



### **Drought impact on the diversity, function and functioning of the forest microbial communities**

Principle investigator: Stéphane UROZ / Aurélie DEVEAU, UMR Interactions Arbres/Micro-organismes (IAM) 1136

LabEx partners: Marie-Pierre TURPAULT, UR Biogéochimie des Ecosystèmes Forestiers (BEF) 1138

Thematic actions concerned: WP1 WP2

Longer periods of drought combined with higher intensity are expected to become increasingly frequent with future climate change. Although many studies focused on the response and adaptation of the aboveground part of forests, comparatively little addressed belowground processes linking soil, plant and microbiome. However, drought strongly reduces nutrient diffusion in soil and slows down biogeochemical processes such as soil organic matter turnover and mineral weathering. It induces also quantitative and qualitative changes of the root exudates, which differently impact the composition of the root-associated microbial communities. Drought consequently affects many central biogeochemical processes, which have critical feedback effects on tree nutrition, development and even survival. In the same time, nutrient availability and pH are known to condition the functional and taxonomic structures of the soil and rhizosphere microbiomes. Determining how drought and the frequency of drought impact

the soil microbiome in relation to soil properties and the plant nutritional requirements is a challenging area of research, but which it should permit to better understand forest ecosystem functioning and to determine whether a coevolution occurs between trees and their associated microbiome to cope with drought. In this context, the aims of the project “Drought impact on the diversity, function and functioning of the forest microbial communities” (**DroughtMIC**) are: i) to determine how an historic of successive droughts impacts the soil properties and the taxonomic and functional structure of the soil microbiome and ii) to test whether the microbiome captured by the trees differ according to an ancient or recent historic of drought and how this impact tree physiology.

**Context** — Climate change and especially variations in the amount and frequency of rainfall are major events that directly impact the forestry sector. Longer and more frequent periods of drought are expected in the coming years. As trees have significant water needs to ensure their nutrition, growth and health, these variations are likely to strongly affect them as well as the forest management sector and the associated industry. While needs may differ from one species to another and depending on soil properties (type, depth, usable water reserve), the drought observed in 2020 affected many forest areas leading to the massive death of spruces and in to a lesser extent of other species such as beech. It is therefore essential to understand how trees adapt to these changing conditions. Significant efforts have been made over the past 20 years in the fields of tree physiology and genetics to understand how trees respond and adapt to drought, but without considering the relative role of fertility and soil microbiota, and their interactions. However, drought strongly disrupts the diffusion of nutrients in the soil and slows down many geochemical processes such as the degradation of organic matter and the alteration of minerals. At the same time, drought leads to changes in root architecture to access water and also to variations in root exudation which consequently differentially stimulate the microbiota surrounding or associated with the roots. In this context, it is essential to determine how drought affects soil microbiota and tree-associated microbiota both taxonomically and functionally, in relation to soil properties and tree nutritional requirements.

**Objectives** — In this context, the objectives of the project 'Impact of drought on the diversity, function and functioning of microbial communities in forest soils' are to: i) Determine how a history of successive droughts affects soil properties and taxonomic structure and function of the soil microbiota, and ii) Testing whether the microbiota captured by tree roots differs according to the ancient or recent history of drought.

**Approaches** — In order to carry out this project, we focused on the instrumented device of Montiers-sur-Saulx. This site has a precipitation exclusion system (Roof) that has been in place for several years. This system is installed on a soil that has already been studied in terms of geochemistry, microbiology and its capacity to support tree growth. To answer the questions related to the project, we considered soil plots with (D) or without (ND) a history of water exclusion. These plots were used: i) to collect the different horizons (litter, organic horizon and mineral horizon) to carry out different analyses (enzymatic, pH, microbiology) and ii) on each of these plots we placed young beech seedlings collected on the site at the foot of the same mother plant in order to limit genetic diversity. An electric fence was installed to protect the site from pests. In parallel to these in situ experiments, greenhouse experiments were carried out with soil and beech seedlings. The same beech seedling (as the one installed on site) with its soil adhering to the root system was set up in the greenhouse (ultimately one plant per pot) for soil moisture control experiments. For these experiments, soil from the plot without water exclusion was taken and used to repot the beech seedlings and ensure their growth. The soil horizons (litter, organic and organo-mineral) were respected for the recovery phase. The drought work was carried out on pots containing mainly the organic-mineral horizon. Due to the mortality of the plants on site and in the greenhouse, the operation had to be repeated several times in order to have sufficient numbers. After a first phase of recovery in the greenhouse, the plants were replanted individually in pots. The first results of the project were obtained in 2022 via the recruitment of 2 interns (C. BEITZ, Master M2 and M. BEDNARECK, Master M1).

**Key results** —

**Collection approach:** The soil samples collected were used to carry out analyses of pH, soil moisture and overall functional potential using BIOLOG plates and enzyme tests. In parallel, a collection of bacterial strains (50 strains per horizon) was made from the organic and mineral soil horizons, for a total of 400 strains. A sub-sample of this collection of strains was functionally characterised using different biotests mimicking processes related to the decomposition of organic matter, the dissolution of minerals and resistance to drought (in the presence of

polyethylene glycol; PEG) and at the taxonomic level via the sequencing of the ribosomal sub-unit (16S). This work did not reveal any significant difference between treatments with or without a history of drought, either at the global or strain level. No significant difference was observed on the density of bacteria between the two treatments (D=ND). A trend towards better drought resistance (tested in the presence or absence of PEG) was observed for strains from soil with a history of drought. Most of the strains effective for one of the functions tested were found to lose this ability under drought conditions. A significant difference was observed between the different horizons considered, both at the level of the global tests (BIOLOG) and at the level of the strains (e.g. solubilisation of inorganic P), with an efficiency varying according to the gradient: Litter > organic > mineral.

**Greenhouse approach:** The greenhouse experiments showed a marked effect of drought on the growth of beech seedlings, their colonisation by micro-organisms and the activities of the latter. The drought led to a reduction in the biomass of the seedlings by a factor of two compared to the controls, as well as an increase in the mortality of short roots (+20%). In addition, drought led to a very strong reduction in the enzymatic activities involved in the degradation of organic matter. This effect was even more pronounced in soils located at a distance from the roots, suggesting a protective effect of root systems on the activity of micro-organisms.

Samples were collected for the microbiota study. DNA has been extracted and libraries of fungal and bacterial marker amplicons are being developed. The sequencing of these libraries and the quantification of fungal and bacterial markers by quantitative PCR will make it possible to quantify the impact of drought on the density of microorganisms in soils and root systems and to identify both drought-sensitive and drought-resistant microorganisms.

#### **Main conclusions including key points of discussion —**

At this stage of the project, our results show that :

- i) the drought history has no obvious impact on the functional properties of the isolated and tested bacteria. This result raises a number of questions about the device, the temporality of water exclusion, the resilience of the microbiota and the sampling time chosen. For experimental reasons and because of the M2 internship, the microbiology work was carried out in March, i.e. several months after the water exclusion period, and moreover after a year in which there was little rain, limiting the differences between the D and ND treatments.
- ii) the control of the drought level is well reflected in the changes we observed of the physiology and growth of the plant and in the soil microbiota functions. The conclusions, although partial, are interesting.

**Perspectives —** In 2023, several experiments related to the manipulation of water availability will be developed to determine its impact on the growth and development of young trees and on the associated microbiota. A Master2 student will start his internship in March on the effect of drought on soil microbiota. The greenhouse experiments are essential because they allow the water supply to be modulated and limit the variation of other factors.

#### **Valorization —**

At this stage, this project is being developed in the form of 2 master's internships and by training these master's students:

- Maud BEDNARECK-Master 1 -Master Microbiology (Nancy) Effect of drought on the growth of beech and its associated microbiota (co-supervisor A. DEVEAU)
- Clement BEITZ - Master 2 - Master Microbiology (Nancy) Effect of a drought history on the taxonomic and functional structuring of the soil microbiota of the Montiers forest (co-supervisor A. DEVEAU)