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Indicator species and co-occurrence in communities of arbuscular mycorrhizal fungi at the European scale

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ABSTRACT

Utilizing a European transect of 54 soil samples, comprising of grasslands, arable and forest sites, we analyzed community composition of Arbuscular Mycorrhizal Fungi (AMF, Glomeromycota) using pyrosequencing of the Internal Transcribed Spacer region. We found a significant influence of environmental factors (soil pH and organic carbon or land use) on the community composition, but these factors did not fully explain the overall amount of AMF diversity. Geographical distance of sites also significantly affected community structure, indicating significant dispersal limitations of Glomeromycota at the European scale. Indicator species have been proposed by land use and physicochemical soil parameters. Generalist species were also identified, that were found occurring in a large proportion of the sample sites. By co-occurrence analysis of species pairs we show that, at this spatial scale, closely-related species are more likely to co-occur than distantly-related ones. This suggests that environmental filtering is a more dominant driving force in community assembly than fungal competition.

1. Introduction

Arbuscular mycorrhiza is an extremely widespread mutualistic symbiosis between plants and fungi from the Glomeromycota phylum. This interaction occurs with at least 65% of land plants (Brundrett, 2009), including many crops, and is essential for many important ecosystem functions and processes, including nutrient cycling and plant productivity. Plant diversity was also shown to be influenced by the diversity of their mycosymbionts (van der Heijden et al., 1998).

About 250 species of the putatively asexual Glomeromycota have been described, mostly based on the morphology of their spores. Recent molecular surveys, however, have indicated that the real number of AMF species may be much higher, comprising many uncultivated taxa (Ohsowski et al., 2014). A number of factors have been shown to act as environmental filters, structuring AMF communities, such as land use, fertilization and soil pH (Hazard et al., 2013; Lin et al., 2012; Oehl et al., 2010; Peyret-Guzzon et al., 2016). Host preferences have also been demonstrated to exist to a certain extent in AMF (Pivato et al., 2007), but strict host specificity seems to be rare (Öpik et al., 2009).

Most studies addressing glomeromycotan community structure have been conducted at a relatively small scale, with only a few authors reporting AMF diversity at the regional scale or larger (e.g. Hazard et al., 2013; Öpik et al., 2006; Davison et al., 2015). Therefore, the understanding of the geographical distribution of these fungi remains somewhat limited. Some AMF taxa have been reported to be surprisingly widespread (Davison et al., 2015), however, many cannot as yet be directly linked to a certain set of environmental conditions.

Geographical influence has long been thought to be absent in microorganisms ("everything is everywhere, but the environment selects") (Baas-Becking, 1934); but molecular approaches are rapidly demonstrating that the apparent lack of structure was at least partially due to the lack of diversity markers at an appropriate resolution (Oakley et al., 2010). Nevertheless, Hazard et al. (2013)

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confirmed the Baas-Becking hypothesis for a sampling scheme across Ireland.

Meta-analyses of database sequences of arbuscular mycorrhizal by Kivlin et al. (2011) demonstrate significant effects of geographical distance, soil temperature, moisture, and plant community on AMF community structure. Davison et al. (2015) used a worldwide sampling of roots to address the question of AMF geographical distance. In contrast to earlier findings (Öpik et al., 2010, 2013), these authors found a large proportion of "Virtual Taxa" (VT) across all continents, but geographical distance and environmental factors were also found to affect the community composition. A negative effect of increasing latitude on diversity was detected (Davison et al., 2015). In contrast, the study of Tedersoo et al. (2014) reported that Glomeromycota were found to have the lowest geographical range of all fungi analyzed, but AMF only accounted for a very small fraction of analyzed sequences in this study.

On the regional/national scale, DNA-fingerprinting techniques have been used to study AMF communities across England (van der Gast et al., 2011) and Ireland (Hazard et al., 2013). These techniques evidently offer less information about the taxa detected, and lower resolution than sequencing. However, AMF community composition was shown to be influenced by abiotic variables (pH, rainfall and soil type), but not land use or geographical distance across Ireland. In contrast, van der Gast et al. (2011) showed significant change in AMF communities with distance at the regional (250 km) scale, and differences between organic and conventional management. Jansa et al. (2014) took a novel approach and generated community profiles using specific qPCR of six widespread AMF species from trap culture plants in soils from 154 arable sites across Switzerland. The quantitative variation of these species was significantly influenced by geographical distance, latitude, pH, soil fertility and texture, but not by available P, and not strongly by land use. This study offered interesting insights into the factors determining AMF community structure, but was obviously limited by the six species analyzed.

Whereas continental-scale data are available for ectomycorrhizal fungi (Talbot et al., 2014; Suz et al., 2014), the present study, to our knowledge, is the first one addressing diversity patterns of AMF by pyrosequencing at a continental-scale. Our study used the sampling scheme of the European project EcoFINDERS. In this project, the biodiversity of a large range of different groups of soil microorganisms was analyzed to assess soil functioning, and to define indicators of soil biodiversity across Europe. The objective of our study was to characterize the diversity of glomeromycotan fungi at the European scale 1) to determine environmental factors influencing it, and 2) to identify indicator species as marker for these environmental factors. We hypothesized that some AMF species can be identified as marker of specific land use, soil properties or geographical distance. This knowledge will contribute to a better understanding of soil AMF biodiversity across Europe.

2. Materials and methods

2.1. Field sites and sampling

For the European project EcoFINDERS, 81 soil samples across Europe were sampled between September and November 2012. These samples were chosen for their representativeness of the different land use, climate and soil properties found at the European scale (Stone et al., 2016). The 54 samples for which we obtained a sufficiently high number of sequences and their attributes are summarized in Table S1. These sites mostly cover Western and Central Europe, spanning four climatic zones (atlantic: 22, continental: 19, mediterranean: 4, alpine: 9) and three land use types (arable: 20, grassland: 27, forestry: 7).

Soil was sampled from each site following pre-agreed standard operating procedures (SOPs) within EcoFINDERS, guaranteeing that all sites were sampled in a consistent manner (Stone et al., 2016). Details on the sampling procedure are provided in Stone et al. (2016). Briefly, twelve soil cores of 50 mm diameter and 50 mm depth were taken within a 2 by 2 m area at each site and then pooled to obtain a sample. For each sample, soil was sieved through a 2 mm mesh and stored at −20 °C until DNA extraction. Physico-chemical parameters of the soils were determined as described by Creamer et al. (2016).

2.2. DNA extraction and purification

Genomic DNA was extracted from 1 g of each sample using the ISOm protocol, described in Plassart et al. (2012). DNA extracts were purified in two steps. First, DNA was loaded onto polyvinylpolypyrrolidone (PVPP) minicolumns (BIORAD, Marne-la-Coquette, France) and centrifuged at 1,000*g* for 2 min at 10 °C. Then, the eluate was purified using the Geneclean turbo kit (Q-Biogene, Illkirch, France). Purified DNA was quantified using the Picogreen kit (Invitrogen, Saint Aubin, France) according to the manufacturer's instructions.

2.3. PCR amplification and pyrosequencing

Nested PCRs were performed on all samples, and each DNA extract was amplified in three replicates. A nested PCR approach was selected to both specifically amplify the ITS2 region of AMF and use PCR conditions compatible with 454 sequencing. The primers in the first PCR reaction were specific for AMF (SSUmCf and LSUmBr from Krüger et al., 2009). In the second round, the primer ITS4 was used (White et al., 1990) which is broadly eukaryote-specific, as well as the ITS3m primer which was modified from ITS3 (White et al., 1990), removing some mismatches to optimize coverage of the Glomeromycota. Likewise, ITS3m will also amplify the respective fragment from some other fungal lineages. The first PCR was performed using 0.4U of Phusion High Fidelity DNA polymerase (Thermo Fisher Scientific, Courtaboeuf, France), 1× Phusion HF buffer, 0.5 μM of the primers SSUmCf and LSUmBr (Krüger et al., 2009), 0.2 mM of each dNTPs and 1 μl of genomic DNA, in a final volume of 20 μl. The PCR conditions used were 5 min at 99 °C, 35 cycles of 10 s at 99 °C, 30 s at 63 °C and 1 min at 72 °C, followed by 10 min at 72 °C, using an Eppendorf Mastercycler epgradient S (Vaudaux-Eppendorf, Schönenbuch, Switzerland). Each PCR product was checked on agarose gel, and diluted at 1/50 to be used as template in the nested PCR. The nested PCR was done using 1U of Phusion High Fidelity polymerase, 1× HF buffer, 0.5 μM of the primers ITS3m (GCATCGATGAACAACGYAG) and ITS4 (White et al., 1990) with barcodes, 0.2 μM of each dNTPs and 2 μl of diluted PCR product, in a total volume of 50 μl. PCR conditions were 30 s at 98 °C, 30 cycles of 10 s at 98 °C, 30 s at 64 °C and 20 s at 72 °C, followed by 10 min at 72 °C, in an Eppendorf Mastercycler epgradient S. PCR products of the nested PCR were checked on agarose gel, the three replicates of each sample were pooled and purified using the High Pure PCR Product Purification Kit (Roche Applied Science, Meylan, France) following the manufacturer's instructions. After quantification using Picogreen (Thermo Fisher Scientific), the purified PCR products were equimolarly mixed to prepare the 454 sequencing libraries. The libraries were sent to Beckman Coulter Genomics (Grenoble, France) for sequencing using 454 GS FLX technology. Raw data of 454 pyrosequencing were submitted to Sequence Read Archive under the Bioproject PRJNA313532, and representative sequences of the *Claroideoglomus* sp. and *Glomeraceae* sp. were submitted to Genbank under the accessions KX548891 to KX548898.