *N. dombeyi, N. obliqua*, and *N. pumilio*) and the other four only in Chile (*N. alessandri*, *N. glauca*, *N. macrocarpa*, and *N. nitida*) (Marchelli et al. 2021). Independently of their geographic location, species in the Nothofagaceae form ectomycorrhizas (EcM) (Tedersoo et al. 2009; Nouhra et al. 2013; Fernández et al. 2015; Truong et al. 2017; Barroetaveña et al. 2019; Godoy and Marín 2019). Due to the ecological and economical importance of the *Nothofagus* species and to their close and vital relationship with EcM, several studies have focused on describing their mycorrhizal associations (Nouhra et al. 2019), including diverse ecological aspects as well as the diversity and distribution of the associated ectomycorrhizal fungi (EcMF). In this chapter, we describe different approaches used for studying this symbiosis in *Nothofagus* from South America and the influence of different biotic and abiotic factors on it.

14.2 Mycorrhizas in Nursery-Cultivated *Nothofagus*

Anthropogenic activities have caused persistent and prominent losses of forest cover all over the world. Natural regeneration of forest trees is usually challenging due to the lack of available seeds and to the harsh conditions for germination and seedlings' establishment (e.g., plant competition, unfavorable climatic and microsite circumstances, consumption by herbivores). Therefore, artificial reforestation using nursery-grown seedlings is usually required (Haase and Davis 2017). Moreover, estimations of future effects of global climate change suggest that the need for forest restoration will increase, as environmental conditions become harsher, and the incidence of megafires increases. In this context, the availability of good-quality nursery-cultivated seedlings will be of crucial importance for delivering effective and successful restoration programs (Haase and Davis 2017; Marchelli et al. 2021).

Forest nurseries comprise a set of facilities (greenhouses) and lands (nursery beds) used to produce seedlings under favorable conditions, until they are ready for planting in the field, either for commercial or restoration purposes. Seedlings are commonly produced using two main cultivation systems: (i) containerized seedlings (grown in different types of pots and substrates) and (ii) bare-root seedlings (grown in open-field soil beds) (Menkis et al. 2011). Nursery managers have known for a long time that nursery-cultivated seedlings should be colonized by mycorrhizas to ensure adequate field survival and growth (Cordell et al. 1987; Rudawska and Leski 2021). Such is so that mycorrhizas should be taken into account as an integral component of nursery production and considered when determining seedlings' quality. Besides, inoculation with selected growth-promoting fungi is an energy-efficient and environment-friendly alternative to fertilization with inorganic products (Cordell et al. 1987; Domínguez-Núñez and Albanesi 2019). However, a good understanding of the cultivation system and of the mycorrhizal status of the produced forestry seedlings is needed to be able to use mycorrhizal fungi as biotechnological tools (Cram and Dumroese 2012; Rudawska and Leski 2021).

Nursery cultivation is also a good way of obtaining novel information on diverse ecosystem processes, which complements what can be observed or described directly in the field. This is because this type of experimental approach, performed under controlled conditions, simplifies the complex influence of different environmental factors and allows to focus on particular variables (e.g., warming, drought, nutrient availability) or attributes of the species under study (e.g., genetic and phenotypic plasticity, adaptive potential). The description of mycorrhizas in *Nothofagus* seedlings cultivated in forest nurseries as part of domestication programs will be presented in Sect. 14.2.1, while the employment of nursery trials for the study of plant traits and ecosystem processes will be addressed in Sect. 14.2.2.

14.2.1 Domestication of **Nothofagus** *Species*

Domestication of forest trees refers to the development of technologies for high-quality seedling production, for both productive and restoration purposes. Tree domestication is mostly based on the selection of appropriate gene pools and considers other important aspects, such as the improvement of seedlings' growth, outplanting in the field, and plantation management (Pastorino 2021). The forestry industry in South America has been mainly focused on introduced fast-growing species, as those included in the *Pinus*, *Pseudotsuga*, *Populus*, and *Salix* genera (González et al. 2008; Pastorino 2021). However, in the last decades, much more attention has been paid to native forestry species, since there is a social and governmental consensus on the importance of conserving and restoring natural ecosystems and on moving toward more sustainable forestry practices. Among the native species of greatest interest to domesticate and cultivate are those comprised in the genus *Nothofagus* (Pastorino 2021).

There is a growing concern in finding alternative technologies to chemical inputs (pesticides and fertilizers) used for agriculture and forestry production. In South America, Argentina, Brazil, and Colombia are playing a leading role in using bioproducts (e.g., biofertilizers, biocontrol agents). However, most of their effort is focused on agriculture (Goulet 2021), being still necessary to develop more sustainable options for the forestry industry. Since the 1950s, mycorrhizal fungi have been frequently applied as biofertilizers in forestry production, as their effectiveness in seedlings' field survival and growth was experimentally demonstrated (Cram and Dumroese 2012; Domínguez-Núñez and Albanesi 2019). Despite one of the aspects that should be included in *Nothofagus* domestication is the application and management of EcM (Fernández et al. 2013), the occurrence of this symbiosis is generally overlooked. This is mostly due to the lack of information on the mycorrhizal status of seedlings during cultivation, the appropriate ways of inoculating EcMF, and on the evaluation of seedlings' performance after being established in the field. In order to fill these knowledge gaps, some studies have been performed and are described below.

In a two-year experiment, Fernández et al. (2013) analyzed the abundance and diversity of mycorrhizas naturally established in *N. alpina* seedlings cultivated under the same conditions as those used for domestication programs (i.e., seed stratification, containerized seedlings production, fertigation, lack of EcMF inoculation). Seedlings were grown under two different techniques in the nursery of the Instituto Nacional de Tecnología Agropecuaria (INTA) EEA Bariloche (Fig. 14.2): (1) two years in pots inside the greenhouse (containerized) or (2) the first year in the greenhouse followed by a second year in the nursery soil bed (containerized + bare-root). For each plant, EcM colonization percentage in addition to richness and diversity of EcMF were determined. It was observed that seedlings naturally developed EcM between six and 12 months after germination, right after the rustification stage, when nutrient supply (mainly N and P) and irrigation frequency were significantly reduced. Seedlings that continued for a second year in the greenhouse were significantly bigger (higher values of stem length, root diameter, and stem diameter) and had significantly higher EcM colonization (73%) than those transplanted to the nursery soil (65%), which were subjected to transplant stress and harsher environmental conditions (e.g., radiation, frosts, wind). However, bare-root seedlings were colonized by a higher number of EcMF species (six) with respect to those that remained in the greenhouse (two). The most abundant EcMF in seedlings from both cultivation techniques were *Tomentella ellisii* (Basidiomycota, 54–71%) and a still unidentified fungus named Ascomicetos EcM sp. 1 (29–37%) (Fig. 14.2). Four EcMF species were only found in bare-root seedlings, two basidiomycetes (*Hebeloma cavipes* and *Rickenella* sp. 1) and two ascomycetes (*Peziza* sp. 1 and *Peziza* sp. 2). This is in agreement with other studies that also reported that mycorrhizal fungi adapted to greenhouse conditions often remain in the roots after transplantation, but they are gradually replaced by soil-borne species better adapted to natural environmental conditions (Fernández et al. 2013; Rudawska and Leski 2021).

In a different forest nursery, which is also involved in *Nothofagus* domestication programs (INTA-EEA Trevelin) and uses the same cultivation protocols, Barroetaveña et al. (2009) evaluated how the inoculation of three native basidiomycetous EcMF (*Hallingea purpureus*, *Austropaxillus statuum*, and *Setchelliogaster fragilis*) affected the mycorrhization and growth of three *Nothofagus* species. These EcMF species were selected based on the following criteria: (1) the presence of abundant fruiting bodies in native forests, (2) a high number of spores in the sporocarps, and (3) their occurrence in more than one *Nothofagus* species. Seedlings were inoculated using spores of the different selected EcMF. After one year in the greenhouse, it was observed that EcM colonization was relatively low and differed significantly between *Nothofagus* species (mean values: 14.5, 8.6, and 3.6% for *N. pumilio*, *N. obliqua*, and *N. alpina*, respectively). Only two EcMF were found in

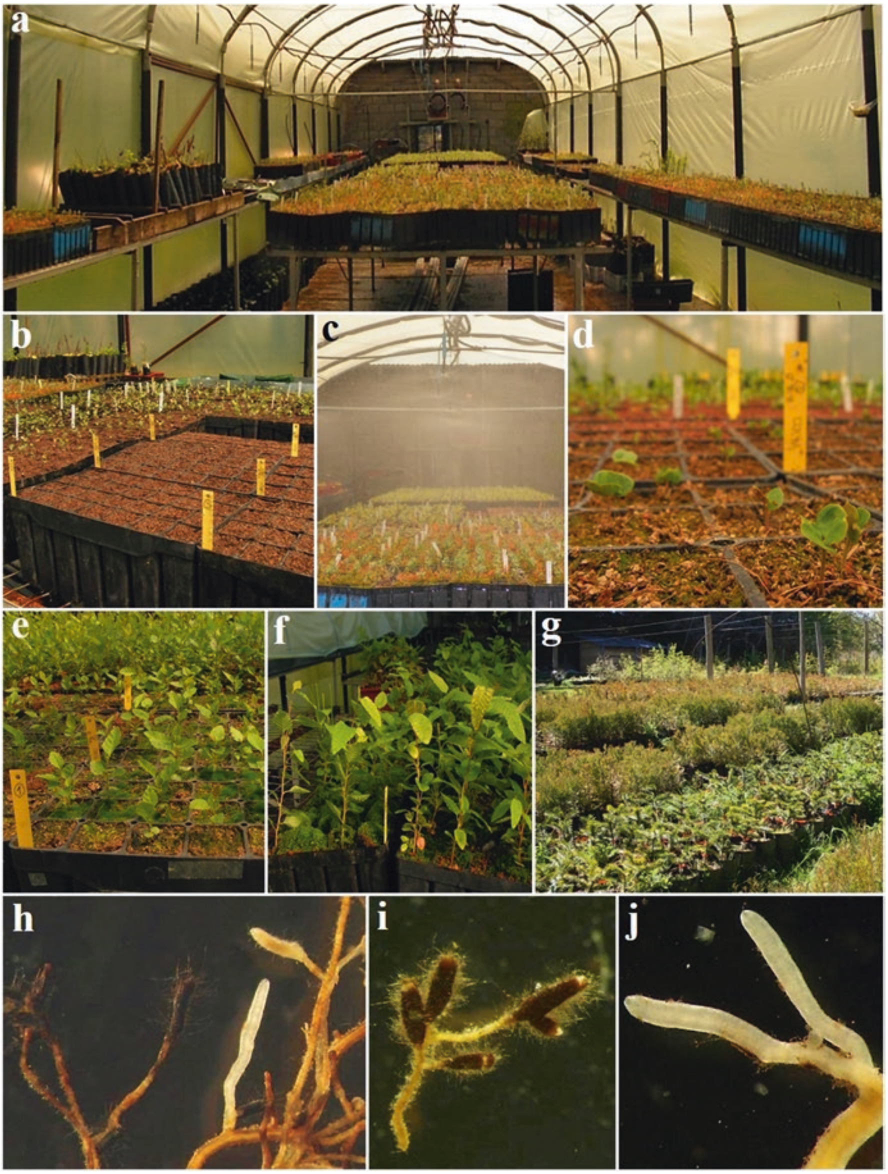

Fig. 14.2 Cultivation of *Nothofagus alpina* seedlings in the greenhouse (**a–f**) and soil bed (**g**) of the INTA EEA Bariloche forest nursery, and most abundant ectomorphotypes observed in their roots and formed by Ascomicetos EcM sp. 1 (**h, i**) and *Tomentella ellisii* (**h, j**). Photo's credit: Fernández N

seedlings' roots, and none of them corresponded to the inoculated species. The most abundant EcMF was *Thelephora terrestris* (Basidiomycota), while *Cenococcum geophilum* (Ascomycota) was registered in just a few root tips. Significant relationships between growth parameters and inoculation treatments were not detected. These results are in agreement with the abovementioned study (Fernández et al. 2013), in which only two EcMF were described in containerized seedlings, being the basidiomycetous species much more abundant than the ascomycetous. The unsuccessful inoculation of native EcMF in *Nothofagus* seedlings was most probably a consequence of their intolerance to fertigation and cultivation conditions in the greenhouse and/or to their incapacity of outcompeting EcMF normally present in forest nurseries and better adapted to these conditions (Barroetaveña et al. 2009). This is supported by the fact that Valenzuela et al. (2008) succeeded in inoculating the native EcMF *Descolea antarctica* (Basidiomycota) in *N. obliqua* seedlings grown without any kind of fertilizer. It is known that most of the techniques applied for intensive seedling production typically suppress or delay mycorrhizal colonization, particularly when high fertilization rates are used (Cram and Dumroese 2012; Fernández et al. 2013), so this information has to be considered for successful EcM inoculation during *Nothofagus* cultivation.

In a different study, Carron (2021) analyzed the EcM behavior of five *Nothofagus* species (*N. alpina*, *N. antarctica*, *N. dombeyi*, *N. obliqua*, and *N. pumilio*) cultivated in three different nurseries located in northern Patagonia, Argentina. These nurseries used different seed provenances and cultivation conditions (e.g., substrate, pot type and size, irrigation frequency, fertigation regimes). All the analyzed specimens had EcM despite not being artificially inoculated, and a total of 16 ectomorphotypes were recorded. Colonization percentages, EcMF richness, and diversity varied widely between the different nurseries. The nursery which produced the seedlings with the highest colonization values (72%) also presented the highest EcM richness. It was also noticed that EcM abundance and richness differed significantly not only between different *Nothofagus* species but also between seed provenances. Therefore, this study highlights the importance of contemplating plant material (e.g., seed provenance) in addition to nursery production conditions (e.g., substrate, fertigation) for managing the establishment and development of EcM in *Nothofagus* seedlings. Later on, some of these seedlings were transplanted into a native *N. antarctica* shrubland, and after four growing seasons, EcM abundance and diversity were analyzed again. Colonization values of the transplanted plants did not vary significantly between the nurseries and the field, but, as previously found by Fernández et al. (2013), EcM richness and diversity were much higher in seedlings growing under natural conditions.

According to the abovementioned studies (Barroetaveña et al. 2009; Fernández et al. 2013; Carron 2021), *Nothofagus* seedlings are naturally colonized by EcMF during their production in forest nurseries. It is possible that the EcMF inoculum is already present in the cultivation substrate or in the irrigation water, that it is caused by windblown spores, or even that resistant propagules remain in the greenhouse from one season to the other (Barroetaveña et al. 2009; Cram and Dumroese 2012; Fernández et al. 2013). It is also evident that colonization and diversity values of the

EcMF depend on the cultivation technique, as has been described for other nursery-cultivated forestry species (El Karkouri et al. 2005; Menkis et al. 2011; Rudawska and Leski 2021). It is important to consider that naturally established EcMF, such as *Th. terrestris*, *To. Ellisii*, and *Ce. Geophilum*, which have been found in forest nurseries worldwide, are often well adapted to intensive nursery systems (Cram and Dumroese 2012; Rudawska and Leski 2021) and might outcompete the inoculated species, probably not tolerant to these conditions. Since the effectiveness of artificial inoculation can vary greatly depending on the host plant, the inoculated fungi, and the environmental conditions in the nursery or planting sites, a careful selection and testing of particular mycorrhizal inoculants able to ensure a positive benefit-to-cost ratio is needed before operational use (Cram and Dumroese 2012). An alternative to artificial EcMF inoculation would be to manage the cultivation system in order to obtain better EcM development and higher seedlings' quality, for example, by reducing the amount of applied fertilizers so that more EcM are naturally formed, perhaps obtaining slightly smaller seedlings but with better adaptability to field conditions (Menkis et al. 2011; Cram and Dumroese 2012).

Altogether, this information emphasizes the importance of considering all the steps of the cultivation process to accurately manage the mycorrhizal symbiosis as an effective and sustainable way of producing high-quality *Nothofagus* seedlings. Although progress has been made in this regard for different *Nothofagus* species, more studies are urgently needed to identify beneficial EcMF species and favorable cultivation conditions to optimize the development of this symbiosis and seedlings' quality.

14.2.2 Description of Plant Traits and Ecosystem Processes

Nursery trials are also useful not only for describing the association between specific EcMF and certain forestry species but also for describing the effect of diverse factors on mycorrhizal development and its influence on plants' growth or survival. For example, morphological and anatomical description of the ectomorphotypes formed by *Laccaria laccata* (Basidiomycota) and *Paxillus involutus* (Basidiomycota) in *N. dombeyi* and *N. alpina*, respectively, was investigated in nursery-cultivated seedlings (Godoy and Palfner 1997). Alberdi et al. (2007) and Álvarez et al. (2009) carried out greenhouse experiments for evaluating the physiological response of *N. dombeyi* seedlings to inoculation with a specific native EcMF (*D. antarctica*) and a nonspecific cosmopolitan EcMF (*Pisolithus tinctorius*, Basidiomycota) under drought stress conditions. They concluded that the inoculation with these EcMF favored plant resistance to water deficit (Alberdi et al. 2007), stimulated plant growth, and increased foliar N and P concentrations, especially under drought stress conditions (Álvarez et al. 2009). Based on these results, they strongly suggested including EcM in afforestation and ecological *Nothofagus* restoration programs in order to optimize their production, reestablishment, and sustainability (Álvarez et al. 2009). In later studies, the importance of using EcM inoculation for carrying out restoration activities with *Nothofagus* seedlings was confirmed (Godoy and Marín 2019). It was observed that the inoculation of *N. alpina*, *N. obliqua*, and *N. pumilio* with *Ps. tinctorius* and/or *L. laccata* improved not only seedlings' growth in the nursery (Marín et al. 2018; Godoy and Marín 2019) but also their establishment and development in the field (Godoy and Marín 2019).

Due to the fertile volcanic soils and to the climatic similarities with the native range of different *Pinus* species (Bradford and Lauenroth 2006), large plantations with fast-growing exotic conifers have been successfully established in the Subantarctic Temperate Forest region. Despite being an important economic resource, the establishment of *Pinus* plantations has seriously impacted native ecosystems, causing significant alterations in soils' physical, chemical, and biological properties, with severe consequences on environmental processes and ecosystem services (Franzese and Raffaele 2017; Castro-Díez et al. 2019; Fernández et al. 2020). Besides, some *Pinus* species can easily spread outside the plantations, therefore becoming successful invaders (Franzese et al. 2016). Biological invasions are a serious global threat. Although it has been demonstrated that belowground invaders can impact ecosystem processes and determine aboveground communities' assembly, the investigation of biological invasions has been mainly addressed from an aboveground perspective (Policelli et al. 2020). Most of the EcMF identified in both, *Nothofagus* and *Pinus*, are basidiomycetes, but fungal communities in their roots are completely different. Members of the Cortinariaceae are the dominant EcMF taxa in *Nothofagus* (Nouhra et al. 2013; Fernández et al. 2015; Barroetaveña et al. 2019; Godoy and Marín 2019; Truong et al. 2019; Mestre and Fontenla 2021), but are not that frequent in *Pinus*. Among the most abundant EcMF in *Pinus*, especially in plantations, are *Rhizopogon roseolus* and *Suillus luteus*. These two EcMF species are native to the northern hemisphere and co-invaded with their hosts (Barroetaveña et al. 2007; Policelli et al. 2018). Therefore, the establishment of a *Pinus* plantation and their associated mutualists might significantly impact native fungal communities. In this context, Salgado Salomón et al. (2013) used a greenhouse bioassay to assess the occurrence and abundance of EcM in seedlings of four *Nothofagus* species (*N. alpina*, *N. antarctica*, *N. dombeyi*, and *N. obliqua*) grown in soils from pure *Nothofagus* forests, from pure *Ps. menziesii* plantations, and from *Nothofagus* forests invaded by *Ps. menziesii*. They found that seedlings grown in soils from pure *Nothofagus* forests had higher EcM colonization rates (57.8%), but those cultivated in soils from the plantations or from the invaded forests were still able to form abundant EcM (44.6 and 42.5%, respectively). Similarly, Policelli et al. (2020) used cultivation approaches for evaluating the growth and EcM colonization of *N. antarctica* and *Pi. contorta* seedlings cultivated in soils from pine-invaded or non-invaded forests. They observed that both tree species grew equally well and had high colonization rates (>60%) in both types of soil. However, it is important to mention that despite similar colonization values, the fungi forming EcM varied widely depending on the soil where each plant species was cultivated (e.g., in *N. antarctica* the most abundant EcMF found in seedlings cultivated in the soil from the invaded area -*Sistotrema* sp.- was not even present in those growing in the soil from the non-invaded area). Out of the 12 EcMF species registered in this work, only one was common for both tree species when cultivated into the soil from pine-invaded area (*Sistotrema* sp., Basidiomycota). This study revealed that the soil from native forests where the invasive host is still absent is already preconditioned for invasion by the presence of compatible EcMF, so the presence of native below-ground fungal mutualists is apparently not hindering the spread of the non-native species (Policelli et al. 2020).

The fact that *Nothofagus* species are able to form EcM in soils from *Pinus* plantations has been registered in some other studies also using cultivation approaches. For example, Fioroni (2020) evaluated in a nursery experiment how plant growth and mycorrhization varied in *N. obliqua* and *Pi. ponderosa* seedlings depending on three different cultivation aspects: (1) soil origin: native *Nothofagus* forest *vs Pi. ponderosa* plantation, (2) cultivation type: monospecific (two *N. obliqua* or *Pi. ponderosa* seedlings in the same pot) *vs* mixed (one seedling of each plant species in the same pot), and (3) application (or not) of a commercial EcM inoculant (Ectovit, Symbiom®, Czech Republic). This inoculant is suitable for most coniferous and some deciduous trees, as those included in the Fagaceae, which are closely related to the Nothofagaceae. It was observed that both species grew better in the forest soil, most probably as a consequence of its higher nutrient content. When cultivated in the plantation soil, seedlings' growth was higher in mixed cultures than in the monospecific ones, being this information promising for considering the establishment of mixed plantations that could replace, at least in part, Pinaceae monocultures. The application of the commercial EcM inoculant usually favored the development of *Pi. ponderosa* seedlings but negatively affected *N. obliqua*, mainly in mixed cultures. In contrast to what was observed by Policelli et al. (2020), Fioroni (2020) found for both plant species that EcM colonization was higher when cultivated in its soil of origin (native forest or plantation soil for *N. obliqua* and *Pi. ponderosa*, respectively). Communities of EcMF differed significantly not only between plant species but also for the same species cultivated in the different types of soil. The most abundant EcMF in *N. obliqua* roots grown in the forest soil were *Ruhlandiella patagonica* (Ascomycota) and Sordariomycetes sp. 1 (Ascomycota) for monospecific and mixed cultures, respectively, while in the plantation soil, it was *To. ellisii*. In the case of *Pi. ponderosa*, the most abundant EcMF in the seedlings cultivated in the native forest soil were *Su. luteus*, *Wilcoxina mikolae* (Ascomycota), and *Meliniomyces bicolor* (Ascomycota), but in the plantation soil, EcMF dominance varied widely between the different treatments. In summary, the three evaluated factors influenced EcM abundance and EcMF composition in both hosts. Other important findings in this study were that: (1) *Ru. patagonica* was first described as forming EcM in *Nothofagus* roots (it had been found only in soil samples, Kraisitudomsook et al. 2019; Nohura et al. 2019), and it improved *N. obliqua* seedlings' growth; and (2) some EcMF were found simultaneously in roots of both forestry species when cultivated in the plantation soil (*To. ellisii*, *Tuber* sp. 1, and one species of Pyronemataceae), demonstrating that they can harbor common fungal symbionts under specific environmental conditions (Fioroni 2020).

Fire is one of the most important recurring disturbances in Subantarctic Temperate Forests, and it might favor *Pinus* establishment and invasion (Franzese and Raffaele

2017). In a recent study, Soto-Mancilla (2022) carried out a greenhouse experiment for investigating how fire intensity and soil origin affect the growth and EcM colonization of recently established *N. antarctica* and *Pi. contorta* specimens. Seedlings of both species were grown in two types of soil (native scrubland *vs* pine plantation), which were subjected to experimental burning of different intensities (High = 900 °C, Low = 500 °C, and Control = no burning). In *Pi. contorta*, the aerial biomass of the seedlings growing in the plantation soil was almost double than in those cultivated in the forest soil, but no differences were observed with respect to fire intensity. On the contrary, the aerial biomass of *N. antarctica* seedlings was almost the same for both soil types but tended to increase in burned soils, especially with high intensity burning. Regarding EcM, and unlike what was observed by Fioroni (2020), colonization values were always above 70% for both species. In *N. antarctica*, EcM colonization increased as the intensity of the fire also increased, while this trend was not observed for *Pi. contorta*. Interestingly, a positive correlation between aerial biomass and EcM colonization was observed for the native species, being this correlation negative for *Pi. contorta*. In brief, *N. antarctica* seems to be quite resilient to this disturbance, and no evidence that fire favors the establishment and growth of recently established *Pi. contorta* seedlings was found (Soto-Mancilla 2022).

As demonstrated above, nursery trials can be used to evaluate the effect of diverse factors on *Nothofagus* establishment and development, their relationship with other forestry species, and the dynamics of mycorrhizal communities in their roots. Thus, nursery trials are an extremely useful tool and the information obtained from them complements what is observed in natural ecosystems, thus contributing to the overall understanding of this symbiosis.

14.3 Mycorrhizas in *Nothofagus* From Natural and Managed Ecosystems

14.3.1 Types of Mycorrhizas in Nothofagus

Plants belonging to the same genus usually have the same type of mycorrhiza. However, some species can harbor more than one mycorrhizal type, either simultaneously or at different life stages or environments. These plants are known as dual hosts (Smith and Read 2008; Teste et al. 2020). This phenomenon was observed among important forestry species of the genera *Eucalyptus*, *Quercus*, *Pinus*, *Tsuga, Pseudotsuga, Populus*, and *Salix*, in which both EcM and arbuscular mycorrhizas (AM) were described (Wang and Qiu 2006; Teste et al. 2020). In the case of *Nothofagus*, structures resembling AM colonization (i.e., typical arbuscules and coils) have not been found, even though several researchers have looked for them in different life stages and environmental conditions (Barroetaveña *com. pers.*; Salgado Salomón et al. 2013; Fernández et al. 2013, 2015; Fioroni 2020). There is one exception, in which an AMF species (*Glomus* sp.) was registered in a unique *N. dombeyi* tree but only based on molecular methods (Bidartondo et al. 2002), which does not exclude the possibility of having amplified AM hyphae firmly attached to the roots. Consequently, and based on the available information, we consider *Nothofagus* species as exclusively ectomycorrhizal.

14.3.2 Natural and Anthropogenic Factors Influencing **Nothofagus** *Ectomycorrhizas*

There are many abiotic and biotic factors that influence different aspects of the EcM symbiosis. Different researchers have investigated how several environmental factors affect EcM dynamics in *Nothofagus* forests. For example, it has been described that both temperature and precipitation strongly affect EcMF richness. In North Patagonian forests, Nouhra et al. (2012) observed that species richness and sporocarp biomass of hypogeous EcMF in *N. pumilio* and *N. dombeyi* forests were positively correlated with precipitation but negatively correlated with altitude. Nouhra et al. (2013) also found that altitude has a significant effect on EcMF community structure, even along a narrow altitudinal range (700 m). On the contrary, Truong et al. (2019) determined in *N. pumilio* forests from Tierra del Fuego (Argentina and Chile) that EcMF community shifts were mainly mediated by soil pH, while elevation had no significant effects. One of the possible reasons for this discrepancy is that the elevation range considered in this last work was lower than in the previous ones (~500 m or less).

Another important factor of analyses has been seasonality, being the obtained results variable between *Nothofagus* species and seasons. For instance, species richness of hypogeous EcMF sporocarps of *N. dombeyi* and *N. pumilio* forests did not vary in relation to season (autumn and spring), but biomass was consistently greater in autumn for *N. dombeyi* and in spring for *N. pumilio* (Nouhra et al. 2012). Richness and diversity of EcMF in *N. alpina* roots was also found to be higher in autumn than in spring, and the relative abundance of most of the identified EcMF species changed seasonally (some EcMF were more abundant in spring and others in autumn) (Fernández et al. 2015). However, the opposite tendency was registered in some *N. pumilio* forests, where EcMF diversity tended to be higher in spring than in autumn (Longo et al. 2011). In a *N. antarctica* shrubland, abundance of EcMF in the soil was higher in summer than in autumn, and despite richness and diversity did not vary between seasons, fungal composition was significantly affected by seasonality. For example, Ascomycetes predominated in autumn, while Basidiomycetes were more abundant in summer (Carron et al. 2020). Evidently, there is not a common or regular pattern of seasonality on *Nothofagus* EcM, which might be related to the fact that different methods of analyses (e.g., fruit body collection, ectomorphotype quantification and identification, metabarcoding approaches) on distinct *Nothofagus* species and environments were used. Much more studies using similar experimental approaches in greater geographical and temporal scales are needed to finally elucidate if there is (or not) a clear effect of seasonality on this important symbiosis.

The impact of different natural and anthropogenic disturbances on *Nothofagus* EcM has also been worthy of consideration. A few field studies have been carried out to describe the impact of forest fires on *Nothofagus* EcM. Palfner et al. (2008) determined that EcM abundance in *N. alpina* seedlings was quite similar between burned and unburned sites, but EcM richness tended to be higher in the last. Besides, in burned sites, there was a striking dominance of *D. antarctica* (43.5% out of the total number of analyzed root tips), an EcMF species that is especially abundant on seedlings or young trees growing at disturbed or ruderal sites (Palfner et al. 2008). Longo et al. (2011) evaluated the long-term effect of fire on EcM colonization and ectomorphotype richness in roots of *N. pumilio* adult trees in three areas differing in precipitation regime (1500–1750 annual mm) and fire age (6–10 years). They found that fire might impact EcM communities and that the effects are mostly context dependent, varying according to different forest sites, fire age, and seasons. Despite these authors strongly suggested to carry out further studies to understand patterns and mechanisms regarding the effects of forest fires on belowground communities (Longo et al. 2011), as far as we know, no other studies have been performed on this topic, so much work is still needed. The fact that climate change predictions foreseen an increase in the frequency of forest fires causes concern for the future of Subantarctic Temperate Forests (Marchelli et al. 2021) and highlights the urgently need to address this type of studies in brief.

Volcanic eruptions are another relatively common disturbance in Subantarctic Temperate Forests, being tephra fall and deposition one of the most widespread effects. After the astonishing eruption of the Puyehue-Cordón Caulle volcanic complex in 2011, the natural regeneration and development of *N. pumilio* seedlings and associated EcM were analyzed in the first two growing seasons after the eruption by Moguilevsky et al. (2021). It was observed that only 40% of the six-month-old seedlings directly germinated on the tephra had EcM, being colonization values (<12%) significantly lower than in non-affected sites (80%). However, all the 18-month-old seedlings growing on thick tephra deposits were as colonized by EcM as seedlings in sites without tephra, but diversity and community composition of EcMF differed significantly between these sites. *Cortinarius* and *Inocybe* species where only found in seedlings from the forest without tephra accumulation, while other EcMF were present only in those growing in the tephra, such as *Aleurina echinata*, *Th. Terrestris*, and *To. elisii*. The latter two, also registered in nursery cultivated *Nothofagus* seedlings (Barroetaveña et al. 2009; Fernández et al. 2015; Fioroni 2020), are known as pioneer mycobionts that may be displaced by other EcMF in competitive natural environments, such as natural forest soils. The early and increasing EcM colonization found in seedlings growing on thick tephra deposits supports the strong dependence of *Nothofagus* on this symbiosis and highlights its importance as an adaptive mechanism under stress conditions (Moguilevsky et al. 2021).

Nothofagus forests also constitute an important economic resource. In the last decades, priority conservation zones have been defined, and new silviculture techniques have been implemented in order to achieve greater forest sustainability. One of these attempts was the implementation of "variable retention managements", which consists of leaving intact patches of primary or secondary forest within a harvested area, where a few trees (~20%) remain as seed trees, regeneration shelter, or for conservation purposes (Hewitt et al. 2018). When EcM colonization and EcMF diversity were studied in these managed ecosystems, no significant differences were observed between primary forests and intact patches, but they were significantly lower in deforested areas, where some EcMF species were very scarce or absent. These findings emphasize the negative effect of this intensive practice on EcM and support the idea that intact patches act as refugee for EcMF diversity more effectively than individual trees in deforested areas (Hewitt et al. 2018). Similarly, Dickie et al. (2009) observed that timber harvesting reduced the presence of EcM in roots and caused significant shifts in fungal communities in *Nothofagus* rainforests, where some *Laccaria* species tended to increase in harvested areas while *Russula* species decreased in abundance. In *N. betuloides* forests, it was described that the higher the disturbance, the higher the soil fungal richness and abundance of plant pathogens and the lower the richness and abundance of ectomycorrhizal and saprophytic fungi (Marín et al. 2017). Considering that mycorrhizas are directly related to vegetation dynamics and several ecosystems' processes and services, this information should be seriously taken into account for outlining sustainable management programs.

In addition to the previously mentioned experiments addressing the interaction between *Nothofagus* and *Pinus* and their EcM (Salgado-Salomón et al. 2013; Fioroni 2020; Soto-Mancilla 2022), it was determined in the field that soil fungal communities associated with roots of *N. alpina* plants implanted beneath a native *Nothofagus* forest and a *Pi. ponderosa* plantation are completely different: Basidiomycetes are the dominating fungal taxa in the native forest and Ascomycetes in the plantation (Fernández et al. 2020). Furthermore, Fernández et al. (2020) determined that EcM colonization rates as well as richness and diversity of EcMF were significantly lower in *N. alpina* individuals established under the *Pinus* plantation with respect to those growing in the native forest (both planted and naturally established). Out of the 26 EcMF identified in this study (Fig. 14.3), only four were present in *N. alpina* individuals implanted under the *Pinus* plantation, and no common fungal species between this environment and the native forest were registered (Fernández 2012). It was also observed that *N. alpina* trees growing in the plantation were much shorter and had a less developed root system. This study demonstrated that despite of being able to establish and grow in ecosystems completely dominated by a *Pinus* species, the conditions for *N. alpina* to establish beneficial EcM are probably not optimal, which might directly influence its development and productivity (Fernández 2012).

Dispersal is another critical factor in the ecology of EcM. Dispersal limitation has been shown to have strong impacts on diversity patterns of mycorrhizal fungi and consequently on their plant hosts. Many EcMF produce fruiting bodies to disperse their spores. Some of them grow aboveground (epigeous mushrooms), shoot their spores into the air, and rely on wind to disperse, but in this case, most spores

Fig. 14.3 Most abundant ectomorphotypes found in *Nothofagus alpina* specimens analyzed in the native forest (**a–j**) and ectomorphotype formed by *Ruhlandiella patagonica* in roots of *N. obliqua* seedlings cultivated in the soil of the same forest (**k**). Some of these ectomorphotypes have also been found in other *Nothofagus* species, such as ectomorphotypes (**j**), (**i**), and (**j**) in *N. obliqua* and *N. dombeyi* and *R. patagonica* in roots of *N. antarctica* and *N. alpina.* Photo's credits: Fernández N, (**a**–**j**) and Fioroni F, (**k**)

travel only a few meters from their source, so long-distance dispersal is relatively rare. Other EcMF produce enclosed belowground fruiting bodies (hypogeous fungi, such as truffles) and have to rely on animals that eat them for spore dispersal. Animal mycophagy is an important process that increases the chances of EcMF, both epigeous and hypogeous fungi, to disperse far from the source. This dispersal mechanism has been mainly studied in mammals from the northern Hemisphere. Insects and other invertebrates have also been occasionally shown to disperse fungal spores (Caiafa et al. 2021 and references within). In Patagonia, it was observed that exotic EcMF associated with *Ps. menziesii* and usually dispersed by invasive mammals (mainly wild boar and deer) are present even in sites where these animals have been historically absent, so it becomes evident that alternative dispersal mechanisms are operating in their dispersion (Policelli et al. 2022). In this sense, Caiafa et al. (2021) have recently shown that two endemic Patagonian birds, chucao (*Scelorchilus rubecula*) and black-throated huet-huet (*Pteroptochos tarnii*), regularly consume and disperse copious viable spores from hypogeous and other EcMF. These findings indicate that birds may act as cryptic but critical fungal dispersal agents not only in Patagonia but also in other ecosystems.

14.3.3 Ectomycorrhizal Fungal Diversity in Native **Nothofagus** *Forests*

The Southern Hemisphere harbors many unique fungal lineages that are absent from the Northern Hemisphere (Tedersoo et al. 2014). In South America, great efforts have been made to describe the structure and diversity of EcMF communities in *Nothofagus* forests. There is a general consensus that in these forests soil fungal communities are extensively dominated by Basidiomycetes (Fernández et al. 2020; Mestre and Fontenla 2021). This is also true for EcMF, either in the soil (Truong et al. 2017), roots (e.g., Nouhra et al. 2013; Fernández et al. 2015; Fioroni 2020; Mestre and Fontenla 2021; Soto-Mancilla 2022), and fruit body occurrence (Nouhra et al. 2012). EcMF communities from Subantarctic Temperate Forests are relatively diverse in terms of taxonomic groups, mostly involving members of the /cortinarius in the first place, followed by /inocybe, /ramaria-gautieria, /tomentella-thelephora, /clavulina, and /tricholoma lineages. The /russula-lactarius lineage is remarkably species poor compared with almost every other EcM region of the world, whereas the /suillus-rhizopogon, /boletus, and /pisolithus-scleroderma lineages are almost completely absent, which is a unique pattern at the global scale (Barroetaveña et al. 2019; Nouhra et al. 2019). These tendencies have been observed even using different experimental approaches, such as sporocarp collection (voucher specimens) or OTU amplification from colonized root tips and soil samples (Fig. 14.4). The overall EcMF community of these South American forests is relatively similar to that of other *Nothofagus*-dominated forests in New Zealand and Australia (Nouhra et al. 2013; Truong et al. 2017; Nouhra et al. 2019). Even more, the Subantarctic Temperate Forests harbor a prominent set of exclusively Gondwanan taxa that includes at least five lineages: /aleurina, /descolea, /austropaxillus, and /porpoloma, as well as species of *Underwoodia* (an exclusively Southern Hemisphere branch of the /tuber-helvella lineage) (Truong et al. 2017; Nouhra et al. 2019).

It is interesting to mention that there are many common EcMF between different *Nothofagus* species (Fig. 14.3). Palfner (2001) described 15 EcM morphotypes, associated with natural evergreen and deciduous *Nothofagus* forest in Southern Chile and Argentina and found no significant differences between the EcMF communities of these distinct forest types. More recently, the biodiversity and community composition of EcMF were simultaneously assessed in

N. dombeyi, *N. obliqua*, and *N. alpina* forests, and no significant differences between these forests types were neither noticed (Nouhra et al. 2013). This is because some of the most common and abundant mycobionts, such as species of *Cenococcum*, *Clavulina*, *Cortinarius*, *Inocybe*, and *Tomentella*, were found in association with the different *Nothofagus* species. Barroetaveña et al. (2019) also showed that *Nothofagus* species share several EcMF but stated that *N. dombeyi* had the highest number of unique EcM species, followed by *N. pumilio*, *N. antarctica*, and *N. obliqua.* One example of this is the EcMF *Co. pyromyxa* that was found across a wide latitudinal range of Subantarctic Temperate Forests but always in association with the evergreen *N. dombeyi* (Lam et al. in review). This type of comparative studies has laid the basis to preselect some EcMF that could be of biotechnological importance, such as *Au. statuum*, *Co. fragilis*, *Co. xiphidipus*, and *Ha. purpurea* (all Basidiomycetes), which have been proposed by Barroetaveña et al. (2019) as candidates for nursery spore inoculations and to further scientific evaluation. We would like to include in this list the ascomycetous fungi *Ru. patagonica* (Figs. 14.3k and 14.4c), which have been found in roots of *N. antarctica* (Soto-Mancilla 2022), *N. obliqua* (Fioroni 2020), and *N. alpina* (Fernández et al. 2013 where it was named as *Peziza* sp. 2) and seems to improve seedlings' growth (Fioroni 2020). Further associations between specific EcMF and hosts are likely waiting to be discovered as more molecular-based studies address the EcM communities of Subantarctic Temperate Forests (Nouhra et al. 2019).

Studies based on environmental sequences have detected in *Nothofagus* forests several previously unknown fungal lineages, thereby demonstrating that fungal diversity is probably much higher than presently known. For example, Truong et al. (2017) revealed surprisingly high species diversity in some fungal lineages where only a few species have been previously described from South America, such as /austropaxillus and /descolea, and detected relatively low diversity in several EcM lineages that are hyperdiverse in other regions of the world (e.g., /amanita, /boletus, /russula-lactarius) (Fig. 14.4). It was also observed that most of the obtained OTUs (46%) matched sequences originating from South America, but many others (20%) had their closest match with sequences from Australasia, highlighting both the scarcity of sequences from South America and the historical biogeographic connection of these two regions. This biogeographic pattern was particularly striking within some fungal lineages, as *Ruhlandiella*, *Amylascus*, and *Gymnohydnotrya* (Pezizales), since members of these genera were so far known only from Australasia (Truong et al. 2017).

Recently, there has been significant efforts to sample and analyze the global distribution of soil and root-inhabiting fungi at a global scale. The pioneer study on this topic was carried out by Tedersoo et al. (2014) and included 365 sites worldwide. Other studies have been performed since then, including some focused on mycorrhizas (e.g., Soudzilovskaia et al. 2015; Truong et al. 2019). Evidence on how well represented are the EcMF from South American *Nothofagus* forests could be estimated by analyzing three sources of available, highly used, global datasets that specifically target soil fungi: GlobalFungi (Větrovský et al. 2020), Global Soil

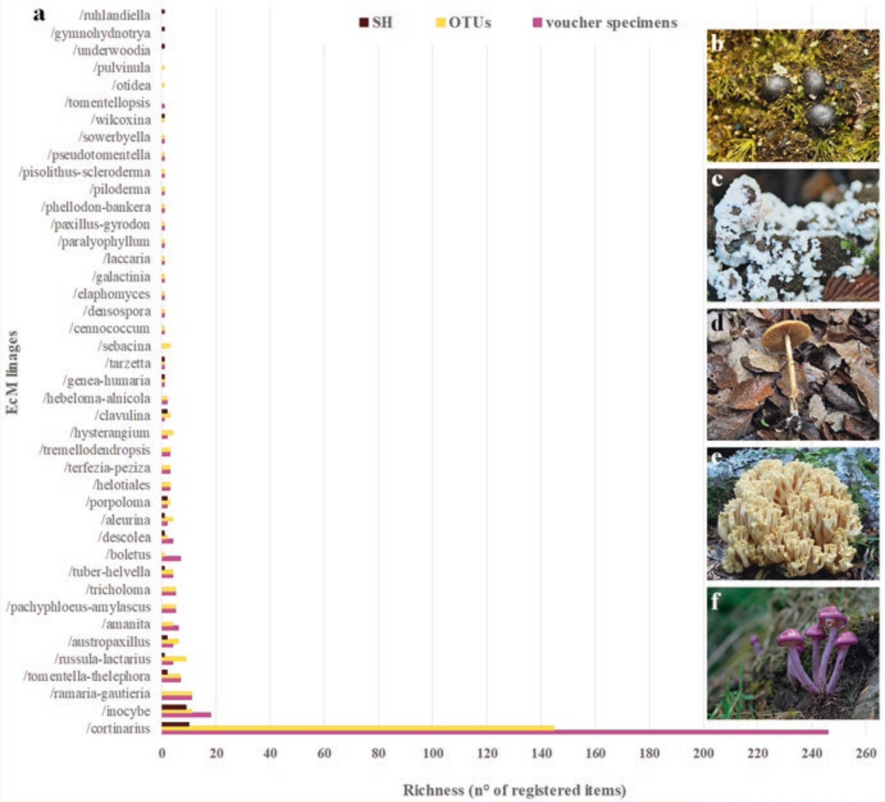

Fig. 14.4 Ectomycorrhizal fungal richness described in *Nothofagus* forests from Argentina and Chile using different experimental approaches: collection of vouchered specimens or molecular and OTUs recovered from EcM roots (data provided by Nouhra E and modified from Nouhra et al. 2019) in addition to the assignment of species hypothesis (SH) on the FungalTraits database (Põlme et al. 2020). Some of the most important ectomycorrhizal fungal species in Subantarctic Temperate Forests from Argentina and Chile: (**b**) *Cenococcum geophilum*, (**c**) *Ruhlandiella patagonica*, (**d**) *Descolea antarctica*, (**e**) *Ramaria patagonica*, (**f**) *Cortinarius magellanicus*

Mycobiome (Tedersoo et al. 2021), and FungalTraits (Põlme et al. 2020). The first one includes all published data on fungal community composition and biodiversity obtained by next-generation sequencing (Větrovský et al. 2020). It allows to obtain different fungal species hypothesis and filter them by geographic region, taxa, and/or sequence. On its third release, this database contained 36,684 samples from 367 studies, and only 3.1% of them were from South America. Among these samples, 222 were collected in *Nothofagus* forests and correspond to only three studies (Tedersoo et al. 2014; Marín et al. 2017; Truong et al. 2019). The Global Soil Mycobiome dataset (Tedersoo et al. 2021) includes information from more than 3200 plots in 108 countries, using standardized and throughout sampling designs and PacBio sequencing of the full-length ITS and 18S-V9 variable regions, which allows a much deeper taxonomic classification with respect to previous methods. Currently, this dataset comprises a total of 905,841 OTUs (Tedersoo et al. 2022). Within this dataset, there is information obtained from 47 plots located in broadleaf forests from Argentina and Chile. A preliminary analysis of this data shows that there is an extremely high endemicity of EcMF present in *Nothofagus* forests as compared to the rest of South America biomes (Nouhra et al. 2019; Tedersoo et al. 2022), thus being this biome one of the most endemic for this fungal guild in the whole southern hemisphere. The third database, FungalTraits (Põlme et al. 2020), contains information on functional assignments and traits of a total of 95,227 species hypothesis, but only 776 (0.84%) have *Nothofagus* as the interacting plant taxon, and just 310 of them were sampled in Argentina and Chile. A total of 146 of these species hypothesis was functionally classified as unspecified/unassigned (47.1%), 61 as different types of saprotrophs (19.7%), and 82 (25.5%) as EcMF, mainly corresponding to the genera *Cortinarius* and *Inocybe* (ten and nine species hypothesis, respectively; Fig. 14.4).

Although environmental sequencing can rapidly detect diversity and elucidate ecological patterns, these approaches depend on informative sequence databases for fungal identification (Truong et al. 2017). In studies using molecular tools to asses EcMF (and other fungal) communities in *Nothofagus* forests, many of the sequences cannot be identified at a meaningful taxonomic level. This is because there is still a lack of accurate taxonomic information on the immense fungal diversity inhabiting these forests (Truong et al. 2017; Salgado-Salomón et al. 2021). Despite it is known that it is critical to compile and validate high-quality environmental sequences, it is also important to consider that "traditional" methods are irreplaceable and complementary to "next-generation" approaches. Consequently, not only barcoding herbarium collections but also high-throughput collect-and-sequence inventories are considered highly effective to document fungal diversity and are instrumental for future studies of plant-fungal symbioses. Besides, herbarium vouchers provide much more than just DNA barcodes, and fresh well-documented specimens remain critical to reconstruct robust phylogenies, linking sequence data to morphology, and supply ecological information on hosts and substrate associations. Specimens can also be used for genomic studies in a way that environmental samples cannot (Truong et al. 2017 and references within). Therefore, we agree with Truong et al. (2017), who stated that given the increasing threats to biodiversity due to habitat loss and climate change, collecting vouchered specimens and associated data, and sharing these resources are more necessary today than ever before.

14.4 Conclusion

The conspicuous and intrinsic relationship between *Nothofagus* species and their EcM is indisputable, as well as the fact that there are diverse biotic and abiotic factors influencing EcM formation and EcMF diversity. In this chapter, we aimed to show not only the importance of carrying out field studies based on different methods of analysis but also the relevance of performing nursery trials under controlled conditions, which complement what can be found or described in natural ecosystems. Together, both experimental approaches provide valuable information on the ecology of this symbiosis in *Nothofagus* forests and the potential application of EcMF as biotechnological tools.

Although much progress has been made in the study of EcM occurrence and dynamics in different *Nothofagus* species and forest types, either natural or managed, there is still a long way to go. On the one hand, Subantarctic Temperate Forests have been relatively little studied in relation to other forest systems in the world. On the other hand, there are some phenomena of great importance at the landscape and ecosystem scale (such as fires, volcanic eruptions, or biological invasions) that severely affect these forests not only aboveground but also belowground. The "belowground perspective" of these high-magnitude disturbances has been quite overlooked, thus deserving further attention and investigation in the near future. Studies at larger geographic and temporal scales and using similar experimental approaches are also needed to elucidate common ecological and biogeographical patterns and to identify how various factors associated with global change might influence EcM and their host plants' distribution. This information would be essential to make future projections and identify possible strategies for mitigating climate change, restoring degraded environments, optimizing assisted migration of forestry species, and improving programs of sustainable forestry production and management.

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Chapter 15
Arbuscular Mycorrhizal Fungi in the Espinal Ecoregion, Gaps, and Opportunities

Emanuel Ontivero and Mónica A. Lugo

Contents

15.1 Forest and Espinal Ecoregion

United Nations Convention on Biological Diversity (UN-CBD 2006) defined forest as a land area of more than 0.5 ha, with a tree canopy cover of more than 10%, which is not primarily under agriculture or other specific non-forest land use. In the case of young forest regions where tree growth is climatically suppressed, the trees should be capable of reaching a height of 5 m in situ and of meeting the canopy cover requirement (Chazdon et al. 2016). Forests are ecosystems formed by plant communities that host great biodiversity not only of plant species but also of animals and microorganisms; hence, they are a critical reservoir of the world's biodiversity and home to most of the terrestrial biodiversity of our planet (MEA 2005; FAO 2020).

On the Earth, these ecosystems occupy a total area of 4.06 billion hectares, and about 1.6 billion people survive thanks to the supply of services they obtain from forests. Despite the importance of these biomes, deforestation and forest degradation continue to occur at an alarming rate, contributing significantly to the loss of

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biodiversity. It is estimated that in the last three decades, 420 million hectares of forest have been lost due to conversion to other land uses. Agricultural expansion remains the main driver of deforestation and forest degradation. Large-scale commercial agriculture accounted for 40% of tropical deforestation between 2000 and 2010 and local subsistence farming for another 33% (FAO 2020). This conversion causes the loss, fragmentation, or degradation of available habitats for most species and is therefore one of the main threats to biodiversity worldwide (Guida Johnson and Zuleta 2013).

South America, like a large part of the Latin American countries, is not exempt from the consequences of the conversion and degradation of forest ecosystems. In Argentina, the increase in the world population and the growing global demand for products were some of the main forces that drove forestry and agricultural changes in the last 150 years (Risio Allione 2016). In this scenario of overexploitation and misuse of resources, a territory with high current or potential agricultural productivity and low population, such as central Argentina, experienced a drastic change in its forest ecosystems and land uses as occurs in the Espinal areas (Viglizzo and Jobbágy 2010).

The Espinal is an Ecoregion of the Argentine Chaco-Pampean plain, which occupies a wide arc of forests surrounding the Pampa Ecoregion to the North, West, and South, with an area of 291,941 km^2. It covers the south of Corrientes province, the northern half of the Entre Ríos province, a central strip of the provinces of Santa Fe and Córdoba, the center and south of San Luis province, the eastern half of La Pampa province, and the south of the province of Buenos Aires (Matteucci 2018). The predominant landscape is flat to gently undulating plain and, to a lesser extent, low mountains, on loess and sandy soils. Given the great extension of the Ecoregion, especially in a North-South direction, it includes several climatic types. In the North, it is hot and humid, while in the South and West, it is temperate and dry with marked water deficits (Matteucci 2018). The soils were formed on loess sediments of lacustrine, marshy, and alluvial origin and are determined by humid, subtropical to temperate climatic conditions, in a variable humidity regime of udic or aquic type. The dominant soils are clayey, and the drainage is generally moderate to imperfect. Given the large extension of the Ecoregion, there are a variety of Orders and Great Groups represented. Among the Orders, the Mollisols, Vertisols, and Alfisols stand out in the Northern Complexes, with somewhat more humid and warm climates; Entisols and Aridisols in the Southeast Complexes, with drier and cooler climates; and sandy soils (Matteucci 2018).

The vegetation is a combination of tree or shrub species. Mimosoid legumes predominate with xerophytic characteristics and an herbaceous stratum dominated by tussock grasses. The dominant types of vegetation are the forest, which in some cases occupies a considerable area, and the savannah, from very open to park-like (Oyarzabal et al. 2018). The typical zonal vegetation is represented by the open sclerophyll forest, dominated by species of the *Prosopis*, with a height of up to 10 m, and by the savannah, with a matrix of megathermal or mesothermal grasses depending on the latitude (Lewis and Collantes 1973; Cabrera 1976; Oyarzabal et al. 2018). Along the Espinal Ecoregion, there are important physiognomic and composition variations of plant species, due to factors such as its biogeographical transition situation, the heterogeneity of the relief and soils, and economic activity, which produces clearing, alterations of the natural fire regime, the introduction of exotic species, and selective forest extraction. As a consequence, forests now form intricate and heterogeneous mosaics with patches of a range of seral states and crop plots (Matteucci 2018). In the Ecoregion, there are some 93 endemic species, *Prosopis caldenia* Burkart ("caldén"), which is endemic to Argentina, and *Condalia microphylla* Cav ("piquillín"), *Senecio subulatus* D. Don ex Hook. & Arn. ("romerillo"), *Gaillardia megapotamica* (Spreng.) Baker (buttercup), among others (SAyDS 2007a). Due to biogeographical and ecological differences, the Espinal Ecoregion has been subdivided into three floristic districts, according to the dominant tree species: Ñandubay District, Algarrobo District, and Caldén District (Cabrera 1976; Matteucci 2018) (Fig. 15.1).

The forests of the Espinal region have been altered mainly as a result of the expansion of the agricultural frontier and the unsustainable use of forest resources, which caused a significant reduction in the area occupied by them (SAyDS 2007a). At the beginning of the colony, in the mid-1800s, with the sole presence of indigenous settlers, the natural environment did not suffer significant alterations. At the beginning of the so-called desert campaign, the settlers begin to take over vast regions of the Espinal, establishing the first agricultural exploitations (SAyDS 2007b). During the end of the eighteenth century and the beginning of the nineteenth century, the installation of the railway caused the indiscriminate extraction of timber vegetation to provide the railway constructions with fuel and sleepers, as well as the delimitation of livestock and agricultural fields that require the use of wood suitable for poles, corrals, and other rural installations causing, even today, the excessive felling of *Prosopis* due to its rotten wood. Fire, used in many cases as a tool for managing natural pastures, can get out of control and cause fires with negative effects on forest formations and natural pastures. Starting in 1950, the agricultural frontier expanded from the humid pampas to the interior, causing extensive clearing in areas of unstable ecological balance due to their scarce water resources (DB-SAyDS 2003).

Deforestation often involves the removal of plant biomass through logging of high-value wood trees and slashing and burning of low-value trees before consolidation into cattle ranching operations or mechanized agriculture with highly disturbed soils. This results in loss of soil organic matter and nutrients and changes to soil physical properties that disrupt resource supply and habitat suitability to a variety of soil organisms (Neill et al. 1997; Garcia-Montiel et al. 2000; Cerri et al. 2004; Smith et al. 2016). Consequently, deforestation can dramatically alter the structure of soil communities (Crowther et al. 2014), commonly through the loss of specialist species (Mueller et al. 2016), which in turn leads to decreased functional diversity and functional homogenization (Clavel et al. 2011; Nordén et al. 2013; FAO et al. 2020).

Arbuscular mycorrhizal fungi (AMF) (phylum Glomeromycota; Schüßler et al. 2001) comprise one of the most ubiquitous groups of soil microbes (Dickie et al. 2014). AM fungi live in symbiosis with the roots of about 80% of terrestrial plant

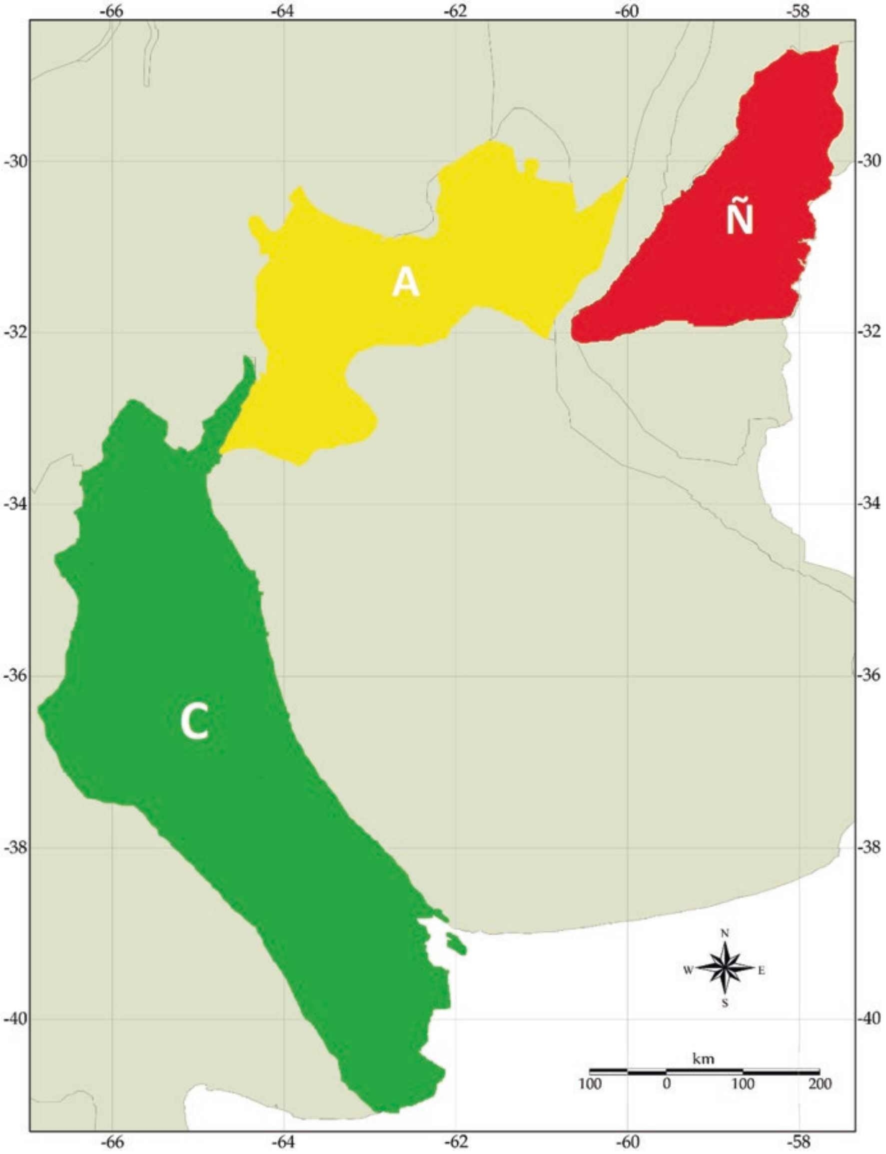

Fig. 15.1 Districts belonging to the Espinal Ecoregion. References: Ñ: Ñandubay District, in red; A: Algarrobo District, in yellow; C: Caldén District, in green. Image modified from the original image by Silvia D. Mateucci, available free at Morello et al. (2018)

species (Smith and Read 2008) and provide nutrients (mainly P and N) to their host plants in exchange for plant-assimilated carbon. AMF alleviate plant abiotic stress and can increase plant resistance to pathogens (Smith and Read 2008; Pozo et al. 2015). Knowledge is increasing on the geographic distribution and community ecology of these fungi (García de León et al. 2018). Therefore, the objectives of this chapter include the review of the data available in the literature on the AMF communities registered in the Espinal Ecoregion and the determination of differences between the AMF communities present in the different Espinal Districts.

15.2 Arbuscular Mycorrhizal Fungi

The arbuscular mycorrhizal fungi (AMF, phylum Glomeromycota) are a relevant group of soil fungi with a worldwide distribution (Stürmer et al. 2018), associated with almost 80% of the vascular plants of the Earth (Smith and Read 2008) and comprise ~343 morphologically defined (http://www.amf-phylogeny.com/) or 350–1000 molecularly defined taxa (Davison et al. 2015). The multifunctional role of AMF in plant nutrition, pathogen protection, stress tolerance, and soil structure provision is well recognized (Chen et al. 2017). The different soil types, crop species, and land use affect the AMF diversity and their function inside the microbial soil communities, and for these reasons, some AMF species may be used as environmental indicators (Bouffaud et al. 2016; Oehl et al. 2017). In fact, it is reported (Boivin et al. 2016) that many anthropogenic factors, both in the past and nowadays, can modify and shape AMF communities, which could be considered a potentially suitable target for studying regional and local effects on soil microbial diversity with potential impact to global level (Pärtel et al. 2017; Ontivero et al. 2020).

External factors (abiotic and biotic) and intrinsic properties of AMF species (dispersal ability, rates of speciation, and extinction) affect the AMF geographical distributions (Chaudhary et al. 2008). For instance, the abiotic factors of temperature and precipitation constrain the AMF occurrence (Davison et al. 2015), while biotic ones such as host preferences determine the rhizospheric AMF community (Soteras et al. 2016; Senés-Guerrero and Schüßler 2016). Moreover, anthropogenic activities like agricultural practices that alter soil conditions could influence the occurrence of AMF taxa (Cofré et al. 2017). At the same time, either external or internal factors may indirectly influence each other, causing changes in AMF taxa occurrence and distribution (Chaudhary et al. 2008). Currently, an increasing number of studies attempt at unravelling the worldwide geographical patterns of AMF (Öpik et al. 2010; Kivlin et al. 2011; Öpik et al. 2013; Tedersoo et al. 2014; Davison et al. 2015). These researchers reviewed AMF descriptions based on DNA methods and showed contrasting results of AMF biogeographical patterns. For instance, Öpik et al. (2010) found that two-thirds of AMF taxa showed restricted distribution, but Davison et al. (2015) postulated that most of the AMF taxa show a cosmopolitan distribution and that species richness of AMF virtual taxa decreases with latitude at the global scale (Cofré et al. 2019).

The factors that influence the structure of AMF communities at both local and regional scales have not yet been studied in sufficient depth. Studying the influence of environmental drivers on AMF community structure at different spatial scales (i.e., local, regional, and global) can give us a more robust overview of the ecology of arbuscular mycorrhizal systems (Vályi et al. 2016). A factor that may influence the distribution of AMF taxa is environmental filtering, including the abiotic factors in a particular location that prevent the establishment or persistence of species. Through studying AMF taxa at higher taxonomic levels, it became apparent that taxa belonging to different AMF families appear to have distinct ecological preferences (Veresoglou et al. 2013). As an example, species of the family Glomeraceae appear to thrive in soils with high availability of nutrients, while fungi in the Gigasporaceae prevail in soils with low nutrients (Lekberg et al. 2007). This may reflect physiological differences found across AMF groups and, depending on the relative composition of the AMF community, give rise to systematic differences in the kind of ecosystem services the plant hosts receive from their mycosymbionts across different environments (Sikes et al. 2010; Sousa et al. 2018).

15.3 Arbuscular Mycorrhizal Fungi Diversity in the Espinal Ecoregion

In the Espinal Ecoregion understudy, only four published works were found, and we add a work that is currently being corrected; for this reason, the analyses that were carried out regarding the regional distribution of AMF species are limited. In the Ñandubay District, three works carried out were found (Velázquez et al. 2008, 2010, 2013), all in the “El Palmar” National Park in which the diversity of AMF species is reported using morphological techniques. In the Algarrobo district, the only work published (García de León et al. 2018) was performed with molecular techniques, and not all virtual taxa found are reported when comparing the richness of AMF species found in native forest soil and soybean crops; however, a greater diversity is reported in the native. In the district of Caldén, no published works were found; for this reason, we provide the data of our work review (Ontivero et al. unpublished data). This work determined the diversity of AMF species, through morphological techniques, found in native forests of *Prosopis caldenia* Burkart.

Comparing the works that determine diversity in natural environments, we found that the highest average diversity was found in the Ñandubay district. The lower species richness found in the other districts could be explained, among other factors, by the floristic differences that exist between the sample collection sites belonging to each district. In the Ñandubay district, the samples were collected in this National Park, which is one of the national parks in Argentina with the greatest floristic diversity, with more than 700 species of vascular plants (Biganzoli et al. 2001; Velázquez et al. 2013). This diversity of plants could increase the probability that an AMF species finds a suitable host (Velázquez et al. 2013), favoring an increase in diversity in this particular ecosystem.

On the other hand, comparing the number of species corresponding to each family (Fig. 15.2), in the districts in which diversity was determined with morphological techniques (Ñandubay and Caldén), it was found that the families Glomeraceae and Acaulosporaceae were dominant. This dominance of Glomeraceae and

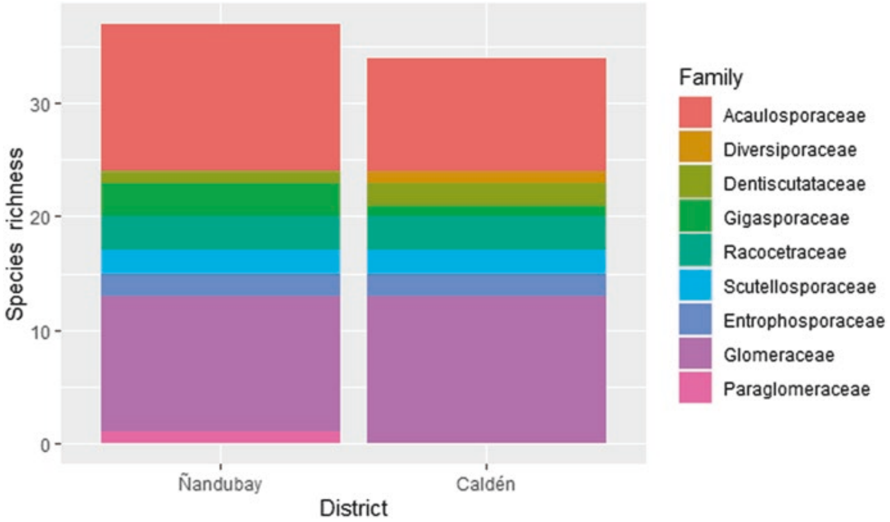

Fig. 15.2 Total species richness and richness in every family of AMF in Ñandubay and Caldén Districts

Acaulosporaceae has been observed at different geographic scales, with records for South America (Cofré et al. 2019) and the whole world (Stürmer et al. 2018). Likewise, Glomeraceae is the dominant family in all continents and cosmopolitan, since it has been recorded in all biomes of the world (Stürmer et al. 2018). However, it is necessary to consider the composition of the species in each District (Table 15.1), because although the number of species is similar, the species shared between both districts corresponds to 16 (Fig. 15.3), that is, they share less than half of the total richness.

The Algarrobo and Caldén Districts are the driest within the Espinal Ecoregion. Comparing the richness of species with other Argentine Ecoregions with similar temperatures and rainfall, we find a greater richness in the Espinal. Coinciding with the results obtained in other ecosystems, in many semiarid ecosystems, researchers have detected exceptionally diverse AMF assemblages in the dual niche occupied by these fungi, plant roots (Wubet et al. 2004; Martínez-García et al. 2015), and soil (Xu et al. 2016; Sousa et al. 2018). The differences found in terms of average diversity may be the result of differences in terms of dominant species and the different soils that exist in each district. However, the richness found coincides with the results obtained in an analysis of the ecoregions carried out at the South American level (Cofré et al. 2019).

Research that compares the effect of deforestation and the subsequent start of agricultural activity is scarce in the Ecoregion. The work of García de León et al. (2018) in the Algarrobo District was the only one found so far. In this work, a decrease in the diversity of AMF species is observed when comparing native forests dominated by the tree genus *Prosopis* compared to soybean crops. This decrease in

Table 15.1 List of arbuscular mycorrhizal fungi (AMF, Glomeromycota) species described or cited in the Districts of Espinal Ecoregion, considering only the morphological descriptions at species level

Family	Morphospecies	Ñandubay District	Caldén District
Acaulosporaceae	*Acaulospora alpina*		x
	Acaulospora bireticulata	x	
	Acaulospora colombiana		x
	Acaulospora delicata	x	
	Acaulospora denticulata	x	
	Acaulospora dilatata	x	
	Acaulospora entreriana	x	
	Acaulospora excavata	x	
	Acaulospora lacunosa	x	x
	Acaulospora laevis	x	x
	Acaulospora mellea	x	x
	Acaulospora morrowiae		x
	Acaulospora nicolsonii	x	
	Acaulospora punctata		x
	Acaulospora rehmii		x
	Acaulospora spinosa	x	
	Acaulospora scrobiculata	x	x
	Acaulospora thomii		x
	Acaulospora tuberculata	x	
Diversisporaceae	*Sieverdingia tortuosum*		x
Dentiscutataceae	*Dentiscutata biornata*	x	x
	Dentiscutata heterogama		x
Gigasporaceae	*Gigaspora candida*	x	
	Gigaspora gigantea	x	x
	Gigaspora margarita	x	
Racocetraceae	*Cetraspora gilmorei*	x	
	Racocetra beninensis		x
	Racocetra castanea		x
	Racocetra coralloidea	x	
	Racocetra fulgida	x	x
Scutellosporaceae	*Scutellospora arenicola*		x
	Scutellospora calospora	x	x
	Scutellospora dipapillosa	x	
Entrophosporaceae	*Claroideoglomus claroideum*	x	
	Claroideglomus etunicatum	x	x
	Entrophospora infrequens		x

(continued)

Table 15.1 (continued)

Family	Morphospecies	Ñandubay District	Caldén District
Glomeraceae	*Funneliformis coronatum*	x	x
	Funneliformis dimorphicus	x	
	Funneliformis geosporum		x
	Funneliformis mosseae	x	x
	Glomus ambisporum	x	
	Glomus badium		x
	Glomus glomerulatum	x	
	Oehlia diaphana	x	x
	Rhizoglomus aggregatum	x	x
	Rhizoglomus clarum	x	
	Rhizoglomus fasciculatum	x	x
	Rhizoglomus intraradices	x	
	Rhizoglomus irregulare		x
	Rhizoglomus microaggregatum	x	x
	Sclerocystis sinuosa		x
	Septoglomus constrictum	x	x
	Septoglomus deserticola		x
	Simiglomus hoi		x
Paraglomeraceae	*Paraglomus laccatum*	x	

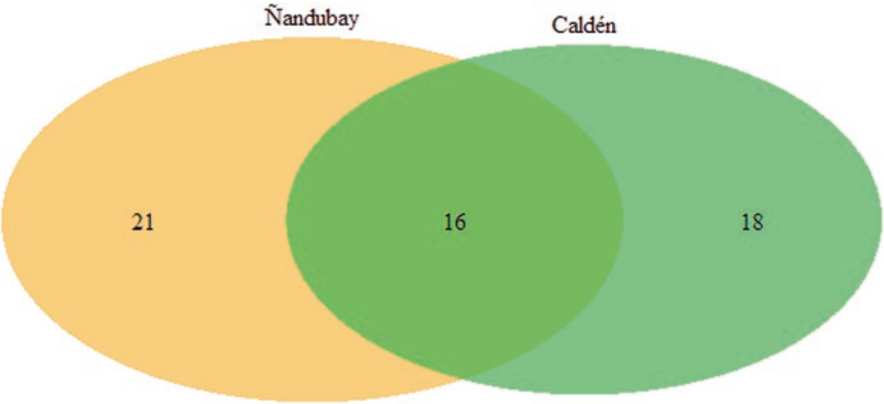

Fig. 15.3 Venn diagram comparing AMF morphospecies occurrence in Ñandubay District and Caldén District

diversity may be caused by the type of agricultural activity carried out in the region, which probably negatively affects the soil and the microorganisms that live in it.

In different investigations, it was demonstrated that the agricultural practices strongly affect soil's physical and chemical characteristics that impact the microbial communities affecting their abundance, diversity, and activity (Kladivko 2001; Govaerts et al. 2007; Lienhard et al. 2014). The effect of the agronomic management practices could have positive or negative effects on microbial biodiversity (Lehman et al. 2015; Pellegrino et al. 2020), affecting also the interaction between different microbial communities including bacteria and fungi (Dodd and Ruiz-Lozano 2012), which are proposed as the key to soil sustainability (de Vries and Wallenstein 2017). Fungi are widely distributed among all terrestrial ecosystems with huge biodiversity and ecological importance by their principal role in ecosystem processes such as soil carbon cycling, plant nutrition, and phytopathology (Hawksworth and Lücking 2017; Ontivero et al. 2020).

Particularly, high intensity agricultural land use, associated with mechanical disturbance and abundant application of chemicals, tends to exhibit lower AMF diversity, having shown that the richness of AMF is reduced with the intensification of agricultural practices (Oehl et al. 2004; Verbruggen et al. 2010; Wagg et al. 2011). In particular, the species richness as well as the abundance of AMF spores can be widely affected by fertilization, cropping sequence, and fallow periods (Douds and Millner 1999; Köhl et al. 2014) and tillage practices (Kabir 2005; Alguacil et al. 2008).

15.4 Conclusions and Final Comments

The AMF community characterization is historically based on the measurement of morphological features of spores; although, in recent years, this technique has been often replaced with DNA-based methods (Sousa et al. 2018). However, in South America (SA), molecular characterization of AMF communities is fairly scarce (Grilli et al. 2015; Soteras et al. 2016; Senés-Guerrero and Schüßler 2016; Cofré et al. 2019). Both approaches have limitations but can provide complementary information for taxonomic and ecological studies. Spore-based approaches yield important information concerning land management on AMF propagules, abundances, and richness in the soil (Säle et al. 2015; Overby et al. 2015). Furthermore, morphological spore identification may allow better differentiation of fungal taxa and can be quite sensitive in terms of detecting changes in AMF community composition and diversity (Wetzel et al. 2014; Sousa et al. 2018).

In the Espinal Ecoregion, a species of Acaulospora has been described that was unknown to science until the development of the investigations of Velázquez et al. (2008, 2010, 2013). In addition, in all the works analyzed, there are species that could not be determined. Particularly, in the only work that was developed using molecular techniques, they determined that there is a significant number of species considered key, which have not yet been cultivated or described. For this reason, and considering that there are more than 90 species of endemic plants in the Ecoregion, it is likely that there is a significant number of AMF species in the region that have not yet been discovered or described, considering the close relationship that exists between the plants and AMF. Therefore, the Ecoregion could host a significant number of AMF species that are unknown to science. Considering that some species are more sensitive than others to changes in plant species and considering the constant deforestation that exists in the Spinal Ecoregion, it is necessary to increase the resources allocated to AMF research, both with morphological and molecular techniques.

In the last 50 years, the Espinal has suffered a significant reduction in the original area occupied by forests. So much so that much of the natural vegetation that existed in the provinces of Entre Ríos, Santa Fé, Córdoba, and Buenos Aires has disappeared (SAyDS 2007a). In provinces such as San Luis and La Pampa, there are still important relics of this Ecoregion; however, the management of these ecosystems is not always adequate, since they are found on private properties that are not always properly advised, so it is necessary to review them. The management of forest ecosystems is important in their conservation because not only deforestation but also most forms of within-forest degradation (such as wildfires and selective logging) can have pronounced impacts on biodiversity (Gibson et al. 2011) in general. Recent research shows that soil biodiversity and related ecosystem processes may be lost after even very-low, reduced impact logging intensities (França et al. 2012; de Carvalho Mendes et al. 2017; FAO et al. 2020). Considering that AMF are key microorganisms in maintaining the health of plant species, especially in arid or semiarid ecosystems (Smith and Read 2008), and the study of the composition and dynamics of AMF communities in forests that have suffered a considerable reduction in the area they occupied, it is necessary to develop adequate reforestation plans, with the main objective of recovering forest ecosystems.

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Chapter 16
Edible Ectomycorrhizal Mushrooms in South America

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16.1 Outline

Edible mushrooms have been part of the human diet almost worldwide since prehistorical times, many of them are formed by mycorrhizal species (Pérez-Moreno et al. 2021). A comprehensive treatise on edible ectomycorrhizal mushrooms from a global perspective, but with special focus on some American countries, has recently been edited by Pérez-Moreno and coauthors (2020). Earlier, a checklist of saprobiotic, parasitic, and mycorrhizal fungi with edible sporomas has been published by Garibay-Orijel et al. (2010) for Mesoamerica and South America. In this chapter, we present a more specific and updated review of habitats, diversity, management, and conservation of ectomycorrhizal edible fungi (hereafter EEMF) in South America, especially in the Andean-Patagonian region of Argentina and Chile.

At species level, many ectomycorrhizal fungal symbionts are characterized by specific requirements in terms of vegetation type and climatic and edaphic conditions (Lilleskov and Parrent 2007); having co-evolved with their respective tree host, their geographical distribution is often more sharply defined than in case of less specific, saprobiotic species. Contrasting the dominance, high diversity, and wide extension of ectotrophic forests in the northern hemisphere, especially in the temperate and boreal zones, South America is characterized by only rather few autochthonous ectomycorrhizal tree taxa in disjunct areas (Singer and Morello 1960; Nouhra et al. 2019). Most of those areas coincide with cool temperate climate zones delimited by altitude or latitude, such as the high Andean oak forests in Colombia, the *Alnus acuminata* belt along the northern to central Andean slope, and the Andean Patagonian *Nothofagus* forests of Southern Chile and Argentina. Especially the more extensive woodlands with the presence of Fagales trees bear a considerable variety of autochthonous fungi, which produce edible mushrooms, although the mycophytosociological background of the respective *Quercus* and *Nothofagus* forests, which are geographically isolated from each other, is very different: The *Q. humboldtii* ectotroph in Colombia shows affinity to Central and Southern North America, whereas the ectomycorrhizal community associated to *Nothofagus* is of Gondwanan origin (Nouhra et al. 2019).

The scarce historical records indicate that collection and consumption of EEMF have a long tradition in local and regional indigenous populations (Coña *apud* Moesbach 1930; Molares et al. 2020). Although mushroom hunting has hardly been as popular among a broader public in South America as it is in many countries of the northern hemisphere (Commandini and Rinaldi 2020; Niveiro et al. 2009), a growing interest in EEMF can be observed in more recent times, as well with a commercial as a recreational background and evidently boosted by increased use of social media via Internet.

Among the most notorious anthropogenic changes of landscape and vegetation in many parts of South America during the last 50+ years is the large-scale replacement of native forests with timber trees, mostly conifers and eucalyptus (Echeverría et al. 2006). An increasing number of adventitious ectomycorrhizal fungal species forms part of these alien ectotroph communities, among them edible taxa, such as *Suillus* or *Lactarius*, which are not compatible with native ectomycorrhizal trees but often grow in large numbers in their monoculture habitat, making them a sought-after non-timber forest product not only for local and regional trade but also for food industry and exportation (Silva-Filho et al. 2020).

Finally, attempts have been made to culture introduced EEMF, such as truffles (Reyna and García-Barreda 2014), or to boost production of certain species in their natural habitat through enrichment of substrate with selected inoculum, a technique of mycosilviculture, which is increasingly applied worldwide (Wang and Chen 2014).

In this chapter, we did not include those wild EEMF from the region, which have either occasionally been categorized as being edible in the sense of being nontoxic and palatable but of low quality, such as *Laccaria* spp., or which represent rare and/or insufficiently known species, but we rather focused on those taxa that are regularly harvested and consumed and/or that have a market potential at local, regional, or international scale.

16.2 Edible Ectomycorrhizal Mushrooms in Native Forests

A detailed taxonomic inventory of ectomycorrhizal macromycetes growing in Colombian *Quercus* forests, which represent the second most important natural ectotroph area in South America, has been provided recently by Vargas and Restrepo (2020). The authors mention 37 edible species for the area. Previously, Peña-Cañón and Enao-Mejía (2014) published a list of 16 edible taxa known and harvested by rural communities of the Northeastern Colombian Andes, of which 11 are ectomycorrhizal, nine of them belonging to the genus *Ramaria*, and one to each, *Tylopilus* and *Russula*.

Although around 1000 species of macrofungi have been described from Andean-Patagonian *Nothofagus* forest (Mueller et al. 2007), only few species are regularly collected and consumed by local populations. Among EEMF, *Ramaria* spp. named “changle” or “chandi” in Southern Chile and Argentina (Coña *apud* Moesbach 1930; Sanchez-Jardón et al. 2017) are most commonly offered at local markets during the season. Species, often sold in mixed collections, have been named as *R. flava* (Schaeff.) Quél., *R. botrytis* (Pers.) Bourdot, *R. patagonica* (Speg.) Corner, and *R. subaurantiaca* Corner, among others, in the literature (Singer 1969; Lazo 2016; Barroetaveña and Toledo 2016); however, taxonomy of the South American Ramariae requires revision as species concepts applied at present are poorly defined (Valenzuela 2003) and date back to publications from the 1950s and 1960s (Singer 1969), lacking input of molecular and morphological data by advanced standards as proposed by Christan and Hahn (2005).

Autochthonous Boletes show low diversity in the *Nothofagus* region: a mere five species of Boletaceae are reasonably known so far from Southern Chile (Horak 1977; Garrido 1988; Riquelme et al. 2019), of which only *Boletus loyo* Phillippi and *B. loyita* E. Horak are considered good EEMF and regularly collected; both species are restricted to Mediterranean and temperate *Nothofagus* forests between the Pacific coast and the Andean foothills, from approximately 35° to 41° s.l.

B. loyo, named accordingly to its indigenous mapuzungún name "loyo" (Coña *apud* Moesbach 1930), resembles the king bolete *Butyriboletus regius* (Krombh.) D. Arora & J.L. Frank from the Northern hemisphere, but a comprehensive taxonomic study in order to justify a reclassification of *B. loyo* at genus level is still pending. Loyo is a rare regionally endemic species, but due to the impressive size of its basidiomata, which can reach 40 cm in diameter, it is easily spotted in the forest and regularly sold at local markets between Valdivia and Concepción, Chile.

Another regionally frequent EEMF in Chilean *Nothofagus* forest is *Cortinarius lebre* Garrido. Its main area of distribution is along the coastal cordillera, approximately between 36° and 39° s.l. (Arnold et al. 2012; Garrido 1988), although isolated records exist as far south as 44° s.l. (Salazar Vidal et al. 2020). A probable conspecificity with *Cortinarius purpurellus* M.M. Moser, E. Horak, Peintner & Vilgalys (syn.: *Rozites violacea* E. Horak), which was originally described from the Argentinian side of the Southern Andes (Moser and Horak 1975), remains to be verified. A specific and characteristic organoleptic attribute of fresh *C. lebre* is its strong and repugnant, naphthalene-like smell whose main component was identified as indol (Arnold et al. 2012) and which fades to a pleasant, perfume-like aroma during cooking. A similar species from the same area, although less frequent than *C. lebre* and with colors and smell strikingly similar to *C. camphoratus* Fr. from the northern hemisphere, is *C. contulmensis* Garrido, according to Garrido (1988) locally collected by the peasants of the Nahuelbuta coastal mountain range. Although *Cortinarius* is by far the most diverse genus of ectomycorrhizal fungi in South American *Nothofagus* forests (Moser and Horak 1975; Nouhra et al. 2013), only a few other common species, such as *C. magellanicus* Speg., are consumed locally (Gamundí and Horak 1994; Barroetaveña and Toledo 2016; Sanchez-Jardón et al. 2017). *Cortinarius austroturmalis* M.M. Moser & E. Horak; *C. effundens* M.M. Moser, E. Horak, & Singer; and a few other large-sized and gregarious Cortinarii described and gathered in the sect. *Xiphidipus* by Moser and Horak (1975) have been reported as edible and of good organoleptic quality by the same authors, but their culinary potential so far has been hardly appreciated, and there is yet no existing market for these attractive species although they are widely distributed. Salazar Vidal and Palfner (2015) calculated the seasonal yield of *C. austroturmalis* up to 7 kg fresh weight per ha for *Nothofagus* forest in the Maule region of Central Southern Chile.

At least two *Amanita* species, *A. diemii* Singer and *A. merxmuelleri* Garrido, have been traditionally collected and consumed in rural areas of Southern Chile (Salazar Vidal 2016a), but as adventitious toxic Amanitas, such as *A. phalloides* (Vaill. ex Fr.) Link and *A. gemmata* var. *toxica* Lazo, are becoming increasingly common in mixed woodlands where introduced trees, such as pines, oaks, or chestnuts are present (Valenzuela 1992), the harvest of *Amanita* spp. should nowadays be avoided due to the high risk of confusion and severe intoxication, especially for unexperienced mushroom gatherers.

Chanterelles (*Cantharellus* and related genera) and milk caps (*Lactarius*), not only common EEMF on the northern hemisphere but also found in Colombian oak forests, are absent in Southern *Nothofagus* forests, and only allochthonous *Lactarius* sect. deliciosi can be found in pine plantations, which today cover large areas previously occupied by native forest (see next section).

16.3 Edible Ectomycorrhizal Mushrooms Associated to Exotic Timber Plantations

Across South America, allochthonous timber tree species have long been introduced and are still expanding as monocultures or, more critically, becoming naturalized and invading native vegetation (Langdon et al. 2010). Although *Eucalyptus* spp. are probably most important in this context, the most relevant hosts for non-native EEMF are *Pinus* spp., which nowadays are spread across all climate zones, from the tropics to the subantarctic steppe (García et al. 2018). Pinaceae in South America are typically found to be associated to few but highly host-specific, adventitious EEMF, such as *Suillus* spp. and *Rhizopogon* spp., among others (Hayward et al. 2015). These low-diversity communities homogenize and simplify the landscape across a vast latitudinal gradient, not only replacing native ectotrophic communities like *Quercus* and *Nothofagus* forests (Simijaca et al. 2018; Palfner and Casanova-Katny 2019) but also establishing invasive alien ectomycorrhizal guilds in previously anectotrophic areas (García et al. 2018).

Although historically, *Suillus luteus* (L.) Roussel is by far the most important and widely distributed commercial species (Barroetaveña and Toledo 2016; Melgarejo 2014, 2015; Molares et al. 2020), less common species, such as *S. granulatus* (L.) Roussel and *S. bellinii* (Inzenga) Kuntze, are increasingly receiving attention by researchers and mushroom gatherers (Chung Guin-Po 2021; Niveiro et al. 2009).

After *Suillus* spp., *Lactarius* sect. deliciosi are the second most important EEMF retrieved from pine plantations in South America. The most commonly encountered milk cap was historically identified as *L. deliciosus* (L.) Gray (Mikola 1969) and reported from different countries (Singer 1969; Barroetaveña and Toledo 2016); however, more recent studies from Chile and Brazil (Chávez et al. 2015; Silva-Filho et al. 2020), backed by molecular, morphological, and ecological data, yielded evidence that this taxon should be correctly named *L. quieticolor* Romagn. and is probably distributed throughout large parts of the continent.

16.4 Putatively Mycorrhizal Edible Fungi: *Morchella*

Morchella ascomata are among the most demanded and high-prized edible mushrooms worldwide. Although there is still controversy about whether or which *Morchella* species are mycorrhizal or saprobiotic (Hobbie et al. 2017), we decided to include this genus in our review due to its extensive presence in South America and the evidently specific association of some species to ectotrophic forest.

Early mentions and descriptions of true morels from South America date from the late nineteenth and early twentieth centuries and were hence based on a eurocentric and today obsolete taxonomic concept. The first records of *Morchella* from Southern Argentina were published by Spegazzini (1909) and named as *M. conica* Pers., based on collections from the Southeastern Andes; later, records under the same name from Central and Southern Chile (Spegazzini 1918, 1921) were added. Espinosa (1929) reported *M. esculenta* (L.) Pers. from a market in Santiago de Chile. A single finding of the supposedly endemic *Morchella patagonica* Speg. by Spegazzini (1909) for Argentina still remains unrepeated and unconfirmed. Gamundí (1975) reported *Morchella elata* from Patagonia as representing a species complex; the same author later also mentioned records of *M. intermedia* and *M. semilibera* (Gamundí 2010) from Argentina. Only recently, taxonomy and phylogeny of *Morchella* in South America have been substantially updated, mainly based on molecular markers (Baroni et al. 2018; Machuca et al. 2021), indicating that probably most species names had been incorrectly applied before. Confirmed and new taxa published for the region are supporting the concept of coexistence of endemic and widely distributed species, and this is for Patagonia: *M. andinensis* A. Machuca, M. Gerding, & D. Chávez; *M. aysenina* A. Machuca, M. Gerding, & D. Chávez; *M. septimelata* Kuo; and *M. tridentina* Bres. (Machuca et al. 2021; Pildain et al. 2014) and for Ecuador, Perú, and Venezuela: *M. gracilis* and *M. peruviana* (Baroni et al. 2018). Furthermore, insufficiently identified *Morchella* spp. belonging to the "elata" and "esculenta" clades have also been reported from Bolivia (Melgarejo Estrada *pers. com.*).

A comprehensive treatise with up-to-date information about biology, diversity, ecology, and nutritional properties of *Morchella* spp. of the Patagonian Andes, together with guidelines for sustainable and environmentally friendly harvest as well as a market analysis, has recently been published by various authors (edited by Lobos and Icarte 2021).

16.5 Management and Culture of Native and Introduced Edible Ectomycorrhizal Fungi

16.5.1 Consumption and Trade Levels

As a common pattern, regionally endemic and/or infrequent species of EEMF are mostly collected, sold, and consumed in limited quantities and at a local scale, whereas widely distributed and/or frequent species tend to be commercialized across larger regions or even internationally, as in the case of *Morchella* spp. or *Suillus* spp., in some regions by technically and logistically rather advanced trade chains. However, there is only little published and updated information about commercial statistics of edible mushrooms in South America, especially of mycorrhizal species (Quiroz et al. 1996; Gysling et al. 2005; Albertó et al. 2010).

16.5.2 Mycosilviculture

Approaches toward sustaining, increasing, or diversifying production of EEMF in native forests or timber plantations are forming part of the concept of mycosilviculture (Savoie and Largeteau 2012; Wang and Chen 2014). Especially in managed, low-diversity, or degraded forest ecosystems, mycosilviculture can be an efficient tool for reinforcement or enrichment of mycorrhizal associations, with special focus on EEMF (Iwabuchi et al. 1994; Palma et al. 2021).

Mycosilvicultural methods aiming at increased productivity of EEMF are based on enrichment or boosting of natural production by inoculating compatible host trees in situ with spores or mycelium of selected species, previously produced under controlled conditions. In a study aiming at optimizing culture conditions for mycelial inoculum, Santelices et al. (2012) found differences in growth rates of geographically distant *Suillus luteus* strains from central Southern Chile. Successful formation and high colonization rates of mycorrhizal roots formed by *Pinus elliottii* Engelm. inoculated with spores of fresh *Suillus granulatus* were reported from Argentina by Nouhra and Becerra (2001). Pereira et al. (2014) induced mycorrhiza formation in seedling roots of *Pinus radiata* inoculated with mycelium of *Lactarius quieticolor* isolated in central Southern Chile and produced under optimized conditions in liquid medium. Also, inoculation trials of *P. radiata* with introduced *Boletus edulis* Bull. and *B. pinicola* (Vittad.) A. Venturi were reported for Chile by Chung et al. (2010); however, although formation of mycorrhizal roots could be observed in laboratory and nursery, no production of sporomata occurred.

A highly promising approach of enrichment of autochthonous EEMF in native *Nothofagus* forest by spore irrigation was recently realized by Palma et al. (2021) in Southern Chile, Valdivia Province: in a long-term collaboration project with a community of rural mushroom collectors and small land owners, spore inoculum of *Boletus loyo* and *Ramaria* spp. was brought out in previously monitored and prepared forest patches, accompanied by an information and training campaign for environmentally friendly EEMF harvest and adequate habitat management. During three consecutive years after spore irrigation treatment (2020–2022), at least one of the experimental sites showed continuously increasing production of *Ramaria flava* (changle) (Fig. 16.1), whereas the land owners reported that before the treatment, they had yielded very little or no harvest of this species from the same site. Although the results are still under evaluation, they provide important evidence that mycosilvicultural techniques can be powerful tools for sustainable use of native fungal resources and *Nothofagus* forest by local communities.

Fig. 16.1 Growth and harvest of edible *Ramaria* cf. *flava* basidiomata (lower photo showing EEMF gatherer Rosario Catripan Lincocheo from Caricuicui) in privately owned managed *Nothofagus* forest in Southern Chile (Valdivia Province) during May 2022, 3 years after spore irrigation treatment. Photo credit: Rodrigo Sagardía Parga, INFOR Valdivia

16.5.3 Truffle Culture in South America

Culture methods for *Tuber* spp., mainly for *T. melanosporum* Vittad. from Mediterranean Europe, were introduced in South America from around 2000 onward. Due to the Mediterranean to temperate climate required by *T. melanosporum* and other species of interest, like *T. borchii* Vittad. and *T. magnatum* Picco, Southern Chile and Argentina so far have proven to be the most adequate countries for truffle culture on the continent (Micofora 2018). First experimental assays with *T. melanosporum* were successfully performed and documented in Central Southern Chile by academic research teams (Pérez et al. 2007; Pereira et al. 2013). Results and perspectives of the attempts to introduce *Tuber magnatum* in truffle orchards in Chile have been recently summarized by Pereira et al. (2021). At present, private entrepreneurs provide a growing number of interested land owners with mycorrhizal tree seedlings inoculated in vitro, mainly with *T. melanosporum,* and, along with guilds of truffle growers, also offer technical guidance for successful management of truffle orchards in both countries (www.atchile.cl, www.trufasdelsur.com); harvests, as far as being documented in public media, have reached profitable levels within a few years (Micofora 2016). An interesting aspect of sustainable mycosilviculture has been the successful in vitro synthesis of ectomycorrhizas between *Nothofagus obliqua* and *Tuber melanosporum* (Pérez et al. 2007), which could open a new perspective for combining conservation and reforestation of native Fagales trees with truffle production, reducing the need of planting allochthonous host trees like *Quercus* or *Corylus*.

16.6 Mycogastronomy and Cultural Importance

During recent years, a rising interest in promoting wild mushrooms as part of local or regional gastronomy can be observed in various regions in South America, but scientific studies about this issue are still scarce. Fernández et al. (2021) recently published the results of an interview campaign performed with local chefs from the Cordillera de Chubut, Argentinian Patagonia, reporting EEFM being commonly offered in restaurants but only a low variety of species, led by introduced *Suillus luteus* and morels, whereas other, especially native fungi, only play a marginal role, mainly due to irregular and unpredictable supply as well as lack of knowledge about species or problems with their identification.

S. luteus is a neophyte in many regions of South America and, as mentioned above, even grows adventitiously in areas where no native EEMF existed before. Its harvest can therefore be an opportunity for local peasants to diversify crop sales and yield additional income, as has been shown for rural communities in Bolivia by Melgarejo Estrada et al. (2018). In central Chile, there exists even a traditional fair since 1988 in the village of Empedrado, dedicated to activities around *S. luteus*, called “Festival de la Callampa.”

Also in Chile, festivals or fairs held around native EEMF can be seen to be spreading along an increasing number of villages and urban areas: The "Changle" (*Ramaria*) Festival takes place in the town of Cañete every year since 2012, featuring cooking workshops by well-known national chefs, artistic shows, a mycological tour known as the "Changle Route," contests, gastronomy, and handicraft sales. In the town of Paillaco, located near the city of Valdivia, the Wild Mushroom Festival created in 2014 is organized during the month of April where dishes based on mushrooms, such as changle, loyo, lebre, among others, can be tasted, in addition to other activities linked to mushrooms. The Morel Festival has been held since 2015, in December, in Villa Ortega, located near the city of Coyhaique in Chilean Patagonia, which not only promotes sustainable harvesting of *Morchella* but also intends to raise awareness to increasing threats such as intentional burning of native forest and overexploitation. In this festival, art is presented together with dishes based on this gourmet edible mushroom. Another example is the "FungiFest" mushroom festival held in Valdivia since 2016, with scientific and popular talks on mushrooms, handicraft sales, photography, and cooking contests, highlighting characteristics and uses of a large number of species for a broad public.

For Argentina, Barroetaveña and Toledo (2016) mention high diversity of EEMF associated with Nothofagaceae in western Patagonia, some of which are little known and, therefore, not collected by the local and regional population; accordingly, the authors seek to promote the sustainable collection and consumption of these mushrooms as a novel non-timber forest product. In Chubut, wild edible and cultivated mushrooms have been incorporated into a gastronomic circuit called "Patagonia Fungi, trails and flavors," co-organized by public and private entities and based on experiences in foreign countries, mainly European, which promotes mycogastronomy and mycotourism. Activities include courses and workshops for collectors, flavor tastings with chefs from the region, gastronomic fairs, joint organization of tasting dinners of novel mushrooms, and the production of preserves and gourmet products (Fernández et al. 2021).

16.7 Nutritional Chemistry and Biological Activity

Edible wild mushrooms have been part of the human diet for centuries due to their attractive nutritional and organoleptic characteristics, such as flavor, texture, and aroma (Aisala et al. 2020). From a nutritional point of view, mushrooms are low in lipids but present high content in proteins, carbohydrates, fiber, vitamins, and minerals (Cheung 2010; Valverde et al. 2015). In addition to being recognized as a nutritious food, certain mushrooms are also an important source of biologically active compounds with medicinal potential, including phenolic compounds, polysaccharides, sterols, and triterpenes, among others (Wasser 2010).

Most chemical analyses of EEMF have been performed with species from the northern hemisphere, only some of which are adventitious in South America (Kalač 2013). For example, Ribeiro et al. (2009) characterized the lipid profile of European species, such as *Amanita rubescens* Pers., *Suillus bellinii*, *S. granulatus*, and *S. luteus*, which are also adventitious in South America (Valenzuela et al. 1998; Niveiro et al. 2009; Chung Guin-Po 2021). However, chemical and organoleptic analyses are generally still scarce for South American EEMF, especially for native and endemic species: High carbohydrate content and low fat/protein ratio was determined for *Boletus loyo* mushrooms from Chile by Schmeda-Hirschmann et al. (1999), as well as for *Cortinarius magellanicus* and *Ramaria patagonica* from Argentinian Patagonia by Toledo et al. (2016); in the latter study, the authors also detected high content of tocopherol in *C. magellanicus*, whereas *R. patagonica* stood out by its high antioxidant activity and phenol content. Barroetaveña et al. (2020) expose in detail the nutritional composition of common EEMF from *Nothofagus* forests as well as from pine plantations. Jacinto-Azevedo et al. (2021) provide not only nutritional data (moisture, protein, fat, ash, and carbohydrate content) but also biological activity (antibacterial, antifungal, and antioxidant) for four EEMF native to Chile and Argentina: *Boletus loyo, Cortinarius lebre, Ramaria flava*, and *R. subaurantiaca,* among which *R. flava* stands out by its high antioxidant activity.

16.8 Outlook: Global Change and Conservation Strategies

Following a worldwide tendency, changes in climate and soil use are putting increasing strain on many natural habitats of EEMF in South America. The most important factors, which negatively affect mycorrhizal trees and associated fungi in the region, are disturbance and deforestation of native woodlands as well as overharvesting of wild mushrooms in the remaining forests. Conservation measures for EEMF should include individual fungal species as well as their tree hosts and specific habitats. Protection should be implemented not only by improving environmental laws but also by recommendations for good practice and sustainable harvesting, made available to mushroom collectors. In Chile, the conservation status of fungi is classified according to IUCN criteria since 2012 by the Ministry of Environment, based on an annual public call for suggestions of species. To date, distribution and vulnerability of the important EEMF *Boletus loyo* (classified as endangered “EN”) and *Cortinarius lebre* (classified as vulnerable “VU”) have been assessed (MMA 2022). A proposal of classification of the respective ecosystems and phytosociological units by IUCN criteria has been published (Pliscoff 2015) but is awaiting legal status. However, protocols and recommendations for sustainable harvesting are being issued by governmental and scientific institutions as well as by NGOs (Salazar Vidal 2016b; Palma et al. 2021).

On the other hand, local invasion of native forest by ectomycorrhizal fungi, which were originally introduced with allochthonous timber trees but changed to autochthonous tree hosts, has been observed. For the special case of EEMF, there is the example of *Amanita rubescens* in Southern Chile, which, being locally adventitious in Monterrey Pine plantations, has also been reported from pure *Nothofagus* forest in the coastal mountains near Valdivia (Valenzuela et al. 1998).

Sustainable harvest of EEMF, especially in native forests, should be based on protection of fungal species (selective and careful extraction of sporomata, minimally intrusive treatment of substrate and mycelia) but also of the habitat (host trees, understory, water courses); in managed forests, grazing livestock should be excluded; on the other hand, pruning and selective cutting of trees may be considered in order to maintain canopy cover and tree density adequate for the respective EEMF taxa (Palma et al. 2021).

There is evidence that at least some of these good practices have been applied and passed on since ancient times by indigenous and rural communities, for example, in Southern Chile and Argentina; these often local experiences should be considered as a baseline for the development of regional, socially acceptable collector's guidelines and conservation frameworks for EEMF in South America (Tacón et al. 2006; Alvarado-Castillo and Benítez 2009; Toledo et al. 2014).

Finally, as outlined by Pérez-Moreno et al. (2021), sustainable use and management of EEMF based on local and regional cultures and traditions can have multiple positive effects on economy, ecology, and social structure, especially on a highly diverse continent like South America.

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Chapter 17
Arbuscular Mycorrhizal Symbiosis in Temperate Grassland Forage Species of Argentina

Ileana V. García and Tomás A. Chippano

Contents

17.1 Introduction

Grasslands, which cover about 23% of terrestrial ecosystems, provide numerous ecosystem services, such as water infiltration and storage, soil erosion control, wildlife habitat biodiversity, and carbon sequestration, and help to mitigate global warming (Suttie et al. 2005; Rangelands Atlas 2021). However, grasslands are ecosystems seriously threatened by agriculture, livestock, and forestry and have been among the most degraded ecosystems worldwide over the last 50 years, with a decline in ecosystem functioning and services (McSherry and Ritchie 2013; Conant et al. 2017).

In temperate zones, grasslands also provide most of the forage for ruminant nutrition (Hopkins and Wilkins 2006; Muir et al. 2011), and since their soils rarely provide ideal conditions for plant growth, they maintain an acceptable forage production. In South America, these temperate ecosystems are known as the Río de la Plata Grasslands and are among the most important in the world, with an area of 760,000 km^2. These occupy a large part of central Argentina, almost all Uruguay, and part of the state of Río Grande do Sul, in Brazil. Also known as the “Pampas”

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or "Campos," these regions have been the base of the economy of commodity-exporting countries such as Argentina, Brazil, and Uruguay. In particular, the Flooding Pampa (90,000 km^2) represents the largest area of remnant natural or seminatural grasslands in the temperate-humid Pampa region of Argentina and in South America (Soriano et al. 1991; García et al. 2018). In this area, agricultural development is limited on edaphic and climatic factors, and thus livestock production depends mainly on natural grasslands (Soriano et al. 1991). The productivity of these grassland communities is strongly affected by the topography, which determines a range of salinity, alkalinity, excess or deficit of water in the soil, and low nutrient availability, which, together with the climate, decreases plant growth (Escudero and Mendoza 2005; García and Mendoza 2008). The land use and/or environmental conditions in these grasslands have led to the dominance of perennial and annual C_3 and C_4 grasses associated with a decline or absence of native legumes, a fact that seriously affects the forage quality and productivity in this area (Cahuépé 2004; Perelman et al. 2001; Burkart et al. 2005).

Some of the alternatives to improve both the quantity and quality of the forage production when undesirable factors affect productivity include irrigation; addition of herbicides, fertilizers, or seeds of forage plants; and the control of the stocking rate (Muir et al. 2011). One of the most deficient nutrients of the temperate grassland soils worldwide is phosphorus (P). This deficiency is also one of the main limiting factors for forage production in the Argentine Pampas. Thus, one of the practices commonly used to increase productivity in these grasslands is the addition of P (Rubio et al. 2012; Mendoza et al. 2016a). Other feasible strategies to increase forage production include selecting plant species adapted to grow under P-deficient conditions (Cavagnaro et al. 2014; Chippano et al. 2020) and taking advantage of the benefits provided by native soil microorganisms involved in P nutrition, such as arbuscular mycorrhizal (AM) fungi (Mendoza and Pagani 1997; Cavagnaro et al. 2014; Ikoyi et al. 2018; Chippano et al. 2021).

Approximately 80% of all land plant species form symbiotic associations with AM fungi (Smith and Read 2008; Tedersoo et al. 2020). In these symbiotic associations, plants colonized by AM fungi obtain nutrients, and in return, AM fungi receive photosynthates from the plant (Smith and Read 2008). In grasslands, AM fungi play an important ecological role in shaping plant communities, by influencing plant growth and nutrient uptake (especially that of P), plant diversity, and competitive ability (van der Heijden et al. 2006; Klironomos et al. 2011; Klabi et al. 2014). On the other hand, AM fungal communities are affected by the changes in the plant community and environmental factors (Eom et al. 2001; van der Heijden et al. 2006; Klironomos et al. 2011). In addition, in the Pampas grasslands, both plant communities and AM fungal communities are modified by the raising of livestock and the addition of P fertilizers to increase the forage resource. Thus, the benefits of AM fungi on both plant development and the whole grassland ecosystem are affected as well. In this sense, AM fungi improve the grazing tolerance of their host plants, although it has also been suggested that AM fungi may place an additional burden on the plant, depending on the intensity and frequency of grazing and the level of nutrients in the soil (Barto et al. 2010). In particular, both grazing and defoliation have been shown to decrease AM fungal colonization in grasslands and in grassland species grown under controlled conditions (Gehring and Whitham 1994; Frank et al. 2003; Saravesi et al. 2014). This negative effect of defoliation on AM colonization has usually been ascribed to a reduced photosynthetic capacity of plants, which, in turn, limits the carbon supply to roots and AM fungi, particularly under heavy grazing conditions (Barto et al. 2010; van der Heyde et al. 2017, 2019; Faghihinia et al. 2020; Yang et al. 2020). However, grazing has also been shown to increase or have no influence on AM fungal colonization (Eom et al. 2001; Hokka et al. 2004; Yang et al. 2013). The context-dependent nature of the mycorrhizal symbiosis association suggests that the effects of grazing or defoliation on AM fungi occur through changes in the soil and root environment in which AM fungi are found. The different responses of AM fungi and their hosts during defoliation could also be associated with variations in plant genotype, climatic factors, soil available nutrients (P and N), and/or differences in the relative growth rates of roots and hyphae of AM fungi inside roots and in the surrounding soil (Barto and Rillig 2010; van der Heyde et al. 2017, 2019; Faghihinia et al. 2020).

Regarding fertilization, in most P-deficient grassland soils, P fertilization is necessary to obtain maximum forage yield (Muir et al. 2011; Mendoza et al. 2016a; Chippano et al. 2020). However, when P availability in the soil increases due to fertilization, both AM root colonization and mycorrhizal response decrease in several forage plant species, even if AM fungi can still be actively contributing to plant P uptake (Smith et al. 2004; Jeffery et al. 2018; Chippano et al. 2021). Furthermore, it has been reported that application of high levels of fertilizers induces a decline of the AM fungal community (Mäder et al. 2000; Kahiluoto et al. 2001; Fornara et al. 2020). Compared with agricultural crops, little is known about the effect of grazing/defoliation or P fertilization on the symbiosis between forage species and AM fungal communities from soils of the grassland Pampas.

This chapter discusses the ecological role of AM symbiosis on the functioning of the temperate grasslands of Argentina, as valuable information to promote better management of forage land sustainably while increasing forage production and preserving the beneficial effects of AM fungal communities in these ecosystems. We focus mainly on the effects of grazing/defoliation and P fertilization on the mycorrhizal status and AM benefits on forage species growing on grassland soils of the Argentine Flooding Pampa.

17.2 Natural Habitat

The mutualism between plants and AM fungi is an essential component of temperate grassland communities. The beneficial effects of AM fungal communities on their plant hosts and the occurrence and abundance of AM fungi are affected by multiple soil parameters, including soil pH (Fitzsimons et al. 2008; Oehl et al. 2017; Van Geel et al. 2018), P availability (Gosling et al. 2013; Camenzind et al. 2014), N level (Camenzind et al. 2014; Fitzsimons et al. 2008; Van Geel et al. 2018), salinity, soil disturbance, vegetation, and soil hydrological conditions (Miller and Bever 1999; Ingham and Wilson 1999; Escudero and Mendoza 2005; García and Mendoza 2007, 2008; García et al. 2017; Fusconi and Mucciarelli 2018).

Despite the importance of AM fungi in the physiology and nutrition of grassland plants, the study of the dynamics of AM fungal communities and the possible role of this fungal group in the modeling of the ecosystem remains a challenge due to the high degree of connection between the different root systems, the wide range of host plants involved in the AM symbiosis and the formation of a common hyphal network that connects root systems of plants within the same or different species. The hyphal network may mediate the plant interaction by the inter- and intraspecific transfer of assimilates (Smith and Read 2008; Tedersoo et al. 2020), although the ecological relevance of this mechanism is not fully elucidated.

The grasslands of the Argentine Flooding Pampa are subject to periodic droughts or floods, and their soils show extreme values of salinity and sodicity. These stress conditions represent a particular situation to study the effect of seasonal variation on AM fungal populations and to understand seasonal patterns of AM colonization morphology and their relationship with plant nutrient demands and soil properties and then forage quality. In this sense, in a 2-year field study, Escudero and Mendoza (2005) analyzed the seasonal variation in AM populations in four grassland sites of the Flooding Pampa, which varied widely in their soil conditions, vegetation composition, and period over which the soils were subject to flooding. These authors found high values of spore density even when the period over which the soil was subject to flooding ranged from 8% to 60% of the year. The spore density also showed a seasonal pattern, with highest values in summer, lowest values in winter, and intermediate values in autumn and spring, and these changes appeared to be controlled mainly by the succession of dry and wet periods. They also observed that, in the four grassland sites, the spore communities were dominated by two species of AM fungi, *Rhizophagus fasciculatum* and *R. intraradices*, and that the spore density of these species was relatively more abundant in summer than in winter, when flooding is most frequent in the Flooding Pampa. These authors also found that the mean value of spore density varied fivefold among soil sites, from 305 per gram of dry soil in the nutritionally well balanced soil to 68 per gram of dry soil in the clay and extremely P-deficient soil (1.38 mg P kg^{-1}) (Escudero and Mendoza 2005). Besides, in agreement with that reported by Abbott and Robson (1991), who observed that, in field soils, spore density appears to reach a maximum when P availability in the soil is less than that required for maximum shoot growth and then decreases with increasing P availability, Escudero and Mendoza (2005) reported that P availability in the soil (1.38–10.93 mg P kg^{-1}) was always less than that required for maximum plant growth. In these P-deficient soils, an increase in P level could increase spore count before the expected decrease occurs. In addition, the P required to reach maximum plant growth generally differs between soils, a fact that could explain the lack of direct relationship found by the authors between soil P and AM fungi measurements. Finally, Escudero and Mendoza (2005) found that the soil pH had an effect on spore density similar to that of P level. They concluded that the spore density of each soil site is a consequence of the influence of many plant community and soil variables on the AM fungal communities rather than of the influence of one specific dominant plant species or soil property (Escudero and Mendoza 2005). Since AM fungal communities may be adapted to different soil conditions, it is not surprising that the abovementioned authors did not find neither a marked trend nor a limiting value for soil pH, exchangeable Na or P availability to increase or decrease spore density. In the Flooding Pampa grasslands, sporulation of AM fungi depends on the season, and this seasonality is an expression of AM fungi to survive when the environmental conditions in the soil are unfavorable.

Following the studies in natural grasslands from the Flooding Pampas, our research group studied the temporal dynamics of AM fungal root colonization in legume and grasses, propagules in the soil, plant tissue nutrients (N and P), and soil properties, along a topographic slope ranging from saline and/or sodic lowlands of high soil moisture content and soil pH to uplands of drier and neutral non-saline soils (pH 6.6–9.5; EC 0.9–0.1 dSm^{-1}; exchangeable Na 28–77%) (García and Mendoza 2007, 2008). The plant species selected were the legume *Lotus tenuis* and the grasses *Distichlis spicata*, *Paspalum vaginatum*, and *Stenotaphrum secundatum.* The results of these studies showed that the *Arum*-type colonization predominated in *L. tenuis* roots and that the co-occurrence of *Arum* and *Paris*-type colonization predominated in grass roots in all the grassland sites studied. The overall mean values of AM root colonization over sites and seasons were 89% for *L. tenuis* and 68% for grasses. The capability of plants to be densely colonized by AM fungi is an important fact in plant nutrition and stress tolerance because it means that the AM fungi-plant symbioses can persist in roots under a wide range of soil moisture saturation levels, nutrient status, salinity, and sodicity in space and time (García and Mendoza 2007, 2008).

Our results also showed that the dynamics of the morphology of AM root colonization presented similar patterns in all the plants studied (García and Mendoza 2007, 2008). Maximum arbuscular colonization occurred at the beginning of the growing season in late winter, with a minimum in late summer, whereas maximum vesicular colonization occurred in summer, with a minimum in winter. This could indicate the preferential production of a morphological structure by the fungus in a specific season. The highest arbuscular colonization was associated with the highest N and P levels in plant tissue, suggesting a relationship between increases in the rate of nutrient transfer and symbiosis partners. Soil water content, salinity, and sodicity were positively associated with AM root colonization and arbuscular colonization in *L. tenuis* but negatively associated with AM root colonization and arbuscular colonization in the grasses (García and Mendoza 2007, 2008). Besides, number of entry points per millimeter of colonized root increased in spring for all species and was higher in the roots of the legume than in the roots of the grasses, showing differences in the affinity for AM fungi between both species. Since soil properties and plant communities along the topographic slope are characteristic of each site, if legume and grasses present similar seasonal dynamics in the number of entry points in all the sites analyzed, it suggests that each AM fungal community is adapted to the particular soil conditions of each site and then responds seasonally in relation to the growth stages and nutritional demands of the plant host (García and Mendoza 2007; García 2008).

As mentioned above, several researchers have shown that AM fungal communities of temperate grasslands can be affected by agronomic management practices, such as fertilization and/or the use of herbicides, including glyphosate (Menéndez et al. 2001; Druille et al. 2015; Cofré et al. 2017). In grasslands of the Flooding Pampa, P fertilization and glyphosate application are frequently used to increase forage production. In relation to herbicide application, greenhouse and field experiments have demonstrated that glyphosate application reduces AM fungi spore viability in the soils of these grasslands and subsequently the mycorrhizal colonization of plants (Druille et al. 2013, 2017). The results of these experiments showed a larger reduction in spore viability even when a dose that damaged but did not kill *Lolium multiflorum* plants (sub-lethal dose) was used, which demonstrates that ecosystem components may have different vulnerability to glyphosate. In recent years, farmers in the Flooding Pampa grasslands have adopted the promotion of *L. tenuis* through glyphosate application, due to the important contribution of this species to forage quantity and quality. In a field study performed by our research group, we found an AM fungal community in the rhizosphere of *L. tenuis* in natural grasslands and in that of *L. tenuis* promotion with glyphosate application (García et al. 2017). The soil sites studied presented different levels of salinity and sodicity, and those with glyphosate-mediated promotion presented an increased relative frequency of *L. tenuis*. Our results also showed that AM root colonization was higher in natural grasslands than in sites with *L. tenuis* promotion and that this colonization correlated positively with pH and exchangeable Na (salinity and sodicity) in grasslands and negatively in sites with *L. tenuis* promotion. In this regard, Druille et al. (2015) reported that the AM fungal community could be less infective in sites with *L. tenuis* promotion than in natural grasslands. The concomitant loss of plant diversity induced by the herbicide application can also affect AM fungal communities. Our studies showed that the proportion of roots colonized by arbuscules in *L. tenuis* plants was high and similar between the different soil management conditions (García et al. 2017). These results suggest that *L. tenuis* plants are functionally mycorrhizal for a diversity of soil conditions and/or managements (García et al. 2017). In this field study, AM fungal species were grouped into seven families, four of which presented the highest spore density: Glomeraceae, Claroideoglomeraceae, Acaulosporaceae, and Diversisporaceae. Spore values at the family level, soil properties, and *L. tenuis* relative frequency showed that Claroideoglomeraceae spores were associated with an increase in pH and exchangeable Na and a decrease in *L. tenuis* frequency (saline/sodic soils in natural grasslands) (García et al. 2017). The spores from Acaulosporaceae and Glomeraceae were associated with high *L. tenuis* frequency and a decrease in pH and exchangeable Na (non-saline/non-sodic soils in sites with *L. tenuis* promotion). Finally, the Diversisporaceae was associated with non-saline/sodic soils (in sites with *L. tenuis* promotion). We concluded that AM fungal families and root colonization are good indicators to differentiate sites by their soil characteristics (pH and exchangeable Na) and/or management conditions (e.g., herbicide application) (García et al. 2017).

17.3 Grazing, Fertilization, and Plant-Plant Interactions

Grazing or defoliation and fertilization lead to changes in the soil conditions and thus to alterations in the plant community structure. Hence, the cause-and-effect relationships between plant nutrient status and AM fungal communities are difficult to attribute primarily to soil or host–plant characteristics. Excessive grazing of grasslands causes not only loss of plant biodiversity but also a decline in ecosystem functioning and services such as those provided by AM fungal communities (Faghihinia et al. 2020). In this sense, previous reports have shown different AM colonization responses in the roots of forage plants subject to grazing or defoliation in temperate Argentine grassland soils (Grigera and Oesterheld 2004; García and Mendoza 2012; Mendoza and García 2019; Chippano and García 2021b; Cavagnaro et al. 2021; García 2021).

In particular, Grigera and Oesterheld (2004) described AM colonization patterns in contrasting grazing situations (exclosure and continuous grazing) of plant community level as a whole and of *Paspalum dilatatum*, a dominant species, in a Flooding Pampa grassland. At the community level, these authors found higher total colonization in the grazed area than in the exclosure. In contrast, *P. dilatatum* roots showed higher total colonization and higher proportion of vesicles and arbuscules in the exclosure than in the grazed area. Animal selectivity on this highly palatable grass could determine higher grazing pressure compared with plant community on average. This not only contributes to explaining the controversial result of AM colonization percentage at the two levels (community and individual plant) but also emphasizes the possibility that the mineral nutrition and growth of palatable species are more affected by grazing through its effect on AM colonization (Grigera and Oesterheld 2004).

In a recent study, the impact of defoliation on the relationship between native AM fungal communities and forage species of the Flooding Pampa was investigated under controlled conditions (García 2021). In this study, *L. tenuis* and *Schedonorus arundinaceus* co-culture was exposed to defoliation frequency plus water stress in a saline-sodic soil (García 2021). The results showed that defoliation frequency increased AM colonization in *L. tenuis* roots under both well-watered and water-deficit conditions. This may be a consequence of increased nutrient demand simultaneously with a decrease in root biomass of defoliated plants. In contrast, the AM colonization in *L. tenuis* roots was affected by the defoliation plus water excess treatment, where the combination of stresses seemed to be the most stressful scenario for *L. tenuis* growth. On the other hand, AM colonization in *S. arundinaceus* roots under water stress and defoliation was minimal, and similar quantities of root biomass were observed in the three water treatments evaluated. These results are in accordance with those of Barto and Rilling (2010), who found similar results and proposed that root biomass resilience to defoliation may explain the lack of effect on AM colonization of grasses.

Regarding defoliation intensity, we found that it changes AM colonization dynamics in *L. tenuis* roots (García and Mendoza 2012). Results showed that vesicular colonization decreased from 19% to 5% for non-defoliated plants to 100% for defoliated plants, respectively, indicating a remobilization of nutrient reserves to support shoot regrowth after defoliation. The high proportion of arbuscular colonization found (ranging from 66% to 77%) suggests that *L. tenuis* plants and AM fungi may establish a functional symbiosis even under intense defoliation in a saline-sodic soil (García and Mendoza 2012). These results are in line with those of Cavagnaro et al. (2021), who found that high defoliation intensity (60% of removed shoot biomass) also decreased vesicular colonization and did not affect arbuscular colonization in *Agropyron elongatum* roots. In our study, we also found that the external hyphal network increased from 10.4 m.g^{-1} dry soil in non-defoliated plants to 14.3 m.g^{-1} dry soil in 100% defoliated plants. This increase would contribute to compensating for the losses of root absorptive area in defoliated plants. Then, the strategy of the AM fungal symbiont consists in investing most of the C resources to preferentially retain arbuscular colonization as well as inoculum density in the soil (García and Mendoza 2012).

In relation to the functionality of the AM symbiosis, in other recent studies, we found that a high defoliation intensity (~75% removed shoot biomass) of *L. tenuis* plants decreased the mycorrhizal growth response under P-deficient conditions (Mendoza and García 2019; Chippano and García 2021b). This could be partly explained by the C limitation hypothesis, which predicts that defoliation should decrease C availability for AM fungi, reducing fungal colonization and mycorrhizal benefits in some situations (van der Heyde et al. 2019). Carbon resources of mycorrhizal and high-intensity-defoliated plants are invested to support regrowth and maintain AM symbiosis, showing a net decrease in regrowing shoot tissue compared to non-mycorrhizal plants (Mendoza and García 2019; Chippano and García 2021b). The lack of C is also reflected in a lower relative growth rate of mycorrhizal than non-mycorrhizal plants (Chippano and García 2021b). Therefore, mycorrhizal and defoliated *L. tenuis* plants are not able to compensate shoot biomass production (Mendoza and García 2019; Chippano and García 2021b). In both studies, the AM fungi could be considered a burden for plant growth due to the drainage of C compounds in a P-deficient soil. Regarding this issue, Cavagnaro et al. (2021) reported discrepant defoliation effects (60% of shoot removed) on plant regrowth of grass forage species related to P supply under P-deficient conditions. These authors found that AM fungi increased the relative growth rate of defoliated *Agropyron elongatum* (C_3 grass) grown under low P supply. In contrast, the effects of defoliation on plant regrowth in *Bachiaria brizantha* (C_4 grass) were independent of AM fungi at any P supply. These results indicate that, even under intense carbon stress, the benefits of AM association exceeded the costs at low P supply and promoted plant regrowth after defoliation (Cavagnaro et al. 2021). In conclusion, the AM symbiosis in defoliated plants is highly context-dependent and varies with growth and P mycorrhizal response of the forage species.

Several authors agree that, in the Flooding Pampa grasslands, P more than N is the main deficient nutrient for plant growth that limits the quantity and quality of forage production (Ginzo et al. 1986; Lavado et al. 1993; Rubio et al. 2012). The main agronomic practices utilized to increase forage production in temperate grassland soils are P fertilization and the selection of plant species adapted to grow in P-deficient soils (Mendoza and Pagani 1997; Muir et al. 2011; Mendoza et al. 2016a, b; Chippano et al. 2020, 2021). However, the increase in soil P availability because of P fertilization decreases the AM root colonization of forage species because P affects soil mycorrhizal infectivity through the inhibition of spore germination and a decrease in edaphic hyphal development, which result in a low ability of AM fungi to colonize plants (Covacevich et al. 2006; Gryndler et al. 2006). In addition, it has been suggested that the increase in P concentration within plants increases the resistance to penetration of the root by AM fungi, contributing to a decrease in the AM colonization percentage (Mendoza and Pagani 1997). In a previous study, we found that AM colonization in the roots of a legume and C_3 and C_4 grasses was related to root P concentration under increasing P availability in the soil (Chippano et al. 2020).

In temperate grasslands of Argentina, the doses of P fertilizer usually applied to increase forage production are in the range of 100–200 kg triple superphosphate (TSP) ha^{-1} (Ginzo et al. 1982; Bailleres and Pirodi 2000). Although these doses are lower than those applied in countries with greater economic resources, little is known about the effect of increasing the soil P availability on the functionality of the symbiosis between the native AM fungal communities and forage species. Another effective and inexpensive management option to improve the quantity and/or quality of grasslands in the Flooding Pampa is mixing grasses with legumes. In a field study performed by our research group, the biomass production of *L. tenuis* and *S. arundinaceus* co-culture increased, and AM colonization decreased in both plant roots under the application of 90 kg TSP ha^{-1} (Mendoza et al. 2016a). However, *S. arundinaceus* roots were much more extensively colonized by AM fungal communities when grown with *L. tenuis* in co-culture than when grown in monoculture without P addition (soil P availability: 12 mg P kg^{-1}) (Mendoza et al. 2016a).

Several studies have demonstrated that the inoculation with AM fungi has better effects on the plant growth and P acquisition of legumes than on those of C_3 and C_4 grasses under P-deficient conditions (Tawaraya 2003; Tran et al. 2019). In particular, our studies have shown that *L. tenuis* presents higher AM root colonization than *S. arundinaceus* (C_3 grass) and *Panicum coloratum* (C_4 grass) despite P fertilization (Mendoza et al. 2016a, b; Chippano et al. 2020, 2021). In line with that reported by Mendoza and Pagani (1997), in a recent study, we observed that AM colonization in *L. tenuis* roots increased at low P supply (5–20 mg P kg^{-1}), which allowed a soil P availability of 6.1–12.7 mg P kg^{-1} in a markedly P-deficient soil (available P: 4.20 mg P kg^{-1}) (Chippano et al. 2020). This implies that the low P availability limited AM colonization and that small additions of P can improve colonization levels in *L. tenuis* roots. Selecting an optimum P dose may stimulate the potential activity of native AM fungi in the soil and increase P-acquisition efficiency in several pasture and agricultural crops (Gosling et al. 2013). Our results in *L. tenuis* have shown that it is possible to increase forage yield (80–90% maximum growth rate) and maintain high values of AM colonization (near 70%) simultaneously with low to moderate P fertilization (30–60 mg kg^{-1}), which corresponds to 10–20 mg P kg^{-1} available (Chippano et al. 2020). Regarding the C_3 and C_4 grasses studied

(*S. arundinaceus* and *P. coloratum*, respectively), it was difficult to obtain high forage yield with the concomitant maintenance of AM root colonization (Chippano et al. 2020). If the objective is to retain AM symbiosis, then forage production should reach lower values than those agronomically acceptable, especially for *S. arundinaceus*. Thus, the presence of *L. tenuis* in grasslands or pastures may contribute to maintaining the native AM fungal communities under a wide range of soil P availability and water stress conditions (Escudero and Mendoza 2005; García and Mendoza 2008; Chippano et al. 2020; García 2021).

Another point to highlight is the solubility of the P source added and the different soil P availabilities reached with each P source. The availability of a P source to plants largely depends on its rate of dissolution. However, the availability of P to plants and thus to AM fungal communities may be altered by the influence of soil properties, soil water level, and P-source management factors. In particular, Covacevich et al. (2006) showed that, in a P-deficient soil (available P: 6.5 mg P kg^{-1}) of a temperate grassland, 60 kg P ha^{-1} as soluble superphosphate decreased both the AM colonization in *S. arundinaceus* roots and the number of propagules. In contrast, 60 kg P ha^{-1} as phosphate rock did not change AM colonization or propagules, associated with a higher solubility of soluble superphosphate than phosphate rock (Covacevich et al. 2006). Regarding this issue, Mendoza et al. (2009) showed that P doses from 0 to 480 mg P kg^{-1} in a P-deficient soil (5.7 mg P kg^{-1}) decreased AM colonization in *L. tenuis* roots by 12% despite the P fertilizer source (P soluble as KH_2PO_4, powdered TSP, or phosphate rock) under controlled conditions. In the same line, more recently, we evaluated the effect of a low P dose (15 mg P kg^{-1} as KH_2PO_4) on the mycorrhizal infectivity of a P-deficient soil (6.27 mg P kg^{-1}) and found that the P dose applied did not affect mycorrhizal soil infectivity with *L. tenuis* as test plant (Chippano and García 2021a). In another study, this P dose was able to increase the forage production of *L. tenuis* and to maintain AM colonization in its roots (Chippano et al. 2020). These two complementary studies (Chippano et al. 2020; Chippano and García 2021a) suggest that low to moderate P doses (30–60 mg P kg^{-1} as KH_2PO_4) may be a sustainable strategy to increase *L. tenuis* forage production without affecting AM root colonization and mycorrhizal infectivity in P-deficient soils of the Flooding Pampa.

Regarding the role of AM symbiosis on plant growth and P uptake, several studies have reported a higher AM response in legumes than in grasses (Tawaraya 2003; Tran et al. 2019; Chippano et al. 2021) and a higher AM response in C_4 than in C_3 grasses (Hetrick et al. 1990; Cavagnaro et al. 2014; Chippano et al. 2021) despite soil P availability. In this regard, in a recent study, we evaluated the different strategies for P uptake between a legume (*L. tenuis*) and C_3 and C_4 grasses (*S. arundinaceus* and *P. coloratum*, respectively) under P-deficient conditions (Fig. 17.1) (Chippano et al. 2021). According to the PCA diagram performed, the main strategy of *L. tenuis* was the association with AM fungal communities, whereas that of *S. arundinaceus* was the highest ability of the root system to explore the soil (highest specific root length (SRL) and root mass fraction (RMF)) (Chippano et al. 2021). In contrast, the strategy of *P. coloratum* could not be explained by changes in root traits (SRL and RMF) or AM association (Fig. 17.1) (Chippano et al. 2021). It is

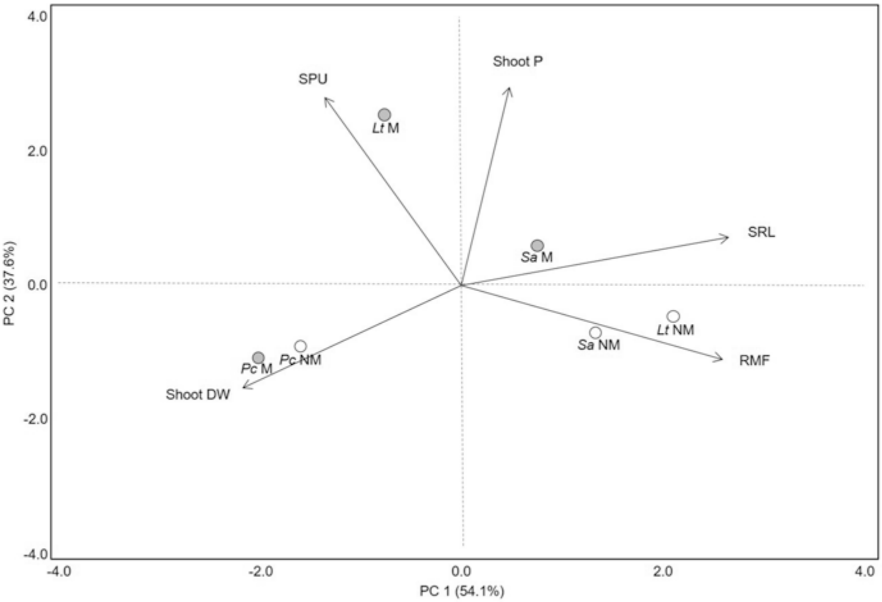

Fig. 17.1 Principal component analysis (PCA) diagram for shoot dry weight (DW), specific P uptake (SPU), specific root length (SRL), root mass fraction (RMF), and P in shoot of *Lotus tenuis* (Lt), *Schedonorus arundinaceus* (Sa), and *Panicum coloratum* (Pc) with (M) or without (NM) AM fungi (gray and white circles, respectively) grown without P added for 68 days. The variables are represented by arrows. (Reproduced from Chippano et al. 2021)

interesting to highlight that, under P fertilization, the association with AM fungal communities caused an increase in the biomass yield of *L. tenuis* similar to that obtained with only the addition of a low P dose (15 mg P kg^{-1}) in the absence of AM fungi. The ability of AM fungi to explore a greater volume of soil than the root system and absorb P beyond the limits of the P depletion zone (Smith and Read 2008) allowed supplanting the net effect of a low P dose on *L. tenuis* growth. At high-P supply (75 mg P kg^{-1}), the mycorrhizal growth and mycorrhizal P response decreased abruptly but with positive values. These results indicated that *L. tenuis* could take advantage of the benefits provided by AM fungal communities with and without P addition (Chippano et al. 2021). Regarding grasses, a high P dose (75 mg P kg^{-1}) caused a negative mycorrhizal growth and P response on *P. coloratum* and a positive mycorrhizal P response on *S. arundinaceus*, associated with the maintenance of a low percentage of AM root colonization (3.47%) (Chippano et al. 2021). These results are in accordance with those of Hetrick et al. (1990), who suggested that C_3 grasses could maintain low levels of AM root colonization despite a high-P fertilization, since the maintenance of low levels of root colonization could ensure rapid proliferation of an already established symbiosis when a period of active nutrient uptake by the plant demands it.

In temperate grasslands, competitive and facilitative interactions between plants of the same or different species operate simultaneously. The balance between interactions largely varies as a result of the intensity of management practices, environmental conditions, plant developmental stage, and species composition (Valladares et al. 2015; Rehling et al. 2021). In addition, plants can also enhance or reduce the AM fungi colonization of neighboring plants, thus conditioning the outcomes of plant interactions (Klironomos 2003; van der Heijden and Horton 2009). In temperate Argentine grasslands and pastures, some reports have evaluated the role of AM fungi in both inter- and intraspecific interactions (Di Bella et al. 2019; Chippano and García 2021b). In these interactions, AM fungi can enhance the rhizospheric environment of plants adapted to grow under salinity and/or alkalinity to promote the presence of species unable to grow under these stress conditions. This has been demonstrated by Di Bella et al. (2019), who found that *P. coloratum* established in a saline-sodic soil of the Flooding Pampa allowed creating suitable microsites through AM symbiosis for the establishment of *L. tenuis* in its neighborhood. In addition, several authors (Facelli et al. 1999; Smith and Read 2008; Tedersoo et al. 2020) have shown that the mycorrhizal hyphal network is able to regulate plant-plant interactions through the nutrient flux between symbiosis partners and tends to intensify intraspecific competition more than interspecific competition. Moreover, a recent study of our group has shown under controlled conditions (Chippano and García 2021b) that AM fungi can increase intraspecific competition between legumes at different growth stages. In this study, *L. tenuis* seedlings grown in the neighborhood of conspecific adult plants defoliated at different intensities showed a negative mycorrhizal growth response, despite the defoliation intensity of neighboring adult plants (Chippano and García 2021b). This is partly because the AM benefits may be unequally distributed if the plants differ in their sink strength for the shared resources in the hyphal network (Kytöviita et al. 2003). Then, we concluded that mycorrhizal *L. tenuis* adult plants modified the competitive balance with seedlings in their favor (Chippano and García 2021b). Our results also showed that non-mycorrhizal adult plants did not affect seedling growth since seedlings grown alone and near adult plants reached the same biomass production and the competition balance was near zero (neutral interaction) despite the increase in defoliation intensity of adult plants (Chippano and García 2021b). These results suggest that, in P-deficient soils, AM symbiosis is a strategy of *L. tenuis* adult plants to compete and regulate the establishment of conspecific seedlings. All these studies are a valuable starting point to better understand the role of AM fungi in intra- and interspecific interactions that allow increasing the presence of legumes in grasslands and pastures of the Argentine Flooding Pampa.

17.4 Conclusion

This chapter provides information about different aspects of the AM symbiosis in temperate grasslands. In particular, it provides data on the effect of grazing/defoliation and P fertilization on the dynamics of the association between plants and native

AM fungal communities, as well as on the role of AM fungi in plant-plant interactions of forage species grown in soils of the Flooding Pampa grasslands.

The information gathered in this chapter suggests that the AM symbiosis in natural grasslands of the Argentine Flooding Pampa is influenced by different factors, including soil characteristics, plant species, and climate factors. In addition, the AM symbiosis and its benefits on forage plant growth and nutrition in the grassland soils of the Flooding Pampa are strongly affected by agronomic practices, P fertilization, and grazing/defoliation. In this sense, it is difficult to conclude which of these factors is more important in controlling changes in AM fungal variables, and it is thus necessary to study a natural system in a holistic form along the time.

In relation to the forage species in symbiosis with the native AM fungal communities of the Flooding Pampa grasslands, *L. tenuis* stands out. This species, which is a perennial herbaceous legume naturalized in the Flooding Pampa, is appreciated by farmers because of its plasticity, productivity, and nutritional forage value for livestock. According to the information presented in this chapter, promoting the presence of *L. tenuis* through low defoliation frequency and a low-P fertilizer dose would improve forage yield and quality with the maintenance of AM symbiosis in legume-grass communities.

Finally, this chapter highlights that it is necessary to investigate the resilience of the AM fungal communities under realistic field conditions in order to implement management practices (e.g., low to moderate P fertilization and rational grazing) that would increase forage production and minimize the detrimental effects on the soil resource and native AM fungal communities of the Flooding Pampa grasslands.

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Chapter 18
Current Trends and Challenges in Viticulture Using Arbuscular Mycorrhizal Fungi

Paula Aguilera, Ninozhka Becerra, Juan Karlo Romero, Nancy Ortiz, Claudia Castillo, and Fernando Borie

Contents

18.1 Introduction

Grapevines (*Vitis vinifera* L.) were one of the first fruit species to be domesticated and nowadays are the world's most economically important fruit crop (Keller 2010). Grapes can be consumed as fresh or dried fruits, as well as grape juice, marmalade, or wine. Worldwide, the global production surface reached 7.5 million ha in 2018 (IOV 2019), mostly for winemaking, with 292 million hL produced in 2018 (IOV 2019). At present, grapevines are extensively cultivated in six of the seven continents, under different and contrasting climates. In South America, three countries stood out in vine crops in 2020, Argentina (215 thousands of ha), Chile (207 thousands of ha), and Brazil (80 thousands of ha). Regarding wine production, Argentina produced 10.8 million hL, Chile produced 10.3 million hL, and Brazil produced 1.9 million hL (IOV 2020).

Nowadays, viticulture is facing several challenges that need to be addressed, based on scientific support. On one hand, water scarcity in the frame of climate change scenarios is of major concern (Miura et al. 2019) since climate models to

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2050 predict that droughts would increase by the middle of the century (IPCC 2014; Santillán et al. 2020) accompanied by increases in temperature. These environmental variations will impact agricultural crops; in an important way, it will also affect viticulture, affecting the production and the potential quality of the grape (van Leeuwen et al. 2019); in addition, there are varieties that have presented greater sensitivity to environmental changes such as Cabernet Sauvignon, Chardonnay, and Pinot noir (Jackson and Schuster 2007; Muñoz et al. 2008; Hadarits et al. 2010). A key element is the improvement of the sustainability of vineyards in terms of water stress, since it has been projected that the availability of water may be a limiting factor (Santos et al. 2020; Marín et al. 2021). Low levels of available water and long periods of drought cause water stress in vines, limiting the absorption of water and nutrients, preventing the plant from developing properly (Sardans et al. 2008), altering both biochemical and physiological processes (Palliotti et al. 2014). However, in specific stages of the vine, the water deficit is favorable for the quality of the wine, since it produces a decrease in the size of the berry and an increase in the phenolic compounds in the skin of the grape (van Leeuwen et al. 2009; Ollé et al. 2011; Triolo et al. 2019; van Leeuwen et al. 2019). In the context of climate change, the impact of increased water deficit and heat stress has been extensively studied in vineyards in the northern hemisphere (van Leeuwen et al. 2019; Santillán et al. 2020), but not in the southern hemisphere. Consequently, this also means new climatic conditions in areas recognized as too cold for the establishment of vineyards, leading to an extension of new cultivation areas due to the expansion of production (Santillán et al. 2020).

Viticulture in Chile as a case study is very interesting due to the existence of different edaphoclimatic conditions in its nearly 4300 km from north to south, where prestigious world-class wine companies are found. This agroindustry in Chile has experienced an extreme drought with a drastic drop in precipitation (estimated between 30% and 45% in the following decades) causing an increased intensity and frequency of fires (González et al. 2020). The latest fire of vast magnitude, the so-called extreme fire event or megafire, occurred in the 2016–2017 summer season in central and south central areas (32–40° S Latitude) and reached a historical maximum of affected area of about 600,000 ha (de la Barrera et al. 2018; González et al. 2020). The Mediterranean areas of the country, where grapevine production concentrates, have been especially affected by this mega-drought and extreme fire events. It has to be underlined, however, that a common oenological practice in many viticultural regions is controlled deficit irrigation because of its benefits to plants as well as the growth and metabolism of grapes, which improves the final wine product (Chaves et al. 2010).

Interestingly, the wine production areas have been moving fast toward the Southern Chile, to areas with higher winter and spring precipitations but lower temperatures. As a result, the South has experienced an increase in planted areas, mostly with cool climate varieties, mainly Chardonnay followed by Pinot noir. Even though plantations have been slowly increasing in the South, viticulture is facing a new constraint such as the production on Andisol, very acidic, phosphorous fixing, and prone to induced aluminum (Al) toxicity.

Considering that new rootstock material specific for soil threats, even though appropriate, is a long-lasting work involving plant breeding, alternative approaches should be explored. It is relevant to consider that, facing the existing restrictions in acid Andisol of Southern Chile, one of the practices used corresponds to the use of calcareous amendments that are applied in large areas. In viticulture this practice could be used from a location located in the cultivation row. However, the high pluviometry ends up washing the existing bases in the soil. On the other hand, P is fixed in low availability, and, in turn, Al in low pH appears in its phytotoxic form. Also, since vineyards are subjected to a significant pressure by applying numerous agrochemicals to reduce pests and weeds (Popescu 2016), many of which have an environmental impact (Pascual 2016; Popescu 2016); environmentally friendly strategies would be more convenient. In fact, there is a growing interest from wine producers, universities, research centers, and in general in the scientific community, in improving the understanding of the ecosystem services that could be granted by products based on microorganisms such as biostimulants that correspond to bioformulations with a scientific-technological base in which the base is made up of species of one or more microorganisms whose name in the research stage is inoculant, inoculum, or inocula. Special emphasis on high-tech agriculture has acquired arbuscular mycorrhizal fungi (AMF)-based biostimulants (Aguilera et al. 2022).

18.2 Mycorrhizal Symbiosis: Role in Agricultural Management Systems

The AMF, belonging to Glomeromycota phylum (Wijayawardene et al. 2018), are obligate symbiotic partners of up to 78% of land plants (Brundrett and Tedersoo 2018). This symbiosis is crucial for the plant community's health and the functioning of nutrient cycles at the ecosystem and landscape level (Castillo et al. 2006; Azcón-Aguilar and Barea 2015). In a recent study, Marín et al. (2021) showed that for Chile, 59 AM species have been reported, mainly documented in agroecosystems as reported elsewhere in Aguilera et al. (2017 and reference therein). Globally, this symbiosis also presents very low levels of endemism (Davison et al. 2015). The AMF symbiosis is based on the bidirectional exchange of nutrients (Smith and Read 2008). Basically, the fungus improves the host plant nutrition via an extensive mycelium network proliferating in the soil, while the plant provides the energy, from photosynthates to the fungus (Bago et al. 2002; Barea and Richardson 2015). In this context, several studies have also shown that AMF favor the adaptation of host plants exposed to abiotic stressors (Bissonnette et al. 2010; Janoušková and Pavlíková 2010), including phytotoxic Al^{3+} (Seguel et al. 2016, 2019), one of the main constraints for crop production in acidic Andisols as here targeted.

The AMF acquire special relevance in agricultural management systems, because they are ubiquitous in soil and play an important role in plant nutrition and health, water uptake, ecosystem services, and soil quality (Barea 2015). Additionally, AM fungi can improve the soil structure through the formation of stable aggregates by a specific group of proteins called glomalin, usually measured as glomalin-related soil protein (GRSP) (Rillig 2004; Rillig and Mummey 2006; Kumar et al. 2018; Wang et al. 2018). These proteins can be produced by the AMF or extracted directly from the soil or even from artificial environments when these AMF are included in soilless systems (Nichols 2010). The environment where the fungus and the root coexist is currently named as mycorrhizosphere and has been defined as the zone in the soil where the processes that affect the root and mycelial structure are developed (Barea 2015). The mycorrhizhosphere, however, is indirectly affected by agricultural practices by modifications on the soil physics, chemical composition, and other aspects (Barea 2015). In this sense, it is important to gain deeper knowledge on how agricultural practices can promote the AM fungi activity leading to the management of the AMF communities (Aguilera et al. 2019). Even though the approximately 300 morphospecies currently recognized have been described mainly based on morphological and anatomical differences of the spores, a large part of the AMF structures (arbuscules, vesicles, and hyphae) are found within the root of the plant or just do not form spores, making this method unreliable (Błaszkowski et al. 2015; Oehl et al. 2017; Sieverding et al. 2014). More recently, molecular techniques have made possible the precise identification of various species either in plant roots or soil samples, through rRNA nuclear genes (Kohout et al. 2014; Öpik et al. 2014; Renaut et al. 2020; Stevens et al. 2020). With this approach it has been found that there is a greater diversity of AMF than previously thought by analyzing only morphospecies from only soil spores and more data on the molecular diversity of HMA or phylogroups is now available. The AMF phylogroup has been proposed to be called "virtual taxa" (TV), in order to have a standard taxonomy of the molecular diversity of the AMF known to date (Öpik et al. 2010, 2014). Given this, today approximately 425 AMF TVs are recognized (MaarjAM database, status October 2018); of which, approximately 60, can be assigned to the morphospecies concept (Öpik et al. 2014). All these indicate that there is a greater AMF diversity than previously thought. Finally, when describing the AMF that make up a given community, it is necessary to consider both the roots and the soil. AMF inhabit both compartments, and when soil has been compared to root for the description of AMF communities, it has been found that the fungi present in the soil are not necessarily the same than those present in the roots and vice versa (Avio et al. 2020; Jansa et al. 2020).

18.3 Current Trends and Projection in Viticulture Using AMF

The AMF have been gaining space in modern agriculture as a sustainable strategy for plant production. Indeed, there is science-based supportive evidence that AMF symbiosis can favor plant fitness when exposed to salinity, drought, diseases, nutritional deficits, and heavy metals, among others, providing ecosystem benefits and improving the stability of soil aggregates (Vlček and Pohanka 2020). Recently, an increasing interest has been devoted to AMF in the field of ecology and plant physiology, reporting benefits of the symbiosis in various crops, dealing with biomass accumulation, translocation of nutrients, changes in heavy metals homeostasis, and exudation of compounds by the mycorrhizal root, among others (Aguilera et al. 2011; Miransari 2017). The application of plant biostimulants in viticulture based on AMF as an eco-friendly strategy has proved to be of benefit for viticulture. Mycorrhizal symbiosis has been reported to promote P absorption in grapevines (Schreiner 2005; Khalil 2013; Trouvelot et al. 2015), by spreading Pi through its extensive extraradical mycelium. Also, N uptake is promoted by AMF (Jansa et al. 2019) generating an increase in the biomass of vine plants (Karagiannidis et al. 2007). Fellbaum et al. (2012) reported that AMF can mobilize N in the form of arginine. In addition to macronutrient absorption, AMF supports the absorption of different chemical elements. Several AMF species have been used as inoculants in vine plants, in which species belonging to *Glomus* genus (*G. constrictum*, *G. desertícola*, and *G. mosseae*) have been highlighted, which have increased the contents of Ca, K, Mg, Fe, and B, among others (Schreiner 2005). These fungi can not only increase nutrients absorption, but also they can attenuate serious constraints that water deficit generates. Water stress is a factor that can be limiting in grapevine production affecting phenological development and consequently production quality. Although AMF are described in literature mainly because they favor water and nutrient absorption (Püschel et al. 2020), mechanisms that confer an increase in water absorption are not yet elucidated, only the expression of putative aquaporin genes (Giovannetti et al. 2012; Kikuchi et al. 2016). In this sense, it has been observed that mycorrhized vine plants have a more efficient use of water (Valentine et al. 2006). On the other hand, AMF provide protection to plants exposed to metals such as Al and heavy metals such as copper (Cu). Although mature vines that could be exposed to this type of phytotoxicity could be adapted to this condition, new plants could be affected, and here AMF could play a mitigating role (Cornejo et al. 2013). Along with the above, it has been reported that AMF can induce defense capacity of plants against pathogens, nematodes, and herbivorous arthropods (Pozo and Azcón-Aguilar 2007; Cameron et al. 2013). AMF species, genus, or strains that they have been adapted to several biotic or abiotic stresses could have great potential use as inoculants oriented to develop biological formulations for use in sustainable agriculture. However, field data is still scarce (Tsvetkov et al. 2014; Szczałba et al. 2019), and local knowledge is much needed, particularly for chemical traits, such as those described for acidic Andisol, and for grapevines managed under controlled deficit irrigation regimes. In particular, grapevines have a high affinity for AMF, as seen by high levels of colonization (Sas Paszt et al. 2019), and by means of biostimulants, colonization rates can reach up to a 30% (Petit and Gubler 2006; Sas Paszt et al. 2019) or even 70% (Fig. 18.1, Flores et al. unpublished data). The high affinity between the fungus and the grapevine has been attributed to the increase in chitinase activity (Li et al. 2006). In addition, it has been reported that the use of AMF in grapevines positively affects yield, water use efficiency, and growth after replanting (Trouvelot et al. 2015). Interestingly, it has been shown in Pinot noir that the diversity of AMF communities varies according the phenological stages of the plant (Massa et al. 2020). Also, some AMF species such as *Funneliformis mosseae*

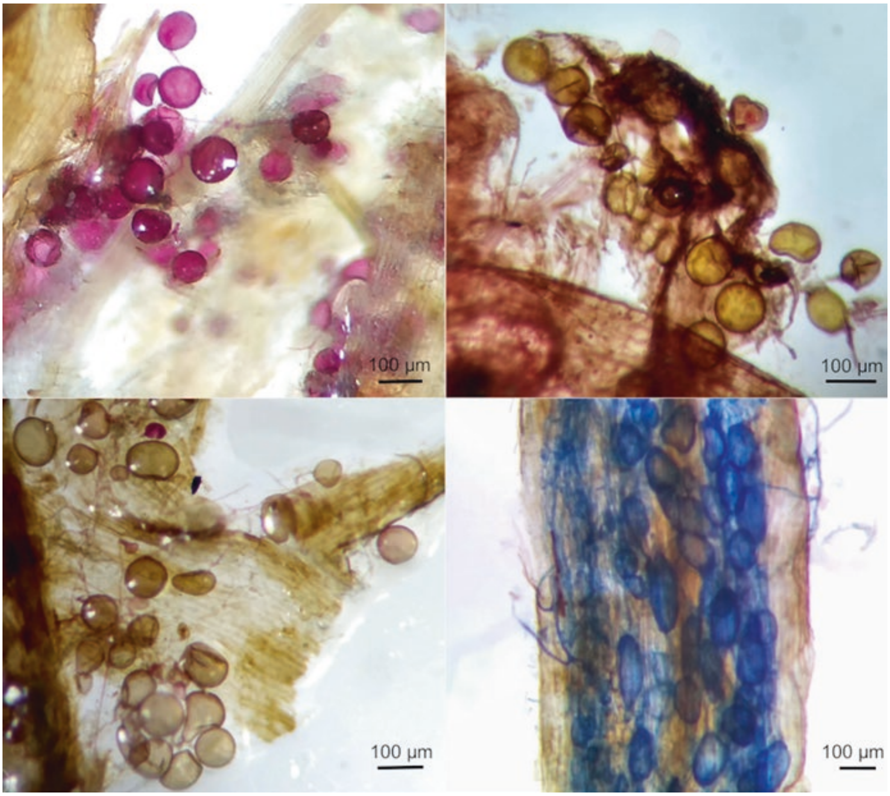

Fig. 18.1 AMF visualization of grapevine plants after 4 months of application of inoculum in commercial vineyards. Photo's credits: P. Aguilera and N. Becerra

and *Rhizophagus irregularis* have been promising in vineyards growing in soils with high concentrations of heavy metals (Nogales et al. 2019).

It has to be said that in grapevines cultivated for oenological purposes, besides yield, the quality of the grape berries is of paramount importance. The chemical composition of the grape berries, regarding primary and secondary metabolites, determines the wine quality and retail price. In this regard, it is interesting to note that roots inoculated with AMF have resulted in a higher concentration of antioxidant compounds, such as phenols, anthocyanins, carotenes, and tocopherol, as well as flavor compounds in cultivated species such as tomato, strawberry, and lettuce (Castellanos-Morales et al. 2010; Baslam et al. 2011, 2013; Han et al. 2012; Zeng et al. 2014; Aguilera et al. 2021a, b). Also, the observed effects of AMF have been reported to be more dramatic in plants under water deficit (Mena-Violante et al. 2006; Baslam and Goicoechea 2012). Some evidence is available on grapevines, where a positive link between AMF and secondary metabolism at the berry level has been suggested (Rouphael et al. 2015; Schreiner 2020), in particular regarding terpenes (Trouvelot et al. 2015). In fact, species, such as *F. mosseae*, have been suggested to favor "terroir" properties in wines, i.e., organoleptic properties very distinctive of a specific productive site (Aguilar et al. 2017).

As suggested previously, one important aspect to take into account regarding eventual plantations in acidic Andosols is the chemical properties of these soils inducing Al phytoxicity and low P availability. Tools have been sought to solve this problem, and conveniently, it has been reported that AMFs, besides acting as biostimulant, biofertilizer, bioregulator, bioprotector, and biocide, favor nutritional aspects in plants, increasing nutrient translocation, especially P, and at the same time decrease translocation of metals (Al) and heavy metals (Cu). In fact, it seems that AM colonization occurs mainly under low levels of soil fertility, particularly P (Smith and Read 2008), and a positive effect of established AMF has been observed in P-deficient soils (Smith et al. 2011). The P is a macronutrient of vital importance for metabolic processes in crop production, being key in photosynthesis, respiration, and energy transduction and as a structural component of membranes, nucleic acids, and enzymes (Ramaekers et al. 2010; Plaxton and Tran 2011; Seguel et al. 2017). However, acid soils are characterized by P deficiency and aluminum (Al) toxicity, which limit root growth and increase susceptibility to drought. Therefore, the use efficiency of P (PUE) is essential, which is defined here as the amount of P accumulated in the tissue per unit of biomass (sprout and/or root) or grain produced (Rose and Wissuwa 2012), which is related to metabolic modifications that can reduce the demand for P during plant development (Hammond et al. 2009; Veneklaas et al. 2012). Therefore, improving internal PUE will lead to more resource-efficient use of P instead of increasing the absorption of potentially scarce forms of P, since, in theory, crops will acquire less P, minimizing the need of P fertilizers (Campos et al. 2018). Furthermore, the microbiota associated with plant roots can improve soil P acquisition through adaptive strategies such as changes in root geometry and architecture that improve the exploitation of soil volume (Gahoonia and Nielsen 2004; Lambers et al. 2006; Seguel et al. 2017). Symbiosis with arbuscular mycorrhizal fungi (AMF) can give the plant a relative tolerance to Al, which has been directly attributed to a greater acquisition of P, Ca, or Mg (Aguilera et al. 2018, 2021a; Campos et al. 2018), by promoting the exudation of organic acids by the roots of the plants to chelate Al (Klugh-Stewart and Cumming 2009; Seguel et al. 2013). In addition, gene expression that could be involved in the mechanisms of Al tolerance and P acquisition efficiency in vine plants will be studied. Aluminum has been identified by several authors as a cause of the increase in reactive oxygen species (ROS), which in large quantities generate cellular damage in plants (Giannakoulas et al. 2010; Xu et al. 2012); however, plants have defense systems that keep ROS levels low (Gad et al. 2010), which include antioxidant enzymes such as peroxides dismutase (SODs), peroxidases (PODs), and catalase (CAT). Various studies show that the presence of HMA increases the activity of antioxidant enzymes in plants under abiotic stress conditions (Huang et al. 2014; Mirshad and Puthur 2016; He et al. 2020); in addition, AMF has the ability to alleviate oxidative damage to plants by hyphae that accelerate the rate of peroxide flow from the host plant to the rhizosphere (Huang et al. 2017). The antioxidant capacity of enzyme components is probably an important factor that determines tolerance to Al in plants (Xu et al. 2012).

Finally, viticulture for high-quality wine is normally accompanied by regulated deficit irrigation in order to improve the grape berry composition. However, deficit irrigation leads to negative effects such as a reduced photosynthetic capacity, lowering yield in the medium term. If additional water deficit is applied to vineyards currently managed with controlled deficit irrigation, it has been observed that AMF frequency increases, simultaneous to a decline in the fine root density, suggesting a compensation (Schreiner et al. 2007). Furthermore, in 1-year-old vines colonized with AMF, physiological traits related to water deficit, such as stomatal conductance and net CO_2 assimilation, were shown to be higher than in non-colonized plants (van Rooyen et al. 2004). The underlying mechanisms of such effects, however, are still not clear but are suggested to depend on soil type, rootstock, and the AMF genotypes involved (Trouvelot et al. 2015).

18.4 General Statements

Vine cultivation in Chile, due to factors associated with global change, has expanded in areas where it had not been developed. The southern edaphoclimatic conditions, such as low temperatures, high rainfall, and characteristics of Andosol, present great challenges for cultivation, both for the selection of productive varieties and for the choice of rootstocks. We have observed that AMF have enormous potential for use as bioinoculants. The uptake of nutrients and the modulation of the secondary metabolism of the crop allow the AM symbiosis to contribute so that the plant can face biotic and abiotic stress, so that an increase in crop vigor and fruit quality is generated. Our research group began with studies in morphotaxonomic identification of arbuscular mycorrhizal fungi, and then with the implementation of molecular tools, we have achieved a multiphase approach to these organisms. In recent years we have dedicated ourselves to scrutinizing species of arbuscular mycorrhizal fungi throughout Chile, and we have generated a germplasm bank, both of generalist and specialist species, positively observing their potential for the future development of biostimulants. Currently our research line is focused on innovation and technology transfer toward the development of inoculants for agricultural production. Agriculture will benefit from improving its quality, productivity, and sustainability. Even so, it is necessary to deepen these foundations in the current scenario of global change, all these under a paradigm of sustainable agriculture.

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Chapter 19
Communities of Glomeromycota in the Argentine Arid Diagonal: An Approach from Their Ecological Role in Grassland Management and Use

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19.1 Introduction

19.1.1 The Argentine Arid Diagonal: Climatic Features, Phytogeography and Glomeromycota Community Importance

In South America, the arid land regions include the South American Dry Diagonal (SADD) that are distributed in two large diagonal of land stripes (Luebert 2021), one involved with the Gran South American Chaco that includes the domains of Caatinga, Cerrado, and Chaco (Olson et al. 2001) and the other one also named South American Arid Diagonal (SAAD) which is a vast area of drylands of deserts which is covering different bioregions such as the Andean region (including high Andes or Altoandino, Puna, Atacama, and Peruvian deserts), Monte, Patagonia, and Chaco (represented by Prepuna) (Abraham et al. 2020) covering an extensive area along the Argentine territory. These two dryland diagonals have a structural and crucial role to determine the patterns of distribution of South American biodiversity, due to their functions as barriers and corridors for species dispersion, which also results in a great difference of flora and fauna between them (Luebert 2021 and references therein). It is important to emphasize that none of the biogeographic and phytogeographic studies in these both SADD have included fungi in general and mycorrhizal fungi in particular in their analysis.

Among the SAAD desertic areas (Abraham et al. 2020), the Argentine Arid Diagonal (AAD) constitutes an area with low precipitations (less than 500 mm) and extends from the north of the country (on the border with Bolivia) to Patagonian Atlantic coasts (González Loyarte 1995; Fig. 19.1). It integrates a set of dry zones that include arid, semiarid, and sub-humid zones with dry periods related to 28 vegetation classes adapted to hot and cold desertic environments (Martínez Carretero 2013).

The arid and semiarid zones represent two-thirds of the Argentine continental surface, and phytogeographic provinces within this surface have a great variety in plant community structure (Cabrera 1976; Busso and Fernández 2018; Fig. 19.1).

According to Argentine phytogeographic description of Cabrera (1976) and the compilation of Busso and Fernández (2018) about studies performed in the last 20 years, 7 provinces are partially (*High Andes*, *Prepuna*, *Chaco*, *Patagonia*, and *Espinal*) or completely included (*Puna* and *Monte*) in AAD (Fig. 19.1). In the northeast of the country is the arid *Puna* province. It constitutes one of the coldest regions in the world (mean annual temperature is 10 °C), and annual precipitation is usually less than 200 mm (Busso and Fernández 2018). The dominant vegetation is shrubby steppe, and in some areas, there are observed herbaceous steppes; there is a high abundance of aphyllous shrubs or with reduced leaves and resinous plants (Carilla et al. 2018). The *high Andean* phytogeographic province has a cold and dry high-mountain climate; vegetation is characterized by grassy or cushion chamaephyte steppes. The *Prepuna* province presents a dry and warm climate, with precipitations concentrated in summer. The dominant vegetation is xerophytic shrub steppe, with

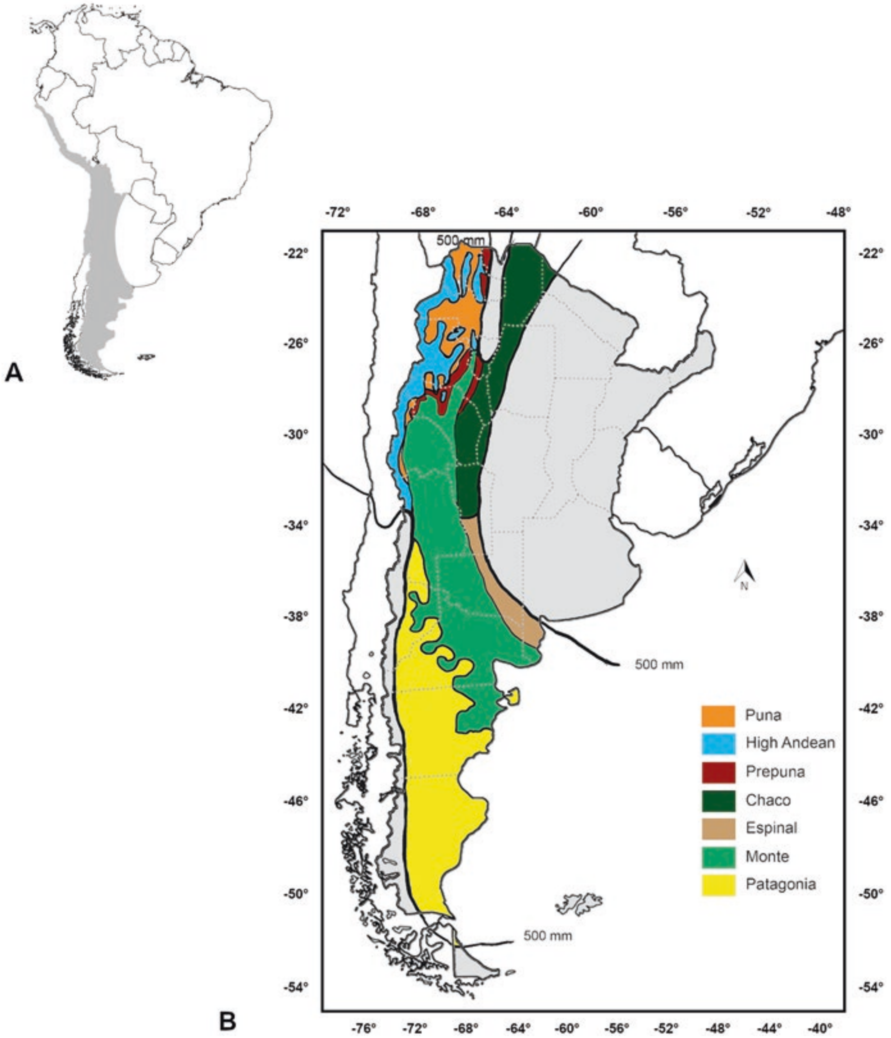

Fig. 19.1 (**a**) South American Arid Diagonal (SAAD, in gray). (**b**) Phytogeographic provinces included in the Argentine Arid Diagonal (AAD). Adapted from Cabrera (1976) and Abraham et al. (2020)

"cardonales" (arborescent cacti species of the genus *Trichocereus*), dwarf forests (Kuentz et al. 2007), and cryptofruticetum communities (buried vegetation, with the cups on the soil surface) (Halloy 2002; Halloy et al. 2008; Carilla et al. 2018). The western portion of *Chaco* phytogeographic province is characterized by warm and humid summers, dry and temperate winters, mean annual precipitation of 320 mm, and high thermic amplitude (Cabrera 1976); plant community is characterized by a medium to low forest of mesophytic or xerophytic trees, with meadows of forage grass species and isolated shrubs (Cabido et al. 1993). The *Monte* phytogeographic province presents a dry climate, being warmer in the north and colder in the south, and records annual precipitations from 60 to 500 mm (Morello 2012). The plant community is associated with a shrubby steppe with xerophytic, sammophilic, or halophilic shrubs (Oyarzabal et al. 2018). The southern portion of the *Espinal* has semiarid climate; the aridity increases from west to south and records annual precipitations from 350 to 550 mm; the dominant vegetation is a xerophytic forest of *Prosopis*, savannas, and steppes with grass species of good forage quality (Lewis and Collantes 1973). Finally, within the *Patagonia* phytogeographic province are the contemplated areas corresponding to the Patagonian steppe. It has a dry and cold climate with intense winds and annual precipitations between 100 and 200 mm (Paruelo et al. 1998; Busso and Fernández 2018). The dominant vegetation is shrubby steppe, with aphyllous shrubs, reduced or spiny leaves, and the presence of cushion species; herbaceous species with good forage quality grow among shrubs (Gaitán et al. 2019).

In these arid and semiarid environments, the economic activities are mainly related to livestock production (Estelrich et al. 2005; Giorgetti et al. 2006; Quiroga Mendiola and Cladera 2018), which has resulted in increasing desertification processes at an extremely high level for these ecosystems (Vorano and Vargas Gil 2002). Also, other activities are carried out related to agriculture, forestry, oil exploitation, and tourism (Abraham et al. 2020). Excessive grazing has degraded many of the areas and has caused changes in the type of dominant vegetation (Giorgetti et al. 2006; Distel 2016). In addition, environmental factors such as droughts, strong winds, irregular precipitations, and anthropic factors, such as clearing and excessive tillage, have led these environments to degradation state and desertification processes (UNEP 1997). Many of these processes are linked to erosion and decrease in soil fertility (MEA 2005; FAO 2020).

Fungi of the phylum Glomeromycota have an important ecological role in poorly fertile environments used for livestock purposes as fungal partners of arbuscular mycorrhizal symbiotic associations with plant roots (Smith and Read 2008). The hyphae of these fungi help the host to absorb nutrients, increase photosynthetic rates, and recover aerial biomass lost after grazing (van der Heyde et al. 2019). In addition, they provide greater resistance to water and nutritional stress and are involved in the establishment, assembly, and succession of plant species (Renker et al. 2004). In the soil, the mycelium of arbuscular mycorrhizal fungi (AMF) is involved in aggregate formation to facilitate adhesion of particles and contribute to giving structure and stability, reducing erosion, and improving the water retention capacity (Finlay 2008).

In this chapter, we have focused on different studies performed in AAD about the ecological role of AMF in natural grasslands used for livestock purposes. Establishing relationships between the AMF diversity, mycorrhizal colonization, and plant communities under different grazing pressures is a factor key in the conservation of forage resources and ecosystem functions in fragile environments with low resilience capacity.

19.1.2 Use and Management of Natural Grassland in AAD

In Argentina, livestock production industry is based on the grazing of native vegetation on arid and semiarid grasslands. The activities are mainly linked to raising of goats and llamas in the northeast of the country, cattle and goat in the central area, and sheep in the south (Busso and Fernández 2018; Quiroga Mendiola and Cladera 2018). Grazing constitutes a modifying and modeling factor not only of the affected species but also of the plant community. Moreover, it alters the structure and functionality of the ecosystem (Peri et al. 2016). Management practices that involve conservationist and rotational grazing have been shown to be efficient in maintaining the preferred grass species, without affecting plant diversity (Giorgetti et al. 2006). However, inadequate grazing management (continuous and with high stocking rates) and adverse climatic conditions have led to replacement of preferred grass species by unpreferred ones, deteriorating the productive potential of natural grasslands (Distel 2016; Oñatibia et al. 2020). Moreover, these floristic changes have a direct impact on soil fertility, because they affect litter decomposition and nutrient mineralization (Ambrosino et al. 2019).

Argentine grasslands are affected by shrub encroachment process (D'Odorico et al. 2012; Carilla et al. 2018). It is defined as an increase in density, cover, and biomass of plant species with a shrub or semi-shrub growth habit, occurring in environments dominated by herbaceous species (van Auken 2009). These woody species are native and were originally present in low-density or in restricted areas (Dussart et al. 2011). However, various factors (climate change, intensive grazing, changes in historical fire regimes, and the dispersal of woody plant seeds by livestock) have increased density and cover of shrub species and favored the development of species with low light requirements (Rauber et al. 2014). Different management practices are used to reverse this situation (Kunst et al. 2012). Some of them involve controlled burning and mechanical controls. In this way, the system "is open" (from a closed physiognomy to a savannah), allows a greater entry of light and water, favors the development of herbaceous biomass, and increases the forage supply and accessibility to livestock (Adema 2006).

Natural grasslands in good state of conservation have a high abundance and diversity of Glomeromycota species (Hartnett and Wilson 2002). Given that, these fungi have multiple attributes and functional traits; this event has a great ecological and environmental value (Chagnon et al. 2013; Longo et al. 2016; Weber et al. 2019).

19.2 Ecological Role of Glomeromycota Communities

Mutualisms are considered key interactions in structuring ecological communities (Bronstein 1994). Mycorrhizal mycelium communicates and integrates plants within a community, facilitating colonization by other fungi and transferring compounds among plant roots (van der Heijden and Horton 2009). These processes promote the establishment, growth, survival, and defense of individual plants in a wide variety of ecosystems (Simard et al. 2012). However, the outcome of such interactions can range from mutualism to parasitism, depending on the identity of the symbiotic species and environmental factors (Bronstein 1994). Plant communities affect AMF distribution and composition in the soil (Johnson et al. 1992) and favor fungal species presence with different ecological attributes maximizing benefits on root architecture (Sikes et al. 2010). In turn, AMF may exhibit “ecological host specificity” (McGonigle and Fitter 1990) where roots of the same plant species are preferentially colonized by some fungi in contrast to others when grown in the presence of the same Glomeromycota community (Li et al. 2014).

There are ecological differences among Glomeromycota species that influence the cost-benefit of this association to their plant symbionts. These functional traits are mainly related to carbon demands toward their plant hosts, competitive abilities, P translocation toward roots, intra and extraradical mycelium production, and sporulation rates (Ijdo et al. 2010; Chagnon et al. 2013; Weber et al. 2019) that are also related to different families of Glomeromycota. Thus, members of Glomeraceae have characteristics typical of r-strategist species; they have high spore production in a short time period and regulate their growth depending on the host plant activity (Sýkorová et al. 2007; Ijdo et al. 2010). They are associated with disturbed and ruderal environments, have rapid growth, and produce anastomosed extraradical mycelium close to the root with abundant intraradical mycelium (Parniske 2008). Together with Claroideoglomeraceae and Paraglomeraceae, they are considered “rhizophilic” symbionts because they allocate most of the resources for hypha development in the radical interior (Weber et al. 2019). Resistance to pathogens and herbivores is the main benefit that these fungi offer to their plant symbionts (Chagnon et al. 2013). Some genera have been described for other semiarid environments in the world (Aliasgharzadeh et al. 2001; Carvalho et al. 2001). *Rhizophagus* is characterized by high tolerance to environmental stress (Liu et al. 2009) and grazing (Yang et al. 2013). On the other hand, *Glomus*, commonly found in environments with low precipitation (Lovelock et al. 2003), shows great adaptation to soils of different quality and has a high capacity to establish symbiotic relationships with different plant species (Cai et al. 2014).

Members of Gigasporaceae have characteristics of strategic K organisms and allocate most of the resources for growth and survival, producing a low spore number of prolonged viability and bigger size than Glomeraceae (Ijdo et al. 2010). Together with Diversisporaceae, they are considered “edaphophilic” symbionts because they produce abundant loose extraradical mycelium and invest few resources for intraradical hypha development (Weber et al. 2019). Gigasporaceae have a high competitive capacity and increase the uptake of nutrients (mainly P) from plant symbiont due to their abundant mycelium (Giovannini et al. 2020). However, due to its low hyphal healing capacity, this family is susceptible to disappearing when environmental conditions are unfavorable (Verbruggen and Kiers 2010; Giovannini et al. 2020).

The Acaulosporaceae has low growth rates and produces long-lived hyphae with low turnover rates (Chagnon et al. 2013). These fungi are poor colonizers of soil and plant roots because they produce little intra- and extraradical mycelium (Powell et al. 2009). Considering this feature, together Archaeosporaceae, Ambisporaceae, and Pacisporaceae are grouped within a guild called "ancestral" (Weber et al. 2019). They are considered stress-tolerant fungi and have reported in natural grasslands of Argentina (Lugo et al. 2003; Velázquez et al. 2013; Ambrosino et al. 2018). Members of this family can survive in adverse conditions such as low temperatures, soil acid pH, and drought (Chagnon et al. 2013). The initial cost to their plant symbiont can be high because these fungi can be slow to provide nutritional benefits to their hosts; however, plants may be compensated by symbiosis benefits in the long term (Chagnon et al. 2013).

19.3 Glomeromycota Communities in AAD Natural Grasslands

The Glomeromycota species that have been cited for AAD and included in this chapter come from both morphological and molecular identification due to the importance of using both approaches as it has been highlighted by Covacevich et al. (2021). Moreover, considering the low sporulation of these microorganisms in arid soils, these authors suggest employing trap plant multiplication strategies to improve the knowledge of AMF richness. We used the AMF morphospecies classification proposed by Schüßler and Walker (2010) and Wijayawardene et al. (2020). To date, 44 AMF taxa have been reported in the phytogeographic regions of the AAD, *High Andes*, *Puna*, *Monte*, and *Patagonia*; those taxa that were identified to species level are recorded in Table 19.1.

Along the AAD, Glomeromycota species were grouped in three orders and eight families: Archaeosporales, Ambisporaceae; Diversisporales, Acaulosporaceae, Diversisporaceae, Gigasporaceae, and Pacisporaceae; Glomerales, Claroideoglomeraceae, Entrophosporaceae, and Glomeraceae. The highest number of AMF species belonged to Glomeraceae (17 species), followed by Acaulosporaceae (10 species), Gigasporaceae (6 species), Ambisporaceae (3 species), Diversisporaceae, Claroideoglomeraceae, Pacisporaceae, and Entrophosporaceae (2 species each) (Fig. 19.2).

These results are in agreement with Stürmer et al. (2018) and Cofré et al. (2019), who reported that Glomeraceae is the dominant family in South America and then Acaulosporaceae and Gigasporaceae, considering the species number per family. The highest number of AMF species was recovered in *Patagonia* (52%) followed by *Puna* (36%), *Monte* (31%), and *High Andean* (23%) regions. In a recent review, Cofré et al. (2019) recorded 83 morphospecies for Argentina. The number of AMF species isolated from the different biogeographic regions included in this work constitutes a significant contribution to the total country diversity and highlights the importance of continuing to carry out studies that promote the knowledge and conservation of biological diversity of Glomeromycota associated with these extreme environments.

Table 19.1 List of arbuscular mycorrhizal fungi (AMF, Glomeromycota) species described or cited in the biogeographic regions included in Argentine Arid Diagonal (AAD)

	Biogeographic regions			
AMF taxa	High Andean	Monte	Puna	Patagonia
Archaeosporales				
Ambisporaceae				
Ambispora leptoticha		X		
Am. fecundispora				X
Am. gerdemanii		X		X
Diversisporales				
Acaulosporaceae				
Acaulospora delicata				X
A. denticulata				X
A. excavata		X		X
A. foveata				X
A. laevis		X	X	
A. mellea		X		X
A. paulinae				X
A. rehmii				X
A. scrobiculata				X
A. spinosa			X	X
Diversisporaceae				
Corymbiglomus globiferum			X	
Diversispora spurca		X		
Gigasporaceae				
Dentiscutata heterogama				X
Gigaspora margarita			X	
Gi. ramisporophora			X	
Scutellospora biornata			X	
S. calospora				X
S. dipapillosa				X
Pacisporaceae				
Pacispora scintillans				X
P. patagonica				X
Glomerales				
Claroideoglomeraceae				
Claroideoglomus etunicatum	X	X		X
C. lamellosum	X			
Glomeraceae				
Dominikia iranica	X			
Funneliformis caledonium	X			
Funneliformis geosporus		X	X	X
F. monosporus			X	
F. mosseae	X	X	X	X

(continued)

Table 19.1 (continued)

	Biogeographic regions			
AMF taxa	High Andean	Monte	Puna	Patagonia
Glomus ambisporum			X	
G. fuegianum				X
Rhizophagus aggregatus			X	
R. clarus		X		X
R. fasciculatus			X	X
R. intraradices	X	X		X
R. irregularis	X	X		
R. microaggregatum	X	X	X	
Sclerocystis coremioides	X			
S. sinuosa			X	
Septoglomus constrictum			X	
Se. viscosum			X	
Entrophosporaceae				
Entrophospora baltica		X		
E. infrequens	X			X
Total number of AMF species	**10**	**14**	**16**	**23**

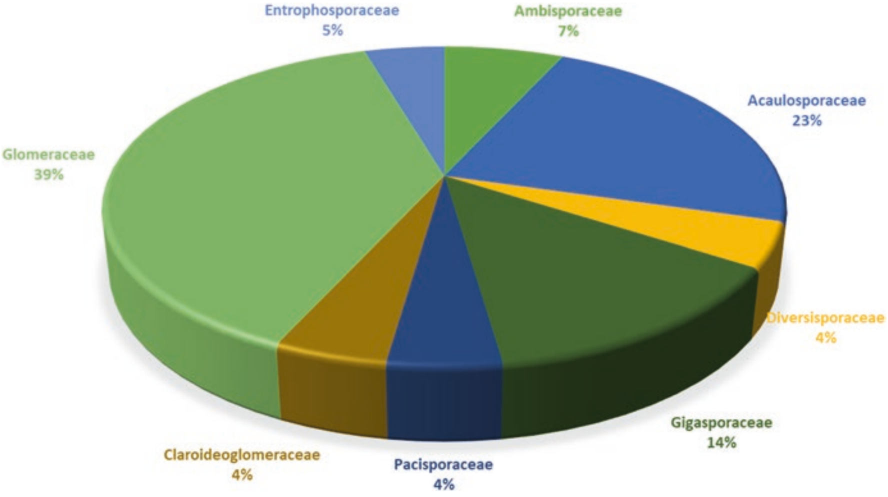

Fig. 19.2 Percentage (%) of the number of species per family of Glomeromycota reported in grassland of the Argentine Arid Diagonal (AAD)

The AMF presence has been reported in grasslands around the world (Hartnett and Wilson 2002), as well as the impact caused by human activities on vegetation and AMF. Studies performed in different arid environments in Asia reported *Glomus* as dominant AMF genus (Su and Guo 2007) and an effect of different grazing intensities on AMF richness and diversity (Su and Guo 2007; Ba et al. 2012; Kusakabe et al. 2018). Stutz et al. (2000) found similarities in the AMF species composition from 54% to 79% and limited mainly to members of the Glomeraceae and Acaulosporaceae in desert areas of North America and semiarid grassland of Namibia. Moreover, in arid regions of South America, *Funneliformis geosporum* prevails in thedry south-central Andes and in Brazil, *Claroideoglomus etunicatum* is associated with the Caatinga ecodivision, and genera *Ambispora* and *Rhizophagus* are associated with the Cerrado ecodivision (Cofré et al. 2019).

Glomeromycota diversity reported in this chapter comes from the analysis of scientific works carried out in natural grassland under real or simulated grazing (Mendoza et al. 2002, 2011; Ambrosino et al. 2018; Dudinszky et al. 2019) and undisturbed environments of AAD (Lugo et al. 1995, 1997, 2008; Silvani et al. 2017; Covacevich et al. 2021). Livestock affects soil structure and reduces AMF sporulation (Allen and Allen 1980; Daniels and Trappe 1980; Thurow et al. 1986). Grazing alters plant species composition (Distel and Bóo 1996; Augustine et al. 2017; Porensky et al. 2017). The effects of grazing on AMF are controversial because the responses of these biotrophic symbiotic fungi to herbivory are context dependent and would be directly related to the carbon flux among plant-AMF-soil and the intensity and extent of grazing over time, the mycorrhizal dependence of grazed plants, adaptation of AMF, and their host plants to grazing (van der Heyde et al. 2019). Furthermore, AMF responses considering soil hyphal length, host root colonization, composition of soil communities, and their sporulation by grazing were mainly driven by the extent of grazing time (van der Heyde et al. 2017). For example, in *Puna*, longtime grazing has caused intense shrubland expansion to the detriment of grassland habitats. Overall, a global meta-analysis of livestock effect on AMF has shown the negative effect of heavy or moderate grazing on AMF abundance; this AMF diversity variable was also reduced due to low annual precipitation, longtime grazing, and, consequently, host aboveground biomass reduction (Yang et al. 2020). Intensive grazing affects AMF negatively because it reduces the photosynthetic capacity of plants (Harley and Smith 1983) and limits the supply of carbon to the roots (Barto et al. 2010). On the other hand, under moderate grazing, symbiosis with fungi would increase plant species tolerance by increasing the nutrient supply, promoting compensatory growth, and favoring plant competitive capacity (Hartnett and Wilson 2002). However, the grazing effects on AMF diversity in grasslands of arid and semiarid zones remain in discussion.

In some cases, changes in AMF communities have been independent of host plant identity (Mendoza et al. 2002, 2011; Dudinszky et al. 2019). However, Ambrosino et al. (2018) demonstrated that simulated grazing did not affect the density, richness, and diversity of AMF in semiarid soils of northeastern Patagonia. The authors argued that grazing intensity might not have been sufficient to decrease photosynthetic tissue significantly and reduce the availability of resources to inhibit AMF colonization rates or induce mortality (Trent et al. 1988). Rather, the particular characteristics of the grass species such as plant size, litter quality, and plant developmental morphology stage were influential in altering AMF communities (Ambrosino et al. 2018).

As mentioned above, AMF species can be classified according to the relationships between life history traits and environmental abiotic filters, competitors, stress tolerant and ruderal (Chagnon et al. 2013); or according to biomass allocation to extraradical mycelium, intraradial mycelium, and spores, rhizophilic, edaphophilic, and ancestral (Weber et al. 2019). Many researchers argue that it is important to consider these AMF functional traits in the study of changes in the abundance and diversity of AMF spores. Members of Glomeraceae and Claroideoglomeraceae showed the highest spore abundance in natural grassland of AAD. These families are considered ruderals and rhizophilics and have strategies related to a short life cycle, rapid and abundant spore production, and high colonization rate. These features allow AMF to re-establish a symbiosis following grazing. Acaulosporaceae species that have low growth rates but efficient carbon use exhibited erratic behavior with respect to grazing intensity. These functional traits might be a way to tolerate abiotic stress factors and grow with carbon-limited amount provided by its plant symbiont. Conversely, Gigasporaceae species were generally absent in sites with high grazing intensity. The notable decrease in photosynthetic tissues would not be enough to cover the high demand for carbon by species belonging to this family and might to explain the absence or decrease in the record of spores.

Although the different functional groups recently mentioned are a component of the Glomeromycota diversity, the benefit that each AMF family provides to its host is different. Fungi belonging to edaphophilic guild (i.e., Gigasporaceae species) provide higher capacity to explore the soil and acquire nutrients than rhizophilic fungi (i.e., Glomeraceae). Under limited availability of nutrients and water, the exclusion or reduction of some family might alter the benefits for the plants.

19.4 AMF Colonization: Effect of Management and Ecophysiological Characteristics of Plant Species in Semiarid Grasslands

Arbuscular mycorrhizal fungi establish an obligate symbiosis with most land plants of ecosystems in the world (Schüßler et al. 2001; Hempel et al. 2007; Spatafora et al. 2016). Arbuscular mycorrhiza is distributed from the equator to the Antarctic and Arctic regions (Trappe 1987; Smith and Read 2008) and is even found at high altitudes until 5250 m in the Andes (Schmidt et al. 2008). The symbiosis between plants and AMF favors plant persistence in unfavorable conditions (Koltai and Kapulnik 2010). In arid environments, this association improves tolerance to drought, allowing access to soil nutrients and promoting plant growth (Dhillon and Zak 1993; Körner 1999).

Colonization of AMF in *extreme* environments of AAD is confirmed in this chapter. The reports include studies performed in: (i) Sierras de las Quijadas National Park, located between the biogeographic regions of *Monte* and *Chaco* (Lugo et al. 2015), (ii) native highland grasses in the *Puna* (Lugo et al. 2012, 2015, 2018; Menoyo et al. 2020), and (iii) hypersaline Andean wetlands (Silvani et al. 2013).

Grazing alters root morphology, soil physical-chemical properties, and structure and composition of the plant communities (Hiiesalu et al. 2014). These modifications alter the root colonization by AMF (Kojima et al. 2014). Some studies mention a decrease (Eom et al. 2001), while others report increases (Frank et al. 2003) or even no changes in the percentages of AMF colonization (Torres et al. 2011). Moreover, grazing can affect the colonization quality increasing the presence of arbuscules or vesicles on plant roots and causing the symbionts to face stressful situations (Parodi and Pezzani 2011; Piippo et al. 2011).

In the south of the Province of Buenos Aires, Torres et al. (2011) studied the simulated grazing effect on mycorrhizal colonization in grasses (native, naturalized, and introduced). In all cases, levels of mycorrhizal colonization were similar after defoliation. However, higher colonization percentages were recorded in native species, parameter that contributes to defoliation tolerance. Similar results were found by Ambrosino et al. (2020), who reported that moderate defoliations did not affect total colonization in the roots of three species of perennial grasses with different palatability. However, changes in structure colonization were observed. Higher percentages of vesicles and arbuscules were detected in *Poa ligularis* (palatable specie) and *Amelichloa ambigua* (unpalatable specie), respectively. These results evidenced plant identity effect on mycorrhizal association and higher dependence on its fungal symbionts in unpalatable grass species.

Studies performed in Patagonia differ from those mentioned above and showed that palatable species was associated with the highest percentages of colonization. Colonization percentages were the highest during active season of grass species and the lowest under overgrazing conditions. These results suggest that under excessive grazing conditions, the benefits of mycorrhizae for the plant could be reduced (Cavagnaro et al. 2019). In agreement, García et al. (2012) demonstrated that low grazing intensity favored AMF colonization in degraded grasslands of Tierra del Fuego.

Shrub presence in natural grasslands causes spatial differences in nutrient availability and water and soil biological activity (Maestre et al. 2011). Cavagnaro et al. (2017) conducted a study in the Patagonian steppe and compared sites with different types of dominant vegetation, shrub patches and grasses in bare soil patches. They observed that mycorrhizal colonization was higher under shrubs and palatable grass species. Shrub patches were a critical point of fertility and may be functioning as refuge for beneficial associations between plants and AMF. This phenomenon should be considered in study of symbiotic associations; spatial heterogeneity in the AMF composition might reveal possible mechanisms for program development that promote the conservation and revegetation in arid ecosystems (Cavagnaro et al. 2017).

Prescribed burning influences arbuscular mycorrhizal colonization in semiarid grasslands. The direct effects of burning on the propagules of arbuscular fungi depend on the intensity, duration, and frequency of said disturbance (Hartnett et al. 2004). Aguilar Fernández et al. (2009) reported that controlled and moderate burning would not have a significant impact on mycorrhizal colonization. This would be the result of the low thermal conductivity in soil and the rapid combustion of herbaceous vegetation (Aguilar Fernández et al. 2009). Ithurrart et al. (2018) analyzed the effects of controlled burning and defoliation on root morphology and AMF colonization levels in three species of native perennial grasses. The authors showed that defoliation after controlled burning did not affect the AMF colonization. However, characters related to root morphology (as diameter and length) determined interspecific differences among the percentages obtained.

19.5 Conclusion

The analysis of the different scientific studies showed that AMF play a key role in the conservation of the natural grasslands of AAD. Considering that arid and semiarid environments are reservoirs of microorganisms adapted to live in extreme conditions, future studies that promote the isolation and identification of AMF with biotechnological potential are important for the establishment of plant species and rehabilitation of degraded areas.

The use of forage resources must be carried out with caution to conserve the attributes and functional traits of Glomeromycota communities and ecosystem services in the natural grassland of AAD (Fig. 19.3). Mycorrhizal colonization is beneficial to recover photosynthetic tissue lost after moderate grazing and to promote compensatory growth of grass species (Fig. 19.3). Future research should identify Glomeromycota species that are effectively colonizing the roots and develop management practices aimed to maintain acceptable levels of AMF colonization in unfavorable environmental conditions.

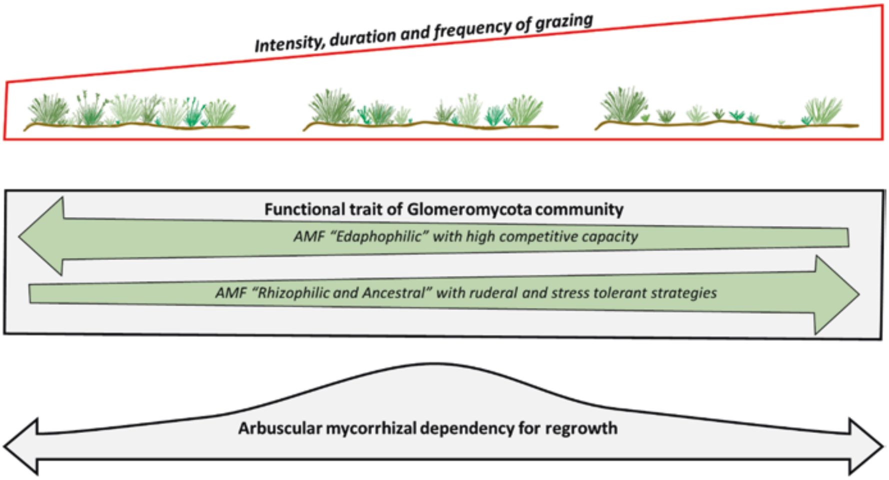

Fig. 19.3 Schematic of the ecological role of arbuscular mycorrhizal fungi (AMF Glomeromycota) in grasslands of the Argentine Arid Diagonal (AAD)

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Chapter 20
The Current Scenario of the Distribution, Functionality, and Ecosystemic Role of the Arbuscular Mycorrhizal Symbiosis in Chile

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Contents

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20.1 Introduction

About 85% of vascular plant species establish relationships with arbuscular mycorrhizal fungi (AMF) (Jeffries et al. 2003; Brundrett 2009), existing in all biomes of the Earth (Soudzilovskaia et al. 2017), including 90% of agricultural plants (Finlay 2008; Smith and Read 2008). Based on the above, AM constitute the most widespread plant-microorganism association on the Earth's surface (Smith and Read 2008). Detailing, the AM association is present in a wide range of habitats, from hydromorphs to deserts, tropical forests, and even at high latitudes and altitudes (Casanova-Katny et al. 2011; Davison et al. 2015; Santander et al. 2021b). The AM symbiosis is crucial for nutrient absorption and cycling in woody species (Smith and Read 2008), as well as in carbon cycling (C) (Read and Pérez-Moreno 2003; Godbold et al. 2006; Averill et al. 2014), even constituting C transfer networks between different plants (Simard and Durall 2004). The role of AM in the acquisition, transport, and transfer of phosphorus (P) to plants is also well known (Smith and Read 2008), being the most frequent symbiosis in temperate and tropical ecosystems (Acuña et al. 2020). In acidic soils, like most soils from central and southern Chile, they are equally dominant, especially improving the acquisition of low available P forms (Urzúa et al. 1993; Seguel et al. 2017), as well as contributing to the resilience of plant communities. Moreover, the presence, richness, and abundance of AM fungi are commonly used as indicators of ecosystem conservation (Castillo et al. 2006b; Torres-Mellado et al. 2012; Chávez et al. 2020).

In a context of climate change and anthropic alteration of the Earth's surface, AM also provides an increase in the tolerance of plants to drought and various diseases (Smith and Read 2008; Santander et al. 2017), while they can also improve the state of soils contaminated with phytotoxic elements such as aluminum (Al) (Seguel et al. 2013; Borie et al. 2019), copper (Cu) (Castañón-Silva et al. 2013), persistent organic compounds (Cornejo et al. 2013, 2017a), or heavy metals (Fuentes et al. 2016b). Additionally, the AM symbiosis can modify the physiological processes of plants growing at low temperatures, through the synthesis of metabolites that promote water balance (Acuña et al. 2020), even providing benefits for plant growth in highly salinized environments (Santander et al. 2019a, b).

Despite the previous background constitutes an overview of the studies that have been conducted in different latitudes regarding the AM symbiosis, in Chile the studies related to this subject are relatively recent, although they have acquired notoriety for their multiple ecological relationships and the possible biotechnological applications that they can originate. Therefore, it is needed to systematize the existing information and organize it locally in terms of areas, thus allowing to identify information gaps regarding the AM symbiosis. For this reason, this chapter aims to analyze the information on the distribution, functionality, and ecosystem role of AM symbiosis in Chile to identify study needs at the national level. We hope this systematization could serve as a starting point for highlighting the importance of integrating the different uses of a territory in an ecological and sustainable way, enhancing the natural characteristics of ecosystems in a framework of maximization of the provision of ecosystem services, where the AM is a key component.

To accomplish our aim, we performed a review of the scientific works in the bibliometric platforms Scopus, Web of Science, SciELO, and Google Scholar for AM-related studies associated with *Chile* as affiliation country. Then, the reports that were elaborated with experimental systems within the territory of Chile were selected, considering both native plant species and agricultural plants. This information was systematized according to five geographical zones: (i) far north, (ii) near north, (iii) central Chile, (iv) south, and (v) far south, in addition to the interest of the study: (i) human-managed ecosystems or (ii) in natural ecosystems. Following, the studies were categorized according to the main focus: (i) phytotoxicity by pollutants, (ii) plant growth and nutrition, (iii) water stress, (iv) factors affecting AM in agroecosystems, (v) factors affecting AM in natural ecosystems, (vi) role of AM in natural ecosystems, (vii) taxonomy of AM, (viii) salinity tolerance, (ix) fire effects, and (x) AM and genetics. Likewise, an analysis of the ecological role of the presence of MAs in the various ecosystems of Chile, both natural and modified by humans, was generated, revealing the importance of promoting the protection of natural ecosystems, as well as promoting the use of sustainable management in agricultural systems to improve their productive efficiency. Furthermore, for AMF taxa was followed the nomenclatural system of the Glomeromycota phylogeny page (http://www.amf-phylogeny.com/). Finally, we identified where the greatest research efforts are concentrated and where deficiencies are evident in, suggesting research areas to be covered in the future. The need to address these studies according to their applications to contribute to improving conditions of both natural and human-modified environments was highlighted.

20.2 Systematization of Studies Regarding the Arbuscular Mycorrhizal Fungi in Chile

By means of the above-described procedure, a total of 93 studies were found that consider the AM symbiosis in Chile, among which 49 are focused on an agronomic approach or alternatively oriented to propose solutions to problems derived from mining activities (soil pollution by potentially toxic elements). Other 38 are framed in the study of properties, functionality, and taxonomy of AMF in natural ecosystems, finally corresponding the remaining 4 studies to a mix orientation of both interests. In terms of zonalization, 11 studies corresponded to the north zone, specifically 7 carried out in the far north, 4 in the near north, and 1 that includes both geographical areas since it covers a wide extension of sampling of several populations of the genus *Atriplex*. On the other hand, 21 studies correspond to central Chile; 58 were performed in the southern Chile, and only 2 were performed in the far south (Fig. 20.1).

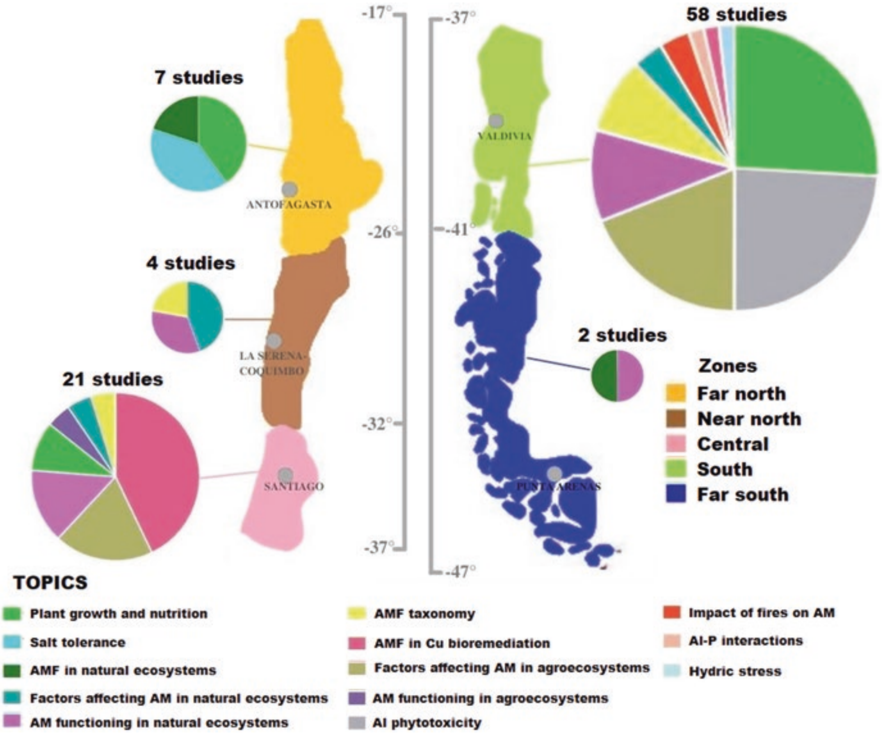

Fig. 20.1 Distribution by geographical macrozone and focus on the studies regarding to the arbuscular mycorrhizal symbiosis in Chile. Map author: P. Yates

20.2.1 Far North Zone

In this zone, considering the eight studies found, six of them included agricultural issues; other three describe the role of AM in plant growth and nutrition, while three others address the effect of AM symbiosis in salinity tolerance. Another study presented the distribution of AMF in natural ecosystems, and a recent report covers both the far and near north zones describing the function of AM symbioses in natural ecosystems.

20.2.1.1 Studies on Plant Growth and Nutrition

In relation to the effect of AMF on plant growth and nutrition, Santander and Olave (2012a, b, 2014) conducted studies on melon during the seedling stage, for evaluating the effect of the fungus *Rhizophagus intraradices* on the plant growth and precocity, evidencing earlier phenological changes in plants colonized by AMF. The inoculation with different doses of spores revealed that the optimal amount is about 40 AMF spores per plant, despite the authors also tested inoculation with half and twice that amount. The optimal spore's density produced a greater accumulation of root biomass, stem diameter, and ratio between the dry weight of the root and the dry weight of the shoot. Likewise, the use of AMF also favored the tolerance to transplantation after the production on nursery conditions (Santander and Olave 2012a, b). The authors also tested the co-inoculation of AMF and *Trichoderma harzianum* through co-inoculation, but no growth-promoting effect was observed; on the contrary a detrimental effect was observed by part of *T. harzianum* on the mycorrhizal colonization (Santander and Olave 2012a, 2014).

20.2.1.2 Studies on the Role of AMF in Salinity Tolerance

The three studies carried out to analyze the role of AMF under saline stress used lettuce (*Lactuca sativa*) as host plant, one of them using in two varieties of lettuce, "Grand Rapids" and "Lollo Bionda," inoculated either with a consortium of AMF isolated from the Atacama Desert (Fig. 20.2) or with the fungus *Claroideoglomus claroideum* obtained from agricultural soils in the southern Chile. In this study, it was shown that the colonization by AMF allowed the modulation of saline stress in lettuce through changes in antioxidant enzyme systems that reduce oxidative damage, favoring the plant growth (Santander et al. 2019a). Additionally, Santander et al. (2021a) in a study with the same lettuce varieties inoculated with two AMF isolated from the Atacama Desert, *Funneliformis mosseae* and *Claroideoglomus lamellosum*, reported a greater AM colonization in lettuce roots at high levels of salinity (Fig. 20.2), which was shown to have implications at the morphogenetic level, regulating genes involved in the maintenance of the internal balance of K^+/Na^+ concentrations and favoring the homeostasis through the accumulation of Na^+ in vacuoles or their extrusion to the outside of root cells (Santander et al. 2021a). These results reinforced previous observations, which suggested that AMF structures, both in the soil and within the roots, can act as a biofilter preventing the entry of high concentrations of Na^+ ions into the plant (Santander et al. 2019b). In this sense, the improvement in the growth of plants, their nutrition, and water capture allow to highlight the role of AMF adapted to conditions of high salinity, which ultimately can improve the conversion to a sustainable agriculture in saline soils when the AMF can be used as bioinoculants (Santander et al. 2021a).

20.2.1.3 AMF in Natural Ecosystems

A recent study reported the presence of AMF along three altitudinal belts in the Tarapacá Region (Atacama Desert), ranging from the hyperarid desert (700–2000 m a.s.l.), the Prepuna (2000–3100 m a.s.l.), and the Puna (3100–4500 m a.s.l.) (Fig. 20.3). Considering a total of 111 plant samples, all of them showed AM colonization or AM fungal structures in the rhizosphere, varying the colonization rates from 3.5% to 87%, and presenting in the rhizosphere of

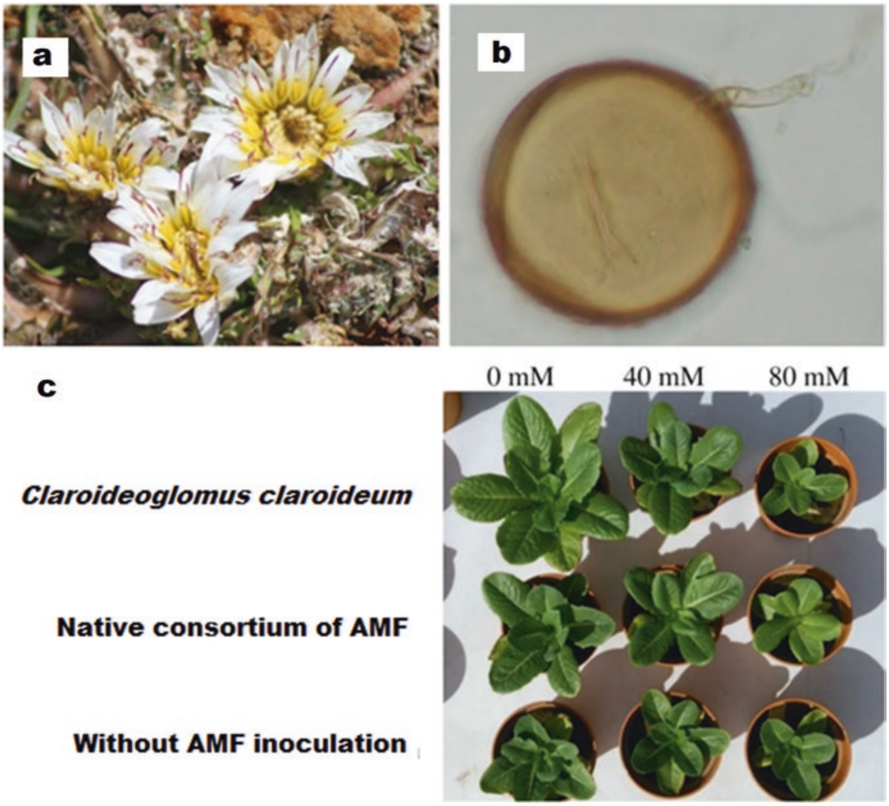

Fig. 20.2 Obtaining of arbuscular mycorrhizal fungi (AMF) spores native from the Atacama Desert (**a**) *Werneria pinnatifida* in the "Salar de Huasco," from which spores were isolated. (**b**) Native AMF spore isolated from *W. pinnatifida.* (**c**) Results obtained with the inoculation of the above AMF or the reference fungus *Claroideoglomus claroideum* in lettuce plants growing at increasing salt stress conditions (0, 40 and 80 mmol NaCl L^{-1}). Photo's credit: C. Santander

tamarugo (*Prosopis tamarugo)*, *Baccharis scandens*, *Werneria pinnatifida*, *Deyeuxia curvula*, and *Festuca deserticola* the largest colonization rates (Santander et al. 2021b). Finally, in the study of Aguilera et al. (1998) in the far north, it was reported that although the genus *Atriplex* has been commonly recognized as non-microtrophic, *Atriplex atacamensis*, *A. nummularia*, and *A. deserticola* presented high levels of AMF spores in the rhizosphere soil, as well as high root colonization, both traits positive and significantly correlated with the level of available P in the soil. Additionally, a negative correlation with the N content. Contrary to the above, in the rhizosphere of *A. repanda*, a complete absence of AMF spores was found (Aguilera et al. 1998).

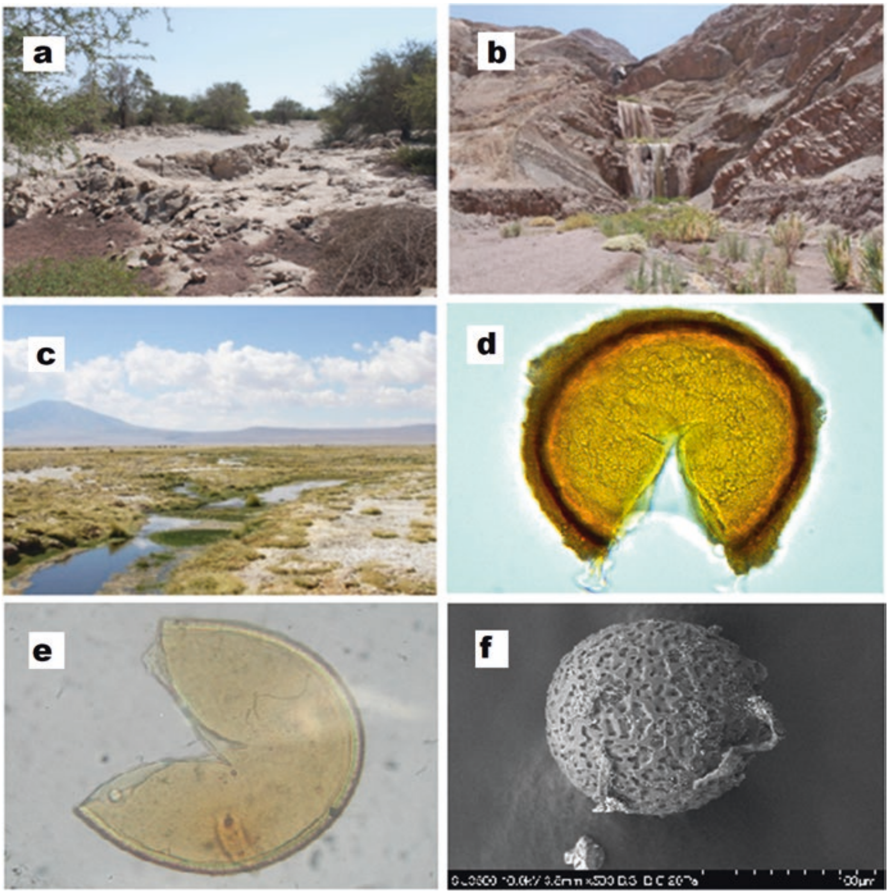

Fig. 20.3 Elevation belts of the Tarapacá Region. (**a**) Hyperarid desert in the Pampa del Tamarugal, which is the driest nonpolar environment worldwide. (**b**) Prepuna, in the "Quebrada de Huatacondo"; (**c**) Puna, Bofedal in the "Salar de Huasco". (**d**) and (**e**) Spores of AMF isolated from the rhizosphere of *Baccharis scandens*, plant species with distribution in the Prepuna from the far north in Chile. (**f**) SEM microphotograph of a spore obtained from "Salar de Coposa" associated to *Festuca* sp. roots, environment in the Puna with high salt contents. Photo's credits: (**a**–**c**), P. Cornejo; (**d**–**f**), C. Santander

20.2.2 Near North Zone

The four studies that are framed in this area are mainly oriented to ecosystemic interests, studying the factors that affect the establishment of AMF in natural ecosystems, the function of the AM symbiosis, and taxonomy of AMF species that have developed these interactions.

20.2.2.1 Presence of AMF in Natural Ecosystems

Dhillion et al. (1995) presented one of the first reports regarding the AM status of annual and perennial plants in shrublands from the Coquimbo Region, finding that more than 90% of 38 species (into 19 plant families) formed exclusively AM associations. In this study, it was also observed that plant species belonging to typically non-mycorrhizal families, such as Portulacaceae and Chenopodiaceae, do not develop the AM symbiosis, evidenced by the almost total absence of propagules and very low levels of AM root colonization. Additionally, in two studies conducted in the Fray Jorge Forest National Park, Aguilera (2004) investigated the presence of phytopathogenic microorganisms associated with the olivillo (*Aextoxicon punctatum*) forest, finding that there is an inverse relationship between the number of phytopathogenic fungi and the AM root colonization. This may be due to the competition for nutrients and space that is generated, as well as the production of antimicrobials and enzymes that degrade microbial pathogenic cell walls (Green et al. 1999). Based in the above, the use of AMF should be considered in the park's restoration strategies, especially in forest reforestation programs with native species. On the other hand, Aguilera et al. (2016), in a 10-year study, found that rainfall is a determining factor in the abundance of AMF, being inversely proportional to soil moisture, suggesting the role of AMF in improving the resilience of plant communities against environmental stresses, such as nutrient deficiency and drought (Barea et al. 2011; Santander et al. 2017). In addition, the presence of small mammals was found to positively influence the AM colonization through spore dispersal, either by epizoocoria (transport of rhizosphere soil) or endozoocoria (root consumption) (Aguilera et al. 2016).

20.2.2.2 Role of AMF as Phytostabilizers

In a study on the phytostabilizing capacity of "romerillo" (*Baccharis linearis*) in soils affected by the accumulation of tailings, it was found that the AM root colonization improved the water retention capacity of the soil and allowed the development of exploratory plant tissues, favoring the colonization and propagation of this species. However, an important factor that decreases the infectivity of the AMF propagules corresponded to high levels of Zn in tailings (Menares et al. 2017).

20.2.3 Central Zone

A total of 21 studies were found, of which 6 presented an agronomic interest, 11 referred to ecosystemic topics, and another 4 covered both interests. Of all these, nine are related to environments contaminated with Cu, four referred to the factors that affect AMF in agroecosystems, three focused on the function of AMs in natural ecosystems, two analyzed the effect of AM in plant growth and nutrition, one addressed the role of AM in agroecosystems, one addresses the factors that affect the MA presence in natural ecosystems, and only one referred to AMF taxonomy in natural ecosystems. It should be noted that the Chile's central zone concentered 85% of the horticultural activity, being the metropolitan region (27.5%), the main area devoted to this activity, followed by Maule Region (19.2%), O'Higgins Region (17.9%), Valparaíso Region (9.5%), and Bío Bío and Ñuble regions (5.6% each).

20.2.3.1 AMF in Plant Growth and Nutrition

In central Chile, a wide variety of vegetables is grown, such as the onion (*Allium cepa*), which has been proven with the inoculation of two commercial products containing AMF (Aegis® and Raizfort-M®), on the transinvernal variety Sonic, showing not significant increases in growth, yield, or quality of the crop. However, the author of this study pointed out that the use of AMF in those species should be studied in greater detail, in addition to monitoring the persistence of the fungus throughout the development of the crop and in conditions of nutritional stress (Díaz 2004). Moreover, in tomato plants (*Solanum lycopersicum*), the co-inoculation of a consortium consisting of saprophytic fungi and *Rhizophagus irregularis* increased the growth of plants, synergistically improving the biochemical activity of the soil, being concluded that co-inoculation decreases plant stress by improving the defense systems and nutrient homeostasis (Fuentes et al. 2016b). A strategy to evaluate the influence of symbiosis between *Rhizophagus irregularis* and tomato plants was to select and probe reference genes, which will depend on the tissue under study and the conditions of experimentation (Fuentes et al. 2016a).

Regarding the wine industry, a large part of the national production is located in the central zone, whose tradition dates back to the sixteenth century, and there are currently many commercial varieties that generate different types of wine (Aguilar and Becerra 2017). In detail, a study in vineyards under different agricultural management reported that the greatest diversity of AMF was found in those of organic management, also evidencing that this symbiosis modifies and increases the concentration of secondary metabolites (Aguilar and Becerra 2017), which is important for its possible implications in the *terroir*. Additionally, other studies in vineyards of the Cabernet Sauvignon variety inoculated with the commercial inoculant Mycosym Tri-ton® reported increases in the percentage of root growth at the second year, suggesting that AMF populations increase year by year if the appropriate conditions are present, such as minimal disturbances in the soil, low application of synthetic phytosanitary products, and adequate fertilization, among others (Bennewitz et al. 2008).

20.2.3.2 Factors Affecting AM Symbiosis in Agroecosystems

In reference to soil tillage strategies, ploughing is still widely used, which can adversely affect soil properties, microbiological characteristics, the presence and activity of AMF, the structure of biotic communities and the production of glomalin, thus affecting the function of the soil as a sink of C (Cornejo et al. 2009; Curaqueo et al. 2010, 2011; Ávila-Salem et al. 2020). In relation to the above, Curaqueo et al. (2010, 2011) pointed out the crucial role of AMF and glomalin in soil aggregation processes, contributing to the stability of organic matter in a Mollisol from the Metropolitan Region. A 6-year study in a wheat-corn rotation crop showed that no-till produced higher densities of AM hyphae, glomalin, and water-stable soil aggregates, increasing by 44% the total C in the soil compared to the amounts accumulated in soils subjected to conventional tillage (Curaqueo et al. 2010). Likewise, Curaqueo et al. (2011) highlighted that in the long-term soil compaction under zero-tillage systems causes a detriment to several of its properties, negatively influencing crop production in annual rotations. Therefore, they suggest carrying out studies in greater depth to establish the situations in which tillage is necessary in soils of medium and coarse texture after some years under no-tillage system.

20.2.3.3 AMF in Copper-Contaminated Environments

It is well-known that in Chile the mining activity generates large amounts of toxic wastes that can contaminate the soils, even affecting the quality of those surrounding mining activities, which are characterized by high concentrations of potentially toxic elements (PTE) such as Cu (Cornejo et al. 2008a; Castañón-Silva et al. 2013; Ávila et al. 2010) (Fig. 20.4). Cu is an essential micronutrient for the growth of plants and soil microorganisms, but when found in excess, it can be highly toxic (Cuillel 2009; Vidal et al. 2020, 2021). Several studies have demonstrated the ability of AMF to alleviate toxicity against this element in plants, such as the compartmentalization of excess Cu in the cytoplasm of fungal spores (Fig. 20.4), which are metabolically inactivated, thus serving as a survival mechanism of AMF in environments contaminated by Cu (Cornejo et al. 2013). Other mechanisms of AMF to cope against the Cu toxicity correspond to the generation and accumulation of glomalin in the soil, which can sequester various PTE, such as Cu and Zn, also contributing to the stabilization of highly contaminated soils (Cornejo et al. 2008a, 2017a, b).

Some plant species have shown the capability to store high amounts of Cu in their structures, such as the sunflower (*Helianthus annuus*). This plant can also accumulate Cd, Ni, Cu, Zn, Pb, and radioisotopes in its roots, being observed that when inoculated with AMF from the genus *Glomus* enhanced its growth (height and biomass), also contributing to the accumulation of Cu in the shoots, which favors the processes of soil recovery through phytoextraction (Castañón-Silva et al. 2013; Meier et al. 2012a).

Additionally, some plant populations have naturally generated tolerance mechanisms against the presence of PTE in the soil, which are known as metallophytes. Among them are *Oenothera picensis* and *Imperata cylindrica*, plant species which grow spontaneously in metal-contaminated soils in central Chile (Meier et al. 2012b). In a study where the effect of inoculation of a consortium of AMF adapted to Cu was compared with a fungus that presumably did not present tolerance

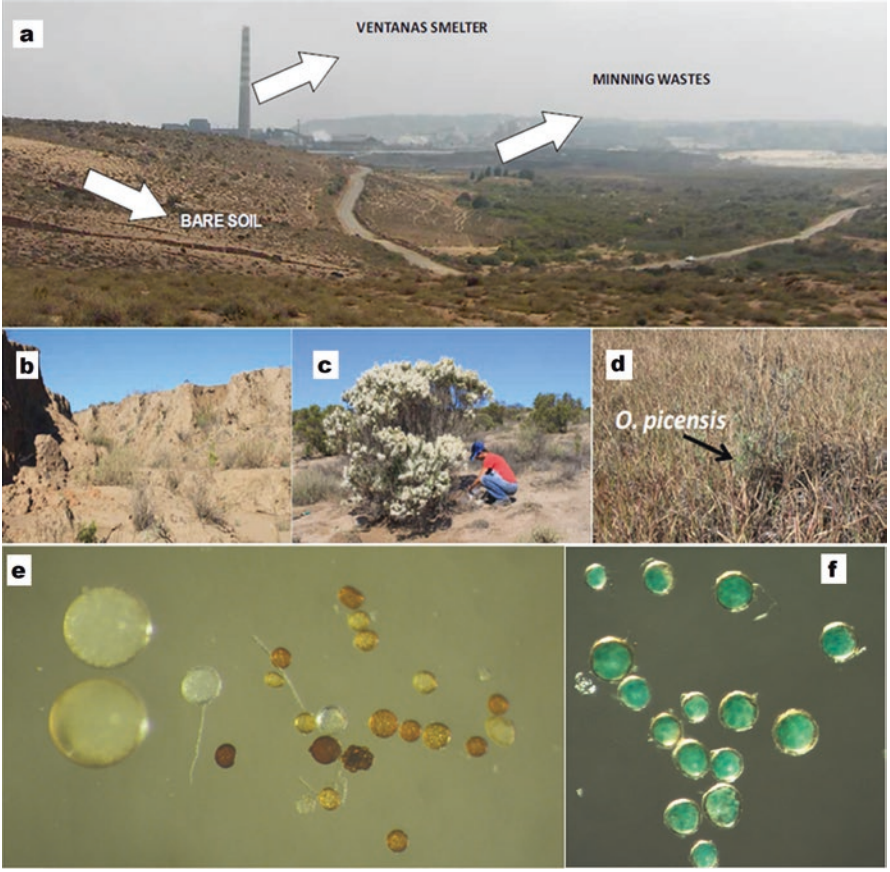

Fig. 20.4 Mediterranean ecosystem of the central zone of Chile. (**a**) Mediterranean scrub in the vicinity of CODELCO's Ventanas Refinery where is visible the industrial installations. (**b**) Pronounced soil degradation that has generated a noticeable gully erosion. (**c**) Specimen of *Baccharis linearis* (Asteraceae), typical scrub AM host present in a wide range in the Mediterranean degraded shrublands of central Chile. (**d**) Lawn of *Imperata cylindrica* (Poaceae), where a specimen of *Oenothera picensis* (Onagraceae) is established. (**e**) AMF spore's diversity obtained from rhizosphere soil of *B. linearis* growing in soils Cu contaminated. (**f**) Spores of *Claroideoglomus claroideum* obtained from Cu-contaminated soils, where is evident the accumulation of Cu salts. Photo's credit: P. Cornejo

(*Claroideoglomus claroideum*), in plants of *O. picensis*, *I. cylindrica*, and sunflower, it was observed that *C. claroideum* provided better results in terms of plant establishment and growth, so it may be useful for implementing phytostabilization programs (Meier et al. 2012a). This is interesting given that microorganisms isolated from contaminated soils are commonly considered to have a better degree of tolerance when compared to other organisms isolated from non-limiting conditions. Another study indicates that high concentrations of Cu in the soil generate an increase in radical exudation, which may serve as a mechanism for plants to settle in sites with high concentrations of this element, among which *I. cylindrica* presented the best response given its great capacity for citric acid exudation (Meier et al. 2012b). Additionally, Cornejo et al. (2017b) observed that the use of *O. picensis* together with *C. claroideum* is an optimal alternative to carry out the phytostabilization of Cu in contaminated soils.

In addition to AMF inoculation, other alternatives to promote plant growth in Cu-contaminated environments are the use of organic amendments to increase the efficiency of phytostabilization programs. One of these alternatives corresponds to the addition of agricultural wastes, such as sugar beet wastes in conjunction with the inoculation of AMF adapted to Cu. In a study using the aforementioned materials, it was observed that the interaction of both technologies allowed a better establishment of *O. picensis* seedlings under high concentrations of Cu in the soil, mainly due to the increase in root colonization (Meier et al. 2011, 2015). Therefore, it is relevant to highlight that the use of native AMFs extracted directly from soils with high concentrations of Cu may have a significant advantage in the establishment of seedlings, compared to the inoculation of AMF that do not have tolerance to this element (Medina et al. 2015). Additionally, the incorporation of compost also contributes to improving the response of *O. picensis* plants inoculated with AMF when growing in Cu-mining tailings, decreasing Cu bioavailability and promoting the production of photosynthetic pigments, which finally generate notable increases in plant growth (Pérez et al. 2021). Finally, other studies highlighted that, in addition to inoculation with AMF in *O. picensis* plants, the addition of biochar contributes to decrease the Cu bioavailability, which generates the decrease of Cu concentrations in the plant while generating improvements in the rhizospheric environment for soil microorganisms and plant growth, suggesting the use of this input to sustainably improve bioremediation of Cu-contaminated soils (Meier et al. 2017).

20.2.3.4 Factors Affecting AM Symbiosis in Natural Ecosystems

Central Chile is the only area in South America which presents a Mediterranean-type ecosystem, which is usually known as “Matorral Chileno,” an important biodiversity hotspot that hosts a large number of endemic species, many of which are seriously threatened by anthropic action (Myers et al. 2000). A study conducted in the O’Higgins Region in two sclerophyllous forests reported that in the forest dominated by “boldo” (*Peumus boldus*), “litre” (*Lithraea caustica*), “bollén” (*Kageneckia oblonga*), and “corontillo” (*Escallonia pulverulenta*), clay content and electrical conductivity correlated positively with AMF spore density, while P availability showed a negative correlation. On the other hand, in the forest dominated by boldo, litre, “quillay” (*Quillaja saponaria*), and “peumo” (*Cryptocarya alba*), the clay content and the total N were positively correlated with the density of AMF spores, while the soil organic matter showed a negative correlation (Silva-Flores et al. 2019). In both sites higher densities of AMF spores were found in the growing season (spring-summer-autumn), while their lower density was recorded in winter, indicating a high regulation of AMF spores as an effect of seasonality. This is the first analysis of the factors influencing the abundance of AMF spores in the Chilean matorral (Silva-Flores et al. 2019). On the other hand, Benedetti et al. (2018) described the species of AMF present in natural boldo formations, identifying a total of 23 species belonging to Acaulosporaceae (*Acaulospora*), Entrophosporaceae (*Entrophospora*), Glomeraceae (*Funneliformis*, *Glomus*, *Rhizophagus*, and *Sclerocystis*), and Paraglomeraceae (*Paraglomus* aff. *laccatum*), highlighting the presence of *Funneliformis badius* and *F. constrictus* in all the sites studied, species that are generally rare.

20.2.3.5 Role of AMF in Natural and Modified Ecosystems

A study conducted under controlled greenhouse conditions with six commercial AMF inoculants (*Gigaspora margarita*, *R. fasciculatus*, *R. intraradices*, *R. aggregatus*, *G. versiforme*, and *Funneliformis monosporus*) in red eucalyptus (*Eucalyptus camaldulensis*) and quillay showed that *F. monosporus* generated the highest number of viable spores along with *G. versiforme* and *R. aggregatus*, presenting *R. fasciculatus* the lowest viability (Godoy et al. 1991). Additionally, *Gi. margarita* responded with a low presence in both plant species. In the case of red eucalyptus, the AMF with the greatest effect on the variables considered were *G. versiforme* and *R. intraradices*, while in the case of quillay the fungal species with the greatest effects were *R. intraradices* and *R. aggregatus*. Another family of AM-forming plants present in the Mediterranean area associated with tree species, such as peumo, quillay, and boldo, corresponds to Gilliesieae (Torres-Mellado et al. 2012). The five known genera of this family, *Gethyum*, *Gilliesia*, *Miersia*, *Solaria*, and *Speea*, show common presence of AM, being the only form of fungal symbiosis present in this group and varying the presence of fungal structures such as arbuscules, vesicles, intraradical hyphae, or coils depending on the species in question (Torres-Mellado et al. 2012).

Another study carried out in the central zone investigated the presence of AMF associated with cushion growth plants, such as the “yareta” (*Azorella madreporica*), which facilitates the establishment of herbs and pastures on them by generating better conditions of humidity and nutrients under the soil in which these nurses are located (Cavieres et al. 2005, 2018). In this study it was found that about 65% of plant species growing above 3000 meters in height have AM-colonized roots, confirming other observations that AMFs can accompany their symbionts without an altitudinal limit (Casanova-Katny et al. 2011; Oehl et al. 2006, 2011; Santander et al. 2021b). The absence of AM was also found in species whose families do commonly present these structures, such as *Chaetanthera lycopodioides* and *Perezia carthamoides* (Asteraceae) and *Viola philippii* (Violaceae). Moreover, the results of the study confirm that yareta cushions promote the development of dense networks of AMF, compared to plants that do not lodge on them, which may be an essential factor contributing to the diversity of species and the structure of the plant cover mediated by the presence of yareta (Casanova-Katny et al. 2011).

20.2.4 South Zone

The vast majority of studies involving AMF have been developed in this area, especially in soils used for agricultural production, being equally important the work in natural ecosystems. In detail, of the 58 studies corresponding to this zone, 15 were focused in plant growth and nutrition, 14 were oriented to aluminum phytotoxicity, 11 included factors that affect the functioning of AM in agroecosystems, 6 described the function of AM in natural ecosystems, 5 addressed the taxonomy of AMF in natural ecosystems, 2 investigate the factors that affect AM in natural ecosystems, 2 referred to the impact of fires on MAs, 1 related the interactions between Al and P, 1 included aspects related to Cu phytotoxicity, and, finally, 1 referred to the role of AM in water stress.

In Chile, more than 50% of the arable soils have been originated from volcanic ash, predominating in the south the Andisol and Ultisol orders that, despite their many edaphic-climatic advantages, present limitations for plant growth. These include low pH, high P-fixing capacity, high levels of organic matter with varying levels of humification, and, in some cases, phytotoxicity by Al and Mn due to frequent acidity (Borie et al. 1998, 2019; Borie and Rubio 1999). Therefore, the use of AMF propagules is essential in conjunction with appropriate management practices, which can maximize the effects of MA symbiosis on plant establishment, nutrition, and growth (Borie et al. 2010, 2019).

20.2.4.1 Plant Growth and Nutrition

In the early 1990s, the first studies on AM emerged, identifying their presence in legumes and grasses, and concluding that the former develop higher levels of colonization, in this case comparing white clover (*Trifolium repens*) and ryegrass (*Lolium perenne*) (Urzúa et al. 1992). Another study conducted by the same authors using white clover found that inoculation with AMF produced an increase in biomass production due to a better P nutrition, as well as an increase in the absorption of other nutrients such as K, Ca, Mg, Zn, Cu, Fe, and Mn (Urzúa et al. 1993). Likewise, other more recent studies showed that both AM root colonization and phosphatase activity are complementary mechanisms used by the plant to generate a better P availability and uptake (Castillo et al. 2008b), which is of great importance due to its low availability in volcanic soils from southern Chile, as previously mentioned.

In other studies, the joint inoculation of *Claroideoglomus claroideum* with native strains of selenobacteria generated a synergistic effect reflected as an increased uptake of selenium (Se) in wheat that allowed its accumulation in grains, thus constituting a sustainable alternative to improve the Se biofortification of cereals (Durán et al. 2013). A more recent study in which a consortium of endophytic bacteria was inoculated together with the fungus *C. claroideum* confirmed the viability of its use as a biofortification strategy of Se in wheat, by producing greater effective colonization of roots, better antioxidant activity, and the increase of the content of Se in the grain (Durán et al. 2018). On the other hand, the inoculation of *Rhizophagus intraradices* in wheat cultivars has been shown to increase the accumulation of Ca, generating a greater exudation of organic acids in the rhizosphere compared to the inoculation with *C. claroideum*, evidenced in the host plant as an increase in the P content (de Souza Campos et al. 2021). Additionally, a study on the nutritional properties and effect on strawberry growth (*Fragaria* x *ananassa*) reported that the inoculation with the fungus *C. claroideum* increased the antioxidant capacity in the fruits (Parada et al. 2018). In addition, in the same study, Parada et al. (2018) found that the roots of strawberry plants exuded important quantities of oxalic acid, which may be an effective mechanism to exclude phytotoxic Al, which in conjunction with the use of AMF can increase the plant P uptake in P-deficient acidic soilsient. This study stressed the need to investigate in depth the effects of the use of AMF in strawberry crop, since the functionality of the AM symbiosis could become more evident in the second or third year of cultivation.

Considering other plant hosts, it has been shown an increase in the production of coriander (*Coriandrum sativum*), parsley (*Petroselinum hortense*), and chili pepper (*Capsicum annuum*) with the inoculation with *Glomus* sp. and *Claroideoglomus etunicatum*, by 70%, 200%, and 400%, respectively (Borie et al. 1997). Similarly, Borie et al. (1998) reported that in volcanic soils the inoculation with *C. etunicatum* produced a significant increase in biomass by ten wheat cultivars, highlighting eight of these that proved to be significantly dependent on the symbiosis generated by indigenous AMF populations. Finally, the inoculation with AMF has shown to be beneficial even in species not commonly recognized as AM plant hosts, such as blueberry (*Vaccinium corymbosum*), where its inoculation produced an increase in biomass in the early stages of plant growth. Likewise, the inoculation with the saprobe fungus *Coriolopsis rigida* increased the colonization of *Gigaspora rosea* and *R. intraradices*, and in addition the saprophyte fungus *Phanerochaete chrysosporium* produced a synergistic increase in the root colonization with the fungus *G. viscosum*, evidencing a mutualistic interaction between AMF and saprophytic fungi, which can be used as a biotechnological tool to improve the adaptation of blueberry plants in their acclimatization phase after the nursery production (Arriagada et al. 2012).

20.2.4.2 Protective Role of AMF Against Phytotoxicity

The largest area of cereal crops in Chile, such as oat (*Avena sativa*), wheat (*Triticum* sp.), and barley (*Hordeum vulgare*), is located on volcanic soils, characterized by high levels of exchangeable Al, which negatively affects plant production (Aguilera et al. 2014, 2018). To address this problem, the use of different wheat cultivars with greater tolerance to Al has been implemented (Seguel et al. 2014), as well as the application of lime to reduce their phytotoxicity, although treatments with AMF turn out to be the most efficient (Borie and Rubio 1999). Even so, the application of liming materials, such as $CaCO_3$ and $CaMg(CO_3)_2$, reduces the phytotoxicity of Al favoring the establishment of the AM symbiosis and the persistence in the soil of the AMF propagules (Mendoza 1997). The mechanism by which AMF facilitate the establishment of crops in acidic soils is through their ability to sequester Al in the structures of the fungus, such as glomalin and hyphae, thus preventing the Al translocation by roots toward the shoots (Seguel et al. 2016, 2019; Mendoza 1997; Aguilera et al. 2018), generating the immobilization of Al (Aguilera et al. 2011).

It is important to consider that AM root colonization is relevant from the initial crop stages, evidencing a greater colonization in the varieties that have a greater Al tolerance, as reported by a study where different varieties of barley and wheat were used (Seguel et al. 2012), observing a greater plant biomass, P acquisition, and acid phosphatase activity in the soil cultivated with Al-tolerant wheat genotypes (Seguel et al. 2017). On the other hand, Al-sensitive wheat genotypes showed a higher concentration of Al in stems and roots (Seguel et al. 2017). In general, Seguel et al. (2017) suggest that Al tolerance is a combination of factors, among which the plant's own ability to tolerate the element, the efficiency in the P uptake, the exudation of chelating compounds by the root, and the AM colonization work together to overcome these adverse conditions typical of acidic soils. Finally, Marín et al. (2016) suggested that some AMF species may be more adapted to very high levels of Al saturation, as well as extremely low amounts of available P.

On the other hand, the structure of the AMF communities varies even according to the genotype within the same plant species, implying a richness of AMF species particular to each variety, which can influence the effectiveness of the inoculation with AMF (Aguilera et al. 2014; Seguel et al. 2016). This differential degree of functional compatibility has also been described for different cereal species (Aguilera et al. 2017) or even in species of other plant families, such as clover (*Trifolium* sp.) in the case of legumes (Castillo 1997; Castillo et al. 2008a). In the same way, Seguel et al. (2016) stated that the application of AMF inocula can contribute to the Al tolerance of crops located in acidic soils only in some wheat varieties, in particular those less tolerant to this element.

Another study referring to the growth of white eucalyptus (*Eucalyptus globulus*) in soils amended with biosolids with high Al levels showed a higher growth of plants inoculated with the AMF *Rhizophagus irregularis*, the saprophytic fungi *Coriolopsis rigida*, and *Phanerochaete chrysosporium* or a combination of both treatments, increasing the concentration of P and the biomass of the stems (Arriagada et al. 2013). In conjunction with the AMF inoculation, the addition of lupine or wheat residues demonstrated the reduction in the absorption of Zn, Cu, Mn, and Al by AM-colonized plants, highlighting the importance of the use of AMF as the main element to treat toxicity problems in acidic soils (Borie et al. 2002). On the other hand, a study related to the establishment of white eucalyptus in soils with high Cu levels showed an increase in the tolerance of the plant against this element once inoculated with the AMF *Septoglomus deserticola* and the saprobe fungus *Coriolopsis rigida*, so the authors recommend this combination for phytoremediation practices. However, this same study recognizes the detrimental effect of Cu on AMF, causing a decrease in the growth of extraradical mycelium (Arriagada et al. 2009).

20.2.4.3 Factors Affecting AMF in Agroecosystems

Several studies in Chile have shown how agricultural practices, such as tillage, rotation designs, and quantity and type of fertilizer, can affect the AM functionality, as well as spores' density, mycelium, and colonized roots (Borie et al. 2010). In particular, tillage affects the physical and chemical structure of the soil in which microorganisms coexist, affecting their abundance, diversity, and activity (Borie et al. 2006; Cornejo et al. 2009). Several studies showed that no-tillage significantly induced a higher AM root colonization than conventional tillage and there are also higher values of AMF spores under this type of soil management (Borie et al. 2006; Castillo et al. 2006a). No-tillage and reduced tillage also improved the accumulation of glomalin, which even increases over time. With these antecedents, it is concluded that reduced tillage positively influences the vast majority of soil characteristics, including higher levels of C, N, S, total P, and pH, increasing the acquisition of P for the next crop in rotation systems (Castillo et al. 2006a; Borie et al. 2000). Consequently, more conservationist practices are associated with higher densities of active AMF mycelium and colonized roots, which has been observed in rotations with wheat and oats (Cornejo et al. 2009).

Another practice that improves the presence and activity of AMF is the application of compost, a common agricultural management among small farmers from central and southern Chile. The application of this material increases the pH, the density of AM-colonized roots, the length of the mycelium, the levels of glomalin, the water-stable aggregates, and the water holding capacity, being recognized as a substitute to chemical fertilizers, in addition to contributing to avoid soil erosion in organic production systems (Valarini et al. 2009). Likewise, the addition of plant residues in acidic andisols increases the pH level, the P availability, and the development of AMs, as well as the growth of plants and the acquisition of minerals (Borie et al. 2002; Lara et al. 2004; Redel et al. 2006). There has also been evidenced a greater AM colonization in host crops such as maize (*Zea mays*), bean (*Phaseolus vulgaris*), and prairie constituent species (grasses and legumes), which in turn increases biomass and nutrient availability in the soil (Millaleo et al. 2006). Additionally, the incorporation of lupine plant residues in crops also enhances mycorrhization, with increases of 49 to 61% in wheat roots (Redel et al. 2006). In this sense, in another study that considered the application of plant residues incubated with *Trichoderma harzianum* and *Coriolopsis rigida*, together with biosolids in a tomato crop inoculated with the AMF *Rhizophagus irregularis*, it was observed that *C. rigida* inhibits the positive interaction between AMF and organic residues in plant growth. On the contrary, plant residues incubated with *T. harzianum* and added to mycorrhizal plants evidenced the greater production of shoot biomass (Almonacid et al. 2015).

Regarding the fertilization of soils with nitrogen sources, a study that investigated the interaction between nitric (NO_3^-) and ammonia sources (NH_4^+) together with the inoculation of the fungus *Claroideoglomus etunicatum* showed that the length of mycorrhizal roots of wheat plants increased with the use of NO_3^-, also improving the density of extraradical mycelium and the number of spores in the rhizosphere (Cornejo et al. 2007, 2008b). With the use of *C. etunicatum*, a higher rate of specific absorption of P and Zn was also evidenced, specifically with the use of NH_4^+ as the nitrogen source. On the other hand, considering the use of NO_3^- and the same inoculum of AMF, a greater accumulation in shoots of N, K, and Cu was evidenced, also decreasing the accumulation of Al and Mn (Cornejo et al. 2008b). Likewise, Cornejo et al. (2009) identified that the use of NH_4^+ as fertilizer was associated with a higher density of AMF hyphae, although the use of NO_3^- was associated with a greater alkalinization of the rhizosphere, as well as a higher density of AMF propagules in wheat beyond postharvest stages, which may facilitate colonization by AMF in early stages in the next crop within the rotation.

In another study, Durán (2007) compared eight ecotypes of wheat from small Mapuche farmers with respect to a commercial variety, evidencing in the former a significantly higher AM colonization and a greater global microbial activity in the state of pasty grain, presenting greater shoot and root biomass in the different phenological stages studied. Additionally, a comparative study of inoculation of a commercial AM fungus (*R. intraradices*) and another native (*C. claroideum*) in local ecotypes of chili pepper (*Capsicum annuum*) variety “cacho de cabra” from Mapuche farmers demonstrated a marked effectiveness of the native strain in contrast to the commercial one, by reducing the stress by transplantation thus accelerating the ripening stage and producing fruits of better quality (Castillo et al. 2009). Previous studies showed that plant-AMF interactions related to nutritional dynamics are highly influenced by the species of fungus that is colonizing the root, as well as intraspecific variations at the genetic level of the host, while growth responses related to nutrition depend on the intrinsic capacity of acquisition efficiency of these nutrients by the plant (de Souza Campos et al. 2021).

In oat, wheat, and barley crops, in the first stage of growth after sowing, Castillo et al. (2012) observed that the intensity of AM colonization is low at 15 days, which increases slightly in the period of 15–30 days, increasing markedly between 30 and 45 days, which also evidences a high production of biomass in the roots until day 30, which remains constant until the 45th day. With this, the authors conclude that in the early stages of growth, the cereals invest energy in root production and then in the production of biomass for the growth of AMF. Finally, a study on various species of forage lotus in samples of andisols and vertisols showed that AM colonization was lower in the latter, increasing in all cases the AM colonization with low levels of P in the soil (Castillo et al. 2013).

20.2.4.4 Presence and Role of AMF in Natural Ecosystems

With regard to natural ecosystems, the native flora from southern Chile has about 84 tree species with 50% endemism of great genetic, phytogeographic, and ecological significance (Armesto 1992). The exploitation of renewable terrestrial natural resources, which involve the extraction of tree cover, erodes the soil and causes imbalances at the ecosystem level (Godoy et al. 1994), being especially fragile the endemic conifers from southern Chile, several of which are limited to small distribution areas, so many of them have a relictual character (Golte 1978). Godoy (1989) documented for the first time the presence of AM in the species of conifers "araucaria" (*Araucaria araucana*), "ciprés de la cordillera" (*Austrocedrus chilensis*), larch (*Fitzroya cupressoides*), "ciprés de las Guaytecas" (*Pilgerodendron uviferum*), "ciprés enano" (*Dacrydium fonckii*), "mañío macho" (*Podocarpus nubigenus*), "mañío de hoja larga" (*Podocarpus salignus*), "lleuque" or "uva de la cordillera" (*Prumnopitys andina*), and "mañío de hoja corta" (*Saxegothaea conspicua*).

Several studies have delved into the relationship between araucaria and AMFs, since they are located in areas exposed to different natural events such as wind, volcanism, fires, and low rainfall, which have been accentuated as a result of climate change, in addition to adverse physicochemical characteristics of the soil; however, they have demonstrated a great capacity for adaptation (Chávez and Rivas 2020). Given the above, an important strategy that has allowed the resilience of araucaria forests is the symbiosis with AMF (Cartes 2008), in which it has been recognized that 90% of the mycorrhizal associations of vascular plants in these forests correspond to the AM type. Even so, AMF communities are prone to being altered by fire, and after a fire event, species of the genera *Archaeospora*, *Acaulospora*, and *Gigospora* have been shown to be the most representative (Paulino 2006). However, over the years after a fire event, the production of glomalin is maintained, which could respond to an ecological-evolutionary strategy of symbiosis in this type of temperate forests, being key in the recovery and restoration of degraded ecosystems (Rivas et al. 2016; Chávez et al. 2020). In this sense, Chávez et al. (2020) showed that the levels of glomalin of a soil are a good indicator of the conservation status of araucaria forests, identifying that among various sites where this species is located, the best state of conservation is presented in the Nahuelbuta National Park, compared to the Conguillío National Park, Tolhuaca National Park, and the China Muerta National Reserve. On the other hand, a study of inoculation with AMF and endophytic fungi (HE) in araucaria concluded that the inoculation of AMF alone or in interaction with HE is beneficial for the seedlings of this species, even improving the ability to tolerate prolonged drought, maintaining stable physiological variables such as photosynthesis, CO_2 assimilation, and foliar water content (Chávez and Rivas 2020).

Another study in araucaria carried out in three forested communities of the La Araucanía region, two sectors in the Cordillera de la Costa, Villa Las Araucarias and PN Nahuelbuta, and one sector in the Conguillío National Park in the Cordillera de Los Andes (Fig. 20.5), showed that in the latter there was a more acidic pH, in conjunction with a greater number of AMF propagules and the greater number of spores, although this does not necessarily ensure a greater and better colonization since it also depends on the efficiency of the AMF that colonizes the root (Cartes 2008). Additionally, Godoy et al. (1994) showed that inoculation in araucaria with *Rhizophagus intraradices* obtained positive results in the growth of seedlings, being also the first study that identified the mycotrophic status of the vascular flora present in four forested communities of native conifers from southern Chile, among which highlighted the araucaria forests, ciprés de la cordillera, larch, and ciprés de las Guaytecas. Among the 83 vascular species investigated, 61 presented AM

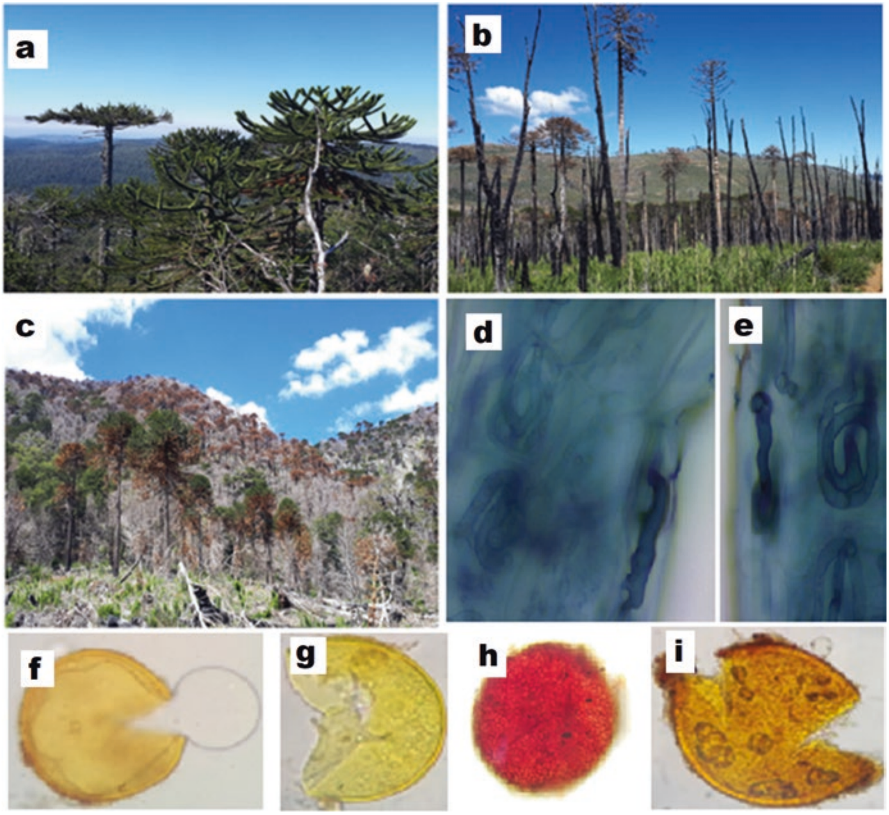

Fig. 20.5 *Araucaria araucana* trees in different states of conservation. (**a**) Good state of conservation in Nahuelbuta National Park. (**b**) *Araucaria* trees in Tolhuaca National Park after fire in the summer 2001–2002. (**c**) China Muerta National Reserve after fire 2015. (**d**) and (**e**) Root colonization by AMF forming profuse coils with the intraradical mycelium. (**f**) to (**i**) Different AMF spores isolated from rhizosphere soils of *Araucaria.* (Photo's credits: (**a**–**c**), P. Cornejo; (**d**–**i**), D. Chávez)

associations, between them were present species belonging to the genera *Baccharis*, *Berberis*, *Adesmia*, *Chusquea*, *Gaultheria*, *Senecio*, *Pernettya*, and *Blechnum* and the tree species *Drimys winteri*, *Fitzroya cupressoides*, and *Maytenus magellanica*, among others (Godoy et al. 1994).

Communities of AMF have also been recognized in forests of the "lenga" tree species (*Nothofagus pumilio*), among which the species of the genus *Acaulospora* are the most abundant (Marín et al. 2016). Among some edaphic traits determining the establishment of AMF, the pH, P, and Al saturation were identified. Regarding the latter, *A. laevis* and *A. punctata* were the most abundant in soils with high percentages of Al saturation, with values close to 70%. On the other hand, the species *F. badius* and *R. invermaius* were identified as potential pioneers in the successions of disturbed habitats, especially in poor-nutrient environments (Marín et al. 2016).

In evergreen forest ecosystems, it has been reported that of 53 plant species analyzed, 77.4% formed the AM symbiosis (Castillo et al. 2006b). Likewise, of the 18 tree species studied, 12 presented AMs: olivillo, "luma" (*Amomyrtus luma*), "meli" (*Amomyrtus meli*), "ulmo" (*Eucryphia cordifolia*), "tepa" (*Laureliopsis philippiana)*, myrtle (*Luma apiculata*), "chilco" (*Fuchsia magellanica*), and "maqui" (*Aristotelia chilensis*) also ferns such as *Blechnum hastatum*, *B. blechnoides* and *B. chilense.* In comparison, a secondary deciduous forest ecosystem presented the lowest number of AMF spores and diversity, compared to an evergreen forest ecosystem and a prairie ecosystem. On the latter, more than 90% of the species were identified as AM hosts (Castillo et al. 2006b). A total of 39 species of the division Glomeromycota were found in this study, predominating species of the genera *Acaulospora* and *Glomus* in the forest ecosystems and *Acaulospora* in the prairie ecosystem. Also, the presence of arbuscules, vesicles, and hyphae in the root system of "chacay" (*Discaria serratifolia*) was verified, as well as the appearance of AMF propagules in the rhizosphere soil (Salinas and Carrillo 2008).

On the other hand, Seguel et al. (2008) studying an adult mixed forest of olivillo, ulmo, "roble" (*Nothofagus obliqua*), "laurel" (*Laurelia sempervirens*), and "lingue" (*Persea lingue)* belonging to the forest type "Roble-Raulí-Coigüe" determined the contribution of glomalin as a sink of C in the soil, finding that the C associated with this glycoprotein represents between 8.9 and 10.4% of the total C of the surface horizon, as well as a high correlation between soil organic C and C glomalin. In addition, despite the ecosystem is dominated preferentially by ectomycorrhizae, the soil showed a high amount of AMF propagules, especially spores, which closely correlated with the levels of glomalin deposited in the soil (Seguel et al. 2008). In this sense, Etcheverría et al. (2009) studied the factors that contribute to the enrichment of ^{13}C and ^{15}N in the soil organic matter of native forests in different degrees of preservation. As a result, glomalin was found to contribute significantly to such enrichment. The authors also emphasize that, although the mechanisms by which this process occurs must be deepened, it is likely that this isotopic enrichment is due to the internal fractionation of the AMF during the capture and transport of C and N, thus concentrating ^{13}C and ^{15}N as part of the AMF biomass (Etcheverría et al. 2009).

Among some factors that determine the variable presence of AMF communities in prairie ecosystems highlight the climate and the regulation by plant species (Montero et al. 2013), implying variations in herbaceous communities between the different seasons in the annual cycle and in the communities of non-mycorrhizal microorganisms in the soil. On the other hand, Oehl et al. (2011) described the morphology of the spores and the taxonomic and genetic-molecular phylogeny of a new species into the genus *Acaulospora* (*Ac. punctata*), present both in the Swiss Alps and in the Andes Mountains, in Los Ríos Region. Likewise, Medina et al. (2014) presented the description of a new species belonging to the order Glomeromycota, *Corymbiglomus pacificum*, the first species of the genus of which direct germination has been observed from the cell wall, which was isolated from the mouth of Lake Budi, inhabiting the rhizosphere of *Ammophila arenaria*, highlighting in the area the periodic salinity given its proximity to the Pacific Ocean. Finally, Medina et al. (2015) conducted a study on the AM status of pioneer plants in Lake Budi, both in roots and rhizosphere of *Polygonum maritimum*, *Carpobrotus chilensis*, *Ambrosia chamissonis*, and *A. arenaria*, the latter presenting the greatest AM root colonization. Likewise, a low richness of AMF species was found, predominating the association of *Corymbiglomus pacificum* and *A. arenaria*, suggesting an important role of AMF in its establishment, as well as the establishment of *Amb. chamissonis* in saline ecosystems, being relevant the potential use of AMF as a biotechnological tool for the stabilization of coastal ecosystems, dunaria, or plants growing under conditions of salinity or drought (Medina et al. 2015).

20.2.4.5 Far South Zone

Only two studies have been carried out in this geographical area. The first of these focuses on the function of AMs in natural ecosystems, specifically the factors that facilitate the rapid ecological successions of *Nothofagus* forests in the vicinity of the Pia Glacier, Tierra del Fuego. The results suggest that the symbiosis of *Gunnera magellanica* ("nalca") with cyanobacteria and of trees and shrubs with AMF is the key process that allows plant succession, with AMF having a small incidence in the uptake of N by the nalca (Benavent-González et al. 2019). Another study highlighted the presence of AMF in the soils of King George Island in the south Shetland Islands, Antarctica, identifying three new species of AMF belonging to the genus *Glomus* and the species *Acaulospora mellea*, being the first time that the latter is identified colonizing plants in the soils of Antarctica (Barbosa et al. 2017).

20.3 Information Gaps Regarding Arbuscular Mycorrhizal Symbiosis in Chile

It is evident the concentration of studies in the central and south zones, predominating subjects such as plant growth and nutrition, Al phytotoxicity, diverse factors that affect the AMF in agroecosystems, phytoremediation of Cu-contaminated soils, and AM functioning in natural ecosystems, where the predominance of studies with agronomic approach or remediation of anthropic alterations is also evident, over the knowledge of the presence, functionality, and environmental factors involved in the MA symbiosis in natural ecosystems.

20.3.1 Far North Zone

As is evident from the systematization, in the far north zone, studies with an agricultural approach are predominant, where have been studied melon and lettuce species, with three studies in each case. On the other hand, a study has recently been published that describes the presence of AMF in plant species from the hyperarid desert (Pampa del Tamarugal), the Prepuna and the Puna in the Tarapacá Region (Santander et al. 2021b), an important advance that allows to know the AM status of the species inhabiting those extreme environments. Reinforcing, this area is the driest temperate desert in the world, with very old soils, unique in its kind and well developed, with horizons rich in nitrates and other soluble salts such as halite and iodine salts, characteristic of the intermediate depression of the Atacama Desert (Ericksen 1983). As altitude increases and to the east, there are some sectors of depression covered by evaporative accumulations of salts, salt flats, and wetlands, with soils rich in organic matter, which are essential for unique and fragile wetland ecosystems (Norambuena et al. 2011). In this scenario, it is essential to generate cadastres of AMF communities that could be facilitating the establishment of plant covers under conditions of high salinity and scarce water availability, serving as a guide to restore ecosystems that are affected by such limitations or favor the incipient agricultural activity.

On the other hand, agriculture in this area is developed mainly in valleys that cross the Atacama Desert, such as the valleys of Lluta, Azapa, Camarones, Camiña, and Huatacondo (Fig. 20.6), characterized by poorly developed soils (Norambuena et al. 2011), so the beneficial effect of the introduction of inoculants based on AMF in the crops that are located there to enhance the evolutionary development of the soils must be evaluated, as well as plant nutrition and growth.

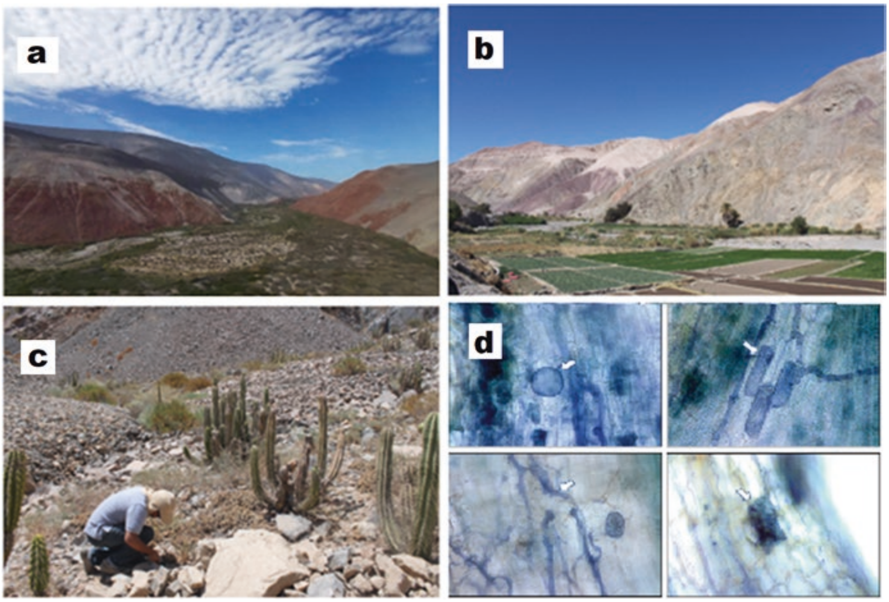

Fig. 20.6 Agroecosystems from the far north. (**a**) Agricultural production in the Camarones Valley, Arica y Parinacota Region. (**b**) Agriculture in the Camiña Valley, Tarapacá Region. (**c**) Sampling rhizosphere soil in endemic cacti from the Huatacondo Valley, Tarapacá Region. (**d**) Profuse AM root colonization in local ecotype of onion produced in Camiña valley. Photo credit: Pablo Cornejo

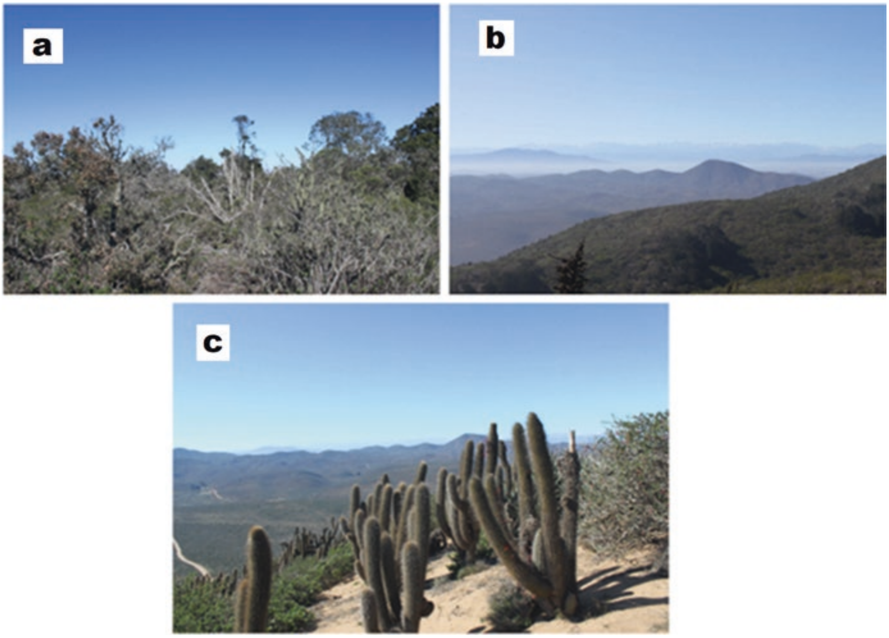

Fig. 20.7 Relict forest from Fray Jorge National Park, Coquimbo Region. (**a**) "Selva Valdiviana" from Fray Jorge Forest National Park. (**b**) Hydric support based in the "camanchaca" originated in the Pacific Ocean. (**c**) Transition from "Selva Valdiviana" to xerophytic ecosystem representative from the near north. Photo's credit: P. Cornejo

20.3.2 Near North Zone

The studies that have been carried out in the near north correspond mainly to ecosystemic interest, when studying the relationship of AMF with native plant species. Two of the four studies presented findings on AMF present in the Fray Jorge Forest National Park (Aguilera 2004; Aguilera et al. 2016), which provided a clear example of the importance for conserving relict ecosystems to conserve resources and disseminate scientific knowledge that allows understanding the interrelationships that are generated in these ecosystems. This is especially important considering the macro-climatic context of the camanchaca, which ultimately results in an extremely particular ecological exception as the Valdivian forest (Fig. 20.7). Likewise, it is noticeable that the other studies have focused on recognizing the AM status of several native species such as the romerillo (Menares et al. 2017), the olivillo (Aguilera 2004), and *Atriplex* sp. (Aguilera et al. 1998), among others, so continuing to deepen the ecosystem role of AMF, which can be of great importance to promote ecosystem restoration in the area. On the other hand, it is important to note that this area is dominated by transverse valleys, the Cordillera de la Costa and the Cordillera de los Andes, so the intermediate depression is not markedly present (Pfeiffer et al. 2019). In addition, it presents young soils of little development in the lower areas, while in the highest fluvial and marine terraces, there may be well-developed soils, existing even areas with relict ecosystems of paleoclimates, as they present wetter climates (Pfeiffer et al. 2019).

20.3.3 Central Chile

In the central zone, there are highly fertile soils, so agricultural activity predominates, which affects a greater pressure on this resource, leading to the development of a high-intensity irrigated agriculture (Armesto et al. 2007). Likewise, in the central zone, there are studies of AMF in several species of agronomic interest, such as onions, vines, wheat, and corn, although there is also a history of the use of AMF in tree species, such as avocado and orange, especially referring to the use of commercial inocula to enhance their production, so it may be interesting to study the potential of using AMF both in other tree species as well as in herbs of agronomic interest, especially if the use of native AMF in the area is considered. On the other hand, it has been documented that most of the native and endemic tree species of the central zone establish the AM symbiosis, whose use given the high pressure of urban expansion and the agronomic use of soils can enhance reforestation and restoration plans for the fragments of native forest and the Chilean matorral. Likewise, the study by Casanova-Katny et al. (2011) on the abundance of AMF in rhizosphere soil of the yareta, on which many more herbaceous species inhabit, highlighted the importance of AMF to maintain ecosystems with high plant diversity and even the networks that can contribute to the formation of plant communities in which energy cooperativism allows the establishment of a large number of species, through the transfer of C between individuals even of different species (Simard and Durall 2004). This is especially relevant in Mediterranean ecosystems because they are recognized as biodiversity hotspots. Likewise, the high colonization of the roots of the yareta, despite growing only a few millimeters per year, could imply that mycorrhizal reinforcement is even a requirement for the establishment of this plant under conditions of extreme altitude (Casanova-Katny et al. 2011).

20.3.4 South Zone

In this area there is the largest amount of information regarding the AM symbiosis in Chile, representing more than half of all the available bibliography. In this zone, studies carried out on wheat and other cereal species, such as barley and oats, as well as tomato, blueberry, goat pepper, and lupine, predominate strongly. Regarding lupine, despite its inability to generate the AM symbiosis, it is interesting to study it as part of the annual rotations in the area, demonstrating that its use can be compatible with agricultural crops of AM hosts plant species, thus constituting more diverse ecosystems that contribute to maintain the soil biodiversity. In this way, it is possible to promote the sustainability of ecosystems by decreasing erosion rates as a result of intensive agronomic use. Likewise, there are studies that detail the detrimental nature of some traditional agricultural practices, especially in the biotic communities of the soil, such as tillage which in turn affects the retention capacity of C (Seguel et al. 2008). This is especially relevant in the context of the current climate emergency, in which it is essential to promote, through appropriate management practices, the diversity and functionality of populations of native AMF (Borie et al. 2010).

The peculiar properties of the volcanic soils from southern Chile determine that native AMF populations play a key role in the sustainability and productivity of the plant ecosystems located there, both agronomic and natural. The strong agricultural and forestry production in this area determines the need to redirect current managements toward more sustainable systems, which are supported by the development of biotechnological tools, among which the selection of efficient native AMF and their incorporation as biofertilizers emerges as a highly favorable alternative (Borie et al. 2010). For natural ecosystems, as well as for agricultural studies, it should be noted that the largest number of studies correspond to the La Araucanía Region, so expanding the existing information in other regions of the south zone will allow a better understanding of the ecosystem dynamics that imply the AM symbiosis, contributing to the formation of databases that allow sustaining reforestation and ecosystem restoration plans, among others. Additionally, in this area, as well as in the central zone, there is a great substitution of natural ecosystems by forest monocultures and also by grassland ecosystems, so it is essential to integrate the different uses at landscape level, taking into account that the establishment of native species can be compatible with other uses.

20.3.5 Far South Zone

This area is evidently understudied in terms of the AM symbiosis, as well as being one of the most unknown in terms of the soils there present, which product of a complete covering by ice fields during the last glaciation (22 thousand years ago) has generated very young soils (Pfeiffer et al. 2010). In this area there is a continuous mantle of peatlands of varying thickness in the fjords, which constitute the largest reserve of organic C in the country (Loisel and Yu 2013), so it is essential to investigate the role of AMF in such ecosystems, which currently suffer great anthropic threats due to the absence of legal regulations on the extraction of moss (León et al. 2012). Likewise, although the ecosystems found in this area are mainly ectomycorrhizal, it would be important to evaluate the degree of functionality of the AM symbiosis in herbaceous or shrub species, to contribute to understanding its ecosystem complexity.

20.4 Projections and Study Needs Regarding to the AM Symbioses at the National Level

International studies detailed the advantages of using AMF in species such as bean (*Phaseolus vulgaris*) or rice (*Oryza sativa*), although also indicate the ability of AMF to acquire nutrients from the soil can be inhibited at low temperatures (Acuña et al. 2020), which is important to address since there are no studies in Chile focused on plant production under low temperatures. The above is crucial, because among factors that are essential to consider, temperature, seasonality, soil fertility, and the C/N ratio can explain about 50% of the variation in the intensity of AM colonization (Soudzilovskaia et al. 2017). Another topic that needs to be deepened is the beneficial use of AMF in conditions of osmotic stress, either due to environmental conditions of drought or salinity, where both agricultural production and the stability of natural ecosystems can be affected (Santander et al. 2017), which is a threat of increasing magnitude in Chile. At present, the beneficial effects of the use of AMF in species of agronomic interest, such as corn, strawberry, clover, and lettuce, among others, have been studied, recommending the use of AMF inocula adapted to osmotic stress (Santander et al. 2017). Given the above, it is evident that the potential of the AM symbiosis in agricultural applications and natural ecosystems in Chile is still incipient. This may be due mainly to the fact that the first studies emerged relatively recently in the early 1990s, with research mainly focused in some specific situations, such as phytostabilization in Cu tailings or the installation of agricultural systems in acidic soils. These topics have the largest number of studies by topic, probably because they imply a great environmental urgency for biotechnological applications. Likewise, in terms of agricultural production systems, studies predominate in cereals, mainly wheat and barley, generating guides that systematize the knowledge already collected around these species, which could serve to disseminate the application of AMF inocula according to each environmental situation or plant material.

There are very few species of agricultural interest in which studies have been carried out apart from the aforementioned cereals, knowing the compatibility of AMF only in some geographical and environmental conditions, and even with few species of AMF, which makes it difficult to extrapolate the results to other conditions as a result of the uncertainty and economic risk they represent. On the other hand, it is complex to carry out experimental analysis of compatibility between AMF and plant species, since the possibilities of symbioses are multiple and the effects on plants may vary depending on the species or variety in question (Aguilar and Becerra 2017). For this, an appropriate alternative would be to focus on the use of molecular tools in future studies, to evaluate the compatibility of each AMF with the plant species of interest, through omics analysis, such as genomics (Redecker 2020), transcriptomics (Venice et al. 2020), or metabolomics. Similarly, it is important to consider that the use of exotic inocula can cause invasions or co-invasions by plants and fungi, in addition to generating competitive relationships with native resources, so the use of local inocula should be prioritized (Hart et al. 2017). On the other hand, a combination of different AMF species will potentially be more effective than a monospecific inoculum (Gogoi and Singh 2011), as long as these interactions are in turn compatible. Likewise, the beneficial effects on plant nutrition will depend on fungal diversity and soil conditions (Avio et al. 2006), depending on the agricultural system used and the plant species cultivated.

In natural ecosystems, efforts should continue to identify indigenous AMF species associated with native or endemic plant species, as it is important to build a database that contributes to management plans to strengthen ecosystem resilience in case of disturbed ecosystems or for ecological restoration programs in case of degraded ecosystems (Rivas et al. 2016). Likewise, it is difficult to generalize a pattern of action, since each particular situation must be evaluated, being important to understand the specific plant-AMF interactions and ecosystem modifications in response to climate change (Smith and Read 2008). This may be relevant for preservation purposes, particularly of vulnerable or threatened species, which may serve to know the influence of AM symbiosis on the structure and stability of forest communities (Godoy et al. 1994). Therefore, knowing the mycotrophic status of native plant species can allow to promote optimal conditions of soil microbial communities to enhance the establishment of seedlings (Godoy et al. 1994), support their growth, development (Medina et al. 2015), and nutrition, promote ecological successions (Benavent-González et al. 2019) and preservation and conservation of ecosystems or endangered species, or even influence the development of agroforestry ecosystems that harmoniously combine the different ecosystem services that can be obtained from them.

Also, it should be noted that the greater diversity of types of mycorrhizal associations and the higher rates of diversity in plant families in the plant cover suggest that different types of mycorrhizae in an ecosystem have a significant evolutionary effect on plant diversity (Mujica et al. 2019). Likewise, deepening on the study of each ecosystem, it will be important to identify associations between different AMF taxa (Torres-Mellado et al. 2012) to preserve forest ecosystems and maintain their mycorrhizal potential (Seguel et al. 2008). Likewise, in both agroecosystems and natural ecosystems, it is important to develop intensive studies of different soil samples, paying attention both to fungal structures adjacent to the rhizosphere, as well as to the extraradical structures located beyond the limits of the rhizosphere, which play a key role in physiological, biochemical, and molecular aspects that determine plant growth (Cornejo and Aponte 2020). In this sense, it is essential to study the density of the AMF structures (hyphae and spores) in the mycorrhizosphere, as well as their vitality, activity, and functionality (Cornejo and Aponte 2020), aspects practically uncovered in studies regarding AMF.

Finally, it should be noted that currently in Chile there is not a solid legal body that protects the soil resource, being essential to move toward a framework soil law, which updates all existing legislation on the matter, taking care that the use of this resource is done in a rational way with the aim of preventing its loss and degradation (Pfeiffer et al. 2019). Thus, along with this, it must be understood that the soil is a system that houses life, which is crucial for the maintenance of an adequate ecosystem functionality and of the ecosystem services they provide, in which the MA symbiosis plays a central role.

20.5 Conclusion

Arbuscular mycorrhizal fungi (AMF) play a key ecological role in the establishment and development of various plant species, by facilitating the plant's acquisition of nutrients and water from the soil. In addition, they allow the reduction of biotic and abiotic stresses such as drought, salinity, and high levels of heavy metals, improving the structure of soil aggregates. This shows that AMF can improve the conditions of a soil system, which is largely favorable given the current climate emergency context largely caused by anthropic alteration of terrestrial ecosystems.

Based on the systematization of the information collected, the predominance of studies with an agricultural focus on monoculture systems in Chile is evident, which indicates the great importance that AMF have for the improvement of plant productivity. Moreover, based on the international literature that indicates that both plant diversity and ecosystem benefits are close related with the presence of AMF, it is important to expand the studies of the AM symbiosis in Chile in unconventional agricultural production systems such as syntropic agriculture, edible forests, and organic agriculture, among others. This is extremely urgent to implement at national level food production systems that adapt harmoniously to the new environmental conditions, in special in a scenario of climate change.

In natural ecosystems, there are still many gaps in information; Therefore, to collect antecedents regarding the AM status of native and endemic plant species will allow in the present and future the elaboration of management, conservation, or restoration plans of intervened or degraded ecosystems, based on a healthy soil, which is the main support on which plant and animal life is located, thus favoring ecosystem resilience. Likewise, focusing on research efforts with the support of molecular tools will increase knowledge regarding the compatibility between AMF species and different plant species, which is essential to reduce experimentation efforts.

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Chapter 21
Mycorrhizas and Ecological Restoration in South America

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21.1 Introduction

Restoration ecology is a discipline that aims to return a degraded ecosystem to an historical trajectory, in order to sustainably persist for a long term, by using the natural history knowledge of a particular ecosystem as well as the ecological theory (Huxel and Hastings 1999; Hobbs and Harris 2001; Van Andel et al. 2012). Currently, internationally exists a growing development of this area of knowledge, with the existence of journals such as *Restoration Ecology*, which publish several investigations about ecological restoration, from different regions of the world with the support of the Society for Ecological Restoration (SER; www.ser.org).

The concern of restoration ecology is highly ambitious, since it requires previous ecological knowledge and the participation of different disciplines such as ecology, botany, zoology, mycology, and edaphology, among many others, and to have a reference ecosystem, which is the one with the characteristics that are expected to reach after the restoration actions. However, the ecosystem dynamic is probabilistic; hence, it can develop many different trajectories. Many of them can occur at the same time in different parts of the spatial dimension since the landscape can have environmental heterogeneity. Based on this, it can be acknowledged that usually it is not appropriate to perform only one restoration plan. Therefore, a restoration plan should have several sub-plans that consider the heterogeneity of the reference ecosystem.

There are two types of ecological restoration strategies: (a) passive restoration, which is the action that implies preventing, controlling, or modifying the degradation factors of a certain ecosystem, such as tree felling, hunting, wildfires, and livestock, among others, in order to allow the recovery of biodiversity and ecological functioning through natural succession (Becerra et al. 2018); and (b) active restoration, which are the actions performed when a component of the ecosystem or an ecological process cannot be recovered, those actions might be, for example, habitat management, sowing of propagules, trees, shrubs or herbs, planting, watering, fertilization, using artificial shadow, among others (Becerra et al. 2018). Which type of restoration is used depends on the site conditions, and any restoration plan should

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evaluate, a priori, the type of restoration needed, as well as where and when the restoration should be performed, following what is known about the focal ecosystem.

In the recent years, new conceptual elements and theoretical frameworks have led to generating restoration proposals by using natural resources from the same site that will be restored. In this context, nature-based solutions (NbS) arise, improving restoration effectiveness and generating several contributions of nature to people, through the recovery and maintenance of ecosystems. Those benefits can reduce the negative socioeconomic and environmental impacts of the different change precursors of ecosystems. Given that any strategy or sustainable action that aims to be effective and scaled to a social level should incorporate the sociocultural context of the communities (Bartels et al. 2013), the effectiveness of the NbS significantly increases when a co-production of a NbS approach is followed, in where the communities knowledge and experiences are enriched with the science-technical knowledge (Lavorel et al. 2020).

21.2 Ecological Restoration and Mycorrhiza: A General View

Mycorrhizal associations have benefits for both partners that establish the symbiosis, since the mycobiont helps the plant with the uptake of nutrients and water from the soil and receives carbohydrates in return (Smith and Read 2008). Also, the mycorrhizal symbiosis allows the plant to tolerate biotic and abiotic stresses, such as drought and pathogens (Delavaux et al. 2017). These functions escalate to population, community, and ecosystem levels, since these enhance plant recruitment, increase plant diversity and ecosystem productivity, and drive carbon and nutrient cycling (van der Heijden et al. 2015). It is also a matter of high importance to consider mycorrhizal symbioses in the context of climate change and its mitigation. Plants with mycorrhizal associations are responsible for significantly higher carbon sequestration compared to non-mycorrhizal plants (Soudzilovskaia et al. 2019). Therefore, since ecological restoration is one of the main mitigation solutions for climate change, through the logic of increasing carbon sequestration (Bastin et al. 2019), mycorrhizal symbiosis consideration will support and upgrade the above-mentioned solution (Soudzilovskaia et al. 2019). In consequence, several studies around the world have considered the use of mycorrhizal fungi to improve the outcomes of ecological restoration.

A relatively recent global meta-analysis found that when mycorrhizal symbiosis is considered and applied in restoration contexts, plant biomass increases, with the greatest effects observed in N-fixing woody plants, C_4 grasses, and plants growing at low soil availability of P, and that the increment of biomass also increases in the first 3 years after inoculation (Neuenkamp et al. 2019). Species richness of the restored communities is also higher when mycorrhizas are used and the restored communities being much more similar to the reference ecosystem in the same contexts (Neuenkamp et al. 2019).

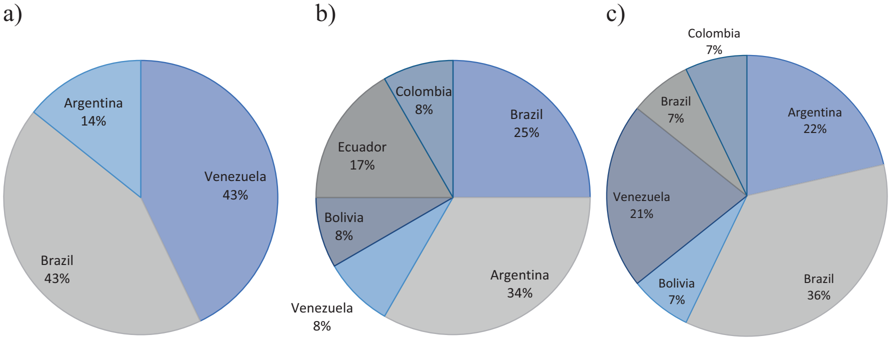

Fig. 21.1 (**a**) Proportion of field experiments by country in contexts of ecological restoration. (**b**) Proportion of nursery experiments by country with an interest in ecological restoration. (**c**) Proportion of descriptive studies by country searching for mycorrhizal behavior in contexts of ecosystem recovery

21.3 Ecological Restoration and Mycorrhizas: State of the Art in South America

In the valuable work of Neuenkamp et al. (2019), as usually occurs with meta-analyses, they do not consider all the available information, mainly due to fit selection criteria for the particular questions of the study. In fact, in the general patterns found by Neuenkamp et al. (2019), only two studies from South America were considered. It is acknowledged that South America can show different ecological patterns compared to those found in the northern hemisphere, especially in terms of mycorrhizal symbioses (Bueno et al. 2017). Thus, searching deeper for other studies conducted in South America might show some deviation of this region from the general patterns already found. This can have important consequences when an ecological restoration experiment within the South American region might be developed. Consequently, for the present chapter, data search was performed in the Web of Science (WoS), using the keyword string mycorrhiza* AND (restoration or reclamation or rehabilitation) and between the years 1980 and 2022. This search identified 1509 studies. Then, within the results of the first search, a filter by South American countries was applied, one by one: Chile, Bolivia, Peru, Ecuador, Colombia, Venezuela, Guyana, Suriname, French Guiana, Brazil, Paraguay, Uruguay, and Argentina. Among them, only seven published studies involved the use of arbuscular mycorrhizal (AM) symbiosis in field experiments in contexts of ecological restoration were found from Venezuela, Brazil, and Argentina, (Fig. 21.1a). In the same search, other studies indirectly related to ecological restoration were found, such as 11 studies in nursery conditions focused to apply the obtained results in field ecological restoration contexts that were also found for South American countries (Fig. 21.1b). Finally, 14 studies explored what occurred with mycorrhizal symbiosis either in space, time, or both after some years of restoration in perturbed sites (Fig. 21.1c).

It is worth highlighting the pioneer studies in the topic of ecological restoration in South America. Those come from Venezuela and as such, they will be further mentioned in the next section. Then a new study from Chile will be presented, and even though is a nursery experiment, the same experiment was replicated in the field, and data are currently under analysis to be further published as the first case of a field experiment of ecological restoration by using arbuscular mycorrhizal fungi (AMF) in Chile.

21.4 Study Cases for South America

21.4.1 Role of Arbuscular Mycorrhiza in the Ecological Restoration of Venezuelan Degraded Ecosystems

Five years after the restoration of a small area (0.16 ha) of a tropical dry forest at Península de Macanao (Venezuela) (Fajardo et al. 2013), Fajardo et al. (2015) compared the AMF communities present in restored plots with those found in plots with 5 years under natural regeneration. The results showed that restoration may have promoted a greater richness and diversity of AMF, in particular, in those plots where a hydrogel was applied (the more effective treatment), although the differences between restored and no restored plots were not significant. It is possible that 5 years after restoration were not enough to define these trends. However, the species composition of AMF between restored and no restored plots was different with the presence of species belonging to the Gigasporaceae in the restored plots, which is a family usually found in undisturbed habitats.

It has been widely reported that the successional rate of degraded ecosystems could be faster through inoculation of plants with AMF of interest or management of their populations (Janos 1980; Allen 1991). In this regard, the use of AMF as a bioinput to propagate tree species to recover disturbed areas is a practice that, in Venezuela, has been increasingly implemented in recent years.

Early works using this important biological interaction for the restoration and rehabilitation were carried out in the oligotrophic savannas of southern Venezuela (Cuenca et al. 2002, 2003). More recently, Cáceres et al. (2014) evaluated the effect of inoculation with AMF from different successional localities and a xeric scrub (reference ecosystem) on the growth and survival of two legumes species with potential to be used in the restoration. For *Piscidia carthagenensis*, the experiment consisted of the application of four treatments that combined three soil types and four inoculation conditions. At the greenhouse, inoculum from two successional sites (2 and 20 years old of abandonment) produced the greatest effects on height, total biomass, and leaf area when plants were grown in the successional soil, but not when grown in the scrub soil (Kalinhoff 2012). In the field conditions and after a drought season, it was found that *P. carthagenensis* plants grown in successional soil inoculated with native AMF showed a survival rate higher than 80% compared to non-inoculated plants (Cáceres et al. 2014). For *Coulteria mollis*, two soil types and three inoculation conditions were combined. The growth response (height and total biomass) of inoculated plants that were grown in the scrub soil was significantly higher than those grown in the successional soil regardless of the inoculation received (Cáceres et al. 2014). It is possible that functional compatibility between different arrays of fungi and plants associated, in turn, with the AMF diversity of each inoculum used, explains the differential responses observed between both tree species (Cáceres et al. 2014). These authors concluded that the production and use of native inoculum of AMF is a favorable strategy for assisted restoration.

Continuing with the activities of recovery of dry forest areas destroyed by sand mining, in 2014, it was decided to incorporate AMF as an additional treatment to improve the propagation of several tree species and leave, as a fixed treatment, the application of hydrogel for its proven effectiveness (Fajardo et al. 2013). To do this, it was selected two distinct areas for planting. One of them consisted of an area under natural regeneration (SA) that began once sand extraction ceased, and the other one was an area where sand mining had finished recently, and hence it was an area with practically bare soil (DA). Regarding plant species, it was selected four native tree species: *Prosopis flexuosa*, *Parkinsonia praecox*, *Coulteria mollis*, and *Bulnesia arborea*. In the nursery, 75 pre-germinated seeds, of each species, were sown in two soil types– one of them collected from the degraded area (DA) and the other from the successional one (SA)–and subjected to 3 inoculation treatments, non-inoculated control (C), inoculation with a mix of AMF from the degraded area (DI), and inoculation with a mix of AMF from a no disturbed area (NI), 25 seeds for each treatment. The mycorrhizal inoculum had been produced 4 months earlier. After 6 months of growth in the nursery, a total of 432 individuals were planted in 9 plots established both in DA and SA. The height and survival of all individuals planted were recorded three times a year.

In general, in the DA, 50% of all plants that were inoculated with DI had survived, while 45% of all no inoculated plants remained alive. Only 39.7% of all plants inoculated with NI survived. Regarding plant species, *P. praecox* had the higher number of live individuals in all treatments, especially when it was inoculated with the NI. Meanwhile, *B. arborea* had the highest mortality. *P. flexuosa* and *C. mollis* had an intermediate survival percentage, highlighting the fact that *P. flexuosa* might not need to be inoculated judging by the results. In the SA, total survival was below 10% at the end of the evaluated period, and the majority of live individuals were inoculated with NI. Again, *P. praecox* was the plant species with the highest percentage of survival, especially when it was inoculated with NI.

With respect to growth, which could only be determined in the DA, both *P. flexuosa* and *C. mollis* showed the highest height values in the control treatment, while *P. praecox* grew slightly more when it was inoculated with NI, though the differences with the other treatments were not significant. In summary, neither *P. flexuosa* nor *C. mollis* would not be necessary to inoculate them, while it would be recommended to do so for *P. praecox*.

Planting mycorrhizal trees or shrubs in degraded areas is a strategy that favors the creation of the so-called fertility island. These plants (also considered as nurse plants), besides contributing to recovering the natural inoculum of soil lost during degradation processes, which will favor the incorporation of other mycorrhiza-dependent plant species, would also act as elements generating wind turbulence around the individual that would produce an increase of the propagule recruitment both from other plant species and AMF (Cuenca 2015). This would contribute to reactivate and accelerate the natural regeneration (succession) that could otherwise be extremely slow, which could lead to further degradation of the site.

*21.4.2 Effects of Individual and Consortia of Arbuscular Mycorrhizal Fungal Species in the Quality Index of the Endangered Conifer from Chile (*Araucaria araucana*)*

In the coastal Nahuelbuta mountain range (37° 40’S to 37° 50’S) in south-central Chile, the temperate forests of the conifer *Araucaria araucana* are classified as endangered (MMA 2020) as a result of the expansion of the agricultural frontier and an increase in the frequency and intensity of forest fires, product of climate change (Garreaud et al. 2020). This makes it necessary to generate nursery seedling production tools, to strengthen future ecological restoration plans. In this context, it has been suggested that the underground microbiological and plants’ metabolic processes (and their effects in the overall ecosystem functioning) would be more adapted to in-site and local conditions in a scenario of ecological restoration using microbial inoculum (Godoy and Marín 2019). Plants and their rhizosphere, including their AM fungal symbionts, constitute a close spatial-temporal connection with ecosystem age, reflecting the state of ecological succession on a large scale. Previous research has also shown that in such restoration programs, applying consortia of AMF works better regarding plant growth and survival than applying single species inoculum (Aguilera et al. 2017; Godoy and Marín 2019).

In order to test such predictions, an experiment to assess the effects of single and mixed-species commercial AM fungal consortia, as well as native AM fungal consortia in the quality index of *A. araucana* seedlings, was performed in Nahuelbuta National Park, south-central Chile. For this, *A. araucana* seeds were collected from the same park (and stored at 4 °C) to maintain the genetic variability of the species and not alter the ecosystem processes of the rhizosphere in the experiment (Fernández et al. 2020). Prior to germination, seeds were disinfected with 1% NaOCl for 1 min and rinsed twice with distilled water before sowing. Germinated seeds were arranged in 2 L plastic pots with composted bark substrate. In addition of a control (C) treatment without inoculum, three treatments with 1 g of inoculum material per seedling (which contained approximately 900 AMF spores) were established: T1, single AMF species (*Claroideoglomus claroideum*) from a commercial inoculum; T2, four AMF species (*Acaulospora laevis*, *Scutellospora calospora*, *Clariodeoglomus etunicatum*, and *Rhizoglomus intraradices*) from a commercial

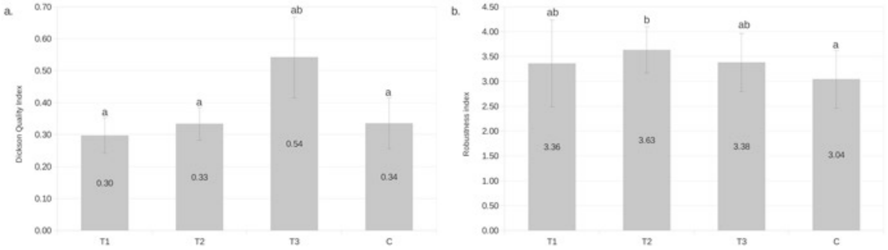

Fig. 21.2 Average values and standard deviations for Dickson's plant quality index (**a**), and robustness index (**b**) for *Araucaria araucana* seedlings ($n = 25$) in treatments with different mycorrhizal inoculations (T1, singular commercial AMF species; T2, four commercial AMF species; T3, native soil containing 17 AMF species) and control (C). Different letters above the mean value indicate statistically significant differences ($p < 0.05$), according to the Games-Howell test

inoculum; and T3, corresponded to native soil containing 17 AMF species (Becerra 2019). A total of 100 seedlings were evaluated (25 per treatment). After 11 months of the bioassay under controlled conditions in the nursery, the evaluation was carried out by harvesting the seedlings for each treatment and the control, to calculate the Dickson quality index and the robustness index (Rueda Sánchez et al. 2018).

The Dickson quality index was significantly higher in T3 (0.54) than in the other treatments (0.30–0.34), which did not differ among them (Fig. 21.2a). In contrast, the robustness index was significantly higher in T2 (3.63), followed by T3 (3.38) and T1 (3.31), and the control (3.04) was significantly lower than the rest (Fig. 21.2b). There is scarce research in the use of the rhizosphere and native symbionts (and their interactions with plants), as restoration tools for native Chilean flora (Godoy et al. 1995; Godoy and Marín 2019; van Galen et al. 2021). A study by Godoy et al. (1993) using the AMF species *Rhizoglomus intraradices* as inoculum for four native conifer species under greenhouse conditions (20 weeks) shows that all morphometric variables analyzed had higher values than the non-inoculated control, indicating compatibility and efficiency of the mycorrhizal inoculation. In this experiment, it is shown that consortia of AM fungal species lead to better results as compared to single-species inoculum and the control. But the evaluation of the inoculation effects depends on the metric used; as here, different indexes were higher under different treatments. Native AMF species inoculum increased the overall plant quality, while AMF commercial consortia increased their robustness, probably indicating differential roles and/or functional redundancy of the particular AMF species involved. Future forest ecological restoration plans in altered ecosystems should include the use of native mycorrhizal inoculates, to increase plant adaptation and survival, ultimately and hopefully recovering tree populations. In this sense, currently, a field experiment with the same setting is being analyzed.

21.5 Ectomycorrhizas and Ecological Restoration with a Focus in the Neotropical Region

Currently, at a regional scale, it was possible to find one short-term study, and outside the WoS search previously mentioned, on the use of ectomycorrhizas (ECMs) in a field experiment in contexts of ecological restoration, with positive effects on plant quality index when ectomycorrhizas are used to inoculate plants (Godoy and Marín 2019). At a global scale, few restoration studies include soil microbiota, and research that explores the landscape does not look so much different. Effects of ECMs in plant success are rare, especially in the neotropical region. Research about restoration has grown in the last decades, but studies usually explore ecological theories that consider only plants. Considering other organisms will allow a better understanding of the plant communities and avoid unsuccessful restoration. How communities assemble, both naturally and artificially (via restoration), has received worthy attention (Belyea and Lancaster 2012; Temperton and Hobbes 2004), but only a few studies have evaluated belowground patterns and the role of soil microbiota (e.g., mycorrhizal fungi) in the restored communities (Sun et al. 2017). Belowground interactions may play an important role in restoration projects, especially in ecosystems that deal with environmental challenges such as high irradiance, salinity, nutrient-poor soils, or contaminated soils (Barcelos et al. 2012).

Inoculation of arbuscular mycorrhiza (AM) has been used as a biological tool for plant establishment and development in agriculture (Solaiman and Mickan 2014) and in restoration (de Moura et al. 2022) practices. However, little is known about the role of ECMs and whether they can facilitate restoration in the same way AMF does. The studies on tropical ECMs are fairly recent (Corrales et al. 2018; Vanegas-León et al. 2019), and the role of ECMs in the neotropical region is not very well known. The use of ECMs in the restoration of native forests in the neotropical region deserves more attention.

Ectomycorrhizas are considered pioneer associations during the colonization of plant communities (Lindig-Cisneros et al. 2019). Introduced native seedlings (via restoration) may not succeed if the native microbial communities are not introduced as well, especially those associated with initial colonization. Restoration studies often involve controlling invasive species (Weidlich et al. 2020), which may depend on more (Moyano et al. 2020) or less (Vogelsang and Bever 2009) on mutualistic species. Very little is known about the consequences of having exotic soil microbiota in areas where exotic plants have been removed. Since plants actively alter the soil microbiome to their benefit in a species-specific way (Huang et al. 2019), aboveground biological invasions also impact the underground. Specifically, for ECMs, interactions between native plants and exotic fungi do not necessarily cease after the removal of exotic plant species (Lofgren et al. 2018), and this fact adds an unknown competition problem between native and introduced fungi.

In the neotropical region, there are records of ectomycorrhizal fungi (Singer et al. 1983; Sulzbacher 2013; Roy et al. 2016) even though not much is known about their function in forests. It is safe to say that ECMs provide ecosystem services, act in processes that help to recover native environments, and, therefore, promote restoration (Ortega et al. 2004). It is assumed this must be the case for forests in the neotropical region as well. Specific ectomycorrhizal fungi enhance the survival rates and early growth performance of several plant species (Carey 2016). Inoculation of nurse seedling species with appropriate fungal partners is one of the most efficient environmental approaches, especially in disturbed ecosystems. The presence of endemic symbionts in an ecosystem is an indicator of a well-diversified community (Mueller and Halling 1995) either above- or belowground. Ectomycorrhiza formation could be one of the reasons species become established and survive in harsh environments. The benefits of these interactions may go beyond single individuals. It is believed that nurse plants facilitate other plant species to become established by (1) improving microclimatic conditions on-site, (2) changing the soil microbiota to favor other ECM-forming plants, or (3) acting as a hub tree, directly transferring resources through ectomycorrhizal connections. Ongoing research in southern Brazil is studying if part of these complex interactions can be attributed to ECMs (Weidlich et al. 2020).

It is, therefore, essential to know more about the diversity and ecophysiology of ECMs in tropical regions and find out which are the ECM hosts and how frequently these associations occur. Also, it is necessary to investigate how the neotropical ECM function and what are the benefits to the partners. This is challenging because it forces us to look outside the box, searching for undescribed patterns. A better understanding of ECMs in the neotropical and tropical regions will allow us to infer how it can be included in restoration projects in the same way arbuscular mycorrhizae have been used.

21.6 Conclusion

The experiments from South America are scarce and come from Venezuela, Brazil, Argentina, and Chile most of them showing positive effects in plants as well. The scarce evidence from South America comes mainly from arbuscular mycorrhizal symbiosis, with one exception for ectomycorrhizal symbiosis. Also, it was not possible to find evidence of the role of orchid and ericoid symbioses in experiments from South America. More field experiments are needed in countries such as Perú, Ecuador, Colombia, Guyana, Suriname, French Guiana, Uruguay, and Paraguay, and it is also urgent to explore the effects of orchid and ericoid mycorrhizas in plants in the context of ecological restoration.

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Chapter 22
Gaps in South American Mycorrhizal Biodiversity and Ecosystem Function Research

César Marín, Roberto Godoy, and Javiera Rubio

Contents

22.1 Introduction: Soil Biodiversity and Ecosystem Functions

Soils represent the largest deposit of organic matter on land, storing ~1500 Gt carbon, which is almost as much as the vegetation (~560 Gt) and atmosphere (~750 Gt) together (Crowther et al. 2019). Soil microorganisms play crucial roles in this storage and in many other ecosystem functions, processes, and services. Studying these roles is one of the main objectives of soil ecology. Over the last

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15 years, soil ecology has significantly expanded to a continental and global scale, with a strong focus on broad collaborations and networking among scientists from many countries. For example, several studies have targeted the whole soil microbial community (Bahram et al. 2018; Luan et al. 2020), soil bacteria (Delgado-Baquerizo et al. 2018), earthworms (Phillips et al. 2019), protists (Singer et al. 2019), fungi (Tedersoo et al. 2014, 2021, 2022; Egidi et al. 2019), arbuscular mycorrhizal fungi (AMF; Davison et al. 2015; Vasar et al. 2022), and root mycorrhizal colonization worldwide (Iversen et al. 2017), among others. Most of these macro-ecological-scale studies were summarized and analyzed by Guerra et al. (2020), who found that in just about 0.3% of 17,186 sampling plots on the planet, both soil biodiversity and the ecosystem functions/services provided by those organisms were jointly investigated.

Although studying the causal relationships between biodiversity and ecosystem functioning has gained attention in the last three decades, at least regarding soil, this issue has been studied since the publication of *The Formation of Vegetable Mould Through the Action of Worms* by Darwin (1881). Despite this, it is surprising how little soil biodiversity and ecosystem functions are, in practice, integrated and jointly studied (Guerra et al. 2020), although analyses at national scales (in Chile) show a more optimistic scenario of integration (Marín et al. 2022). Still, sound inferences can be made with the current data, for example, Delgado-Baquerizo et al. (2020) show that climate (specifically, aridity and mean annual temperature), plant richness and cover, soil pH, and carbon and clay soil content were the main factors determining global soil biodiversity – within their dataset. In turn, this global biodiversity and soil total carbon content explained ecosystem multifunctionality – defined as "the simultaneous performance of multiple functions" (Byrnes et al. 2014; Manning et al. 2018).

When studying biodiversity and ecosystem functions, one central ecological quest is to find causal links between them (Xu et al. 2020). This has been also a major research priority recently in soil macro-ecological research (Crowther et al. 2019; Wagg et al. 2019; Delgado-Baquerizo et al. 2020; Zhou et al. 2020), in particular asking, does an increase in soil (and/or mycorrhizal) biodiversity at a particular site results in increased ecosystem functioning? (Marín and van der Heijden 2020). It is particularly important to experimentally test this question, the global patterns mentioned above (i.e., Tedersoo et al. 2014, 2021; Crowther et al. 2019; Delgado-Baquerizo et al. 2020) at finer scales, and evaluate temporal changes in soil biodiversity and ecosystem function (Guerra et al. 2021). When metrics of functional diversity and community structure of soil microbial communities are added to environmental and climatic variables, the explanatory power of models for carbon and nitrogen cycling significantly increases (Graham et al. 2016). How to go from these correlations to causality? Hall et al. (2018) suggest that microbial processes (i.e., nitrogen fixation, denitrification, nitrification) more directly affect a nutrient pool or flux (i.e., NO_3 or NH_4), while the effects of community properties (i.e., emergent properties as biomass C: N ratio or community-aggregated traits as functional gene abundance, functional diversity overall) and microbial membership (i.e., taxonomic and phylogenetic diversity, community structure, co-occurrence networks) are more indirect, mediated by their concatenate effect on microbial processes. There is already some evidence for this approach, as for example, Wagg et al. (2019) have shown that microbial network complexity and mycorrhizal abundance were the most significant predictors of ecosystem multifunctionality in experimental grassland microcosms.

22.1.1 Mycorrhizal Biodiversity: Functions, Types, and Their Relationship

The mycorrhizal symbiosis is crucially important in the soil food web, its physical structure, and its ecosystem functioning (Soudzilovskaia et al. 2019; Steidinger et al. 2019; Tedersoo et al. 2020). These fungi are central to nutrient and water uptake, soil structure formation, plant productivity, and alleviating pathogens and metal pollution (Tedersoo et al. 2020), among other functions. Establishing how mycorrhizal fungi affect plant growth, nutrition, and defense, has been a central task in mycorrhizal ecology since its beginnings. Establishing these relationships on a global scale constitutes the next frontier (Soudzilovskaia et al. 2017; Martin et al. 2018). For example, Davison et al. (2015) and Steidinger et al. (2019) have shown that AMF and AMF plants' biodiversity reach their maximum in the tropics, respectively, while ectomycorrhizal fungi (EMF) and EMF plants have higher biodiversity in temperate and boreal ecosystems – although they are not always linearly related (Tedersoo et al. 2014, 2022). Few climatic variables related to the decomposition process control the transition from EMF- to AMF-dominated forests worldwide (Steidinger et al. 2019). Similarly, Barceló et al. (2019), while analyzing 37 climatic and edaphic variables, found that temperature-related variables better predicted the global distribution of different mycorrhizal hosts: AMF plants are favored by warm climates, while EMF plants are favored by colder climates. Lastly, it has been shown that AMF generally has very low endemism (i.e., 93% of taxa in multiple continents; Davison et al. 2015) and that climatic and soil parameter gradients control their global community structure more than dispersal limitation and historic factors (Vasar et al. 2022).

For several decades now, it has been suggested that to link mycorrhizal biodiversity and ecosystem functioning, newer conceptual and fundamental ecological theories should be developed, particularly for AMF (Hart and Klironomos 2003). This is a necessity as the biology of this group is very particular, from their multiple nuclei and particular nuclear and genomic dynamics (Young 2015; Kokkoris et al. 2020) to processes and/or management that could be understood in a multilevel selection theory framework (Johnson and Gibson 2021). Several research paths of mycorrhizal functioning have been proposed: quantify the role of mycorrhizal networks at the individual, population, and community levels (Simard and Durall 2004), clarify the functional specificity of different fungal taxa, particularly AMF families and/or genera (Marro et al. 2022), test the effects of different host plants (Hart and

Klironomos 2003), and look beyond nutritional functions. Trait-based approaches have also gained much attention (van der Heijden and Scheublin 2007; van der Heijden et al. 2015; Genre et al. 2020), for example, with some authors proposing biological stoichiometry (AMF N: P ratio), as a functional parameter indicative of facilitation, and weak and strong competition (Powell and Rillig 2018).

Under controlled experimental conditions (MyDiv experiment), it has been shown that the most productive tree communities are those associated solely with AMF and not (as the authors hypothesized) those associated with both AMF and EMF (Ferlian et al. 2018). In addition to controlled experiments, to understand the nature of the mycorrhizal fungal diversity–ecosystem function relationship, it is also important to conduct more in situ assessments. A survey by Lekberg and Helgason (2018) of articles published in *New Phytologist* over 30 years shows that most mycorrhizal functioning studies are conducted under controlled conditions, targeting mostly AMF and EMF (and not the other mycorrhizal types), and with a strong focus on nutrient and carbon dynamics (and not other mycorrhizal functions). Thus, significant work on other mycorrhizal types, non-nutritional functions, and especially in the field is crucially important to revisit or change old paradigms (Albornoz et al. 2021), quantify realized – and not only potential – functions, and discover new functions (Lekberg and Helgason 2018). Another unexplored area is understanding how the genotypic and species diversity of both the plant hosts and their fungal symbionts affects ecosystem functioning (Hazard and Johnson 2018).

22.2 Meta-Analyses of Mycorrhizal Biodiversity and Functioning in South America

22.2.1 Literature Search and Coordinates Extraction

In November 2021, a Web of Science search of articles published between 1945 and 2021 was conducted focusing on four mycorrhizal types (arbuscular, ectomycorrhizal, orchidoid, ericoid) and non-mycorrhizal plants according to Moora (2014) and on nine mycorrhizal ecosystem functions (plant growth, soil aggregation, N uptake, P uptake, chemical defense, allelopathy, other nutrients uptake, soil water content, and disease resistance) according to Lekberg and Helgason (2018). Three different sets of keywords were used in order to increase the number of publications obtained.

For publications centered in *mycorrhizal biodiversity* (classification, distribution, taxonomy) in South America, the following keywords were used: "((South AND America) OR Colombia OR Venezuela OR Brazil OR Chile OR Paraguay OR Guyana OR Argentina OR Bolivia OR Surinam OR Ecuador OR Uruguay OR Peru) AND (clasification* OR diversity* OR distribution*) AND ((arbuscular AND mycorrhiz*) OR mycorrhiz* OR ectomycorrhiz* OR (orchid AND mycorrhiz*) OR (ericoid AND mycorrhiz*) OR (non-mycorrhiz*)))."

For publications centered in *mycorrhizal ecosystem functions* in South America, the following keywords were used: "((South AND America) OR Colombia OR Venezuela OR Brazil OR Chile OR Paraguay OR Guyana OR Argentina OR Bolivia OR Surinam OR Ecuador OR Uruguay OR Peru) AND function* AND ((arbuscular AND mycorrhiz*) OR mycorrhiz* OR ectomycorrhiz* OR (orchid AND mycorrhiz*) OR (ericoid AND mycorrhiz*) OR (non-mycorrhiz*)) AND ((interplant AND signal) OR (soil AND aggregation AND hyphae) OR (pathogen AND (resistance OR protection)) OR (allelopath*) OR (('water uptake') OR ('water transfer') OR (water AND uptake AND transfer)) OR (carbon AND dynamics) OR (nutrients AND dynamics) OR (productivity) OR (microbial AND interaction)))."

For publications that integrate *both mycorrhizal biodiversity and ecosystem functioning* in South America, the following keywords were used: "((South AND America) OR Colombia OR Venezuela OR Brazil OR Chile OR Paraguay OR Guyana OR Argentina OR Bolivia OR Surinam OR Ecuador OR Uruguay OR Peru) AND function* AND (clasification* OR diversity* OR distribution*) AND ((arbuscular AND mycorrhiz*) OR mycorrhiz* OR ectomycorrhiz* OR (orchid AND mycorrhiz*) OR (ericoid AND mycorrhiz*) OR (non-mycorrhiz*) AND ((interplant AND signal) OR (soil AND aggregation AND hyphae) OR (pathogen AND (resistance OR protection)) OR (allelopath*) OR (('water uptake') OR ('water transfer') OR (water AND uptake AND transfer)) OR (carbon AND dynamics) OR (nutrients AND dynamics) OR (productivity) OR (microbial AND interaction)))."

Each article was checked individually, discarding those that did not conduct a direct analysis of plant roots or mycorrhizal fungi in South America, per the mycorrhizal types and functions defined. After compiling the articles, a database including coordinates (UTM system), citation, DOI identifier, and mycorrhizal type and ecosystem function investigated was constructed (available upon request to the authors).

22.2.2 Spatial Data Processing and Analyses

Data was georeferenced using Qgis 3.6 (QGIS.org 2021) to create three-point layers projected in WGS84. These were used to elaborate four spatial distribution cartographies, using in three of them the national administrative limits of South America (from the site: https://www.efrainmaps.es/) and a fourth shape layer of ecoregions extracted from the RESOLVE Ecoregions (Dinerstein et al. 2017) dataset (https://ecoregions.appspot.com/).

The first three cartographies show distribution patterns according to the administrative limits of South American countries. The first cartography used three shape layers, the first one contained the sampling sites of mycorrhizal biodiversity, the second one contained the sampling sites of mycorrhizal ecosystem functions, and the third one for sites containing both. For each layer, the parameters of points grouping (or cluster) were applied in Qgis 3.6 properties, assigning a tolerance distance of 50 km. For the second and third cartographies, the same tools and parameters were used but applying four mycorrhizal types and non-mycorrhizal plants shape layers (second cartography) and nine mycorrhizal ecosystem functions shape layers (third cartography). For the fourth cartography, which used the South American biome limits, the same three shape layers from the first cartography were used. For each layer, the Qgis 3.6 tool grouping assigns a tolerance distance of 50 km to cover the concentrations of sites in that range. The color gradient was adapted to the design of previous cartographies. All cartographies were projected in WGS84/EPSG:4326.

22.3 Main Trends of South American Mycorrhizal Biodiversity and Ecosystem Functions Research

A total of 532 Web of Science articles were obtained for the South American continent, from which 266 investigated mycorrhizal biodiversity, 121 targeted mycorrhizal functioning, and 145 investigated both. The first registered article in this search is from 1983, and up to the mid-2000s, there were very few studies, mostly focusing on mycorrhizal biodiversity (Fig. 22.1). Over the last decade, however, there has been a significant jump in South American mycorrhizal research, with more and more studies also assessing mycorrhizal ecosystem functions and their integration with the biodiversity of the fungal symbionts, reaching up to 50 studies in 2019 (Fig. 22.1). This clear transition from more descriptive, baseline studies to more function and applications-related studies has been shown before for global soil biodiversity (Guerra et al. 2020), Chilean soil biodiversity (Marín et al. 2022), and even for South American mycorrhizal fungi (Marín and Bueno 2019). Several

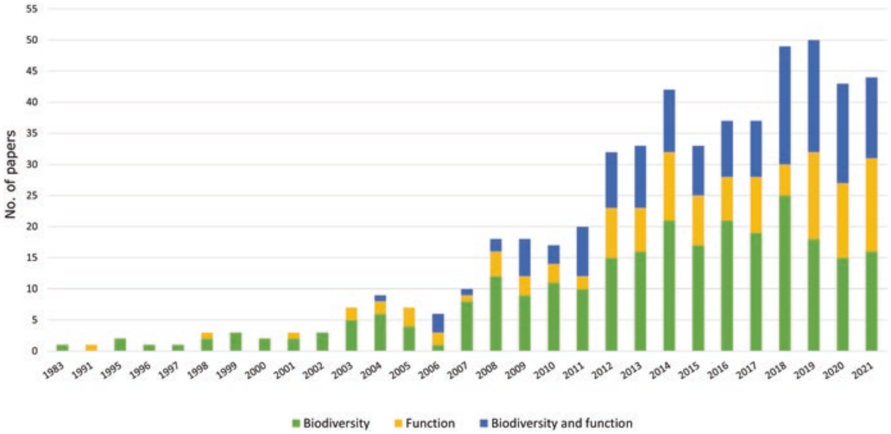

Fig. 22.1 Number of Web of Science articles (no. of papers) published for the South American continent investigating mycorrhizal biodiversity (green), ecosystem functions (yellow), and both (blue). The Web of Science search was conducted for the period 1945 to 2021, but the first study appears in 1983

recent insights (Aguilera et al. 2017, 2019, 2022; Becerra et al. 2019) about the different applications of this symbiosis in South America support this view.

From the 532 Web of Science articles obtained, a total of 1528 sampling sites were extracted (Fig. 22.2). This means that on average, each study targeted less than three sampling sites. The top three countries with the most sampling (almost 80% of the total sampling sites) were Brazil (609), Argentina (416), and Chile (190). Colombia had 92 sampling sites, while this number was 61 and 55 for Ecuador and Venezuela, respectively. Guyana had 33 sampling sites, Bolivia 29, Peru 26, and Uruguay 2 sites. Our search for countries like Paraguay and Suriname did not return any results, but this is probably an artifact of the search itself; further inquiries in both countries should be conducted. It seems that in the countries with more mycorrhizal research, the sampling sites are concentrated next to the most developed areas with big universities, like in Brazil, with a high concentration in the south from Rio de Janeiro to Porto Alegre but also a significant concentration in the northeast, around Natal and Recife (Fig. 22.2). This pattern is in accordance with previous analyses for Glomeromycota in Brazil (Maia et al. 2020) and South America – especially the concentration of points in the northeast (Cofré et al. 2019). In Chile, the

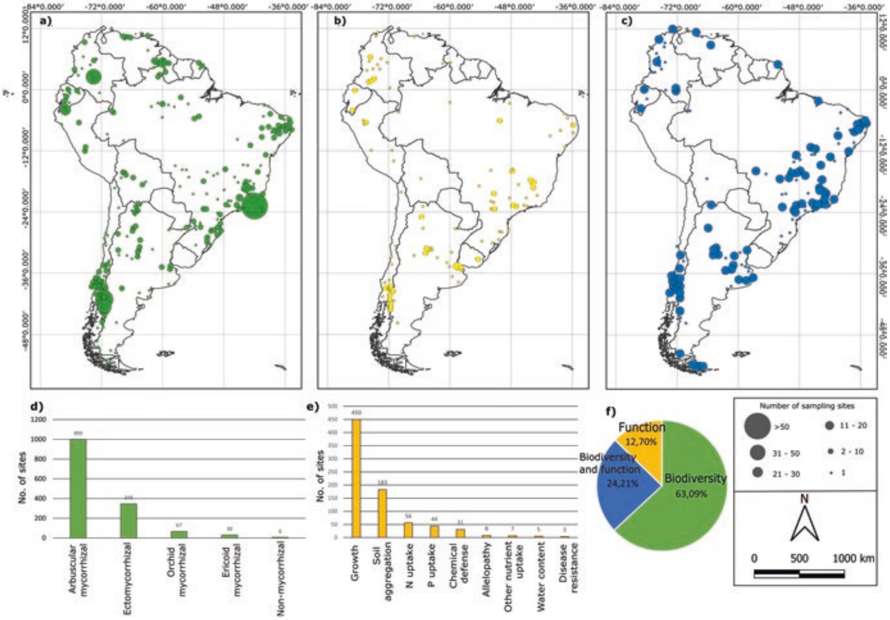

Fig. 22.2 Distribution of sampling sites for mycorrhizal types and ecosystem functions in South America. (**a**) Mycorrhizal types and non-mycorrhizal plants (biodiversity) sampling sites. (**b**) Mycorrhizal ecosystem functions sampling sites. (**c**) Sampling sites where both mycorrhizal type biodiversity and ecosystem functions were conjointly studied. (**d**) Number of sampling sites per mycorrhizal type. (**e**) Number of sampling sites per mycorrhizal ecosystem function. (**f**) Percentages of sampling sites investigating soil mycorrhizal type biodiversity, ecosystem function, and both. The size of the circles is based on a 50 km grid

most mycorrhizal sampling sites concentrate in the southern-central region (northern Patagonia), in the regions of La Araucanía, Los Ríos, and Los Lagos (Fig. 22.2), a pattern previously reported (Marín et al. 2017, 2022) and attributed to an almost two-centuries-old mycological and soil ecological research tradition in those regions (Marín et al. 2018; Godoy and Marín 2019). Ecuador concentrates most of its sampling sites across the Andes, from Quito to the Peruvian frontier (Fig. 22.2), where most universities and croplands are located, while Colombia has its capital (Bogotá) and surrounding areas, as the mycorrhizal research epicenter, although significant research has also been conducted in the Amazon, near Leticia (Corrales et al. 2018; Peña-Venegas and Vasco-Palacios 2019). Another Amazonian region with an important number of studies is the Guiana Shield (Fig. 22.2), particularly the frontier between Venezuela and Guyana (Smith et al. 2011, 2013; Henkel et al. 2012; Husbands et al. 2013; Roy et al. 2016), a highly diverse, unexplored, and difficult to access area. Most Argentinian mycorrhizal sampling sites were located in the north of the country, near a chain of cities starting in Buenos Aires (and through Rosario, Córdoba, Mendoza, San Miguel de Tucumán) and ending in Salta and in the limits with Bolivia (Fig. 22.2).

In most of the sampling sites (63.09%), mycorrhizal biodiversity was assessed, while mycorrhizal functioning was targeted in 12.70% of the sites (Fig. 22.2). In 24.21% of the sampling sites, both mycorrhizal biodiversity and ecosystem functions were studied together (Fig. 22.2), a surprisingly high percentage compared to global-scale analyses on soil biodiversity and ecosystem functioning (where this percentage was 0.3%; Guerra et al. 2020) but comparable to similar analyses at a national scale (Chile, where this percentage was 18.10%; Marín et al. 2022).

AMF was the mycorrhizal type most investigated (999 plots), followed by EMF (345 plots) (Figs. 22.2d and 22.3). Particularly, the distribution of sampling sites for both of these types matches the above-described patterns regarding where are located the main research centers/universities, historical research hotspots, or capital cities (Fig. 22.3). The remaining mycorrhizal types and non-mycorrhizal plants have been barely studied in the continent, with 103 sampling sites overall. This pattern can be partially explained because of the broad phylogenetic distribution of AMF and EMF and the fact that the other two mycorrhizal types are more restricted to specific plant families (Ericaceae and Orchidaceae). Furthermore, research into ericoid mycorrhizas is relatively new on a global scale, with guides and protocols for their isolation and identification being recently developed (Vohník 2020). As expected, most studies on mycorrhizal functions focused on plant growth and surprisingly, in soil aggregation (Fig. 22.4).

As the main role of the mycorrhizal symbiosis is directly related to plant growth, the result obtained in this regard (Fig. 22.4) was expected and matches previous mycorrhizal functioning analyses on a broader scale (Lekberg and Helgason 2018). Furthermore, there are databases dedicated to assess the effects of mycorrhizas on plant growth, like MycoDB (Chaudhary et al. 2016). There are also some insights and an important amount of research regarding the effects of mycorrhizas in soil aggregation (Duchicela et al. 2013), with some emphasis on glomalin research

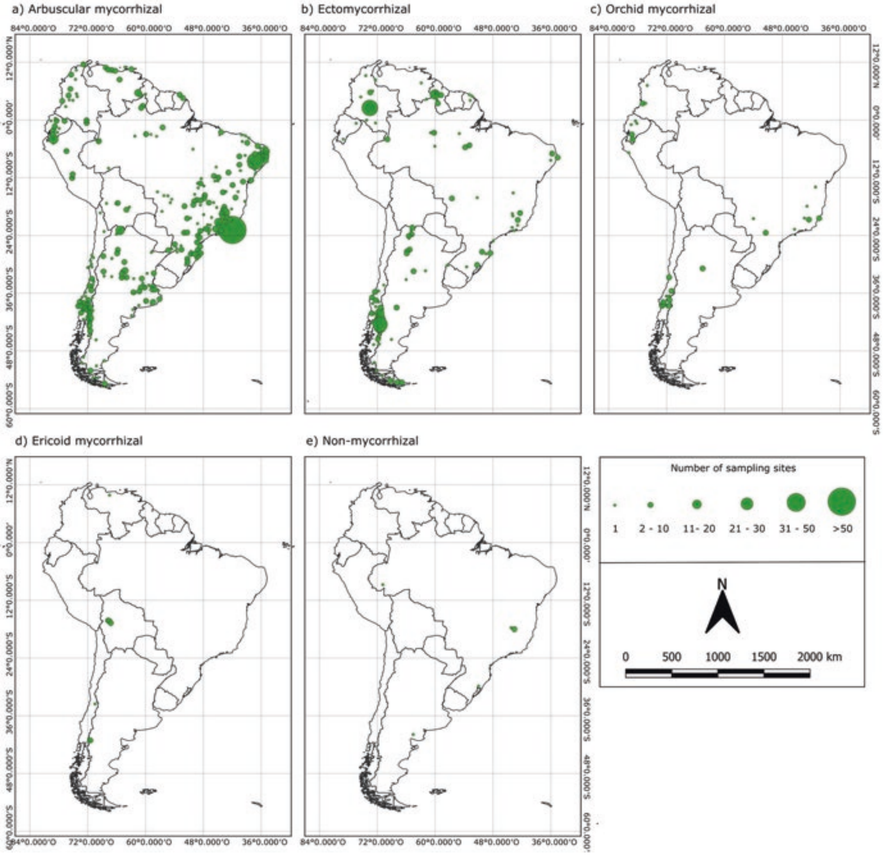

Fig. 22.3 Distribution of the sampling sites for four mycorrhizal types and non-mycorrhizal plants in South America. (**a**) Arbuscular mycorrhizas. (**b**) Ectomycorrhizas. (**c**) Orchid mycorrhizas. (**d**) Ericoid mycorrhizas. (**e**) Non-mycorrhizal plants. The size of the circles is based on a 50 km grid

(Cornejo et al. 2008) – which requires significant further development and better techniques (Irving et al. 2021).

There were several South American biomes highly understudied, like the desert and xeric shrublands of the Atacama Desert; the temperate grasslands, savannas, and shrublands of the Patagonian Steppe; the tropical and subtropical grasslands, savannas, and shrublands of the Cerrado and Chaco; and the tropical and subtropical moist broadleaf forests of the Amazon (Fig. 22.5). This pattern coincides with previous reports about research gaps in South American mycorrhizas (Cofré et al. 2019; Marín and Bueno 2019; Nouhra et al. 2019). As belowground biodiversity hotspots usually do not match aboveground hotspots (Cameron et al. 2019), it is recommended to conduct more baseline studies especially directed toward places with a transition of vegetation (Tedersoo et al. 2014). A recent study by Cazzolla

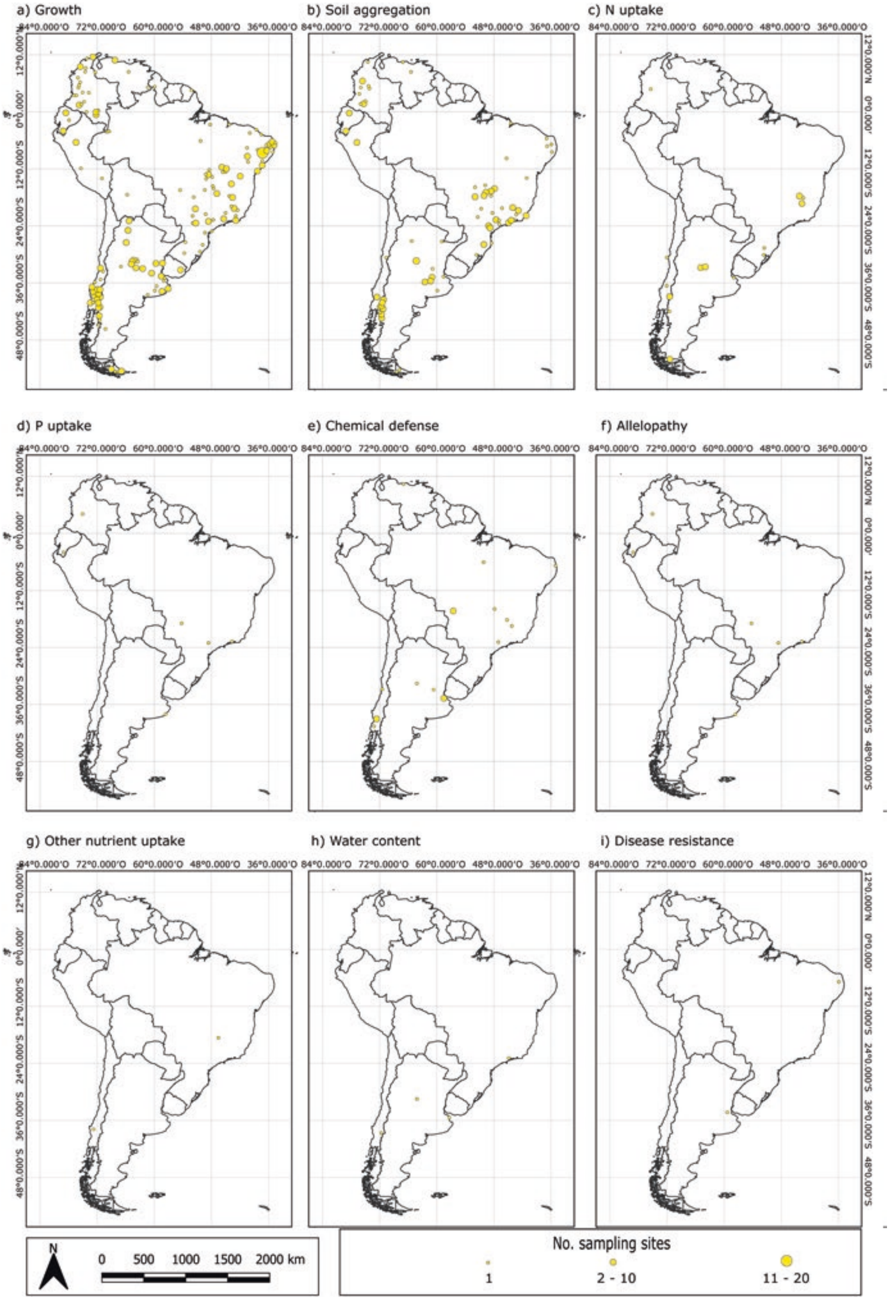

Fig. 22.4 Distribution of the nine mycorrhizal ecosystem functions in South America. (**a**) Plant growth. (**b**) Soil aggregation. (**c**) N uptake. (**d**) P uptake. (**e**) Chemical defense. (**f**) Allelopathy. (**g**) Other nutrients uptake. (**h**) Soil water content. (**i**) Disease resistance

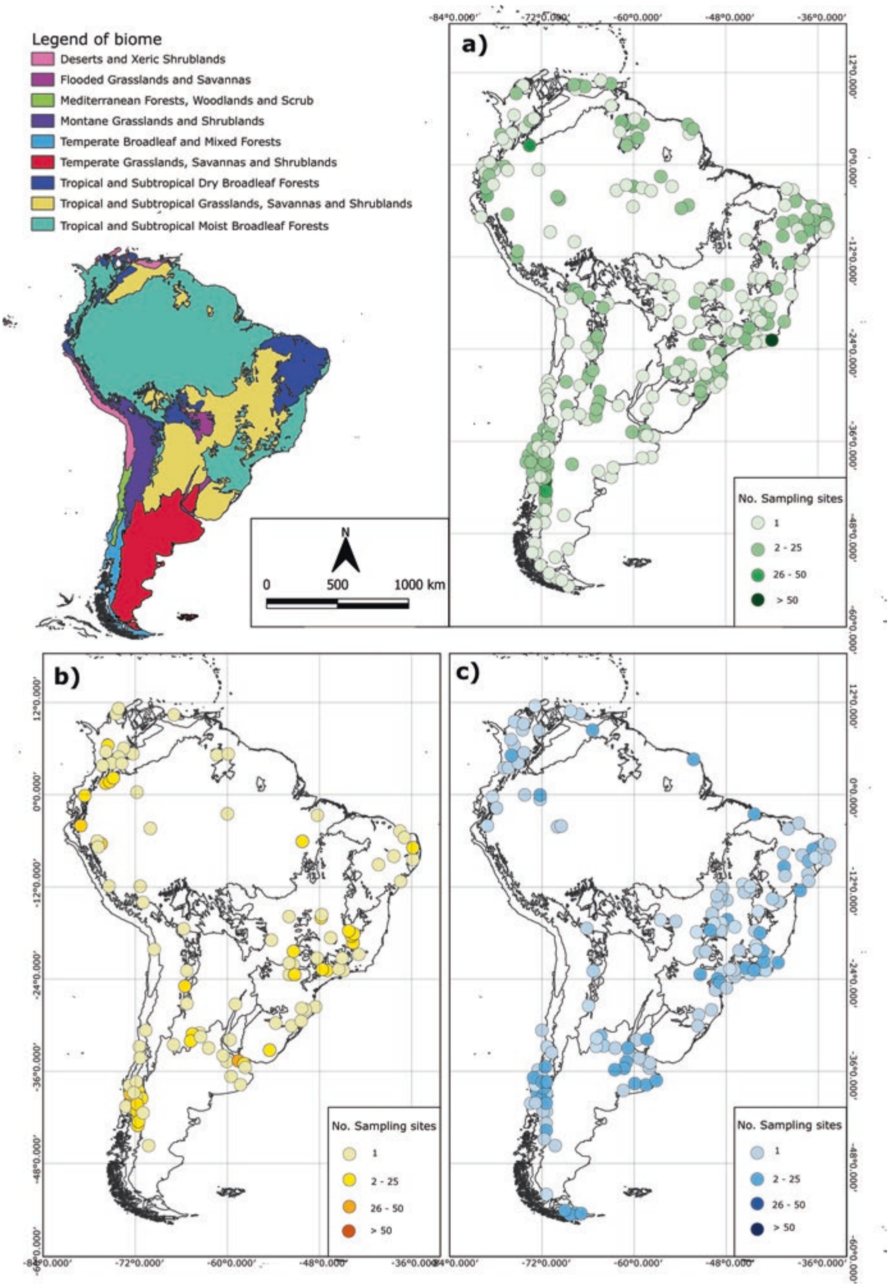

Fig. 22.5 Distribution of the sampling sites across South American biomes. (**a**) Mycorrhizal biodiversity sampling sites. (**b**) Mycorrhizal functions sampling sites. (**c**) Sampling sites where both mycorrhizal biodiversity and functions were jointly investigated

Gatti et al. (2022) estimates that there are about 9200 tree species in the world, 40% of them in South America, especially in the Amazon rainforest. This obviously adds to the knowledge gaps in mycorrhizal research in the continent. Science funding agencies across the continent should be more open to financing baseline studies aimed to discover more species and describe their mycorrhizal types and hopefully in more than one country, as in the European system. Broad collaborations and networking are novel scientific strategies to solve many gaps at the countries in South America in the link between mycorrhizal biodiversity and ecosystems functions, as through the South American Mycorrhizal Research Network and the International Mycorrhiza Society, which are powerful tools for achieving such goals.

The contribution of mycorrhizal fungi to plant nutrition and growth is the key to the formation of the symbiosis but not their only function. For example, on a global scale, it was found a significant correlation between the abundance of mycorrhizal fungi and P mineralization, soil respiration, available N, and in reducing antibiotic resistance genes (Delgado-Baquerizo et al. 2020). Similarly, it is known that decomposition processes (and therefore saprophytic fungi) and the abundance of AMF and EMF are related (Steidinger et al. 2019). It is not clear yet, at a global scale, which specific mycorrhizal types are related to which ecosystem functions. Some functions, like soil aggregation, metal alleviation, and pathogen defense, among others, have not been targeted at a global or even continental scale. Despite this, many regional and local-scale studies have shown different relationships between mycorrhizal biodiversity and other ecosystem functions such as biogenic weathering (Koele et al. 2014) and different components of the nitrogen cycle (Veresoglou et al. 2012). Also, in addition to global-scale analyses and studies and more baseline explorations, conducting controlled mycorrhization experiments is still crucial to understanding and quantifying mycorrhizal functions.

As mentioned earlier, trait-based ecology could make the relationship between mycorrhizal types/biodiversity and functions clearer (Wurzburger and Clemmensen 2018). In this sense, Chen et al. (2018) have suggested using a plethora of mycorrhizal traits like hyphal exploration distance, hyphal turnover, and hyphal uptake capacity and efficiency as proper functional traits because they heavily influence the search for nutrients. Many of these traits, and others like hyphal life span or mycelium structure (van der Heijden and Scheublin 2007), have been proven very difficult to measure are still not widely used. Several “simpler” methods have been suggested to solve these obvious methodological difficulties, AMF N:P stoichiometry (Powell and Rillig 2018) and relating different particular taxa to the acquisition or bioavailability of a particular soil nutrient (Treseder et al. 2018; Marro et al. 2022). Several other traits and the feasibility of easily measuring them at broad scales should be explored, like functional gene arrangements, enzymes, fatty acids, and nutrient consumption profiles, among others. All these increasing efforts show that the mycorrhizal scientific community is aware of the central role that trait-based ecology will play in understanding the ecosystem functions of this symbiosis.

22.4 Conclusion and Further Directions

In these analyses, we have shown that mycorrhizal research has many gaps in South America. Just a few sites in the south-center of Chile, south and northeast of Brazil, around a chain of cities in northern Argentina, around Bogotá (Colombia), south of Quito (Ecuador), and in the frontier between Venezuela and Guyana presented a good amount of sampling points, mainly targeting AMF and EMF, and plant growth and soil aggregation as mycorrhizal types and ecosystem functions, respectively. Thus, all other regions, mycorrhizal types, and mycorrhizal functions seem to be understudied. Furthermore, much more baseline research of plant and fungal biodiversity and on mycorrhizal types and controlled experiments (i.e., response to mycorrhization) are very needed. Additionally, we should ask in which of our nine South American biomes are mycorrhizas playing more ecosystem roles (i.e., multifunctionality) and how different are the roles provided by each type. It is also important to understand how the relationships (either causal relationships or correlations) change in plant communities dominated by different mycorrhizal types, which in many South American ecosystems are relatively close (thus, controlling for environmental conditions). Importantly, we should ask how to relate different mycorrhizal traits with different ecosystem functions. And finally, it is also important to assess the interactions between other soil and rhizosphere organisms and mycorrhizal fungi and the effect of such interactions on ecosystem functioning (Marín and van der Heijden 2020).

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