

## **Research project: Investigating plant-fungus interaction networks in natural ecosystems**

### **Short summary:**

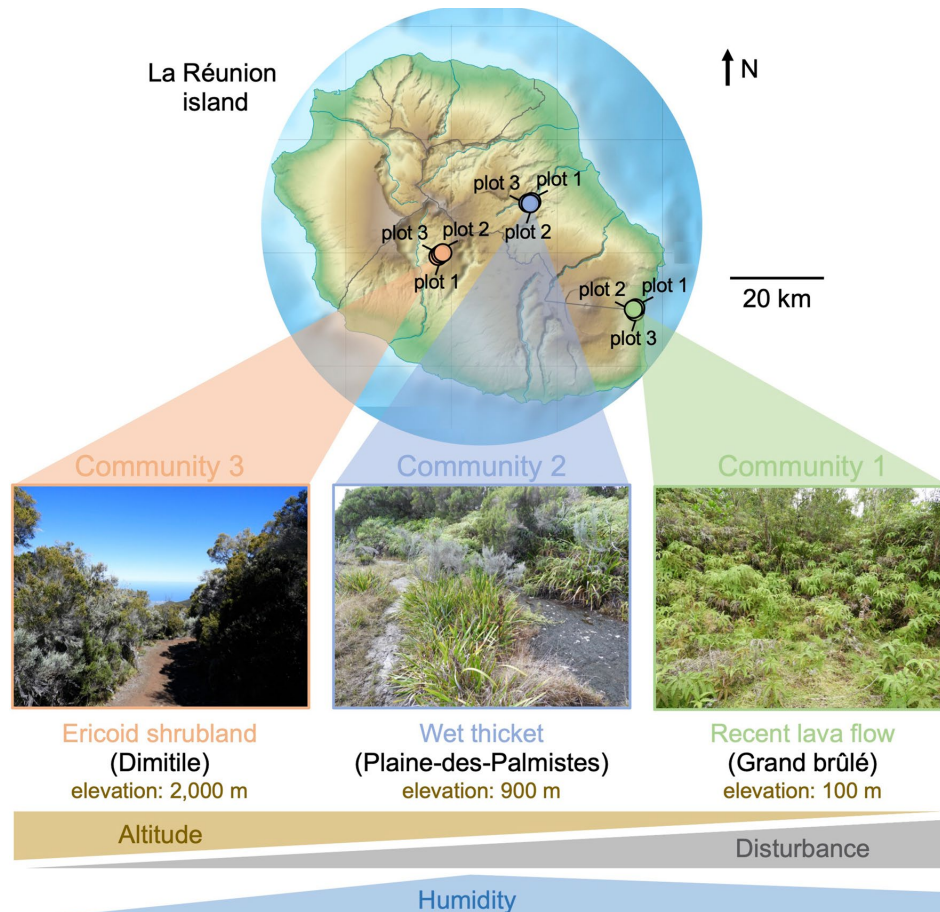
The root mycobiome plays a fundamental role in plant nutrition and protection against both biotic and abiotic stresses. In temperate forests and meadows dominated by angiosperms, many of the fungi involved in root symbioses are shared among neighboring plants, giving rise to complex plant-fungus interaction networks characterized by weak specialization. Whether such weak specialization also occurs in species-rich tropical communities, which harbor more phylogenetically diverse plant lineages, remains unclear. To address this question, we sampled the roots of 30 plant species, including angiosperms, ferns, and lycophytes, across three contrasting habitat types on La Réunion Island: a recent lava flow, a wet thicket, and an ericoid shrubland. Root-associated fungi were then identified through metabarcoding of both the 18S rRNA and ITS2 regions.

### **Methods:**

#### *Study sites and sampling*

The study was conducted on the tropical volcanic island of La Réunion in July 2019 in three ecosystems. The first community, in Grand brûlé is derived from a recent lava flow that occurred in the nineteenth century. The soil in Grand brûlé is therefore shallow, poorly differentiated, and colonized by many non-indigenous invasive plant species. This community is typical of the earliest stages of ecological succession. Near the sea and at a low elevation (100 m), Grand brûlé has frequent precipitations (annual rainfall of 4000-5000 mm). The second community, in Plaine-des-Palmistes, is a wet area very dense in shrubs, called a 'thicket', in the central valley. The soil in Plaine-des-Palmistes is derived from old lava flows that happened between 80,000 and 20,000 years ago. The very frequent precipitations in Plaine-des-Palmistes (annual rainfall of 5000-7000 mm) generate many ponds in these thickets and greatly leached nutrients out of the soil. The third community, in Dimitile, is an ericoid shrubland formed on the high-elevation ridge of the Cilaos cirque, dominated by the endemic species *Erica reunionensis*. Compared with the two previous communities, Dimitile experiences less precipitation (annual rainfall of 1000-2000 mm). The soil in Dimitile, derived from >400,000-year-old lava flows, is particularly enriched in acidic humus.

Three plots distant from 50 to 250 meters having similar sets of plant species were sampled. For each plot, the roots of all the plant species that were present were harvested: within a radius of 1.5 meters, up to 3 individuals per plot per plant species were sampled if available. For small non-woody plants, the entire root systems were carefully removed from the soil, cleaned with sterile water, and immediately dried in silica gel.



**Fig. 1** The three sampled communities correspond to habitats with contrasted environmental conditions. A map of La Réunion island indicating the three sampled communities in this study. The sampling sites were characterized by different vegetations and abiotic conditions with different elevations, levels of disturbance, humidity, and soil conditions: (right) Grand brûlé (recent lava flow close to the ocean on the wet East coast), (middle) Plaine-des-Palmistes (wet thicket on old lava flows in the central valley, elevation 900 m), and (left) Dimitile (ericoid shrubland on old lava flows in the dry crests of Cilaos cirque on the West side dominated by ericoid vegetation, elevation 2000 m). In each community, we replicated the sampling in three plots distant from 50 to 250 m. The photos illustrate the overall vegetation in each sampled community and the gradients at the bottom resume the main variations in the environments. The raw map in the background was generated by Eric Gaba (Wikimedia Commons user: Sting)

### Molecular analyses and bioinformatics

Fungal DNA was extracted from root powder using the NucleoSpin 96 Plant II kit (Macherey-Nagel). Two pairs of tagged primers, AMADf-AMDGr and ITS86F-ITS4, were used for PCR, **amplifying the 18S and ITS2 regions** respectively. The former marker gene rather detects Glomeromycotina and Mucoromycotina, whereas the latter marker is more specific to Ascomycota and Basidiomycota. PCRs were performed using the AmpliTaq Gold 360 kit (ThermoFisher Scientific) and ran for a total of 35 cycles at an annealing temperature of 50°. To ensure better characterization of the mycobiota, the PCR step was replicated at least 2 times. PCR products were purified using magnetic bead-based clean-up, quantified using Qubit, and 3 ng of DNA amplicons per sample were sequenced using Illumina 2x250 bp MiSeq technology.

The sequencing reads were processed using VSEARCH. In short, paired-end reads were assembled, quality checked, demultiplexed using cutadapt, and clustered into operational taxonomic units (OTUs) with a similarity threshold of 97%. Chimeras were removed and a taxonomy was assigned to each OTU using Silva and UNITE databases.

## Available files

All the files for the research projects are available in the following link:

[https://github.com/BPerezLamarque/Scripts/tree/master/Metabarcoding/tutorial\\_M1\\_TULIP/](https://github.com/BPerezLamarque/Scripts/tree/master/Metabarcoding/tutorial_M1_TULIP/)

It includes:

- **metadata\_project\_La\_Reunion.csv**: metadata associated with the different root samples.
- **OTU\_table\_La\_Reunion\_18S\_OTU97.csv** and **OTU\_table\_La\_Reunion\_ITS\_OTU97.csv**: the OTU tables for the 18S and ITS2 metabarcoding respectively.
- **script\_project\_example\_invasive\_species.R**: the script for running on example of analysis (see below)

## Example of script: Investigating the effects of biological invasions on plant-fungus networks:

The aim of this script is to assess how biological invasions affect root-associated fungal communities in the disturbed plant community of Grand Brûlé (La Réunion). To this end, we want to compare both native and invasive plant species. Their root-associated fungi were characterized through 18S rRNA metabarcoding, enabling the identification of diverse fungal taxa across host plants.

After preparing and cleaning the data, our objectives are threefold:

- 1) Fungal composition: to compare the taxonomic profiles of root mycobiota between invasive and native plants.
- 2) Alpha diversity: to assess whether invasive plant species harbor higher or lower fungal diversity within their roots relative to native ones.
- 3) Beta diversity: to investigate whether invasive plants alter the overall community structure of root-associated fungi and disrupt existing plant-fungus interaction networks.

To run this script, you will need to have R and RStudio installed, along with the following R packages: ggplot2, vegan, nlme, ape, reshape2, and igraph.

## Research project assignment:

Using the provided dataset of root mycobiota from La Réunion, you will work in groups of two to develop and investigate an original research question on plant-fungus interactions. Your task is to introduce your research question, analyze the data, interpret the results, and summarize your findings and discuss them in the form of a short scientific article (2 pages maximum).