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

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A B S T R A C T

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. © 2016 Elsevier Ltd. All rights reserved.

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 (Olsowski 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 (Opik 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; Opik 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)
confirmed the Baas-Becking hypothesis for a sampling scheme across Ireland.

Meta-analyses of database sequences of arbuscular mycorrhizal fungi 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 (Opik 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 Teddyso 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 texture), 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 project, the biodiversity of a large range of different groups of soil sampling scheme of the European project EcoFINDERS. In this 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 sufficient 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. Physicochemical 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 Hassart 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 1,000g for 2 min at 10 °C. Then, the eluate was purified using the Gene clean 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 (GCATCGATGAACAGGYAG) 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, and 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 (Greensbole, 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.
2.4. Sequence and data analysis

The sequences were demultiplexed according to their multiplex identifier (MID) using the sffinfo command of Mothur v.1.30.2 (Schloss et al., 2009), allowing one mismatch per MID. The raw flowgrams were filtered using the trim.flows command to a minimum flowgram length of 360 cycles, and were truncated at 720 cycles. Sequences from forward and reverse primers were sorted according to their primer sequences using the trim.seqs and split.ggroups commands of mothur, allowing two mismatches per sequence, and then sequences from reverse primers were converted into their reverse complements. Sequences were checked using Fungal ITS extractor v.2 (Nilsson et al., 2010), and non-ITS sequences were removed. The resulting sequences were clustered using Uclust (Edgar, 2010) at 97% identity threshold to create Operational Taxonomic Units (OTUs), and singletones were excluded from further analysis.

For taxonomic assignment, firstly a Blast search against UNITE database v5.0 (Abarenkov et al., 2010) was performed, using an e-value > 1 x 10^-5, in order to eliminate non-Glomeromycota sequences. Secondly, the EPA algorithm of RAXML v8.0 (Berger et al., 2011) was used to correct and improve the taxonomic assignment of the OTUs and define “Molecular Taxa” (MTs) of Glomeromycota. This method assigns short reads to edges of a reference phylogenetic tree under the maximum-likelihood model, providing a more accurate identification of short reads than the Blast method. All samples were subsampled to the same sequencing depth (1100 reads) before performing further analysis. The species composition was used to perform rarefaction analysis, and to calculate diversity (Shannon) and richness (Chao1) indices, with EstimateS software v9.0.0 (Colwell R. K., http://viceroy.eeb.uconn.edu/EstimateS/).

Bray-Curtis distances were calculated for the relative abundance of AMF communities using the vegan package (Oksanen et al., 2013) of R software (version 2.15.1, R development core team 2013). Geographic distance was calculated and compared to AMF species composition between each pair of samples. Correlations between Bray-Curtis distances of AMF communities and geographical distance or soil properties were analyzed using R software using a Mantel test and Pearson’s method.

Variance partitioning of AMF community composition attributed to environmental properties was calculated after forward selection of environmental variables using vegan package (Oksanen et al., 2013) of the R software and the function varpart. Indicator species were calculated using the indicspecies package of R software (De Cáceres and Legendre, 2009) and the function multipatt.

To analyze pairwise AMF species association preferences at the European scale, non-random association between species pairs were assessed using the PAIRS program (Ulrich, 2008). Only relationships between species that were present in at least ten samples were kept to avoid rare species bias. Random matrices for generating standardized scores (C-scores) and p-values were obtained using the fixed row and equiprobable column constraints algorithm, and 100 random matrices were computed using the presence/absence data matrix. The resulting comparisons were visualized using Cytoscape 3.2.1 (Shannon et al., 2003).

3. Results

3.1. AMF diversity at the European scale

We obtained at least 1100 sequences for 54 soil samples across Europe. OTUs were defined at a threshold of 97% identity, and in total 3974 OTUs excluding singletones were found in the 54 soil samples, including 79% of Glomeromycota and 21% of other fungi (mainly Basidiomycota, Ascomycota and unknown fungi). Between 23 and 394 OTUs of Glomeromycota were found per sample (UKM1 and GER8, respectively), and the number of reads ranged from 1115 to 26,624 (FRA1 and FRA20, respectively). These OTUs were taxonomically assigned to species-level taxa, which were not always corresponding to known described species, and called molecular taxa (MTs) to avoid confusion. The number of MTs ranges from 4 (forest, UK) to 29 (grassland in France). Overall, we defined 54 MTs at the species level. The Chao1 index for species richness was equal or close to the number of molecular taxa found in the different samples, suggesting that the sequencing effort performed was enough to characterize AMF diversity and species composition in these soils.

At the family level, a large part of the sequences were identified to belong to the Glomeraceae, with an average of 48% of the total number of sequences for all samples. This family was followed by the Diversisporaceae, Claroideoglomeraceae and Acaulosporaceae, with an average number of sequences of 22%, 10% and 9%, respectively, for all samples across Europe. The other AMF families were more sporadically found in the soil samples.

The presence of some species was observed in a large number of soil samples (Fig. S1). Those found in at least 20 of the 54 soil samples can be classified as generalist species, which were found in the Glomeraceae, Claroideoglomeraceae and Diversisporaceae. Interestingly, one of the two most frequently found MTs (Glomeraceae sp. 2) could not even be assigned to a known genus. On the other hand, members of the Acaulosporaceae, Gigasporaceae, Ambisporaceae, Arachaeosporaceae and Paraglomeraceae were observed in less than 20 samples, sometimes locally with a high number of sequences. The family Pacisporaceae was found in very few samples, never exceeding 0.2% of sequences, and therefore can be considered extremely rare.

Shannon’s index for biodiversity was calculated for molecular species in all samples and ranged from 0.3 to 2.7. These values were tested for correlation to soil parameters (pH, organic carbon), climatic zone and land use, but no significant correlation was observed. Fig. S2 shows the sampling points across Europe with their Shannon index.

3.2. Distance decay relationship and influence of environment

We performed an analysis of distance decay relationships to determine whether differences among the AMF communities can be attributed to geographical distance. A Mantel test indeed revealed a significant correlation between these two parameters (P < 0.05, r = 0.16). The relationship between geographical distance and environmental parameters was tested to identify environmental factors filtering AMF communities. A variance partitioning of soil physicochemical properties, land use and climate was performed, but only climate was shown to have a spatial influence, explaining 45% of the spatial variability.

To explain the contribution of environmental factors to the variation of AMF community composition, a variance partitioning analysis of soil physicochemical properties, land use and climate was performed. Our results showed that 6.2% of total variability of AMF species composition could be explained by these abiotic parameters. Out of this 6.2% of total variability, 3.5% could be assigned to soil pH, 2.1% to land use and 1.2% to the amount of total organic carbon in soil, and included fractions of the total variability shared between these parameters (Fig. S3). In contrast, soil texture (clay, silt and sand composition) or total nitrogen were not identified as significant factors in the variance partitioning analysis at the European scale.
3.3. Indicator species and co-occurrence of species pairs

As outlined in the previous paragraph, three main environmental factors were shown to be drivers of AMF community composition. We identified indicator MTs at the species level (De Cáceres and Legendre, 2009) for these environmental parameters, as shown in Table 1. We found eight indicator species for soil pH: four for pH < 5 (A. brasiliensis, A. alpina, Archaeosporaceae sp. and Rhizophagus sp.), three for pH > 5 (S. constrictum and two Funneliformis sp.), and one indicator for pH > 7 (Claroideoglomus sp.).

Table 1

<table>
<thead>
<tr>
<th>Categories</th>
<th>Indicator species</th>
<th>p-value</th>
<th>Component A</th>
<th>Component B</th>
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<tr>
<td>pH</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>&lt;5</td>
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<td>0.99</td>
<td>0.67</td>
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<td>0.01</td>
<td>0.96</td>
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<tr>
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<td>0.0845</td>
<td>0.83</td>
<td>0.67</td>
</tr>
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<td></td>
<td>Rhizophagus sp.</td>
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<td>0.76</td>
<td>0.67</td>
</tr>
<tr>
<td>5&lt;pH&lt;7 and &gt;7</td>
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<td>0.99</td>
<td>0.88</td>
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<tr>
<td></td>
<td>Funneliformis caledonium</td>
<td>0.03</td>
<td>0.99</td>
<td>0.87</td>
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<tr>
<td></td>
<td>Funneliformis mosseae</td>
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<td>0.91</td>
<td>0.81</td>
</tr>
<tr>
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<td>0.93</td>
<td>0.91</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forestry</td>
<td>Diversispora sp. W5257</td>
<td>0.03</td>
<td>0.85</td>
<td>0.43</td>
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<td>Arable and grass</td>
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<td>Funneliformis caledonium</td>
<td>0.005</td>
<td>0.99</td>
<td>0.9</td>
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<tr>
<td></td>
<td>Funneliformis mosseae</td>
<td>0.005</td>
<td>0.99</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
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<tr>
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<td></td>
<td></td>
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<tr>
<td>&gt;15%</td>
<td>Paraglomus sp.</td>
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<td>0.97</td>
<td>0.5</td>
</tr>
<tr>
<td>&lt;2%</td>
<td>Diversispora celata</td>
<td>0.045</td>
<td>0.98</td>
<td>0.33</td>
</tr>
</tbody>
</table>

AMF are an important component of soil biodiversity, but they are usually not well represented in microbial biodiversity surveys. This is due to the fact, that using broad fungal-specific primers to amplify from soil- or root-derived DNA, only a very small proportion of sequences obtained can be assigned to the Glomeromycota (Vandenkooi et al., 2002). In a recent large-scale molecular study of fungal diversity and biogeography (Tedersoo et al., 2014), only 0.2% of sequences belonged to this phylum. Under these conditions, major components of glomeromycotan diversity may be missed due to methodological constraints.

4.1. Biogeography and environmental factors influence AMF community

In spite of some trends, we did not observe significant overall effects of environmental factors on the Shannon diversity index of AMF. It was somewhat surprising that there was no significant effect of land use effect on AMF diversity in the present study. This lack of statistical support could be explained by the large variability between arable and grassland sites that were analyzed in this study, with a broad range of environmental conditions in each land use category effectively blurring the borders between the categories. Even though there was a clear trend for diminishing diversity along the sequence grasslands > arable > forest, our data showed a large variation of AMF richness even within the same land use category (8–29 molecular species for the grasslands, 7–25 for the arable sites, 4–16 for the forest samples). The relatively low diversity of the forest sites is most likely due to the predominance of ectomyccorrhiza in forest trees. This lack of environmental effect on AMF composition and the environmental factors in forest trees. This lack of environmental effect on AMF occurrence at a continental scale. Previous other approaches to study European biogeography of AMF were based on spore morphology (Oehl et al., 2010) and therefore implied the well-known limitations known for these approaches, such as the difficulty to determine morphospecies using the scant morphological characters of glomeromycotan spores, and the fact that not all species produce spores at the same time. The fungal biomass that was the source of the DNA used in our study can be expected to originate from spores and hyphae in the soil, therefore representing at the same time propagules and metabolically active vegetative structures (Hart et al., 2015).

4. Discussion

This is the first in-depth sequencing study of the distribution of AMF species and the environmental factors influencing their distribution.
diversity index is in contrast to previously reported fungal richness correlations with latitude (Tedersoo et al., 2014), as well as beta diversity relationships with moisture and temperature (Pellissier et al., 2014). Due to their specialized lifestyle as obligate biotrophs of plants, it is evident that AMF diversity may not always follow the same trends as overall fungal diversity. Tedersoo et al. (2014) presented an interesting example for this: these authors pointed out, that in contrast to other fungi, AMF and pathogen diversity were not affected by plant richness.

Community structure, however, was influenced by several environmental parameters as indicated by variance partitioning (pH, land use and soil organic carbon), and notably also by geographic distance as shown by the Mantel test. To our knowledge, this is the first time this correlation between AMF community composition and geographic distance has been shown at the continental scale. Based on an intercontinental sampling, Davison et al. (2015) concluded that AMF possess surprising dispersal capabilities, which is unexpected for soil-borne fungi forming relatively large spores, while at the same time stating significant distance effects on AMF communities. These findings are in contrast to the previous study by Tedersoo et al. (2014), who found that the Glomeromycota had the lowest average geographical range among fungi. In the present study, we demonstrate a strong correlation between genetic and geographic distance at the pan-european scale, even with less pronounced natural barriers and a long history of intensive human migration most likely involving transport of soils and crop plants. This finding implies that also at the scale we studied, the dispersal of AMF is limited by distance. It seems remarkable that a long history of humans transporting soil and plants across this continent apparently did not result in completely eliminating the distance effect.

The fact that overall diversity did not change significantly across the geographical range, while community composition did change, may imply that taxa present in one place must be replaced by others elsewhere with regard to their ecosystemic function, suggesting some degree of functional redundancy in the Glomeromycota, which should be tested in further studies.

4.2. Niche preferences

As in most other studies, the Glomeraceae were largely dominant in the sequence abundance. We identified a set of 19 generalist species found in over a third of all sites, half of which could be assigned to known morphospecies in the families Glomeraceae, Claroideoglomeraceae and Diversisporaceae. However, there were also less widespread members of these families. Some generalists may indeed also have profited from human activities, and might for example have been spread by agriculture as suggested for Funneliformis mosseae by Rosendahl (2008).

On the other hand, species from the remaining families Acaulosporaceae, Gigasporaceae, Archaeosporaceae, Ambisporaceae and Paraglomeraceae, were sometimes locally abundant, but not as widespread, suggesting more specialized life history strategies. The family Pacisporaceae was extremely rare, and to our knowledge, for the first time detected in a molecular field study.

We also identified indicator species for different environmental parameters. Some of these species were identified as indicators of both pH and land use conditions, such as some Acaulospora and Funneliformis spp., which is reasonable considering the possible covariation of these environmental factors. For instance, it is well known that forestry soils are predominantly acidic, and thus AMF species which are indicators for very acidic soils (pH < 5), like Acaulospora brasiliensis, are also indicator species for forestry soils. Until now Funneliformis mosseae has been described as a widespread generalist species (Opik et al., 2006), but here we can refine its niche preference to grassland and arable lands, whereas it does not seem to be adapted to forest soils. These indicators (some of which are molecular taxa without species names) might be useful...
to further define habitat requirements for AM fungi and better understand their ecological niches. Indicator AMF species for disturbed and undisturbed habitat types were recently identified by Moora et al. (2014) at field sites in Estonia. Due to the different gene fragment and the dated classification used by these authors, comparisons are difficult, but an interesting common result is the fact that *Funneliformis mosseae* was identified as an indicator for open, non-forest habitats.

The patterns obtained from the analysis of generalist species suggested a phylogenetic base for common life history strategies. This prompted us to further elucidate the processes behind AMF community assembly by analyzing co-occurrence patterns of the MTs. The results indicate very strongly that closely-related AMF tend to co-occur at a higher probability than distantly-related ones. The majority of positive interactions we found were among members of the same genus or family. The most striking examples are the two *Acuulospora* and the two *Archaospora* MTs which had only positive interactions between themselves, and negative ones with others.

These findings are in agreement with the results of Kivlin et al. (2011) obtained by a meta-analysis of sequence datasets, and by Horn et al. (2014) in their study of a semi-arid grassland site. However, they are in contrast to studies by Maherali and Klironomos (2012, 2007) who interpreted the relatively rare co-occurrence of AMF species. These authors concluded that phylogenetically-conserved traits resulting in increased plant yield in communities consisting of more closely related taxa.

It can be expected that closely-related taxa respond in a similar way to environmental factors and are therefore more likely to occur together. This is a scenario which would be compatible with our data, and it is also supported by the finding that different species of the same genus were found as indicators for the same environmental conditions in this study. Two other possible explanations of the co-occurrence patterns brought forward by Horn et al. (2014) are very unlikely to play a role in our system, namely the selection of related taxa by the host plant, or interactions with the soil biotic community, because of the large variety of plants and belowground biotic communities in this study, with soils from very distant places and different conditions across Europe.

5. Conclusions

Due to their role in ecosystem functioning, AMF community composition and distribution have received considerable interest, but have rarely been characterized at a large geographical scale. In our study we found evidence for dispersal limitation of the Glomomyctota at the European scale. We also identified environmental factors influencing AMF community composition and found species indicators for these conditions, allowing us to define better their habitat preferences. Finally, we demonstrate that, at this scale, closely-related taxa tend to co-occur significantly more than distantly-related ones, possibly because of environmental filtering on AMF taxa. This study has therefore enabled a deeper insight into biogeography and niche preferences of Glomomyctota and will contribute significantly to a better understanding of AMF ecology.

Conflict of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2016.09.022.

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