Project Database of ICP Forests
PROJECT DESCRIPTION

PROJECT INFORMATION

Project title: Differences in mycorrhizal types in determining soil properties and processes and microbial diversity in European forests

Project ID: 115

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PROJECT DESCRIPTION

1. State-of-the-art and objectives

1.1. Rationale

Fungi, protists and prokaryotes play integral roles in terrestrial ecosystems, acting as nutrient cyclers and decomposers of organic material as well as parasites/pathogens or mutualists. Several groups of free-living prokaryotes drive soil nitrogen cycling by governing nitrification and ammonification processes, whereas other groups release inorganic nitrogen into the atmosphere via denitrification. Rhizosphere bacteria, archaea and amoebae consume root exudates, synthesize vitamins and plant growth regulators and promote establishment and functioning of mycorrhiza

Nearly 90% of terrestrial plant species establish root symbiosis with mycorrhizal fungi that provide mineral nutrients, particularly phosphorus (P) and nitrogen (N) to their hosts. There are four basic types of mycorrhizae, viz. arbuscular mycorrhiza (AM), ectomycorrhizal (EcM), ericoid mycorrhiza (ErM) and orchid mycorrhiza (OrM). The latter type represents non-mutualistic association, in which orchid species exploit either EcM or saprotrophic fungi for nutrition, with little if any carbon supply to mycobionts; therefore this type is not considered further in this project.

Mycorrhizal types differ greatly in their plant associations, degradation potential, ecophysiology and ecosystem services belowground. AM is the most ancient type of mycorrhizal symbiosis, which evolved in Glomeromycota >400 million years ago, long before higher fungi developed powerful degradative enzymes; hence AM fungi have very limited capability of organic material degradation. ErM and EcM fungi evolved mostly from

saprotrophic ancestors within more highly developed Ascomycota and Basidiomycota, and retained much of their enzymatic capacity to release macro- and micronutrients from complex organic compounds. These symbionts take up and deliver simple amino acids and oligopeptides to plants, which substantially “closes” the soil nutrient cycling by limiting bacterial pathways and reducing the importance of mineralisation loop. Due to high plant carbon allocation to EcM fungi and use of similar substrates, EcM fungi and saprotrophic fungi compete for soil organic sources, which may result in hampered degradation activity termed as a Gadgil effect. Equipped with a highly complex weaponry of peroxidases, the ErM symbionts are the most efficient mycorrhizal symbionts in accessing nutrients bound in organic complexes. Ericoid plants possess low-quality litter that is decomposed relatively slowly compared with other plant groups. Because Ericaceae are usually limited to understorey and they dominate only sporadically in Mediterranean and arctic tundra ecosystems, very little is known about their biology and competition with EcM fungi and plants.

Differences in nutrient cycling between AM- and EcM-dominated vegetation are relatively better studied, because of the global dominance of these groups. Furthermore, in several excellent plant ecological experiments, field studies and metastudies, differences in decomposition and nutrient cycling among tree species have been demonstrated, without accounting for mycorrhizal status of the trees due to limited knowledge or *a priori* consideration of its lack of importance, although differences among species representing various mycorrhizal types strike the eye in illustrations. Recent case studies and metastudies have shown that EcM trees and habitats tend to have relatively greater litter and humus C/N ratio, protease and phosphatase activities, mineral Ca weathering, soil fungal biomass, but lower litter quality, cellulase, nitrification and N mineralisation activities, leaching and bacterial biomass. Unfortunately, a detailed look into a majority of these studies reveals substantial biases related to poor replication (few taxa compared), sampling effect (selection of taxa), and/or unaccounted confounding effects of soil pH, C/N ratio and plant life form (angiosperm vs. gymnosperm; deciduous vs. evergreen) that all have strong impacts in more inclusive studies and metaanalyses. While most of the above described mycorrhizal type effects *per se* have been documented at moderate reliability in North America, information is completely missing about direct and indirect effects of mycorrhizal type on soil chemistry, nutrient cycling, multifunctionality, diversity and composition of soil microbes on a continental scale and in Europe.

1.2. Objectives

The principal aim of this project is to determine the direct and indirect effects of mycorrhizal types on soil biodiversity and function from local to European scale. This main idea is divided into three logical and highly interrelated objectives ('work packages' in other terms) as follows:

1. To determine the effect of predominant mycorrhizal type on soil chemistry.
2. To determine the effect of predominant mycorrhizal type on soil processes (respiration, nitrification, denitrification, etc.).
3. To determine the effect of predominant mycorrhizal type on microbial composition (molecular analysis of tiny soil samples; if allowed)
4. To determine whether the mycorrhizal effects are linear (proportional to basal area) or non-linear.

2. Methodology

2.1. Ongoing supporting studies

Before launching this project, I have collected data and maps about the definition of EcM groups and distribution of mycorrhizal fully compiled and submitted these for publication (book in Ecological Studies series). These will form a basis for reliable assigning mycorrhizal types in ICP plots based on vegetation data.

2.2. Metastudy of ICP forest plot data

The main purpose of this project is to use the existing data about vegetation and soil to address differences in soil chemistry and function in relation to mycorrhizal status of plants in the plots. I will assign mycorrhizal types to plant species and calculate the proportion of each mycorrhizal types (by species and by basal area of trees). This continuous variable will be the main predictor in the analyses. To account for multiple potentially confounding variables such as N deposition, tree health, forest age, etc. properly, I need to include these variables as well as proportions of individual species as covariates. This will enable me to disentangle the direct and indirect mycorrhizal type effects from other important predictors. To conclude about the effects about mycorrhizal type on a European scale, we will use spatial linear analysis and Structural Equation Modelling (SEM). To determine and account for any spatial autocorrelation among samples, we calculate spatial PCNM eigenvectors and include these in model selection as covariates. To infer causal relationships and go beyond correlative analyses, we use Structural Equation Modelling (SEM) and Dynamic Bayesian Networks (DBN) for both univariate and multivariate systems. For the latter, we extract Principal

Coordinates to reduce dimensionality. DBN enables to detect directional associations among soil and climatic properties and abundance of taxonomic groups.

2.3. Metabarcoding for soil microbial diversity

If additional sampling is accepted in ICP forest plots, we intend to collect 40 tiny soil samples (10 mm diam. to 50 mm depth) from each 300 pre-selected plots using an electric drill. Homogenised samples of soil and litter are frozen in liquid nitrogen upon collection and preserved deep frozen. To identify prokaryotes, we use Illumina high-throughput (HTS) amplicon sequencing of 16S rDNA fragment using primers 515F and 926R as recommended by the Soil Microbiome consortium. Similarly, eukaryotes are identified using a PCR-based method covering the V9 subregion of 18S rDNA and full-length ITS region. The PCR reactions will be performed in four technical replicates that are pooled thereafter (for method development, these are kept separate to estimate stochastic variability). I use negative (for DNA extraction and PCR) and positive controls and mock communities to check for contamination and accuracy of quantification. DNA samples are further subjected to normalisation of quantity by use of SequalPrep Normalization Plate Kit (Invitrogen) that reduces the 100-fold abundance variation to three-fold variation. More information about the methods can be found in Tedersoo et al. (2015; MycoKeys 10: 1-43). Prokaryotes, protists and fungi are classified according to SILVA, The Protist Ribosomal Reference database and UNITE database, respectively, and assigned to broad functional groups using databases of traits. Bioinformatics analyses are performed according to Tedersoo et al. (2015; MycoKeys 10: 1-43).

Multivariate analyses (composition of communities and gene families) will be performed using the computer program Permanova+ that allows explicit hypothesis testing, factor crossing and hierarchical design in multidimensional space. To determine taxa and functions that particularly influence the overall patterns, we use Random Forests and indicator analysis.

3. Novelty

This project seeks to determine fundamental differences in soil properties, function and biodiversity among types of mycorrhizal symbioses. So far, specifically targeted studies have addressed the effect mycorrhizal types on soil nutrients, enzyme activities and a few soil processes. Even these few findings need to be interpreted with caution due to substantial sampling effect and unaccounted confounding factors and narrow geographic scope. My

team broadens the scope of mycorrhizal effects on functional differences to all major biomes and ericoid mycorrhiza in addition to AM and EcM.

4. Expected significance

- Our models incorporating data of mycorrhizal types and associated shifts in soil processes enable to interpret soil nutrient cycling more precisely and provide more precise predictions about global change factors on shifts in soil biodiversity and function from landscape scale to European scale.
- This project will greatly improve the fundamental knowledge about the biodiversity and function of soil as determined mycorrhizal types of plants. The project provides huge amounts of high-quality information about the biodiversity, taxonomic and functional composition of all soil microorganisms.

5. Costs and extras

The costs related to the project are to be borne from the national and international research grants of the applicant. I have sufficient funds (ESF grant 1399P, Estonian forestry grant RMK16253) to perform both the metastudy and soil microbial diversity study.

I have also carefully read the terms of conditions of the use of ICP Plots and agree to follow these in my research.