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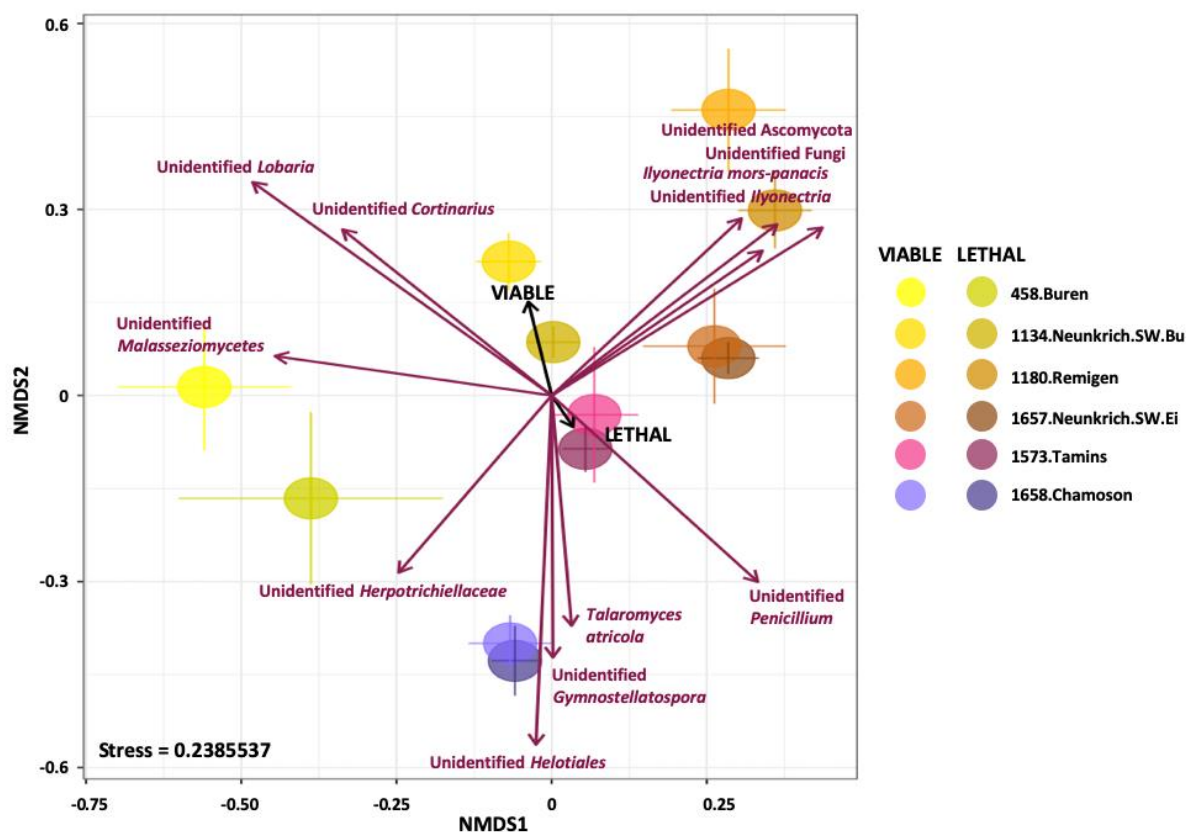
The rescue of beech by well-adapted mycorrhizal fungal partners – Do symbiotic interactions confer adaptive capacity to drought conditions?

Figure 1. Structure of fungal communities associated with vital and damaged beech root systems from distinct sites. NMDS representation of fungal communities structured by sites and tree health (PERMANOVA, p.adj < 0.01). Vectors indicate significant variables after 1000 permutations

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Huayong Wang, PhD student INRAE UMR "Tree-Microbe Interactions"
Félix Fracchia, INRAE/WSL Post-doc hired for the project (12 months INRAE/6 months WSL)

Thematic action 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 will evaluate the fitness of the *C. geophilum* strains from WP1 in agar plates using polyethylene glycol to impose drought stress. We will then 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. Sites and health status of the host tree significantly structured fungal communities. Species richness and diversity were not influenced by the study sites, neither by the host health status. Fungal communities were dominated by the ECM genera belonging to Cortinarius, Tomentella and Hygrophorus. Fungal composition was significantly influenced by the study site but not by tree health. ECM fungi were the most abundant trophic guild detected in beech roots and were significantly associated with healthy trees while endophytes dominated in the root systems of damaged trees. Interestingly, saprotrophs, pathogens and in a lesser extend endophytes relative abundance were correlated with environmental variables such as soil water potential, as well as air and soil temperature, but we did not detect any correlation regarding ECM fungi.

- WP2: Fieldwork for this part was completed last summer 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 started and genome sequencing is planned for this spring. In parallel, we have generated a new chromosome-level *C. geophilum* reference genome assembly for downstream analyses.

- WP3: Preliminary drought tolerance tests of *C. geophilum* strains using polyethylene glycol to impose drought stress were conducted. Several protocols were tested to produce *in vitro* beech plantlets.

Main conclusions including key points of discussion
WP1:
-Fungal community were structured according to the study site and the health of the host tree.
-EcM fungi dominated the fungal communities and were associated with vital beech while endophytes were correlated with damaged trees.
-Saprotrophs, pathogens and in a lesser extend endophytes were correlated with environmental variables (e.g. soil water potential, air and soil temperature…)

Perspectives
A post-doc (Félix Fracchia) was recruited and will work on the project for 18 months (12 at INRAE and 6 at WSL). He participated beginning of February 2023 to the annual Evoltree winter school organised at WSL on the topic of Genomics of Environmental Adaptation and will start now to analyse the whole genome sequencing data from beech trees and the DNA barcoding data of ECM communities and *C. geophilum* present at their root tips generated in WP1. After sequencing he will also work on the genome data from more then 250 *C. geophilum* strains using the new reference genome (that will be annotated in spring 2023 by E Morin (INRAE) using the Joint Genome Institute analysis pipeline). In parallel the drought tolerance screening will be conducted (WP3).

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 will present MAGIC via a poster on the up-coming ECFG16 conference in Innsbruck/Austria in March 2023 (https://www.ecfg16.org/).

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 20 more *C. geophilum* strains.