



Redundancy analysis (RDA) showing distribution of the fungal communities according to a water stress gradient and beech health status. Vectors indicate significant variables and species structuring the microbial communities after multiple regression analyses and 1000 permutations ( $n = 8-10$ , FDR corrected,  $p_{adj} \leq 0.01$ ). The significant effects of environmental variables and beech health status were assessed by PERMANOVA after 1000 permutations ( $p_{adj} \leq 0.01$ ).

## The rescue of beech by well-adapted mycorrhizal fungal partners – Do symbiotic interactions confer adaptive capacity to drought conditions?

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LabEx partners: ARBRE-SwissForestLab proposal

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Thematic action(s) concerned : WP1

### Context

Recent extreme climatic events, such as extended drought periods, impose serious threats to the functioning of forest ecosystems. Forest trees, like beech, are long-lived species that play an essential role in their ecosystem.

To date, the role of symbiotic plant–fungus interactions in their response to drought is poorly understood and its assessment requires an integrative framework linking genotypic, phenotypic, and environmental data from natural populations as well as experimental validation of in situ observations.

### **Objectives**

Recent studies have shown that selection affects the phenotypes and genes of natural beech populations, leading to adaptation to drought conditions. We aim to enlarge this knowledge to belowground adaptation processes. In particular, to understand forest functioning and resilience, we need to know whether the key mutualistic partners of forest trees are adapted to their local habitat and whether, the tree and fungi form a co-adaptation complex that could confer nutrient and water supply benefits during extended drought periods. Our project will bridge genomics and ecology to help predict the fate of beech forests in Europe in the context of climate change by taking in to account their mycorrhizal associates.

In WP1 we aim to identify gene networks responsible for the adaptation of individual beech trees to low water availability and high temperatures and to determine the role that associated ectomycorrhizal (ECM) fungal communities play in these adaptive processes by studying drought-affected beech trees at dry study sites. In WP2 we search for patterns of local adaptation in the main ECM symbiont of beech, *Cenococcum geophilum*, by isolating and sequencing the whole genome of about 250 strains from beech forests situated on a steep water availability gradient across Switzerland. Finally, in WP3 we want to develop simplified mycorrhization systems with beech seedlings and *C. geophilum* in the laboratory to be able to test and understand the effects of different drought-adapted strains on interactions with beech under diverse conditions.

### **Approaches**

In WP1, we will compare pairs of closely situated vital and damaged juvenile beech trees sampled after the severe drought of 2018 at six dry study sites across Switzerland. We will use individual whole genome sequencing of these beech trees and DNA barcoding of ECM communities and *C. geophilum* present at their root tips to investigate significant associations between tree genotype and ECM fungal community composition at six dry study sites along an ecological gradient. In WP2, we will use whole genome sequencing of individual *C. geophilum* strains to assess population allele frequencies and genotypes and relate them to environmental variation using association analyses. In WP3, we plan to evaluate the fitness of the *C. geophilum* strains from WP1 in agar plates using polyethylene glycol to impose drought stress. We will test the mycorrhization capacity and functioning of selected drought-tolerant and non-drought-tolerant *C. geophilum* strains with beech seedlings using both a greenhouse and a sterile in vitro system and sequence a first set of reference transcriptomes of *C. geophilum* and beech using RNA sequencing.

### **Key results**

- WP1: We characterized the fungal communities associated with vital and damaged beech root systems from six study sites across Switzerland. We observed that water stress had a greater effect on the structuring of fungal communities than soil chemical parameters (e.g. pH, clay content, C:N ratio). However, we identified a core fungal community that remained unaffected by water availability among all sampled sites (e.g. *Cenococcum* and *Cortinarius*). On the contrary, some fungal genera such as *Lactarius* were significantly associated with sampling sites more prone to water deficit. Finally, we showed that ectomycorrhizal fungal species were significantly associated with healthy beech root systems while the relative abundance of saprotrophic fungi increased in roots of trees showing severe dieback.
- WP2: Fieldwork for this part was completed summer 22 and over 250 fungal strains from soil samples were isolated (collected along the 14 water availability monitoring sites.) Some adjustments to the protocol were necessary to optimise the isolation of fungal material from the numerous soil samples. DNA extractions of pure fungal cultures have been done and genome re-sequencing has been performed. In parallel, we have generated a new chromosome-level *C. geophilum* reference genome assembly for downstream analyses. E Morin (bioinformatician INRAE IAM) used the JGI annotation pipeline to annotate this new reference and the portal is awaiting portal QC to be released.
- WP3: Preliminary drought tolerance tests of *C. geophilum* strains using polyethylene glycol to impose drought stress were conducted. For one of the drought tolerant strains, *C. geophilum*, isolate 1.093, a transcriptome analysis during stress and recovery period was conducted.



- We used polyethylene glycol (PEG) to mimic the effects of drought. Mycelia growing in medium with and without PEG (-1.5 MPa) were sampled at 6h, 24h, and 48h. After sampling part of the cultures, the remaining mycelia were transferred to no-PEG medium for recovery for 24h and 48h. Triplicate samples were snap-frozen, total RNA was extracted and sequenced using 150bp pair-end DNBSEQ. Further, a Material Transfer Agreement was signed with CSIC in Spain and 5 in vitro clones of *Fagus sylvatica* and *F. orientalis* obtained. First mycorrhization tests with *C. geophilum* are ongoing.

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

WP1:

- Fungal community were structured according to the study site and the health of the host tree.
- we showed that ectomycorrhizal fungal species were significantly associated with healthy beech root systems while the relative abundance of saprotrophic fungi increased in roots of trees showing severe dieback.
- 4 fungal taxa were significantly associated with decaying host (the unidentified saprotrophic fungi Pezizula, Mycena, and Mortierella as well as the unidentified Trimorphomycetaceae), while only 1 fungus was significantly associated with healthy host and the driest experimental sites (the unidentified EcM fungus Lactarius).

### **Perspectives**

Félix Fracchia started the second part of his post-doc at WSL and started to analyse the whole genome sequencing data from beech trees.

After sequencing he will also work on the genome data from more than 250 *C. geophilum* strains using the new reference genome (annotated by E Morin (INRAE) using the Joint Genome Institute analysis pipeline).

### **Valorization**

The project has been presented within the INRAE and WSL teams as well as during the annual meeting of the Nancy-Freiburg-Zurich forest research network in September 2022 (<https://www.nfz-forestnet.eu/>). B Dauphin presented MAGIC via a poster on the ECFG16 conference in Innsbruck/Austria in March 2023 (<https://www.ecfg16.org/>). Félix Fracchia will present MAGIC this year at the ICOM12 in Manchester.

A manuscript Fracchia et al. "Prevalence of drought and soil parameters in structuring fungal communities of healthy and suffering juvenile beech trees" is in preparation.

### **Leveraging effect of the project**

The generation of a new chromosome-level *C. geophilum* reference genome assembly within MAGIC has already resulted in a following-up project with the Joint Genome Institute consisting in the sequencing of additional 25 more *C. geophilum* strains. DNA and RNA samples were prepared and genome sequencing is on-going at the JGI, including for *Pseudocenococcum*.