Diversity of Arbuscular Mycorrhizal Fungi in Grasslands and Arable Fields

Ecological factors related to community composition and dynamics

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Abstract

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This thesis comprises four studies on the identification and ecology of arbuscular mycorrhizal fungi (AMF) in grasslands and in arable soils. Fungi belonging to the phylum Glomeromycota are obligate symbionts in the roots of land plants where they form arbuscular mycorrhiza. The symbiotic association increases the ability of the host plant to take up nutrients from the soil via the extensive mycelia of the fungal symbionts, which have a larger and better distributed surface area for uptake than the roots alone. In turn, the fungi obtain carbohydrates that the plants produce.

Arbuscular mycorrhiza are almost ubiquitous in grasslands, and therefore assumed to play a key role in ecosystem functioning. AMF taxa in the plant roots were identified with the use of different molecular techniques. Their phylogenetic affiliations were investigated and the significance of some relevant ecological factors shaping the AMF community structure was evaluated. In a seminatural grassland, we observed that AMF richness decreased in relation to increasing levels of nitrogen in the soil. The temporal patterns and species composition of the fungal communities colonizing the roots of two co-existing plant species were also shown to be different. We also found that AMF communities colonizing a single plant species varied between different localities within the same region.

We used a long-term field experiment to show how different management practices affected the microbial soil biota. We compared the application of different organic and inorganic fertilisers, and their effect upon the AM fungi colonizing maize roots and the bacteria in root-associated soil aggregates. Some amendments induced dramatic changes in the richness and composition of the bacterial and the fungal communities.

In conclusion, our experiments revealed evidence of complex ecological patterns in this cryptic but important group of fungi. Human management practices in grasslands and arable fields can shape the communities of both AMF and bacteria in the soil. Improved knowledge of the spatial and temporal niches of different taxa is a prerequisite in order to design manipulative experiments that can establish the link between taxonomical and functional diversity.

Keywords: arbuscular mycorrhiza, Glomeromycota, rRNA gene, microbial ecology, grasslands, arable fields.

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Appendix

Papers I-IV

This thesis is based on the following papers, which are hereafter referred to by their Roman numerals:

I. **Santos JC**, Finlay RD, Tehler, A (2006). Molecular analysis of arbuscular mycorrhizal fungi colonising a semi-natural grassland along a fertilisation gradient. *New Phytologist* 172: 159–168.

II. **Santos-González JC**, Finlay RD, Tehler, A (2007). Seasonal dynamics of arbuscular mycorrhizal fungal communities in roots in a seminatural grassland. *Applied and Environmental Microbiology* 73: 5613-5623.

III. **Santos-González JC**, Tehler, A, Finlay RD. Regional variation in the arbuscular mycorrhizal fungal communities colonising the roots of *Prunella vulgaris*. (Manuscript).

IV. Toljander JF, **Santos-González JC**, Tehler A, Finlay, RD. Community composition of arbuscular mycorrhizal fungi and bacteria in the maize mycorrhizosphere in a long-term fertilisation trial. (Manuscript).

Introduction

The history of life is also the history of symbiosis. Symbiosis is an intimate association between two different organisms through all or part of their life cycle. During the history of biology, symbiosis has mainly been considered as a curiosity (Sapp, 2004), but in fact it is more the rule than an exception. The continued development of ecology, powered by advances in molecular identification methods has revealed the widespread occurrence and identity of symbiotic relationships with microbes in many groups of organisms (Margulis & Fester, 1991). The plant kingdom has evolved and diverged after establishing a mutualistic symbiosis with cyanobacteria-like prokaryotes (plastidia) inside their cells (Delwiche, Kuhsel & Palmer, 1995; Schimper, 1883). After this historical event that gave origin to the chloroplasts, arbuscular mycorrhiza is probably the most widespread symbiosis in plants. Other well known symbioses in the roots of plants, such as the ones with bacteria in nitroxen-fixing root nodules (Kistner & Parniske, 2002; Sprent & James, 2007) and other types of mycorrhiza, developed much later (Alexander, 2006), when plants were well established in terrestrial ecosystems. The establishment of this symbiosis probably was a key event that allowed some primitive plants to colonise the harsh land environment for the first time. This led to a fabulous process of evolutional radiation that shaped the earth landscape in the whole planet. Arbuscular mycorrhiza is, without doubt, one the most fundamental keys to understanding many processes in plant biology and ecology.

Arbuscular mycorrhiza

Arbuscular mycorrhiza are mutualistic symbioses between plant roots and fungi belonging to the phylum Glomeromycota. The plant provides the fungal partner with carbon and the fungus improves the plant nutrient uptake from the soil.

These fungi have long been considered obligate symbionts with plants, since growing the AM fungus without a host plant has not been possible. Isolated spores can germinate and produce hyphae, but they die if no host root is found. This view has recently been challenged by an experimental study showing that AMF can grow and form spores *in vitro*, if provided with a carbon source and stimulated by particular bacterial strains (Hildebrandt *et al.*, 2006). Whether this can also occur in nature is not yet known.

Spores are asexual, multinucleate and are produced directly by the mycelium, either inside or outside the root. In some species, small sporocarps can be produced, where several spores are surrounded by a peridium-like structure. Typical glomeromycotan spores are globose, relatively big (40-800 μm) and with a multilayered wall that can be smooth or ornamented. Evidence of sexual reproduction has not been reported so far in the Glomeromycota.

The hyphae lack septa (cross walls between hyphal cells) and can grow both outside (extraradical) and inside the roots (intraradical). This coenocytic structure allows the nuclei to move along the hyphae (Bago *et al.*, 1999). The intraradical mycelium typically produces highly branched structures called arbuscules, inside the cortical cells of roots (*Arum*-mycorrhizal type) (Fig. 1). In some other cases, hyphal coils are formed instead (*Paris*-mycorrhizal type). Between this two morphologically types, where many intermediate types can be observed, there is no a clear-cut (Dickson, 2004). The variability of structures along this *Arum–Paris* continuum have ecological, functional and taxonomic significance that are not fully understood yet (Dickson, Smith & Smith, 2007). Many species of Glomeromycota also form large intraradical, globose, storage cells called vesicles. Because of this, glomeromycotan fungi are sometimes also referred to as vesicular-arbuscular mycorrhizal fungi (VAM).

Glomeromycotan fungi do not disseminate solely by spores. New plant hosts can be colonised from hyphal fragments present in the soil or growing from colonised root fragments. Alternatively, roots can be colonised by extraradical mycelium extending from a previously established mycorrhiza. The latter may give rise to an extensive mycelial network connecting the root systems of several plants from the same or different species. Nonetheless, the relative importance of the two dispersal methods and the establishment of hyphal networks in nature have not been evaluated. There are some widespread and frequent AM species detected in molecular studies, without known formation of spores. This suggests that there might be species that rarely or never sporulate.

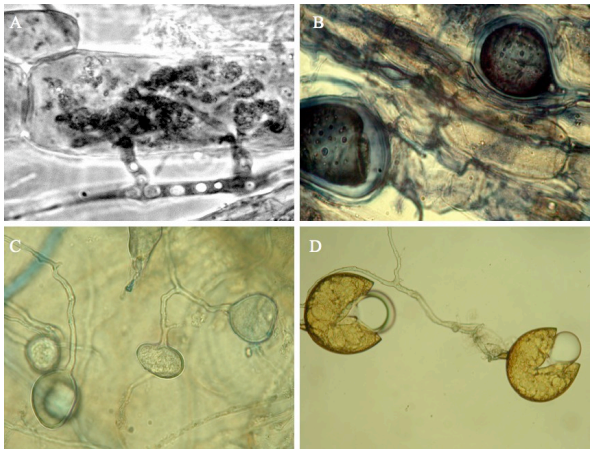

Fig. 1. Different structures formed by arbuscular mycorrhizal fungi. A. Arbuscules inside a cortical cell in roots of *Prunella vulgaris*. B. Vesicles inside cortical cells in roots of *P. vulgaris*. C. Terminal mycelial hyphae forming spores in a culture of *Glomus* sp. D. Crushed spores of *Glomus* sp still attached to the subtending hyphae.

Classification and phylogenetics

The phylum Glomeromycota was recently established by Schüßler, Schwarzott & Walker (2001) as a monophyletic group, distinct from the Zygomycota in which they were previously placed. Phylogenetic studies based on molecular data place

the Glomeromycota as a sister group to the Basidio- and Ascomycota (James *et al.*, 2006; Lutzoni *et al.*, 2004; Tehler, Little & Farris, 2003).

Traditional taxonomy in the Glomeromycota has mainly been based in spore morphology and ontogeny. The structures and characters of the mycelia, *e.g.* arbuscules, vesicles, coils, are of exiguous taxonomical value. Fewer than 200 species, grouped in eleven genera, are described. Most of them have been described after being isolated and grown in pot cultures using a handful of host plant species. This means that most of the described taxa are probable generalists with low host plant specificity. Actually, the application of molecular techniques to identify of AM fungi in field studies has uncovered a large cryptic diversity of new glomeromycotan lineages that are not taxonomically characterised.

The most complete phylogenies in the Glomeromycota are based on the 18S rRNA gene (Schüßler *et al.*, 2001) (Fig. 2). Other sequenced genes have not been systematically sampled through a wide range of taxa (Corradi *et al.*, 2004; Helgason, Watson & Young, 2003; Raab, Brennwald & Redecker, 2005). *Glomus* is the most species-rich genus and under a long time it has remained polyphyletic. Three major clades, representing putative families (*Glomus* group A, B and C), were originally distinguished in Schwarzott, Walker & Schüßler (2001). One of them (*Glomus* group C) has been renamed as the family Diversisporaceae (Walker & Schüßler, 2004), which is monophyletic with Gigasporaceae and Acaulosporaceae, but not all the species included in this family have been renamed and transferred from *Glomus* to *Diversispora*. The other two main *Glomus* clades (*Glomus* group A and B) appear as monophyletic in rRNA gene based phylogenies but they have not been renamed as new families, since this distinction is only based on one single gene and lacks morphological support. Phylogenetical hypothesis about the intraphylum relationships, based in a multi gene approach, have not been formulated yet (Redecker & Raab, 2006).

Evolution and ecology

The evolution of fungal symbiosis probably has an ancient origin. It might date back to a common ancestor that evolved a strategy with factors for recognising autotrophic organisms and structures for infecting them (Tehler *et al.*, 2000). Such an event would make a synapomorphic feature for a group of chitinous fungi, including all Dikaryomycota and the Glomeromycota, informally referred as 'Symbiomycota' by Tehler *et al.* (2003). Palaeontogical evidence supports the hypothesis that fungi developed symbiosis with photoautotrophs long before the evolution of land plants (Yuan, Xiao & Taylor, 2005).

The fossil record shows rhizomes from the Devonian (approximately 400 Mya ago) that appear to contain arbuscules similar to the ones that can be observed in extant mycorrhiza (Remy *et al.*, 1994); and glomeromycotan-like hyphae and spores are reported from the Ordovician (460 Mya ago) (Redecker, Kodner & Graham, 2000). Molecular data suggest a much older origin for the Glomeromycota, going back to 600 Mya ago (Berbee & Taylor, 2000). This

suggests that mycorrhiza-like symbioses between early 'Symbiomycota' representatives and autotrophs evolved and were established prior to the colonisation of land by plants, and not as a result of the colonisation process (Tehler, Little & Farris, 2003).

It is now believed, that this old group of fungi has been instrumental in the process of land colonisation by plants (Pirozynski & Malloch, 1975; Schüßler, 2002; Simon *et al.*, 1993). Glomeromycota can have played a key role in the ability of the first land plants to acquire water, since ancestral plants lacked roots. Another hypothesis suggests that an increased access to limiting resources, such as phosphorus, may have been the most important benefit for plants that are supposed to have evolved in damp habitats (Helgason & Fitter, 2005). Today, the majority of land plant families form arbuscular mycorrhiza. Apart from vascular plants, two of the most primitive extant groups of land plants, hornworts (Anthocerotae) and liverworts (Hepaticae), frequently form symbioses with glomeromycotan fungi in their rhizoids. The functional significance of this 'mycorrhiza'-like association is not yet fully understood (Selosse, 2005).

The main benefit for vascular plants having arbuscular mycorrhiza has traditionally been considered to be increased access to soil nutrients, in particular phosphorus. Phosphates can be taken up directly by the roots but this creates a depletion zone around the root, which is difficult to remove due to low rates of diffusion. Phosphates are highly immobile ions because they tend to build insoluble complexes with most soil cations. Hyphae are thinner than roots and therefore 'cheaper' to produce and more effective at exploiting microsites which are physically inaccessible to larger roots. Apart from phosphorus, AMF have shown to be potentially able to take up both organic (Hodge, Campbell & Fitter, 2001) and inorganic nitrogen from the soil (Govindarajulu *et al.*, 2005).

Some studies also suggest that this ancient symbiosis may also have other important functions. Experimental studies have shown that reduction of pathogen infections (Borowicz, 2001; Newsham, Fitter & Watkinson, 1995), improvement of water relations (Auge, 2001; Porcel *et al.*, 2006) and limiting the uptake of heavy metals (Leyval, Turnau & Haselwandter, 1997) can be potential benefits for mycorrhizal plants.

Apart from the obvious interaction with their host plants, AM fungi clearly interact with other soil macro- and microorganisms. One of the most important interactions that takes place in the mycorhizosphere is between bacteria and the extraradical mycelia of AM fungi (reviewed by Johansson, Paul & Finlay (2004)). Several glomeromycotan fungi have been shown to harbour obligate endosymbiotic bacteria inside their cells (Bianciotto *et al.*, 1996). The most conspicuous of these interactions is the one formed by cyanobacteria living inside hyphal 'bladders' formed by *Geosiphon pyriformis*, an ancestral taxon within the Glomeromycota (Schüßler *et al.*, 1994).

The presence of AM fungi in the roots is not only important for single plant individuals or populations. The establishment of common mycorrhizal networks,

in which the root systems of several plants can be connected through extensive mycelia can also play an important role in the functioning of the whole plant community and ecosystem. Both, plant productivity and diversity, have been shown to be influenced by fungal diversity, and vice versa (van der Heijden *et al.*, 1998; Johnson *et al.*, 2004) .

Experimental aims

The main aim of the experiments described in this thesis was to elucidate some fundamental ecological factors that can influence the structure of field AMF communities in the roots. So far, relatively few studies have examined the identity of the intraradical AMF community in roots, and no such study has ever been carried out in terrestrial ecosystems in Sweden.

The specific objectives can be summarised as follows:

- To use and evaluate different molecular methods to identify the taxa of AMF colonising plant roots and to investigate their phylogentic affiliations within the Glomeromycota.
- To characterise the AMF communities colonising the roots of different plant species, in different Swedish grasslands and agricultural soils, at local and regional levels.
- To determine whether there are spatial and temporal gradients that can be related to the structure of the intraradical AM fungal community.
- To assess how anthropogenic disturbance and different land management practices affect the structure of the AMF communities.

Materials and Methods

This section presents a general and comparative description of the methods used in this thesis. See the Material and Methods sections in the respective papers for a more detailed description.

Study sites

The studies in this thesis were mainly conducted in seminatural grasslands in the south- eastern part of Sweden. The locality studied in papers I and II is Hönsgärde, a seminatural grassland situated in the county of Uppland. This site has still a rich and representative flora typical from a well-managed pasture. The site was selected because it represents a good example of how fertilisation in certain parts of the pasture has changed the structure of the plant community. This has created an evident gradient of soil nutrients that was suitable to analyse the presumptive effect of fertilisation on the AMF community. In paper I the AMF community colonising the root of two forbs, *Festuca pratensis* and *Achillea millefolium,* was analysed along the fertilisation gradient. In paper II, the main objective was to characterise temporal changes in the intraradical AMF community throughout the growing season in two different plants, *Prunella vulgaris* and *Antennaria dioica*, in the non-fertilised parts of the pasture.

Paper III analysed the AMF community colonising *Prunella vulgaris* in seven grasslands located throughout the counties of Södermanland and Uppland. These sites represented semi-natural grasslands with similar characteristics to Hönsgärde, except the Hästhagen site, which had recently been an arable field.

Paper IV focuses on arable soils and presents an analysis of the bacterial and AMF communities in the maize rhizosphere in a long-term field experiment placed at Ultuna in the county of Uppland. This experiment was set up more than 50 years ago to investigate the possible effects of different management regimes on crop production. Six of the available treatments were studied in paper IV (Fig. 4).

Soil and root sampling

Roots of the targeted plant species were obtained by taking soil cores (approximately 10-cm-deep and 7 by 7 cm) from the upper layer of the soil profile containing the identifiable vegetative part of the plants (in papers I, II, and III). Soil cores were transported to the lab and frozen, if samples were not going to be processed immediately after collection.

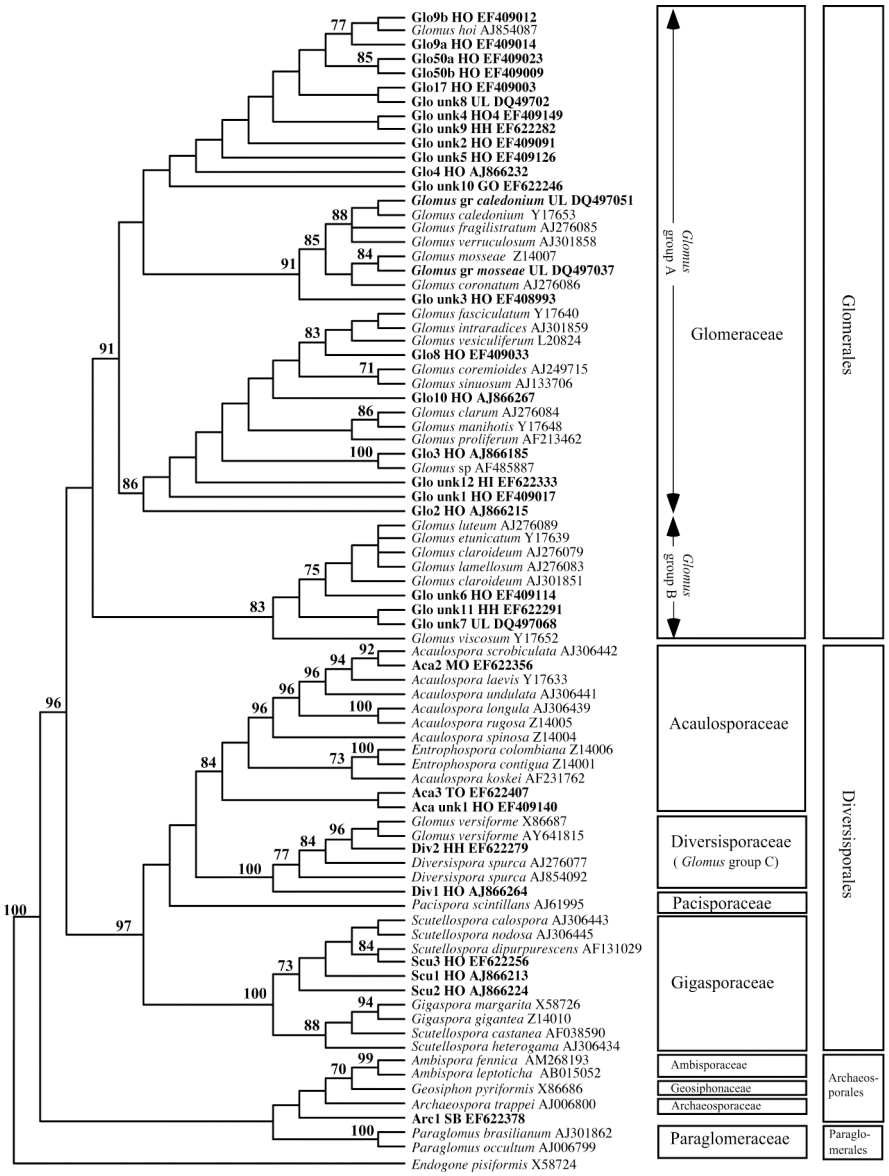

Fig. 2. Phylogenetic tree including taxa in Glomeromycota and sequence groups observed in this thesis (in bold). The cladogram is a 50% majority-rule consensus tree of 3466 equally parsimonious trees, 1523 steps long, obtained in a MP analysis, upon a 1858 bp long alignment of the 18S rRNA gene. A zygomycete (*Endogone pisiformis*) is used as outgroup. MP bootstrap values ≥70% are indicated above branches (1000 bootstrap replicates). They are named after the sequence group they belong to (see respective papers), and their origin site as follows : GO, Gorrhagen; HH, Hästhagen; HI, Himmelsboda; HO, Hönsgärde; MO, Mörby; SB, Stora Benhamra; TO, Torslunda; UL, Ultuna. The MP analysis was performed in PAUP ver. 4.0b10 with the following parameters: `hsearch start=stepwise addseq=random nreps=500 randomize=addseq swap=tbr multrees=yes nchuck=20 chuckscore=1;` and the bootstrap analysis with: `bootstrap nreps=1000/ nreps=10 randomize=addseq swap=tbr multrees=yes nchuck=10 chuckscore=1;`

Soil cores studied in paper IV were collected in bare soil close to the maize plants and were of a smaller size, in order to minimise the disturbance of our sampling in the plots. Maize roots were numerous and easily identifiable, since weeds were almost not existent. Selected roots were thoroughly rinsed under tap water and cut into 1-cm pieces. Only fine, healthy looking-like roots were used for DNA extractions.

Soil analyses in papers I and III were carried out at the Soil Fertility and Plant Nutrition Department, SLU in Uppsala.

Characterisation of the fungal and bacterial communities by molecular methods

Investigations of the diversity of natural AMF communities have traditionally been based on spore identification and quantification. Spore characterisation to species level, and even to the genus levels, is not always possible because of lack of discriminating morphological characters (Redecker & Raab, 2006). Sporulation is a seasonal phenomenon that can be highly related to the physiological status of the fungus, and to environmental factors. Moreover, spore production has different dynamics in different taxa, and some species may sporulate sparsely or not produce spores at all (Sanders, 2004). In many molecular studies, taxa without any known formation of spores have been shown to be dominant in the intraradical AMF community (Helgason, Fitter & Young, 1999; Santos-González, Finlay & Tehler, 2007; Vandenkoornhuyse *et al.*, 2002). This discrepancy is also present in some ectomycorrhizal communities (Dahlberg, Jonsson & Nylund, 1997; Kåren *et al.*, 1997).

The use of molecular tools to characterise the AM fungi colonising root systems of different plant species is not entirely free of problems, but currently provides new opportunities to improve our understanding of the roles these fungi play in plant ecology.

A set of different molecular techniques was used to characterise the different microbial communities in the four studies presented in this thesis. A portion of the rRNA gene was selectively amplified through PCR from environmental complex mixtures of DNA.

In order to obtain DNA templates, root samples were homogenised and disrupted by mechanical means after thawing them. The same DNA extraction kit was used in all the experiments.

A 550 bp long fragment of the 18S rRNA gene was used for characterisation of the AMF community. This fragment comprises the variable V4 region (Nelles *et al.*, 1984), which is the most complex region of this gene (Nickrent & Sargent, 1991). This gene is used in the most comprehensive phylogenetic analyses of Glomeromycota (Schüßler, Schwarzott & Walker, 2001). Moreover, it is a well

sampled gene among taxonomically characterised isolates and natural AMF communities.

In all papers we used the same basic set of primers, with some modifications. In paper I we used the universal eukaryote primer, NS31 (Simon, Lalonde & Bruns, 1992), and AM1 (Helgason *et al.*, 1998), that was originally designed as a glomeromycotan specific primer. In paper I the primer NS31 was modified with a GC clamp (Kowalchuk, Souza & van Veen, 2002) to stabilise the melting behaviour of the DNA fragment during the Denaturing Gradient Gel Electrophoresis (DGGE) analysis. The primer AM1, apart from not targeting the divergent orders Archaeosporales and Paraglomerales, presents mismatches with at least a number of taxa belonging to *Glomus* Group B and Diversisporaceae (*Glomus* Group C). In order to detect these two last groups, we included two modifications (AM2 and AM3) of the primer AM1, plus the primer NS31, in the PCR mixture (papers II, III and IV).

There are several approaches to obtain profiles of complex microbial communities. In this thesis we used three of the most popular molecular methods used in literature (Fig. 3) to screen the diversity of PCR products generated in our molecular analyses.

The fungal communities were characterised by DGGE in paper I and by cloning in the rest. A minimum of eight clones per library was screened trough direct sequencing.

DGGE is a straightforward technique that allows for a rapid screening of major differences among different microbial communities. Dominant sequence groups usually appear as prominent bands, while less common types can be difficult to detect if they appear as faint bands in the acrylamide gels. This may be the explanation of the higher number of AMF sequence groups per root sample observed by us when we compare the cloning with DGGE results. This weakness is a trade-off when assessing the richness of communities composed of a few dominant and common species and a large number of less frequent or rare species.

The bacterial community in paper IV was analysed with PCR and terminal restriction fragment length polymorphism (TRFLP). A 500 bp long fragment of the 16S rRNA gene was amplified using the universal bacterial primers 27f and 534r. The PCR products were digested with two restriction endonucleases, *Cfo*I and *Hae*III, before being separated by capillary electrophoresis. Specific identification of peaks in the TRFLP profiles was performed with a TRF database.

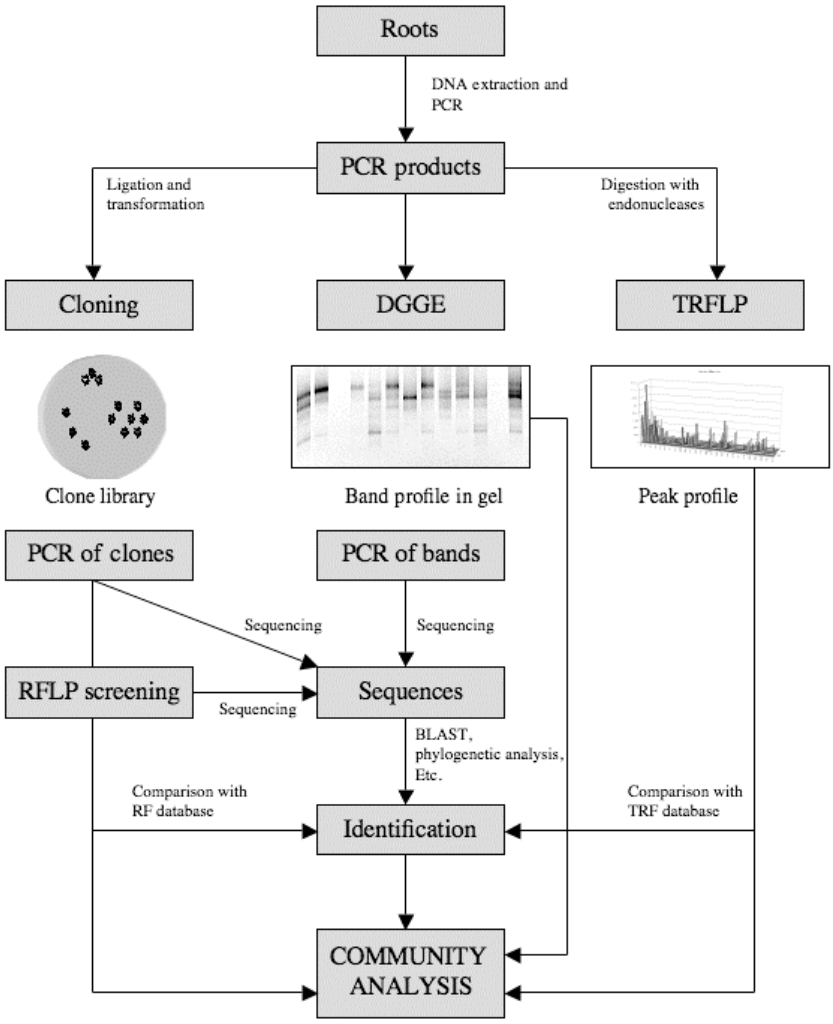

Fig. 3. Flow diagram representing the most common procedures used to characterise microbial communities with DNA based methods.

Identification of AMF sequences

A main problem in AMF ecology is the lack of a molecular definition of species. Glomeromycota are asexual, coenocytic organisms with a peculiar genetic structure. The ribosomal genes in the genome of eukaryotes are usually arranged in tandems of 75-100 repeats. In organisms with sexual reproduction these copies are kept more or less identical through different mechanisms, *e.g.* concerted evolution. The multinucleated spores of Glomeromycota can contain 2000-20000 nuclei (Smith & Read, 1997) and a single spore usually has different variants of the ribosomal gene. How this variation is organised in the nuclei, *i.e.* whether a nucleus has only one gene variant or several, is still a subject of debate among

mycologists (Hijri & Sanders, 2005; Kuhn, Hijri & Sanders, 2001; Pawlowska & Taylor, 2004). Moreover, the degree of variation within spores, isolates and morphospecies is also a much debated issue and still many uncertainties still remain (Clapp, Rodriguez & Dodd, 2001; Rodriguez, Clapp & Dodd, 2004; Schüßler, Schwarzott & Walker, 2003).

This has important implications in AMF ecological studies. Single DNA sequences cannot be unequivocally assigned to single isolates or species. The molecular diversity of the communities is classified in discrete operational taxonomic units (OTU) or sequence groups based on different criteria. Sequence groups (sometimes also referred as ribotypes or phylotypes in the literature) are often defined with a combination of sequence similarity cut-off values and criteria based on phylogenetic analyses. Thus, it is not certain that the sequence groups represent species. Nonetheless, the sequence groups have an operational value when describing and comparing molecular diversity values between different microbial communities.

In this thesis we used Maximium Parsimony (MP) as the phylogenetic approach to analyse our sequence data sets. In paper I we also used Neighbour Joining (NJ). Two software packages were used to carry out these analyses, PAUP* version 4.0b10 for Macintosh (Swofford, 2002) and T.N.T. (version for Windows) (Goloboff, Farris & Nixon, 2003). Maximum Parsimony is a criterion of selection among phylogenetic hypothesis, by minimising the number of evolutionary steps required to explain the data. In most analyses not all the most parsimonious trees were found because of computational constraints.

To represent some of the outcomes of the phylogenetic analyses, we generally chose to illustrate a phylogram of one of the multiple most parsimonious trees found in the respective analysis. One of the reasons for this, is that our main interest was not in the relationships between taxa *per se*, but in the relationships and affiliations of the environmental sequences within their respective sequence groups. A phylogram offers distance information that is important to evaluate the magnitude of sequence divergence between and within sequence groups. The other reason for choosing a phylogram is that is the required standard form of presenting phylogenetic analyses by most microbial ecological journals.

Results and discussion

Identity and phylogenetical affiliation of the detected AMF sequences

In the four papers included in this thesis we defined a total of 33 sequence groups. Because of the high sequence similarity within the defined sequence groups, is very likely that many of these groups represent species. We have tried to adopt a conservative criterion in order to avoid splitting less homogeneneous

sequence groups, in a several minor groupings. The amounts of variability reported in the rRNA gene are largely unknown and disparate among different taxonomical levels and probably also among species (Clapp, Rodriguez & Dodd, 2001; Rodriguez, Clapp & Dodd, 2004; Rodriguez *et al.*, 2005; Schüßler, Schwarzott & Walker, 2003). Although most of the sequence groups we detected could not be related unequivocally to known species, many of them were detected in similar studies using the 18S rRNA gene as a genetic marker (Öpik *et al.*, 2006). Many of these sequence groups probably represent species that are difficult to grow in culture.

As in most studies of similar to ours, *Glomus* group A was the most frequently represented group in the analysed AMF assemblages. *Glomus* group B was not detected in paper I, where we only used the AM1 as the reverse primer. The inclusion of primer AM2 in the primer set enabled identification of sequences clustering in this group in papers II, III and IV. The primer AM3, designed to target the family Diversisporaceae, did not seem to be as effective as the primers cited above. This could be due to lack of specificity, but it might simply reflect that species within the Diversisporaceae are uncommon. A few sequence groups were found in *Acaulospora* and *Scutellospora*. The primer combination used in our studies results in mismatches with species in the basal orders Archaeosporales and Paraglomerales. Nonetheless we were able to detect a single sequence clustering in the Archaeosporales.

The phylogeny obtained in paper III agrees largely with previously published phylogenies using the rRNA gene (Redecker, Morton & Bruns, 2000; Schüßler, Schwarzott & Walker, 2001; Schwarzott, Walker & Schüßler, 2001). In contrast, our earlier analyses (papers I, II and IV) do not support the monophyly of the Glomeraceae. The currently described species in the family Glomeraceae are assigned in rDNA based phylogenies, to two major sister groups: *Glomus* group A and group B (Schwarzott, Walker & Schüßler, 2001). This family is resolved as a monophyletic group in the phylogenies cited above, but not in some of our papers (I, II and IV). The reason for this, is that in the alignments in paper I, II and IV, we trimmed the sequences from characterised isolates (downloaded from GenBank) to fit the length of our own sequences, which had a maximum length of 550 bp. In paper III we used almost the full length (*ca.* 1850 bp) of the small subunit of the ribosomal gene (SSU rDNA) for the characterised isolates. This increased the phylogenetic resolution of our data set and rendered the family Glomeraceae monophyletic, although with low support. It has been argued that *Glomus* group A and group B, could probably be erected as two families, but because lack of discriminating morphological characters they have been kept together (Schwarzott, Walker & Schüßler, 2001).

Effects of fertilisation on AM communities in grasslands

Increasing levels of fertilisation have been shown to be highly correlated to reduced diversity in several groups of organisms in grasslands. In paper I we tested whether the composition of the AMF community in the roots of two perennial

plants (*Achillea millefolium* and *Festuca pratensis*) changes along an artificial fertilisation gradient in a pasture. In this study we tested the power of PCR-DGGE in describing AMF communities in the field. At that point, only one study (Kowalchuk, Souza & van Veen, 2002) had used a DGGE approach to describe AMF communities with promising results. The taxonomical composition of the observed communities was relatively similar to the ones observed in other grasslands (Öpik *et al.*, 2003; Vandenkoornhuyse *et al.*, 2002), but the detected richness was lower, probably because of differences in the methodological approaches, as discussed above.

The main finding of this study was a significant negative correlation between the levels of mineral N in soil and the number of AMF sequence groups in the roots. We did not detect a qualitative change in the composition of the community, as it has been observed by Jumpponen (2005). How increasing levels of N can affect the AMF community composition is not yet understood. One hypothesis is based on a reduced translocation of C from the plant to the fungi when N is not a limiting nutrient. This could reduce the presence of AM fungi in the roots, both in terms of biomass and taxonomical diversity. It is also probable that under these N rich conditions, the roots can become colonised predominantly by a guild of a few nitrophilic AMF taxa.

Patterns of host association in AM fungi

For many years, glomeromycotan fungi have been considered to have low host specificity. This assumption was mainly based on experimental studies using fungal isolates, many times isolated by trap-cultures, and that grew easily in pot cultures in green-house conditions. Most of the described species in the Glomeromycota are these 'easily' culturable isolates, showing low plant host specificity, and therefore, able to colonise a wide range of plant species.

In paper II we showed that there was a significant effect of plant host identity on the species composition of the AMF community when analysing two co-existent plant species. One of the plant species, *Prunella vulgaris*, hosted an unexpectedly rich AMF community in its roots. All the sequence types found in *P. vulgaris* were present in *Antennaria dioica*, but not vice versa. Moreover some of the most frequent sequence types in *A. dioica* were seldom detected in *P. vulgaris*.

This pattern of host preference was not observed in paper I, when analysing a different pair of plant species. This could be because the chosen plant species do not have different AMF community profiles, but another more plausible reason could be that the community profiles in papers I and II were obtained using approaches with different degrees of taxonomic resolution.

Whether host specificity in nature is regulated by species-specific chemotactic signalling (Parniske, 2004) or is just a matter of ecological 'preference', *i.e.*, particular AMF species may grow more or less well in certain types of root cortex, is not yet known.

Temporal dynamics of AM fungi

In paper I we could observe that the number of AMF taxa in the roots had declined dramatically from June to September. This quantitative change was not surprising. Declining levels of fungal colonisation have previously been reported throughout the growing season. Another interesting issue is to know whether there is also a qualitative change in taxa with time, *i.e.* whether the taxonomic composition of the fungal communities shifts throughout the growing season and how rapidly this putative change can occur. In paper II we carried out an intensive, monthly sampling during the growing season. No significant changes in the dominant AMF taxa in the community were observed, except for *Glomus intraradices*. Previously, only weak evidence of seasonal changes in the intraradical AMF community has been presented by Vandenkoornhuyse *et al.* (2002), but the authors could not rule out the possibility that the observed shift was attributable to some changes in land management that occurred during the study. In our study, it was particularly interesting to observe that different co-occurring plants can have very different dynamics of mycorrhizal colonisation. While *Prunella vulgaris* had a relatively rich community throughout the whole growth season, the glomeromycotan fungal community in the roots of *Antennaria dioica* earlier in the growth season seemed to be displaced by a community dominated by Ascomycetes, later in the season.

These Ascomycetes, some of them also referred as dark septate endophytes (DSE) in the literature, could be saprotrophs that take over the roots when they die, but the analysed root pieces looked healthy and without symptoms of necrosis. Another possibility is that Ascomycetes displace glomalean fungi during the autumn when plant growth and mineral nutrient demands are low, and AM fungi cease to obtain carbohydrates from their host. Reductions in light level and thus photosynthesis have been related to decreased mycorrhization (Hayman, 1973; Hayman, 1974), however we can only speculate about the role of these Ascomycetes in the roots. These cryptic endophytic fungi have been shown to have both detrimental and beneficial interactions with their host plants (Addy, Piercey & Currah, 2005). Most interesting are the results of some studies suggesting putative mutualistic associations between DSE and their hosts (Haselwandter & Read, 1982; Redman, Dunigan & Rodriguez, 2001).

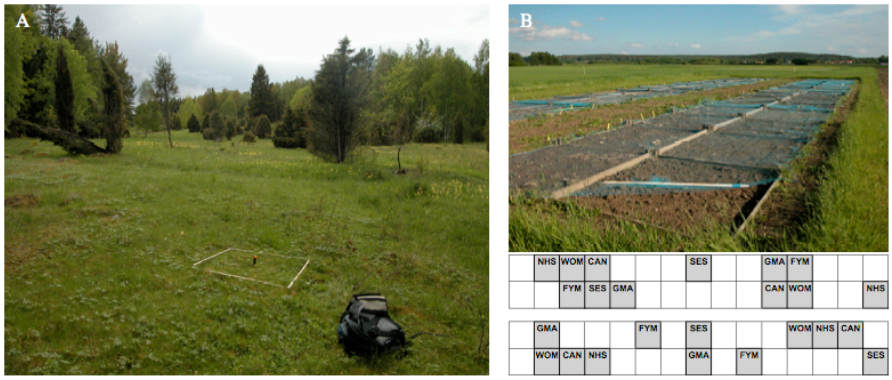

Fig. 4. Two study sites. A. Seminatural grassland at Torslunda (Täby, Uppland) with scattered shrubs and trees. B. Long term experimental field at Ultuna (Uppsala, Uppland) with a map representing the different fertilisation regimes. The six treatments (with four replicates each) studied by us are labelled as follows: WOM, whithout organic treatment; CAN, calcium nitrate; NHS, ammonium nitrate; FYM, farmyard manure; GMA, green manure; SES, sewage sludge.

Spatial patterns of distribution of AM fungi in the landscape

The AMF sequence groups dominating the fungal community profiles in the four plant species in papers I and II were relatively similar. In paper III we showed that this is not always so. The fungal community composition can show significant differences at the regional level, even when comparing the same host plant species in similar ecosystems. These results refute the traditional view of Glomeromycota as a handful of ubiquitous 'generalist' species. We showed that there are obviously some factors, apart from the specific identity of their host plant, that underlie the distribution of particular AMF lineages at a landscape level, and therefore affect AM fungal β-diversity.

Whether microorganisms have biogeography or not is still an unresolved question among microbiologists (Fitter, 2005; Whitfield, 2005). Baas-Becking formulated the popular hypothesis postulating that '*everything is everywhere*, but, the *environment selects*' (Baas-Becking, 1934). Although fungi were not explicitly included in Baas-Becking's concept of microbes, most of the ideas developed around this hypothesis and by Beijerinck (1913), can be applicable to fungi. There is an increasing body of evidence in free-living microorganisms showing that the environment selects (Martiny *et al.*, 2006), but to test whether 'everything is everywhere' is more challenging. The latter implies unconstrained dispersal capabilities in most microorganisms, and that dispersal is a more rapid process than speciation itself (Fitter, 2005). Glomeromycotan fungi have been shown to be dispersed by wind (Warner, Allen & Macmahon, 1987) and animals (Mangan & Adler, 2000), but the relative importance of these and other putative dispersal agents in nature remains obscure.

Experimental evidence suggests that plant diversity can be one of the key environmental factors influencing the belowground diversity of their mycorrhizal

partners (Johnson *et al.*, 2004) and other belowground microrganisms (Kowalchuk *et al.*, 2002). Spore based field studies have shown a correlation between fungal and plant richness (Landis, Gargas & Givnish, 2004). In our paper we failed to find a significant correlation between AMF richness in *Prunella vulgaris* and plant richness at a small scale. Although this could reflect a true lack of correlation, it is very likely that the divergent and unbalanced sample sizes of the fungal communities (6 cm of root of a single plant species) and the plant communities (1 m^2) we measured, do not allow such a correlation to be established.

Effects of different fertilisation regimes on soil microbial communities

Soil microorganisms play key roles in soil processes, both in natural and agricultural systems. However, conventional agricultural practices, *e.g.* tillage, mineral fertilisation, application of biocides, have been shown to be detrimental to many groups of organisms (Gosling *et al.*, 2006). One of the main aims of organic farming is to utilise the ecosystem services of naturally occurring soil microorganisms.

In paper IV we analysed the effect of organic and mineral fertilisers on the structure of the AMF colonising the roots of maize and the bacterial communities associated with the mycorrhizosphere. A long-term experimental field, which had been running for over 50 years was used in this study. The main finding was that both richness and composition of the fungal and bacterial communities were greatly influenced by the type of fertiliser. Plots fertilised with ammonium sulphate, $(NH_4)_2\ SO_4$, had fewer fungal and bacterial species, whereas treatments with higher pH and organic amendments had higher numbers of taxa. The AM fungus *Glomus intraradices* was detected in all treatments, except in the one with ammonium sulphate. In this last treatment, our data also indicate lower densities in the bacterial and fungal populations. Most of the changes in the microbial community composition were related to changes in pH induced by the fertilisation regime.

The AMF community detected in maize had a lower richness than the communities analysed in grasslands. This is a common pattern, previously detected in other studies of agricultural soils (Daniell *et al.*, 2001; Hijri *et al.*, 2006). This lower richness could be due to the effects of tillage in the experimental field (Jansa *et al.*, 2003), the absence of a perennial roots during the winter and the lack of other host plant species. This probably enhances the presence of taxa with good sporulating capacity such as the ones included in the species complex of *Glomus mosseae-G. caledonium* and *Glomus* gr. *intraradices*.

In our study we show that fertilisation has a major effect upon soil microorganisms and therefore, on soil ecological processes. Further knowledge on the functional significance of these effects needs to be gained, before we will be able to design sustainable agricultural practices that make optimal use of ecosystem services carried out by soil microorganisms.

Conclusions

In relation to the initial aims of this thesis it can be concluded that:

- Single plant species can harbour rich AMF communities in their roots. Most of the detected fungal diversity, has not yet been taxonomically described.

- Arbuscular mycorrhizal fungi display different degrees of plant host specificity. This is not a strict species-specific relationship between particular fungi and plants, but it suggests an active choice of mycorrhizal partner and that different fungal taxa have different functions.

- The dynamics of intraradical colonisation is different in different plant hosts, where they coexist with other groups of fungi. The interpretation of the composition of the fungal communities and their temporal and spatial colonisation patterns, can only be possible when we can establish a connection between the identity of particular taxa and their function.

- Different management practices, both in grasslands and arable farmlands, greatly influence the soil microbial communities. This implies unknown consequences in fields like fungal conservation, ecosystem restoration and plant production.

Perspectives

Progress within the discipline of microbial ecology has historically depended upon the application of key technological advances. The development of DNA based methods to study microbial communities in the field, has been one such key advance. Our understanding of microbial ecology, and of fungal ecology in particular, is however, limited in comparison with the knowledge we have on other groups or organisms, such as plants. Size matters in this case. The study of fungal communities necessarily involves the study of small sample sizes and this also limits the scale of the ecological conclusions that can be drawn from their study. This is a problem that will hopefully be overcome by high-throughput sequencing procedures in the future. In the particular case of the Glomeromycota, better understanding of their genetic organisation and basic aspects of their life cycle is needed in order to gain practical benefits from the molecular data that we have already started to generate.

The initial challenge is to gain more complete knowledge about the range of AM taxa colonising both agroecosystems and natural environments. Secondly we need to acquire better information about the degree of host specificity of different AM

taxa and the range of functional roles they play under different environmental conditions. This will help to identify potentially threatened species that may have declining populations. Experimental ecology will help to elucidate what they are doing and discover their functional diversity. This has already been done using some culturable AMF species, but the unculturable taxa that dominate natural communities must also be included. The complexity of natural ecosystems, and of soil ecosystems in particular, makes it both very difficult to discern ecological processes in the field, and nearly impossible to recreate natural conditions in lab experiments. Better methods to apply in the field will have to be developed. At the same time, laboratory and micrcosm experiments need to incorporate more 'ecological complexity' in their designs in order to avoid being dismissed as unrealistic (van der Heijden & Scheublin, 2007; Read, 2002). In particular, bacteria associated with AM fungi and other fungal groups, such as Ascomycetes, in the rhizosphere can play a key role in the interaction between plants and AM fungi. The impressive diversity of invertebrates and their diverse functional groups will also have to be considered in the picture if we want to understand the real ecological roles of AM fungi and how these can be exploited in sustainable agriculture.

References

Addy, H.D., Piercey, M.M. & Currah, R.S. 2005. Microfungal endophytes in roots. *Canadian Journal Of Botany-Revue Canadienne De Botanique 83*, 1-13.

Alexander, I.J. 2006. Ectomycorrhizas - out of Africa? *New Phytologist 172*, 589-591.

Auge, R.M. 2001. Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis. *Mycorrhiza 11*, 3-42.

Baas-Becking, L. 1934. *Geobiologie of Inleiding Tot de Milieukunde*. Van Stockkum & Zoon. The Hague.

Bago, B., Zipfel, W., Williams, R.M. & Piche, Y. 1999. Nuclei of symbiotic arbuscular mycorrhizal fungi as revealed by *in vivo* two-photon microscopy. *Protoplasma 209*, 77-89.

Beijerinck, M. 1913. *De infusies en de ontdekking der backteriën. Jaarboek van de Koninklijke Akademie voor Wetenschappen*. Müller. Amsterdam, The Netherlands.

Berbee, M. & Taylor, J. 2000. Fungal molecular evolution: gene trees and geologic time. In: *The Mycota*. Eds. D. McLaughlin, E. McLaughlin & P. Lemke). Springer Verlag. New York. pp. 229-246.

Bianciotto, V., Bandi, C., Minerdi, D., Sironi, M., Tichy, H.V. & Bonfante, P. 1996. An obligately endosymbiotic mycorrhizal fungus itself harbors obligately intracellular bacteria. *Applied And Environmental Microbiology 62*, 3005-3010.

Borowicz, V.A. 2001. Do arbuscular mycorrhizal fungi alter plant-pathogen relations? *Ecology 82*, 3057-3068.

Clapp, J.P., Rodriguez, A. & Dodd, J.C. 2001. Inter- and intra- isolate rRNA large sub-unit variation in spores of *Glomus coronatum*. *New Phytologist 149*, 539-554.

Corradi, N., Hijri, M., Fumagalli, L. & Sanders, I.R. 2004. Arbuscular mycorrhizal fungi (Glomeromycota) harbour ancient fungal tubulin genes that resemble those of the chytrids (Chytridiomycota). *Fungal Genetics And Biology 41*, 1037-1045.

Dahlberg, A., Jonsson, L. & Nylund, J.E. 1997. Species diversity and distribution of biomass above and below ground among ectomycorrhizal fungi in an old-growth Norway spruce forest in south Sweden. *Canadian Journal Of Botany-Revue Canadienne De Botanique 75*, 1323-1335.

Daniell, T.J., Husband, R., Fitter, A.H. & Young, J.P.W. 2001. Molecular diversity of arbuscular mycorrhizal fungi colonising arable crops. *Fems Microbiology Ecology 36*, 203-209.

Delwiche, C.F., Kuhsel, M. & Palmer, J.D. 1995. Phylogenetic Analysis of Tufa Sequences Indicates a Cyanobacterial Origin of All Plastids. *Molecular Phylogenetics and Evolution 4*, 110-128.

Dickson, S. 2004. The *Arum-Paris* continuum of mycorrhizal symbioses. *New Phytologist 163*, 187-200.

Dickson, S., Smith, F.A. & Smith, S.E. 2007. Structural differences in arbuscular mycorrhizal symbioses: more than 100 years after Gallaud, where next? *Mycorrhiza 17*, 375-393.

Fitter, A.H. 2005. Darkness visible: reflections on underground ecology. *Journal Of Ecology 93*, 231-243.

Goloboff, P., Farris, J. & Nixon, K. 2003. *T.N.T.: Tree Analysis Using New Technology. Available at* http://www.zmuc.dk/public/phylogeny.

Gosling, P., Hodge, A., Goodlass, G. & Bending, G.D. 2006. Arbuscular mycorrhizal fungi and organic farming. *Agriculture Ecosystems & Environment 113*, 17-35.

Govindarajulu, M., Pfeffer, P.E., Jin, H.R., Abubaker, J., Douds, D.D., Allen, J.W., Bucking, H., Lammers, P.J. & Shachar-Hill, Y. 2005. Nitrogen transfer in the arbuscular mycorrhizal symbiosis. *Nature 435*, 819-823.

Haselwandter, K. & Read, D.J. 1982. The significance of a root-fungus association in 2 *Carex* species of high-alpine plant communities. *Oecologia 53*, 352-354.

Hayman, D. 1973. The effects of light intensity on VA mycorrhiza. In: *Rothamsted Report for 1972, Part 1*. pp. 81.

Hayman, D. 1974. Plant growth response to vesicular-arbuscular mycorrhiza. VI. Effect of light and temperature. *New Phytologist*, 71-80.

van der Heijden, M., Klironomos, J.N., Ursic, M., Moutoglis, P., Streitwolf, E.R., Boller, T., Wiemken, A. & Sanders, I.R. 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature 396*, 69-72.

van der Heijden, M.G.A. & Scheublin, T.R. 2007. Functional traits in mycorrhizal ecology: their use for predicting the impact of arbuscular mycorrhizal fungal communities on plant growth and ecosystem functioning. *New Phytologist 174*, 244-250.

Helgason, T., Daniell, T.J., Husband, R., Fitter, A.H. & Young, J.P.W. 1998. Ploughing up the wood-wide web ? *Nature 394*, 431.

Helgason, T. & Fitter, A.H. 2005. The ecology and evolution of the arbuscular mycorrhizal fungi. *Mycologist 19*, 96-101.

Helgason, T., Fitter, A.H. & Young, J.P.W. 1999. Molecular diversity of arbuscular mycorrhizal fungi colonising *Hyacinthoides non-scripta* (bluebell) in a seminatural woodland. *Molecular Ecology 8*, 659-666.

Helgason, T., Watson, I.J. & Young, J.P.W. 2003. Phylogeny of the Glomerales and diversisporales (Fungi : Glomeromycota) from actin and elongation factor 1-alpha sequences. *Fems Microbiology Letters 229*, 127-132.

Hijri, I., Sykorova, Z., Oehl, F., Ineichen, K., Mäder, P., Wiemken, A. & Redecker, D. 2006. Communities of arbuscular mycorrhizal fungi in arable soils are not necessarily low in diversity. *Molecular Ecology 15*, 2277-2289.

Hijri, M. & Sanders, I.R. 2005. Low gene copy number shows that arbuscular mycorrhizal fungi inherit genetically different nuclei. *Nature 433*, 160-163.

Hildebrandt, U., Ouziad, F., Marner, F.J. & Bothe, H. 2006. The bacterium *Paenibacillus validus* stimulates growth of the arbuscular mycorrhizal fungus *Glomus intraradices* up to the formation of fertile spores. *Fems Microbiology Letters 254*, 258-267.

Hodge, A., Campbell, C.D. & Fitter, A.H. 2001. An arbuscular mycorrhizal fungus accelerates decomposition and acquires nitrogen directly from organic material. *Nature 413*, 297-299.

James, T.Y., Kauff, F., Schoch, C.L., Matheny, P.B., Hofstetter, V., Cox, C.J., Celio, G., Gueidan, C., Fraker, E., Miadlikowska, J., Lumbsch, H.T., Rauhut, A., Reeb, V., Arnold, A.E., Amtoft, A., Stajich, J.E., Hosaka, K., Sung, G.H., Johnson, D., O'Rourke, B., Crockett, M., Binder, M., Curtis, J.M., Slot, J.C., Wang, Z., Wilson, A.W., Schussler, A., Longcore, J.E., O'Donnell, K., Mozley-Standridge, S., Porter, D., Letcher, P.M., Powell, M.J., Taylor, J.W., White, M.M., Griffith, G.W., Davies, D.R., Humber, R.A., Morton, J.B., Sugiyama, J., Rossman, A.Y., Rogers, J.D., Pfister, D.H., Hewitt, D., Hansen, K., Hambleton, S., Shoemaker, R.A., Kohlmeyer, J., Volkmann-Kohlmeyer, B., Spotts, R.A., Serdani, M., Crous, P.W., Hughes, K.W., Matsuura, K., Langer, E., Langer, G., Untereiner, W.A., Lucking, R., Budel, B., Geiser, D.M., Aptroot, A., Diederich, P., Schmitt, I., Schultz, M., Yahr, R., Hibbett, D.S., Lutzoni, F., McLaughlin, D.J., Spatafora, J.W. & Vilgalys, R. 2006. Reconstructing the early evolution of Fungi using a six-gene phylogeny. *Nature 443*, 818-822.

Jansa, J., Mozafar, A., Kuhn, G., Anken, T., Ruh, R., Sanders, I.R. & Frossard, E. 2003. Soil tillage affects the community structure of mycorrhizal fungi in maize roots. *Ecological Applications 13*, 1164-1176.

Johansson, J.F., Paul, L.R. & Finlay, R.D. 2004. Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture. *Fems Microbiology Ecology 48*, 1-13.

Johnson, D., Vandenkoornhuyse, P., Leake, J.R., Gilbert, L., Booth, R., Grime, J., Young, J.P.W. & Read, D.J. 2004. Plant communities affect arbuscular mycorrhizal fungal diversity and community composition in grassland microcosms. *New Phytologist 161*, 503-515.

Jumpponen, A., Trowbridge, J., Mandyam, K. & Johnson, L. 2005. Nitrogen enrichment causes minimal changes in arbuscular mycorrhizal colonization but shifts community composition-evidence from rDNA data. *Biology And Fertility Of Soils 41*, 217-224.

Kåren, O., Hogberg, N., Dahlberg, A., Jonsson, L. & Nylund, J.E. 1997. Inter- and intraspecific variation in the ITS region of rDNA of ectomycorrhizal fungi in Fennoscandia as detected by endonuclease analysis. *New Phytologist 136*, 313-325.

Kistner, C. & Parniske, M. 2002. Evolution of signal transduction in intracellular symbiosis. *Trends In Plant Science 7*, 511-518.

Kowalchuk, G.A., Buma, D.S., de Boer, W., Klinkhamer, P.G.L. & van Veen, J.A. 2002. Effects of above-ground plant species composition and diversity on the diversity of soil-borne microorganisms. *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology 81*, 509-520.

Kowalchuk, G.A., Souza, F.A.d. & van Veen, J.A. 2002. Community analysis of arbuscular mycorrhizal fungi associated with *Ammophila arenaria* in Dutch coastal sand dunes. *Molecular Ecology 11*, 571-581.

Kuhn, G., Hijri, M. & Sanders, I.R. 2001. Evidence for the evolution of multiple genomes in arbuscular mycorrhizal fungi. *Nature 414*, 745-748.

Landis, F.C., Gargas, A. & Givnish, T.J. 2004. Relationships among arbuscular mycorrhizal fungi, vascular plants and environmental conditions in oak savannas. *New Phytologist 164*, 493-504.

Leyval, C., Turnau, K. & Haselwandter, K. 1997. Effect of heavy metal pollution on mycorrhizal colonization and function: physiological, ecological and applied aspects. *Mycorrhiza 7*, 139-153.

Lutzoni, F., Kauff, F., Cox, C.J., McLaughlin, D., Celio, G., Dentinger, B., Padamsee, M., Hibbett, D., James, T.Y., Baloch, E., Grube, M., Reeb, V., Hofstetter, V., Schoch, C., Arnold, A.E., Miadlikowska, J., Spatafora, J., Johnson, D., Hambleton, S., Crockett, M., Shoemaker, R., Hambleton, S., Crockett, M., Shoemaker, R., Sung, G.H., Lucking, R., Lumbsch, T., O'Donnell, K., Binder, M., Diederich, P., Ertz, D., Gueidan, C., Hansen, K., Harris, R.C., Hosaka, K., Lim, Y.W., Matheny, B., Nishida, H., Pfister, D., Rogers, J., Rossman, A., Schmitt, I., Sipman, H., Stone, J., Sugiyama, J., Yahr, R. & Vilgalys, R. 2004. Assembling the fungal tree of life: Progress, classification and evolution of subcellular traits. *American Journal Of Botany 91*, 1446-1480.

Mangan, S.A. & Adler, G.H. 2000. Consumption of arbuscular mycorrhizal fungi by terrestrial and arboreal small mammals in a Panamanian cloud forest. *Journal of Mammalogy 81*, 563-570.

Margulis, L. & Fester, R. 1991. *Symbiosis as a source of evolutionary innovation*. MIT Press. Cambridge, MA, USA.

Martiny, J.B.H., Bohannan, B.J.M., Brown, J.H., Colwell, R.K., Fuhrman, J.A., Green, J.L., Horner-Devine, M.C., Kane, M., Krumins, J.A., Kuske, C.R., Morin, P.J., Naeem, S., Ovreas, L., Reysenbach, A.L., Smith, V.H. & Staley, J.T. 2006. Microbial biogeography: putting microorganisms on the map. *Nature Reviews Microbiology 4*, 102-112.

Nelles, L., Fang, B.D., Volckaert, G., Vandenberghe, A. & Dewachter, R. 1984. Nucleotide-sequence of a crustacean-18S ribosomal-RNA gene and secondary structure of eukaryotic small subunit ribosomal-RNAs. *Nucleic Acids Research 12*, 8749-8768.

Newsham, K.K., Fitter, A.H. & Watkinson, A.R. 1995. Arbuscular mycorrhiza protect an annual grass from root pathogenic fungi in the field. *Journal Of Ecology 83*, 991-1000.

Nickrent, D.L. & Sargent, M.L. 1991. An Overview of the Secondary Structure of the V4-Region of Eukaryotic Small-Subunit Ribosomal-RNA. *Nucleic Acids Research 19*, 227-235.

Öpik, M., Moora, M., Liira, J., Koljalg, U., Zobel, M. & Sen, R. 2003. Divergent arbuscular mycorrhizal fungal communities colonize roots of *Pulsatilla* spp. in boreal Scots pine forestand grassland soils. *New Phytologist 160*, 581-593.

Öpik, M., Moora, M., Liira, J. & Zobel, M. 2006. Composition of root-colonizing arbuscular mycorrhizal fungal communities in different ecosystems around the globe. *Journal Of Ecology 94*, 778-790.

Parniske, M. 2004. Molecular genetics of the arbuscular mycorrhizal symbiosis. *Current Opinion In Plant Biology 7*, 414-421.

Pawlowska, T.E. & Taylor, J.W. 2004. Organization of genetic variation in individuals of arbuscular mycorhizal fungi. *Nature 427*, 733-737.

Pirozynski, A. & Malloch, D. 1975. The origin of land plants. A matter of mycotrophism. *Biosystems 6*, 153-154.
Porcel, R., Aroca, R., Azcon, R. & Ruiz-Lozano, J.M. 2006. PIP aquaporin gene expression in arbuscular mycorrhizal *Glycine max* and *Lactuca sativa* plants in relation to drought stress tolerance. *Plant Molecular Biology 60*, 389-404.
Raab, P.A., Brennwald, A. & Redecker, D. 2005. Mitochondrial large ribosomal subunit sequences are homogeneous within isolates of Glomus (arbuscular mycorrhizal fungi, Glomeromycota). *Mycological Research 109*, 1315-1322.
Read, D.J. 2002. Towards Ecological Relevance-Progress and Pitfalls in the Path Towards an Understanding of Mycorrhizal Functions in Nature. In: *Mycorrhizal Symbiosis*. Eds. M. van der Hejden & I.R. Sanders). Springer. pp. 3-29.
Redecker, D., Kodner, R. & Graham, L.E. 2000. Glomalean fungi from the Ordovician. *Science 289*, 1920-1921.
Redecker, D., Morton, J.B. & Bruns, T.D. 2000. Ancestral lineages of arbuscular mycorrhizal fungi (Glomales). *Molecular Phylogenetics and Evolution 14*, 276-284.
Redecker, D. & Raab, P. 2006. Phylogeny of the Glomeromycota (arbuscular mycorrhizal fungi): recent developments and new gene markers. *Mycologia 98*, 885-895.
Redman, R.S., Dunigan, D.D. & Rodriguez, R.J. 2001. Fungal symbiosis from mutualism to parasitism: who controls the outcome, host or invader? *New Phytologist 151*, 705-716.
Remy, W., Taylor, T.N., Hass, H. & Kerp, H. 1994. Four hundred-million-year-old vesicular arbuscular mycorrhizae. *Proceedings Of The National Academy Of Sciences Of The United States Of America 91*, 11841-11843.
Rodriguez, A., Clapp, J.P. & Dodd, J.C. 2004. Ribosomal RNA gene sequence diversity in arbuscular mycorrhizal fungi (Glomeromycota). *Journal Of Ecology 92*, 986-989.
Rodriguez, A., Clapp, J.P., Robinson, L. & Dodd, J.C. 2005. Studies on the diversity of the distinct phylogenetic lineage encompassing *Glomus claroideum* and *Glomus etunicatum*. *Mycorrhiza 15*, 33-46.
Sanders, I.R. 2004. Plant and arbuscular mycorrhizal fungal diversity - are we looking at the relevant levels of diversity and are we using the right techniques? *New Phytologist 164*, 415-418.
Santos-González, J.C., Finlay, R.D. & Tehler, A. 2007. Seasonal dynamics of arbuscular mycorrhizal fungal communities in roots in a seminatural grassland. *Applied And Environmental Microbiology 73*, 5613-5623.
Sapp, J. 2004. The dynamics of symbiosis: an historical overview. *Canadian Journal Of Botany-Revue Canadienne De Botanique 82*, 1046-1056.
Schimper, A. 1883. Ûber die Entwicklung der Scholorophyllkörner und Farbkörper. *Bot. Zetung. 4*, 105-114.
Schüßler, A. 2002. Molecular phylogeny, taxonomy, and evolution of *Geosiphon pyriformis* and arbuscular mycorrhizal fungi. *Plant And Soil 244*, 75-83.
Schüßler, A., Gehrig, H., Schwarzott, D. & Walker, C. 2001. Analysis of partial Glomales SSU rRNA gene sequences: Implications for primer design and phylogeny. *Mycological Research 105*, 5-15.
Schüßler, A., Mollenhauer, D., Schnepf, E. & Kluge, M. 1994. *Geosiphon pyriforme*, an endosymbiotic association of fungus and Cyanobacteria: The spore structure resembles that of arbuscular mycorrhizal (AM) fungi. *Botanica Acta 107*, 36-45.
Schüßler, A., Schwarzott, D. & Walker, C. 2001. A new fungal phylum, the Glomeromycota: phylogeny and evolution. *Mycological Research 2001*, 1413-1421.
Schüßler, A., Schwarzott, D. & Walker, C. 2003. Glomeromycota rRNA genes - the diversity of myths? *Mycorrhiza 13*, 233-236.
Schwarzott, D., Walker, C. & Schüßler, A. 2001. *Glomus*, the largest genus of the arbuscular mycorrhizal fungi (Glomales), is non monophyletic. *Molecular Phylogenetics and Evolution 21*, 190-197.
Selosse, M.A. 2005. Are liverworts imitating mycorrhizas? *New Phytologist 165*, 345-349.
Simon, L., Bousquet, J., Levesque, R.C. & Lalonde, M. 1993. Origin and Diversification of Endomycorrhizal Fungi and Coincidence with Vascular Land Plants. *Nature 363*, 67-69.

Simon, L., Lalonde, M. & Bruns, T.D. 1992. Specific amplification of 18S ribosomal genes from VA endomycorrhizal fungi colonizing roots. *Applied And Environmental Microbiology 58*, 291-295.
Smith, S.E. & Read, D.J. 1997. *Mycorrhizal Symbiosis*. 2nd edition edition London.
Sprent, J.I. & James, E.K. 2007. Legume evolution: Where do nodules and mycorrhizas fit in? *Plant Physiology 144*, 575-581.
Swofford, D. 2002. *Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4*. Sinauer Asssociates. Sunderland, Massachusets.
Tehler, A., Farris, J.S., Lipscomb, D.L. & Kallersjo, M. 2000. Phylogenetic analyses of the fungi based on large rDNA data sets. *Mycologia 92*, 459-474.
Tehler, A., Little, D.P. & Farris, J.S. 2003. The full-length phylogenetic tree from 1551 ribosomal sequences of chitinous fungi, Fungi. *Mycological Research 107*, 901-916.
Vandenkoornhuyse, P., Husband, R., Daniell, T.J., Watson, I.J., Duck, J.M., Fitter, A.H. & Young, J.P.W. 2002. Arbuscular mycorrhizal community composition associated with two plant species in a grassland ecosystem. *Molecular Ecology*, 1555-1564.
Walker, C. & Schüßler, A. 2004. Nomenclatural clarifications and new taxa in the Glomeromycota. *Mycological Research 108*, 981-982.
Warner, N.J., Allen, M.F. & Macmahon, J.A. 1987. Dispersal Agents of Vesicular-Arbuscular Mycorrhizal Fungi in a Disturbed Arid Ecosystem. *Mycologia 79*, 721-730.
Whitfield, J. 2005. Biogeography: Is everything everywhere? *Science 310*, 960-961.
Yuan, X.L., Xiao, S.H. & Taylor, T.N. 2005. Lichen-like symbiosis 600 million years ago. *Science 308*, 1017-1020.

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