

Abstract

How are mycorrhizae communities distributed in the mangrove, restinga, and forest on Santa Catarina Island?

Santa Catarina Island, in southern Brazil, is covered with Atlantic Forest and has mangroves and restinga that act as buffers between the ocean and rainforest. The goals of this project are to understand how many taxa of mycorrhizal fungi are shared between the restinga and forest and to investigate the presence of mycorrhizae in the mangroves. Several woody species grow in the harsh conditions of the restinga, and a few of these also grow in the adjacent forest. These plants often have different habits, such as shrubs in the restinga and tall trees in the forest. Mangroves have their own distinct flora with species that are mostly restricted to this environment. We propose two hypotheses: 1) the community of ectomycorrhizal fungi in the restinga soil is similar to that in the forest; and 2) the diversity of ectomycorrhizal fungi in the mangroves is low. The mangroves and restinga are threatened due to climate change and real estate development. Another problem on the island is the spread of exotic *Pinus* and *Eucalyptus* species that were introduced. It is unknown how the local fungi communities are affected by the exotic fungi that were introduced with these plants. Knowing the soil fungal communities will allow us to better understand the effects of the invasive species on native plants and to select potential native taxa to be used in restoration projects.

We will promote events open to the public so local communities and students can be involved. For example, the Rick Foray and EctoSul will include field expeditions and talks that emphasize the importance of fungi in the soil. We will also teach participants how to look for ectomycorrhizal mushrooms and recognize and collect ectomycorrhizae in the field, as well as show them root tips with mycorrhizae using dissecting microscopes.

Project details

Project Title

How mycorrhizae communities are distributed among mangrove, restinga and forest on Santa Catarina Island?

Project Location(s)

Atlantic Forest, Florianópolis, Southern Brazil.

Project start date

20/08/2023

Project end date

19/07/2024

Total number of samples you plan to collect

50

Description of geographic area and habitat type(s)

Soil samples will be collected in three habitats in the Atlantic Forest in Florianópolis, Brazil: mangrove, restinga and forest. Forest areas are very diverse and composed mostly of shallow soil with litter. The restinga is a buffer habitat between the ocean and the forest, with sandy acidic saline soil where plant species deal with dryness due to the sandy soil, high salinity, and heavy winds. Despite the different habitats, the forest and the restinga share some plant species that are able to grow in both habitats. Mangroves are also buffer areas, but have a distinct flora from the forest and the restinga. The mangrove communities

are subjected to flooding periods by the ocean. Mangroves function as nurseries for local fauna and flora, however the diversity of mycorrhizae in the mangrove soil is not well known, especially for ectomycorrhizae. In Santa Catarina Island these habitats are only a few kilometers apart and are subjected to very similar climatic conditions. To compare the diversity of mycorrhizae in the soil among mangrove, restinga and forest will help us understand how the gradient of the fungi genera change and the role mycorrhizae have in the maintenance of these three environments.

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Hypothesis

Biogeographical studies show that restinga and adjacent forests share several plant species. Only a handful of these species are able to tolerate the harsh conditions in the restinga, often with different growth habits when compared to the species growing in the forest. Mangroves have their own distinct flora and the species are mostly restricted to this environment. Following this pattern, we expect to find that the community of fungi in the restinga soil is similar to the community in the forest, maybe with some differences regarding more resilient mycorrhizae. The mangroves are understudied and we expect to find a smaller or maybe no ectomycorrhizal fungi, but expect the endomycorrhizae community to have several of the same genera of arbuscular mycorrhizae. The geographical proximity among the sampled areas will be key to determine if there is influence of the mangrove communities in the restinga and of the restinga in the forest.

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Description of the molecular and sequencing aspects of the project (metabarcoding rDNA region to be amplified, sequencing platform to be used)

The sampling methods will follow the SPUN protocols. DNA extraction and amplification of the ITS region will be made at the molecular lab at Universidade Federal de Santa Catarina (UFSC). Total DNA extraction will be made using the DNEasy Plant Kit. The selected region to be amplified is ITS using the pair of primers ITS1F and 5.8S with a mutation in 3' that allows amplifying soil Basidiomycota and arbuscular mycorrhizae (fwd ITS5: GGAAGTAAAAGTCGTAACAAGG [White et al. 1990] rev_5.8S: CAAGAGATCCGTTGTTGAAAGTK [Epp et al. 2012]). The sequencing will be done in

Brazil, at Biotecnologia Pesquisa e Inovação, a company from Botucatu (São Paulo state). Sequencing will be done in illumina MiSeq to separate informative barcode fragments from low-variability fragments and to identify fungal OTUs. Blast searches will be done using GenBank and UNITE databases. Suggestions on the methods are welcome and can be adjusted to improve the results.

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Describe the importance of these data and ideas

The impact that these data can have on a small scale in Santa Catarina Island can be estimated by comparing fungal communities in the soil of two habitats, mangrove and restinga, that have an important role as buffers to protect the adjacent forests. Sampling in these habitats and identifying the OTUs will reveal a cryptic diversity of mycorrhizae community that is not known for the most part of the Atlantic Forest. Mangrove and restinga are areas that are threatened due to climate change but more rapidly threatened by real estate development. Another problem faced in the Island is the spread of exotic plant species like *Pinus* and *Eucalyptus* that were introduced in the Island decades ago. It is unknown how the local fungi communities are affected by the introduced exotic fungi that were introduced with these plants. In the forest and the restinga these species are often growing intermingled with the native species. Knowing the local fungal communities and how they interconnect among mangrove, restinga and forest will allow us to select potential taxa to be used in restoration plans. The produced data will improve our knowledge on the communities, complementing the small data we have based on sanger sequencing from basidiomes and root tips from restinga and forest, and will add an important layer of data on yet another buffer habitat, the mangrove.

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Methods (see SPUN's [standardized protocols](#))

Santa Catarina Island is 54 Km long and 18 Km wide, with a total area of ca. 424 Km². There are 42 beaches with restinga adjacent to most of them and 5 mangroves. We selected areas in three points of the Island that have the three habitats that will be sampled: one in the South, one in the East and one in the North. In each habitat we will make 5 samples (except for one that is too small to fit 5 and will have 4 samples instead).

In the South the soil sampling will be made in the municipal park of Lagoa do Peri and the Pirajubaé mangrove. In the park, the trail Guarani crosses the restinga area and the trail Gurita is covered by forest. In the East part of the Island the restinga samples will be made in the park Dunas da Lagoa da Conceição, the forest samples will be made in Morro da Lagoa, and the mangrove samples will be made in Itacorubi mangrove. Because it is located in a more urban area at the center of the Island, the Itacorubi mangrove does not have enough area for 5 samples distant at least 1 Km from each other, so we will make four samples in this habitat. In the North part of the Island we will sample in the park of Costão do Santinho and in the forest Morro das aranhas. The mangrove samples will be made in the Daniela mangrove.

The total number of samples will be 44 distributed in these three large areas. This number will fit the budget for the molecular methods to be performed and will allow us to hire a field assistant to help during the sampling period between August and December. There's room for a few extra samples, if needed.

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Timeline (including at least: 1. Field Work Duration 2. Lab work / DNA extraction 3. Sequencing Period)

Field work for sampling collections will tentatively start at the end of August to be finished in December. The months that are more favorable for the growth of the mycelium in Santa Catarina Island are between October and January when the rainfall is larger (average of 121 mm and 156 mm respectively) and the soil is not too dry. In the restinga, where the water drains fast due to the sandy soil, January is the best month for mushroom collecting. Based on the average climate in the Island for amount of rain and temperature and on the timeline of the project, the first collections will be made in the mangrove areas, where the soil is always wet and therefore less subjected to changes in the communities due to the weather. The forest sampling will be made between October and November and the restinga samples will be collected between November and December. Lab work to extract and amplify the ITS region will be done at UFSC between January and March, allowing time to send out the samples to metabarcoding sequencing between March and April to have at least 30 samples sequenced by May.

Risks of the project

The communities of mycorrhizal fungi will be compared among three habitats: mangrove, restinga and forest. We have data from basidiomes and a few roots that confirm the presence of ectomycorrhizae fungi in the restinga and in the forest. The mangroves in Brazil are very undersampled for fungi and have never been sampled with the aim to look for ectomycorrhizae and the data on arbuscular mycorrhizae are rare. The diversity of trees in the mangrove is really low and there are no records of typical ectomycorrhizal hosts, so the soil samples might show some unexpected results on the metabarcoding. In the FLOR fungarium the basidome records for ectomycorrhizal fungi are non-existent for mangroves and all the fungi records for the mangrove areas in the Island are of decomposing fungi. Therefore, there is a risk that the mycorrhizae fungi are not present or are in a very low number in this habitat.

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Expected impact

Description of local collaborators and how they would benefit from data

I coordinate the Tropico Ectomicorriza project in my lab. The project is multidisciplinary and the collaborators include researchers and students that are plant taxonomists, plant anatomists, plant physiologists and ecologists. Also, we have the permits for the areas that we are proposing to collect. The data produced by the metabarcoding will allow the team to better understand the diversity of fungal OTUs in mangroves, restinga and forest in Santa Catarina Island and give information that will allow the team to ask other questions. The community distribution and the relative abundance of ectomycorrhizae and arbuscular mycorrhizae in these three habitats will compare the areas in a new way. The revealed diversity based on OTUs will allow us to draw projects at the undergrad, graduate or higher research levels to look for patterns and novelties.

I am also involved in the Networks of Fungal Interactions in the Neotropics (NEFINEO) project, coordinated by Melanie Roy (France) and Paulo Guimarães (Brasil). The data generated during the SPUN project will also feed the NEFINEO and will put the information

in a broader perspective, comparing to other regions of Latin America to explore endemisms and specialization.

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Identification of local communities (including Indigenous peoples and Indigenous lands, if applicable)

The three parks where we are sampling in restinga and the forest are surrounded by communities that use the parks for recreational activities. In two of them we already have educational actions to be continued. Lagoa do Peri has good potential because there is a house and they regularly have activities with the local public or schools. We have had mycological events in the park, like the Rick Foray and the Ecto Sul. During these events the community was invited to participate and go on trails with the team to understand the role of fungi in the environment. We will continue to involve the local community in activities to show fungi and mycorrhizae, bringing microscopes to the park (or to local schools) to understand how they feel and how they see fungi where they live. In Lagoa da Conceição there are activities promoted by fellow ecologists that involve the community to help with the removal of exotic plant species, mostly *Pinus*. We intend to straighten the work we are doing with them and raise questions regarding mycorrhizae, both native and exotic, and bring that to the communities to show the impact of fungi in the ecosystems.

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Anticipated outcomes (e.g publications, thesis chapters, conservation status evaluation)

The Tropical Ectomicorriza project has around 25 people involved, among undergraduates, graduate students, post docs and researchers. The large number is a result of the interdisciplinarity of the theme and also the increasing interest in fungi and mycorrhizae in an area that is affected by climate change. During the one year of the development of the project it can be hard to estimate the number of publications and thesis. However, there is no doubt that over a longer period of time the data generated will serve to raise more questions in the future and produce publications about mycorrhizae.

In the short term we intend to write educational posts to be distributed to the parks and schools to highlight the importance of the conservation of biodiversity. We estimate that the

data generated, combined with other methods, will be used by 10 to 12 undergraduates that will publish at least one scientific article or a method protocol, and between 4 and 6 grad students (masters and PhDs) that will use parts of the data in at least one chapter of their thesis.

Me and some of my students have experience in writing conservation status for species and with the barcoding data some of the known species will be better evaluated based on the distribution of both the fungi and the hosts.

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Description of expedition engagement and impact

To engage more students and the general public in the project we will promote a special edition of the Rick Foray with emphasis on the soil diversity to show in the field how to collect soil cores to study mycorrhizae. To make it more clear we will have a dissecting microscope and an optical microscope to show the morphology and the anatomy of root tips. Also, we will promote a special edition of the Ecto Sul to invite speakers to talk about soil diversity and mycorrhizae, including ectomycorrhizae, arbuscular, orchidoid and ericoid mycorrhizae. The diversity of the talks will emphasize the importance of conservation to protect all of the environments.

Besides that we will include the information generated in the website of the Tropical Ectomicorriza project, incrementing the molecular data with photos of the symbiont partners and the localities where the samples were collected. We will feed the laboratory social media channels with updates of the field trips and the partial results. The lab is on Instagram, Facebook, Youtube and Twitter and we are going to register on tiktok as well. As a group we have worked on outreach and several of us have given talks, including in bars, for the Pint of Science project, to promote fungi and their importance for the environment. We intend to continue to do so and with this project, emphasize the soil diversity that will be discovered in Santa Catarina Island.

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Budget and CV

Total amount requested = 9,440.00

Budget Form

Please download the file, fill it out and upload the completed form below.