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Diversity and functioning of arbuscular mycorrhizal fungi in semi-natural grassland ecosystems

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Chapter 1: Introduction

1.1 What are mycorrhizas?

"The study of plants without their mycorrhizas is the study of artefacts. The majority of plants, strictly speaking, do not have roots; they have mycorrhizas." (BEG-Committee, May 25th 1993). This citation pin points the tight relationship between plants and fungi in mycorrhizal associations. The symbiosis is mainly based on the mutual exchange of nutrients, whereas carbon is transferred of from plant to fungus and several nutrients (phosphorus, nitrogen, trace elements et cetera) in the opposite direction (Smith and Read 1997). Two main types of mycorrhizas are differentiated: ectomycorrhiza (EM) and arbuscular mycorrhiza (AM). While EM is commonly found between trees (mostly in the cool temperate and boreal region) and fungi from the orders of the Basidiomycota, Ascomycota or Zygomycota (Smith and Read 1997), AM is formed between almost all fungi of the phylum Glomeromycota and approximately 75% of the plants in all biomes around the globe (Schüßler et al. 2001; Treseder and Cross 2006). Additionally to these two types, several specific kinds of mycorrhizas are formed, namely ectendo-, ericoid, arbutoid, monotropoid and orchid mycorrhiza (for details see Smith and Read 1997).

1.2 Arbuscular mycorrhiza – morphology and phylogeny

This study deals with the AM, the most ancient mycorrhizal form. Fossils records from the Ordovician (460 million years ago; see Redecker et al. 2000) support the hypothesis that AM may have been important for the establishment of first land plants (Simon et al. 1993). The nutrient exchange between the symbiotic partners takes place mainly at the fungal arbuscules, tree-shaped branching structures that invaginate the plant cell plasma lemma and create a large area of membrane-membrane apposition contact (Fig. 1a and b). Arbuscules are formed by the intra-radical mycelium that grows within the plant root (Fig. 1b) and which has also been discussed to take part in the nutrient exchange (Gianinazzi-Pearson et al. 1991; Smith and Smith 1997). Another typical morphological intra-radical structure formed by some but not all AM fungi (AMF) are vesicles (Fig. 1b), lemon shaped storage structures rich in lipids (Cooper and Lösel 1978). Besides the intra-radical mycelium, AMF also form extra-radical mycelia (Fig. 1c and 1d), which explore the soil around plant roots and allow the AMF to take up nutrients behind the root depletion zone. Both, intra-and extra-radical mycelia, can form asexual spores (Fig. 1c and 1d), which serve as propagules. However, no sexual stages of AMF are known.

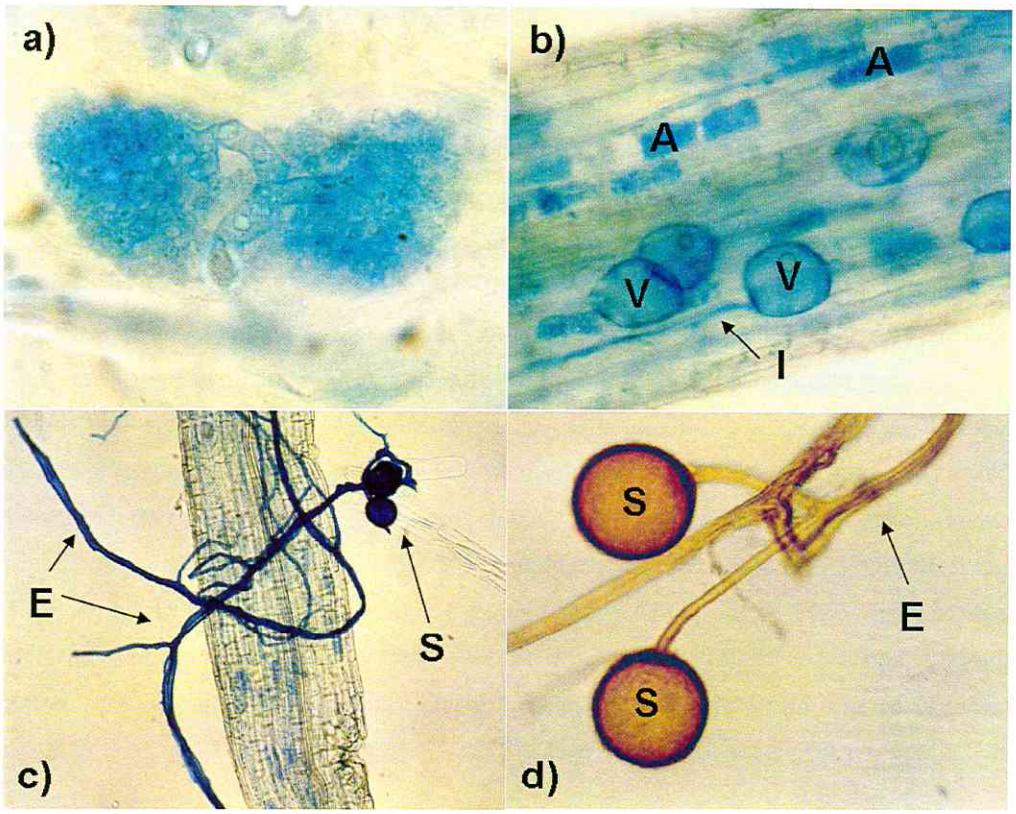

Figure 1: Morphological structures of arbuscular mycorrhizal fungi: a) arbuscules; b) intra-radical hyphae (I), arbuscules (A) and vesicles (V); c) extra-radical hyphae (E) and spores (S); d) extra-radical hyphae (E) and spores (S). Pictures used with kind permission from V. Blanke.

Formerly placed as Endogonales (Gerdemann and Trappe 1974) and later as Glomales (Morton and Benny 1990) in the order of Zygomycota, AMF were recently placed into their own monophyletic group, the Glomeromycota (Schüßler et al. 2001). To date 206 species are listed at the website of Arthur Schüßler (http://www.lrz-muenchen.de/~schuessler/ amphylo/), probably the most complete listing of genetically and/or morphologically described species within the Glomeromycota (Figure 2). However, the phylogenetic relations are under intensive investigations (e.g. Walker et al. 2007a; Walker et al. 2007b). These attempts are complicated by the fact that classical species concepts are not applicable to AMF due to the lack of sexual stages (Rosendahl 2008). Furthermore, molecular studies (see below) suggest a much higher diversity than currently described based on the spore morphology (e.g. Börstler et al. 2006).

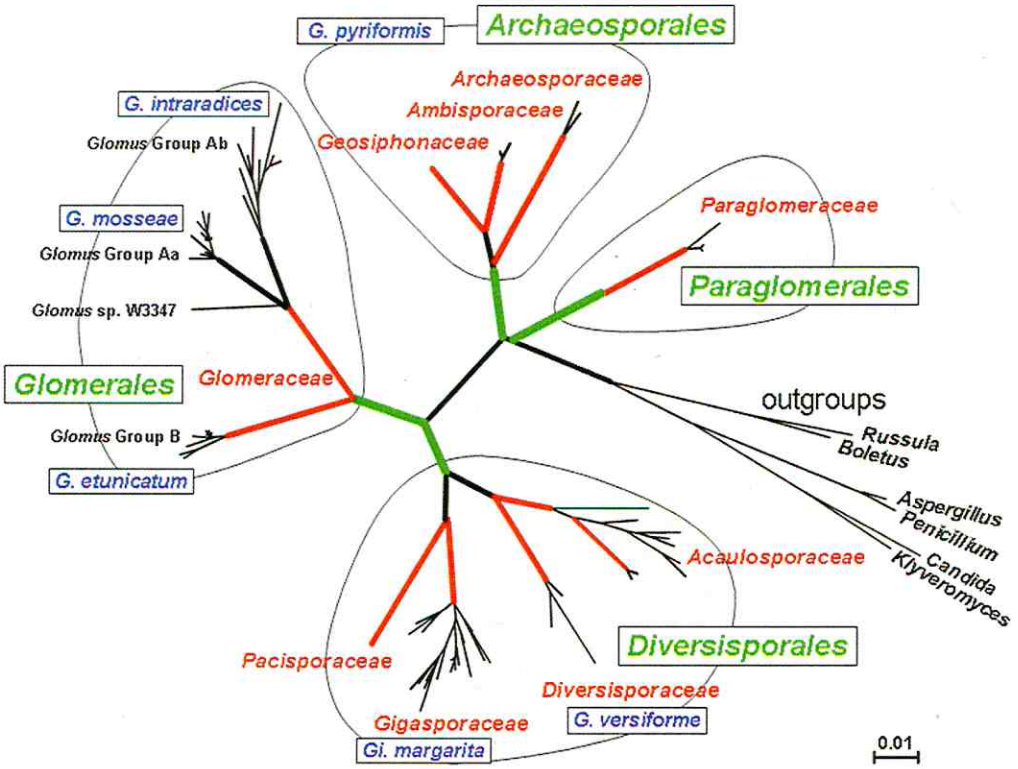

Figure 2: Phylogenetic tree of all families and sequence groups within the Glomeromycota (figure taken from http://www.lrz-muenchen.de/~schuessler/amphylo/).

1.3 Why are arbuscular mycorrhizas ecologically important?

One of the main features making AM an important subject of research for ecology, is their widespread occurrence in all biomes (Treseder and Cross 2006), potentially influencing a wide range of plant traits. In particular, AMF have been shown to impact plant productivity (van der Heijden et al. 1998b, see also chapters 3 and 4) and nutrient acquisition (Grime et al. 1987; van der Heijden et al. 2006b; see also chapter 3), with most publications dealing with the supply of phosphate (e.g. Jakobsen et al. 1992; van der Heijden et al. 1998b) and nitrogen (Hodge et al. 2001; Blanke et al. 2005). It was also shown that AMF can increase the uptake of trace elements (Clark and Zeto 2000) and protect host plants against pathogens (Newsham et al. 1995; Maherali and Klironomos 2007) and drought stress (Augé et al. 2001). In addition, adapted AMF can protect plants against heavy metals (Hildebrandt et al. 2007) and salt stress (Smith and Read 1997).In plant communities with the subordinate species strongly depending on AM, the presence of AMF can support the establishment and competitiveness of subordinate plants, exerting a strong positive influence on plant community composition and diversity (Grime et al. 1987; van der Heijden et al. 2006b, see also chapter 3). However,

when communities are dominated by strongly AMF dependent plant species, AMF can have a negative impact on plant diversity (Hartnett and Wilson 1999; O'Connor et al. 2002). Van der Heijden et al. (1998b) could also show, that more diverse AMF communities can have stronger effects on plant diversity than single AMF species, probably due to functional complementarity (Maherali and Klironomos 2007; Jansa et al. 2008). For instance, Maherali and Klironomos (2007) could show that AMF from Gigasporaceae provided a better phosphate nutrition for *Plantago lanceolata* plants than AMF from the Glomeraceae, whereas the opposite was true for the protection of the plants against root pathogens. However, other studies showed no positive effects of AMF species diversity (van der Heijden et al. 2006b) rather pointing to the importance of the presence of certain AMF species (Vogelsang et al. 2006).

In addition to direct effects of AMF, the fungi were also shown to indirectly influence their host plants in several ways. AMF can impact soil structure (van der Heijden et al. 2006b) with potentially strong effects on the scales of plant communities and individual plants, as discussed in Rillig and Mummey (2006). Singh et al. (2008) recently showed that AMF can affect soil bacterial communities, which might in turn feed back on the plants (van der Heijden et al. 2006a). AMF may also influence the trophic interactions between their host plants and other organisms like herbivores (e.g. Gange 2001; Gehring and Whitham 2002, see also chapter 4) or pollinators (Wolfe et al. 2005); even an influence of higher trophic levels, e.g. parasitoids has been reported (Gange et al. 2003; Guerrieri et al. 2004, see also chapter 4).

1.4 Ecological studies on arbuscular mycorrhizas

Studies on the ecology of AMF are mostly either descriptive studies of natural communities or manipulative experiments under controlled conditions. Descriptive studies survey the natural AMF species composition present in a certain environment. However, AMF are not as easy to study as many plants, animals or even other fungi, as they complete their lifecycle underground. Morphological features of AMF spores, such as the number and construction of spore walls, are used as morphological criteria to describe distinct species within the Glomeromycota (for diagnostic keys see http://invam.caf.wvu.edu/ and http://www.agro.ar.szczecin.pl/~jblaszkowski/). However, the species identification using these criteria requires expert knowledge. Since the first DNA data on AMF became available (Simon et al. 1992) and the phylogenetic relationships within the AMF were determined (see chapter 1.2), several specific primer sets have been designed in order to identify AMF present in the roots of their host plants by molecular methods. These primer sets mostly target the

ribosomal DNA, using either the small subunit (e.g. Helgason et al. 1998; Vandenkoornhuyse et al. 2002), the large subunit (Rosendahl and Stukenbrock 2004) or the internal transcribed spacer region (Redecker 2000; Renker et al. 2003). The development of these methods allowed the assessment of AMF species growing in plant roots. Surveys based on molecular methods reported e.g. on the relationships between AMF diversity and plant diversity (Börstler et al. 2006), nutrient availability (Santos et al. 2006), heavy metal contamination of soils (Zarei et al. 2008) or land use intensity (Hijri et al. 2006) and also revealed a large discrepancy between AMF species found as spores and within roots (Clapp et al. 1995; Börstler et al. 2006; see also chapter 2).

Only a few studies directly manipulated AMF in the field (e.g. Gange and West 1994) by repression or reduction of the plant mycorrhization by application of fungicides. It is to notice that fungicides also impact other soil fungi or serve as nutrient supply for soil microorganisms such affecting plants in various ways (Allison et al. 2007). Manipulation of AMF diversity under field conditions is almost impossible to achieve, as mycorrhizal spores can be dispersed by wind (see Renker et al. 2004). Studies manipulating AMF presence and diversity are therefore almost exclusively done in green houses or growth chambers (see chapters 3 and 4). Although the results of experiments under such artificial conditions might not be extrapolated to natural conditions, greenhouse experiments revealed several ground-breaking features concerning AMF - plant interactions (e.g. Grime et al. 1987; van der Heijden et al. 1998b; Hodge et al. 2001).

1.5 The DIVA Jena program within the BIOLOG framework

The present work about the diversity and ecology of AMF was part of BIOLOG Europe (Biodiversity and Global Change), an interdisciplinary research project studying the impact of environmental and climate change on biodiversity in Europe (http://www.biolog-europe.org/). The project is funded by the German Federal Ministry of Education and Research and consists of five major projects. DIVA Jena, one of these projects, investigates "The relationship between Biodiversity and Ecosystem Functioning in Grassland Ecosystems" (http://www2.uni-jena.de/biologie/ecology/biolog/english.htm).

Six closely cooperating subprojects within the DIVA study the impact of biodiversity on ecosystem services in grasslands on 19 extensively managed meadows in the Thüringer Schiefergebirge and in the Franconian Forest in Central Germany. The sites were selected in

order to represent a plant species diversity gradient, ranging from 18 to 45 species. On these sites several aspects are investigated, e.g. historical land use, soil nutrient status and cycling, plant productivity, diversity and functioning of arbuscular mycorrhizas and the indicative power of the presence of several insect guilds.

Figure 3: Examples of a high yield meadow with low plants diversity (left) and a low yield meadow with a high plant diversity (right) in the study area. Photographs were taken by Claudia Stein.

1.6 Objectives and structure of this thesis

One of the aims within DIVA was the assessment and comparison of the AMF diversity between all meadows. A preliminary study compared AMF diversity between two meadows representing different plant diversity levels with 27 and 43 plant species, respectively (Börstler et al. 2006) and detected a comparable AMF diversity at both sites but differences in the species composition. This study also confirmed the finding by Daniell et al. (2001), that the diversity found in spores does not reflect the diversity within roots. In the present work, it was therefore intended to test which compartment, i.e. spores, intra-radical or extra-radical mycelium should be investigated in order to assess the AMF diversity present at one site (chapter 2).

Another main goal of DIVA lies in the understanding of mechanisms influencing and maintaining biodiversity. Thus, in the second part of this thesis (chapter 3) interactive effects of AMF and parasitic plants on a plant community are investigated, as both, AMF and parasitic plants were singly shown to impact productivity and diversity in grasslands (Koide and Dickie 2002; Ameloot et al. 2005; for details see introduction of chapter 3).

Multitrophic interactions within ecosystems were as well shown to impact plant diversity (van der Putten et al. 2001). In this context it should be tested whether AMF can impact the trophic interactions between plants, herbivores and parasitoids. This aspect was studied in chapter 4.

Chapter 2: Differences in the species composition of arbuscular mycorrhizal fungi in spore, root and soil communities in a grassland ecosystem

2.1 Introduction

Arbuscular mycorrhiza (AM) is one of the most common types of symbiosis globally, and most known terrestrial plant species form relationships with arbuscular mycorrhizal fungi (AMF). Since AMF play important roles *inter alia* in the vigor of plant communities and the restoration of disturbed ecosystems (Renker et al. 2004) and their presence, abundance and composition are strongly related to the composition of the local plant community (van der Heijden et al. 1998b; Börstler et al. 2006) assessment of their species composition is an important issue in several contexts.

Most surveys on AMF assemblies, in which either molecular or morphological approaches have been used, have focused on either mycorrhizal roots (e.g. Helgason et al. 1999; Daniell et al. 2001; Vallino et al. 2006) or spores (Moreira-Souza et al. 2003; Oehl et al. 2003). However, the overlap between the species composition of AMF spores and functionally active AMF within plant roots has been shown to be low (Clapp et al. 1995; Merryweather and Fitter 1998; Renker et al. 2005). Recent studies have revealed a wide variability in colonization strategies of AM fungi (Cano and Bago 2005; Hart and Reader 2005), which strongly affects the relative proportions of the taxa present in the intra-radical and extra-radical mycelia (Hart and Reader 2002b; Drew et al. 2003). PCR-based approaches seem to offer the best current prospects for detecting most of the AM fungi present in an ecosystem. However, the results of PCR-based analyses may be biased by differential amplification (or non-amplification) of target DNA at different concentrations, bearing the risk that PCR analyses focusing exclusively on spores, roots or extra-radical mycelium will fail to detect some important components of the AM community that are weakly represented in the sampled material. Hence, such partial studies may give distorted indications of the composition of the studied communities, and the importance of detected components (Reysenbach et al. 1992; Schmalenberger et al. 2001).

Consequently, a detailed analysis of AMF communities, including assessments of all relevant compartments, i.e. spores and both intra-radical and extra-radical mycelia, is required. Therefore, in the study presented here a molecular approach was used to investigate, in detail, the AMF species composition of the spores, intra-radical mycelia in roots and extra-radical mycelia in the soil of the examined ecosystem, a meadow in central Germany. Following the finding of Hart and Reader (2002b) that there are family-level differences in

AMF colonization strategies, differences in community composition in the AMF spores and two types of mycelia were investigated at this systematic level. Besides providing a more exhaustive characterization of the community, such analyses may provide information on the colonization strategy and life cycle of the different taxa in AMF.

2.2 Experimental procedures

2.2.1 Field site

Soil samples were taken from a farmed meadow (11°37'31"E/50°24'32"N) at an elevation 648 m above sea level in the Thüringer Schiefergebirge (Thuringia, Germany). The soils are flat Haplic Cambisols over presilurian and Devonian schists (Hattenbach 1959). The climate is temperate with an oceanic influence, annual precipitation amounts to 950-1099 mm and the average temperature is 6.0-7.0°C. The potassium, magnesium, sodium, phosphorus and sulphur contents of the soils were 119.37 mg kg^{-1}, 310.33 mg kg^{-1}, 120.38 mg kg^{-1}, 76.28 mg kg^{-1} and 10.39 mg kg^{-1}, respectively. The carbon content was 44.96 mg g^{-1} with a C_{org} fraction of 5.0 wt.-%, and Nitrogen and N_{min} were determined to be 4.21 mg g^{-1} and 5.49 mg kg^{-1}, respectively, resulting in a C/N ratio of 12.46. The soil moisture and pH values were 28.7% and 6.20, respectively. Soil texture is 30.4% sand, 44.8% silt, and 24.8% clay (Börstler et al. 2006).

The vegetation of the site is species-poor (27 plant species in an area of 247 m^2) and plant biomass production reached 640 g dry weight m^{-2} at the beginning of June due to the dominance of highly productive grasses (Kahmen et al. 2005b).

2.2.2 Root, spore and soil sampling from the field

Three independent soil samples (20 cm × 20 cm and 10 cm depth) were taken on July 23, 2004. Soil for DNA and spore extraction was sieved (2 mm) and stored at -20°C. The entire root systems of *Dactylis glomerata* L., *Lolium perenne* L., *Trifolium pratense* L. and *Trifolium repens* L., plants which were abundant at the field site, were excavated and fine roots were fixed in formaldehyde-acetic acid (FAA): 6.0% formaldehyde, 2.3% glacial acetic acid, 45.8% ethanol, 45.9% H_2O (v/v).

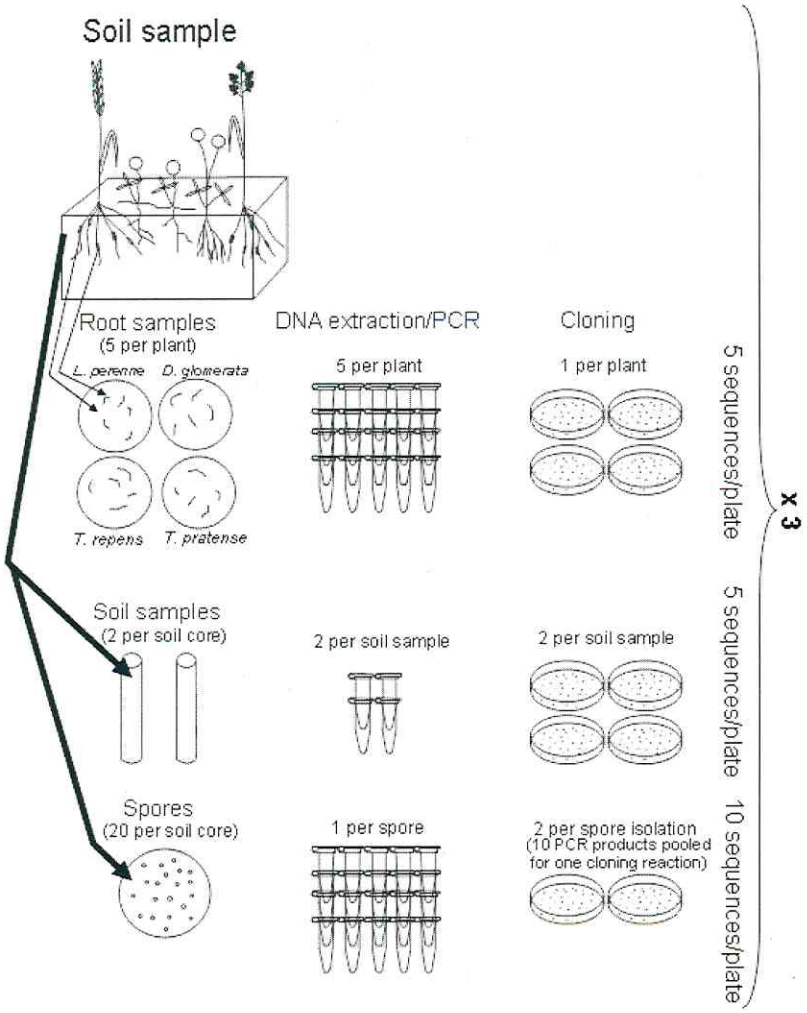

Figure 4. Schematic diagram of the experimental design.

2.2.3 DNA extraction and nested PCR

Genomic DNA was extracted from two 0.5 g sub samples of each soil sample using a FastDNA™ Spin kit for soil (Q-BIOgene, Heidelberg, Germany) as described by Luis et al.(2004) , and from five fixed, 2 cm long fragments of the root system of each plant species using a DNeasy Plant Mini Kit following the manufacturer's recommendations (Quiagen, Hilden, Germany). To amplify DNA from single AMF spores, these were isolated from soils following the protocol of Esch et al. (1994) and separated in a drop of sterile water. The water was removed, then each spore was crushed, pipetted with 20 µl of the PCR-Mix described

below and used directly for PCR (Fig. 4). To amplify the ITS region the nested PCR technique described by Renker et al. (2003) with LSU-Glom1/SSU-Glom1 as AMF-specific primers for the first amplification step and the general fungal primers ITS5/ITS4 for the second step was used. In contrast to Renker et al. (2003) no AluI restriction digestion between the PCR amplifications was included in order to avoid losses of AMF sequences containing this restriction site. For soil DNA, the second PCR step was not necessary since the first step generated sufficient amounts of products.

In addition, soil and root LSU-Glom1/SSU-Glom1 PCR products were amplified using the family-specific primer pair GOCC56/GOCC427 (Millner et al. 2001) for Paraglomeraceae since the abundance of this family appeared to strongly differ between soil and roots (see 2.3 Results). The annealing temperature was raised to 58°C when these primers were used to increase specificity, according to tests using previously sequenced vectors from all obtained families.

2.2.4 Cloning and sequencing

All PCR products were purified by gel electrophoresis and cleaned using a QIAEX® II Gel Extraction Kit (Quiagen, Hilden, Germany). These products were then cloned into the pCR4-Topo vector following the protocol recommended by the manufacturer of the TOPO TA Cloning Kit (Invitrogen Life Technologies, Karlsruhe, Germany) and transformed into TOP10 Chemically Competent *Escherichia coli*. PCR products from spores were also purified by gel extraction, pooled in groups of 10 and subsequently cloned, resulting in six cloning reactions. This pooling process does not reduce the number of AM-families detected (see Renker et al. 2006) but reduces the number of cloning reactions by the factor 10.

All clones chosen for sequencing were tested for the presence of an insert by PCR. Positive clones were sequenced using an ABI PRISM 3100 Genetic Analyzer and an ABI-PRISM BigDye Terminator V3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, California, USA).

2.2.5 Sequence analyses

In accordance with Cullings and Vogler (1998) and Redecker et al. (1999), 5.8S subunit genes embedded between the ITS1 and ITS2 regions of the 180 generated sequences were aligned manually in BioEdit version 5.0.9 (Hall 1999), resulting in an alignment of 156 putatively homologous sites. Additional reference sequences from GenBank (http://www.ncbi.nlm.nih.gov) were included to augment the alignment. A phylogenetic tree

was generated using MrBayes 3.0b4 (Huelsenbeck and Ronquist 2001), a program for Bayesian inference of phylogeny. A Markov chain Monte Carlo (MCMC) run with four simultaneous chains and 5,000,000 generations was performed. The substitution model chosen was the general time-reversible model with invariable sites and gamma-distributed substitution rates (GTR+I+G). At every five-hundredth generation the tree with the best likelihood score was saved, resulting in 10,000 trees. The first 1,000 trees that failed to reach a stable likelihood score were deleted. Remaining trees were condensed in a majority rule consensus tree using PAUP* 4.0b10 (Swofford 2003). Branch supports were assigned as posterior probabilities on the consensus trees. Only support values higher than 0.94 are shown in the presented tree (Fig. 5). Following Larget and Simon (1999) branch supports lower than 0.95 using Bayesian posterior probabilities are considered non-significant. In addition, a neighbour joining (NJ) analysis was conducted based on the Kimura-2-Parameter model. The confidence of the branching was assessed using 1,000 bootstrap resamplings. For additional confidence a heuristic search for maximum parsimony was calculated with stepwise addition and tree bisection-reconnection (TBR) branch swapping based on 60 parsimony informative positions of the alignment. The confidence of branching was again assessed using 1,000 bootstrap resamplings with stepwise addition and nearest neighbour interchange (NNI) branch swapping. Species separations found in the 5.8S analysis were also confirmed regarding alignments of whole ITS regions (data not shown). A full ITS alignment of all species was impossible due to the high variability within the ITS1 and ITS2 spacer regions.

2.2.6 Calculation of rarefaction and statistical analysis

The diversity of the clones was analyzed by rarefaction analysis (Simberloff 1978) using the analytical approximation algorithm (Hurlbert 1971) embedded in the Analytic Rarefaction freeware program from Steven M. Holland (http://www.uga.edu/strata/software/). Based on the assumption that rarefaction curves generally show an exponential rise to an asymptote, the results were fitted to the formula $y = ax / (b+x)$.

Correspondence analysis (CA) was performed and the results were summarized in an ordination diagram using CANOCO software 4.5 (Microcomputer Power, Ithaca, New York). CA is a multivariate statistical method that allows comparisons of community composition between all samples.

2.3 Results

AM sequences were amplified from the roots, spores and soil present in each of three soil samples, until 60 sequences per compartment had been obtained, resulting in a total of 180 AM sequences (Fig. 4). Three different types of phylogenetic analysis (MrBayes, Neighbour joining and maximum parsimony) were conducted. The analyses resulted in concordant phylogenetic trees allowing us to affiliate the sequences to Glomeromycota (see Fig. 5). Sequences of seven families and species groups within mycorrhizal Glomeromycota were obtained (Paraglomeraceae, Archaeosporaceae, Gigasporacea, *Glomus* group Aa, *Glomus* group Ab, Acaulosporaceae and Diversisporaceae). Diversity rarefaction curves and their respective fits (Fig. 6) indicated that there were diversity plateaus of four, four and two Glomeromycota families in the soil, spore and root compartments, respectively. This extrapolation confirmed that the sampling was sufficient for exhaustive characterization of the present families and species groups in each compartment. Remarkable differences between the AMF families and species groups detected in the three compartments were found (Fig. 7). The root fraction was dominated by sequences corresponding to members of *Glomus* group Ab (85%), while 15% of the sequences belonged to Diversisporaceae. When differentiating between distinct plant species proportions of *Glomus* group Ab versus Diversisporaceae of 80% to 20%, 86% to 14%, 78% to 22% and 100% to 0% in *Dactylis glomerata*, *Lolium perenne*, *Trifolium pratense* and *Trifolium repens* roots were detected, respectively. In the AM spore fraction a higher overall diversity of families and species groups was found. Most spore sequences (61.7%) were affiliated to members of *Glomus* group Ab, nearly a third (31.7%) to *Glomus* group Aa and the others to Acaulosporaceae (5.0%) and Gigasporaceae (1.7%). In contrast to roots, no members of the Diversisporaceae were detected in the spore population. In contrast to the high proportions of *Glomus* group Ab sequences detected in the root and spore samples, only 20% of the sequences detected in the soil appeared to be affiliated to this group. The dominating family in this compartment was Paraglomeraceae, followed by Archaeosporaceae and Gigasporaceae, to which 55.0, 18.3 and 6.7% of the detected sequences were affiliated, respectively. All families and species groups detected in the soil, roots and spores were found in at least two different soil or plant samples (see Table 1).

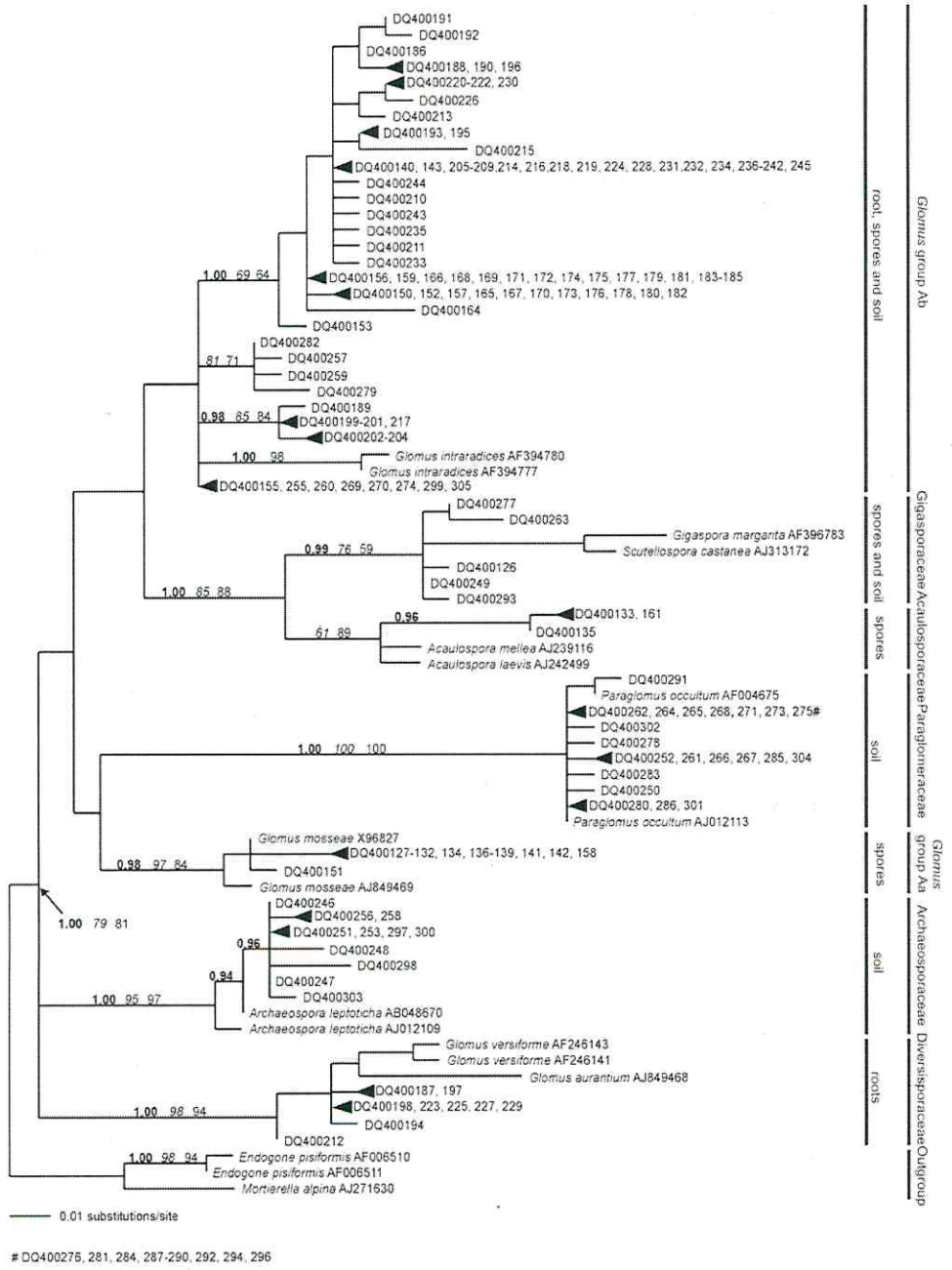

Figure 5 Consensus tree derived from Bayesian phylogenetic inference analysis of 180 5.8S nuclear ribosomal sequences obtained in this study and 18 references sequences obtained from GenBank. Bold numbers above the branches are posterior probabilities derived from 9,000 Markov chain Monte Carlo sampled trees giving an estimate that the respective groups are monophyletic. Numbers in italics are bootstrap values from 1,000 resamplings determined for neighbour joining analysis based on Kimura 2-parameter genetic distances. Non-italicised numbers are bootstrap values from 1,000 resamplings determined for maximum parsimony analysis in the heuristic search mode with simple sequence addition and NNI for branch swapping with consistency and retention indices of 0.633 and 0.906, respectively.

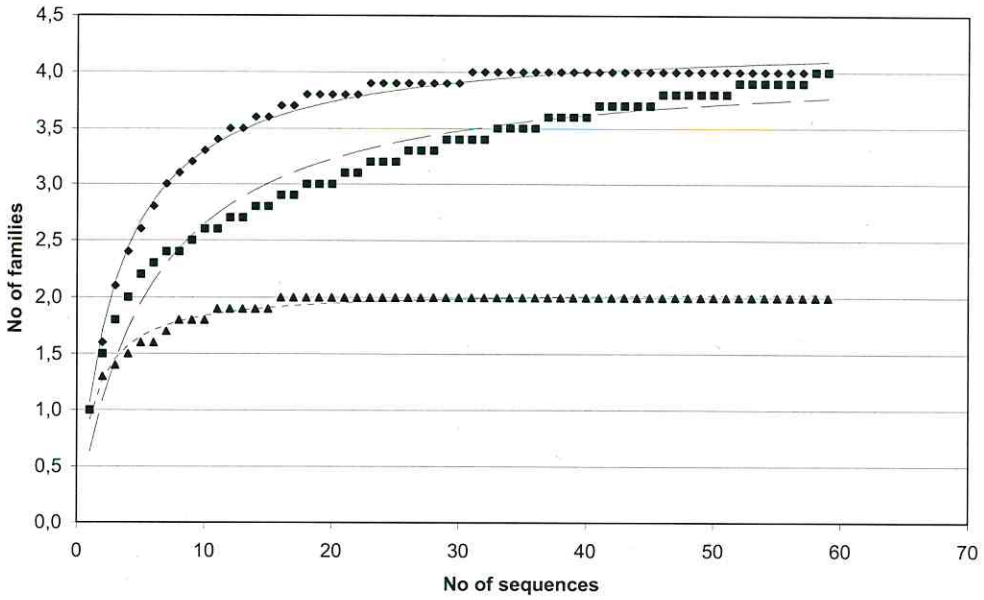

Figure 6 Rarefaction curves and graphs of the fitted formulas for the detected AMF families in soil DNA, root DNA and spores. ◆ rarefaction for soil DNA, ▲ root DNA and ■ for spores. Fitted formulas are as follows; $y = 4.3047x / (3.0452+x)$ ($R^2 = 0.9915$) for soil DNA, $y = 4.1226x / (5.5661+x)$ ($R^2 = 0.9418$) for spores and $y = 2.0722x / (1.2712+x)$ ($R^2 = 0.9652$) for root DNA.

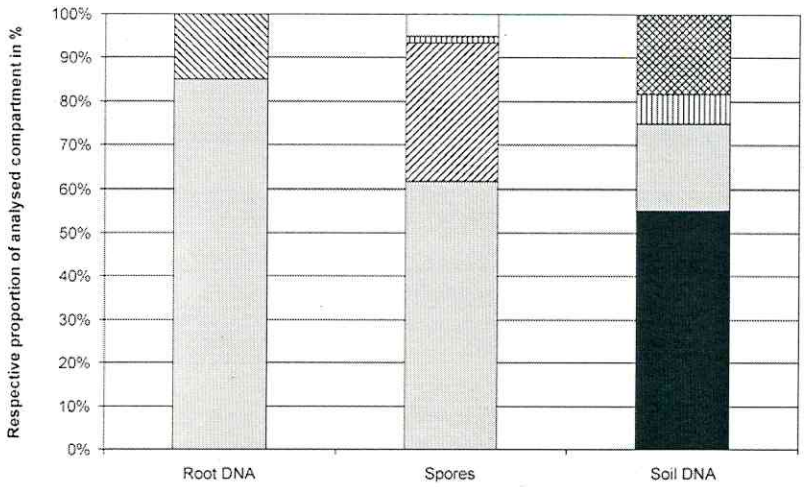

Figure 7 Bar plot showing the relative proportions of the observed families, species groups and genera within the Glomeromycota in the analysed compartments.
□ Acaulosporaceae
⊠ Archaeosporaceae,
▨ Diversisporaceae,
▥ Gigasporaceae,
▨ *Glomus* group Aa,
▩ *Glomus* group Ab,
■ Paraglomeraceae.

The primer pair GOCC56/GOCC427 (Millner et al. 2001) was found to be Paraglomeraceae-specific for the obtained sequences since it only amplified vector inserts from this genus. Nested amplification of LSU-Glom1/SSU-Glom1 products with GOCC56/GOCC427 corroborated the absence of Paraglomeraceae in roots, and confirmed its presence in soil.

To determine the relationship between the source of the respective samples and the AMF families detected, a correspondence analysis (CA) of the AMF communities in the three compartments was carried out using all 180 sequences and the results were displayed in a biplot. The distance between points in this two-dimensional space is related to the difference in composition of the respective AM communities they represent. The communities of the plant root and soil compartments grouped separately, while the AM spore community displayed a wider distribution, clearly overlapping with the plant root communities (Fig. 8). The first two axes explained 71% of the sample variation.

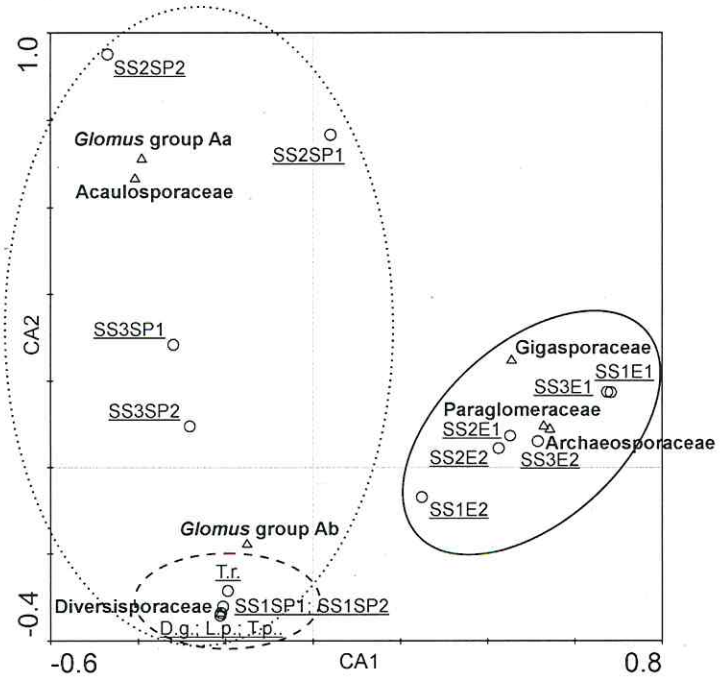

Figure 8. Correspondence analysis of the AM communities of soil DNA, mycorrhizal plant roots and spores. The eigenvalues of the 1st and 2nd axes in the two-dimensional ordination diagrams are as follows: CA1 = 0.77 and CA2 = 0.62. Circles stand for the respective sample (underlined labels), rectangles for the AM families and species groups (labels in bold). The ovals with the solid line, dashed line and dotted line represent the soil samples, root samples and spore samples, respectively. Abbreviations: SS, soil sample; E, soil DNA extract; SP, pool of 10 PCR products obtained from spores; D.g., *Dactylis glomerata*, L.p., *Lolium perenne*, T.p., *Trifolium pratense*, T.r., *Trifolium repens*. The first two axes explain 71% of the sample variation.

Table 1. Distribution of all detected families and species groups of AMF in soil, spores and roots of four plant species (SS, soil sample; E, soil DNA extract; SP, pool of 10 PCR products obtained from spores). Numbers give the relative distribution [%] of the family or sequence group in the respective sample; values sum to 100% within each row. Shading indicates the proportion: light grey < 25%, dark grey < 50%, and black > 50%.

Family	SS1 E1	SS1 E2	SS2 E1	SS2 E2	SS3 E1	SS3 E2	SS1 SP1	SS1 SP2	SS2 SP1	SS2 SP2	SS3 SP1	SS3 SP2	*Dactylis glomerata*	*Lolium perenne*	*Trifolium pratense*	*Trifolium repens*
Paraglomeraceae	9.1	6.1	15.2	27.3	30.3	12.1										
Archaeosporaceae	45.5	18.2				36.4										
Gigasporaceae	20.0		20.0	20.0	20.0				20.0							
Glomus group Ab		4.0	2.0	4.1		2.0	12.0	10.0		2.0	6.0	7.0	16.0	6.0	14.0	15.0
Glomus group Aa									5.3	63.2	21.1	10.5				
Acaulosporaceae										66.6		33.3				
Diversisporaceae													44.4	11.1	44.4	

2.4 Discussion

In this study the diversity of AMF in roots, soils and spores at the AMF family/species group level were compared, since large differences were detected, even at this low level of taxonomic resolution. Furthermore, the validity of a differentiation at species level based solely on sequences from environmental samples would have been debatable (see Wubet et al. 2003; Börstler et al. 2006 and discussion therein).

Validation of the results requires critical consideration of three methodological points. First, since AMF spores were not removed before extracting DNA from the soil, the possibility that some soil sequences may have originated from spore DNA cannot be excluded. However, this is unlikely since the biomass of AM extra-radical mycelium generally exceeds that of AM spores in soils by factors between 10^2 and 3×10^3 (Johnson et al. 2003b; Gryndler et al. 2006). In addition, no *Glomus* group Aa sequences were detected in the DNA extracted from the soil although they represented nearly a third of the sequences obtained from isolated spores. Furthermore, remaining extra-radical AM hyphae adherent to mycorrhizal roots could have contributed to the AM community detected within the roots. Nevertheless, clear differences in the composition of the intra-and extra-radical AM communities were found, despite these possible sources of "contamination" of specific compartments by representatives of other compartments, indicating that the main findings should be valid.

The second methodological consideration is whether generating 60 sequences from each plot was sufficient to characterize the AMF diversity in roots, spores and the soil, at the taxonomic level examined, i.e. families/species groups. This is a legitimate concern since the AMF community is highly diverse at the investigated site. Of the eight families supported to date in Glomeromycota by molecular data (Schüßler et al. 2001; Walker and Schüßler 2004) seven, represented by a total of 19 taxa, have been found at the site in a screening of spores and roots over a year (Börstler et al. 2006). However, the rarefaction analysis (see Fig. 6) indicated that diversity saturation at the family level was approached in the 60 AM sequences per compartment, and the detected diversity levels corresponded to the expected values, i.e. four, four and two families/species groups for the spore, soil and root communities, respectively (Fig. 7). In addition, the proportions of the detected AMF families/species groups were in the same order in the roots of each of the plant species studied. Such consistency would have been unlikely if the sampling had been weak. Furthermore, the non-detection of Paraglomeraceae and Archaeosporaceae in the root samples is consistent with the results of other molecular studies of AM communities in Central Europe (Daniell et al. 2001;

Vandenkoornhuyse et al. 2002; Gollotte et al. 2004; Scheublin et al. 2004; Renker et al. 2005; Börstler et al. 2006). Thus, the results indicate that the sampling was sufficient to characterize the AMF diversity in roots, spores and the soil at this site.

The third critical point to consider is that the observed differences in diversity could reflect biases in the PCR. A first source of potential bias is that variations in the amounts of AMF DNA in the three kinds of extracts could, in principle, have led to substantial differences in the amplification of target regions. However, Dohrmann and Tebbe (2004) have shown that the proportion of PCR products is stable over a wide range of dilutions of complex template DNA extracted from environmental samples. A second source of potential bias was that nested PCR amplification was used for samples with low DNA contents (spores and roots) while a single PCR amplification was used for samples with high DNA contents (soil DNA). However, the possibility that this had any significant effects can be excluded *a priori* since the same AMF-specific primer set was used both in the first step of the nested PCR and in the single PCR, and the ITS primers used in the second step of the nested PCR added no further specificity (cf. Renker et al. 2003). In addition, it was previously shown that pooling different PCR products for a common cloning reaction (see Fig. 4) is a time- and money-saving procedure that does not affect the level of detected diversity provided sufficient clones are sequenced (Renker et al. 2006).

The correspondence analysis revealed a distinct clustering of the AM communities in soils and roots (Fig. 8). The AM spore community showed a wider distribution in the CA biplot, since the spore samples were more heterogeneous, and it strongly overlapped with the community in roots. These results are largely consistent with the distribution of the respective families in the three compartments (Table 1). The large overlap of the communities in root and spore fractions and wider diversity in the spore fraction is in accordance with the findings of Johnson et al. (2003a), who postulated that roots only recruit a fraction of the AMF taxa pool present as spores in soils. The large discrepancy in AM community composition between (i) the extra-radical mycelium fraction and (ii) the spore and root fractions is the most striking finding of the study. As described by Hart and Reader (2002b) for Glomeraceae in general, members of *Glomus* group Ab were found to dominate in DNA extracted from roots but to be rare in DNA extracted from soil, suggesting that this group may generally extend short distances in soils relative to its abundance in roots. Paraglomeraceae were detected in soils and strong indications of their absence in roots were obtained using the specific primer pair GOCC56/GOCC427 (Millner et al. 2001). The apparent absence of Paraglomeraceae in roots raises questions about whether these fungi are able to grow independently of hosts, a

hypothesis that is supported by findings of Hildebrandt et al. (2006) demonstrating the host-independent formation of fertile spores by *Glomus intraradices* in the presence of soil bacteria of the genus *Paenibacillus*. Alternatively, Paraglomeraceae may display strong host specificity and simply be incompatible with the four examined plant species. This second explanation is weakened by numerous reports of at least 36 different host plants from 16 plant families for Paraglomeraceae species (Appendix Table I). Thus, the apparent absence of Paraglomeraceae in all 60 studied root fragments, in conjunction with its contemporaneous presence in all soil samples, may be interpreted as a further indication that arbuscular mycorrhizal fungi are not necessarily obligate symbionts of plants. Although they are of course capable of forming arbuscular mycorrhiza (see Table I), they seem to be able to live in soils without a plant host. Therefore, the results provide additional indications that AMF may have facultative mutualistic life cycles.

Chapter 3: Interactive effects of mycorrhizae and a root hemiparasite on plant community productivity and diversity

3.1 Introduction

Plant diversity and community structure are affected by a large variety of antagonistic and mutualistic interactions. Thus, a comprehensive understanding of the mechanisms determining plant community structure and ecosystem properties requires that interactive effects between different antagonists and mutualists are taken into account (Morris et al. 2007). Antagonistic interactions such as between plants and their herbivores, pathogens and parasites may generate negative feedbacks on plant species abundance, and are therefore known as important drivers for maintaining plant diversity (Tilman and Pacala 1993; Chesson 2000). This is particularly true when plant antagonists exhibit a certain degree of host specialization (Huston 1994). In contrast, mutualistic interactions have long been thought not to generate such negative feedbacks and therefore, they were not expected to be important for the maintenance of diversity (May 1974). However, Bever (1999; 2002) demonstrated that negative feedbacks can also result from mutualistic interactions, in particular when plants are associated with arbuscular mycorrhizal fungi (AMF). A precondition for mutualism to maintain diversity is that the net benefit for both plants and fungi varies across pairs of interacting species (Bever 1999).

Arbuscular mycorrhiza is one of the most common symbioses worldwide (Smith and Read 1997) and an important player for the dynamics of plant communities (Hartnett and Wilson 2002; Koide and Dickie 2002). Results from several studies show that AMF may influence structure and diversity (van der Heijden et al. 1998a), productivity (van der Heijden et al. 1998b; Klironomos et al. 2000) and invasibility (Callaway et al. 2004) of plant communities, and may also affect plant-parasite interactions (Sanders et al. 1993b). However, the association with mycorrhizal fungi is not always favourable to plants, as the effects of AMF can range from benefit to detriment (Johnson et al. 1997). Several studies indicate that these responses depended on the identity of both, the AMF and the plant species (e.g. van der Heijden et al. 1998b; Klironomos 2003). Furthermore, it has been shown that different AMF can exert a functional complementarity (Maherali and Klironomos 2007; Jansa et al. 2008) and therefore an AMF mixture may be more beneficial for host plants than any of the AMF species separately (Koide 2000; Gustafson and Casper 2006).

Not all plants form associations with mycorrhizal fungi and one group that is often considered to be non-mycorrhizal comprises parasitic plants (Brundrett 2004; but see also Li and Guan 2007). Hemiparasites are common species in many nutrient-poor and species-rich

types of grassland, in particular root hemiparasites of the family Orobanchaceae. The species from this group are photosynthetically active to varying degrees and attack their hosts with special contact organs called haustoria. Haustoria are produced by the roots and create vascular continuity of the xylem between host and parasite (Kuijt 1969). Although parasitic plants in grasslands are generally not host--specific (Matthies 1996; Prati et al. 1997), they may exert negative feedbacks by attacking locally abundant plant species and may thereby affect the structure of grassland communities (Press and Phoenix 2005; Bardgett et al. 2006). In addition, as parasitic plants are characterized by high transpiration rates and low water use efficiencies, they also influence the functioning of grassland ecosystems (Press and Phoenix 2005). For instance, the root hemiparasite *Rhinanthus minor* L. has been shown to reduce productivity in grasslands and increase diversity by suppressing dominant grasses (Davies et al. 1997; Pywell et al. 2004; Ameloot et al. 2005). As a result, plant diversity may increase because subdominant forbs benefit from released competitive release. Therefore parasitic plants are increasingly used as a tool for the restoration of grassland ecosystems (Bullock and Pywell 2005; Westbury et al. 2006).

Experimental studies have shown that the mycorrhizal status of a host plant can affect the performance of attached parasitic and hemiparasitic plants (Sanders et al. 1993a; Davies and Graves 1998; Salonen et al. 2001). However, until now, no study has examined the interactive effects of mycorrhizal fungi and hemiparasitic plants on the structure, diversity and functioning of plant communities. When investigating such complex ecological interactions, it is important to account for indirect effects (Morris et al. 2007), in particular mediated by competition among plants (Schädler et al. 2003). The aim of this study was to address this question using two complementary greenhouse experiments. Specifically, it was asked: (1) How does a hemiparasitic plant affect a grassland community, and how is its impact affected by AMF? (2) How do different AMF treatments influence a grassland community? (3) How is a hemiparasitic plant affected by different AMFtreatments? (4) How important are indirect effects when analyzing mycorrhizal effects on a plant community?

In the first experiment, experimental grassland plant communities were either inoculated with two different AMF species (*Glomus intraradices* and *Gigaspora margarita*), with the combination of the two species, or with a field-sampled inoculum. It was hypothesized that inoculation with AMF should generally lead to an increased productivity and diversity of the plant community. Because of functional complementarity, the combination of the two AMF species should produce a stronger effect compared with the single species treatments. Productivity and diversity of the plant community were expected to

be highest when treated with the field-sampled inoculum, which should contain the most complex AMF community. Since AMF usually increase the nutrient status of host plants (Smith and Read 1997) and since hemiparasites of the genus *Rhinanthus* take nutrients from their hosts (Klaren and Janssen 1978), it was hypothesized that *R. minor* should benefit from AMF inoculation of host plants in terms of increased growth and reproduction. Such an effect has been shown, so far, only for *Lolium perenne* inoculated with AMF (Davies and Graves 1998) but not for whole plant communities. It was also expected that nutrient stress of host plants induced by *R. minor* should be mitigated by the different AMF treatments to a varying degree. To distinguish direct AMF effects from indirect effects via plant competition a second experiment was set up, in which all plant species of the first experiment were grown without competition and the same treatments were applied as above.

3.2 Materials and Methods

3.2.1 Study system

The plant material and soil used for the experiments were obtained from a mountain hay meadow in the Franconian Forrest, a plateau-like mountain range in Central Germany. The grassland is situated at an elevation of 606 m a.s.l. (11°26'44''E/50°23'04''N) at an elevation of 606 m above sea level. It has not been fertilized or grazed for at least the past 15 years and was mown once per year in mid-July. The bedrock material consists mainly of schist and graywacke, and produces a carbonate free, nutrient poor soil, categorized as Stagnic Cambisol (siltic). Diversity at this site is about 35 vascular plant species per square meter. Based on species composition the plant community is phytosociologically classified as Geranio-Trisetetum nardetosum Knapp ex Oberd. 1957 (Kahmen et al. 2005b).

The substrate used in the experiments consisted of 50% sieved (1 cm) soil collected from the top 10 cm of the field site and 50% washed silica sand with grain size of 0-2 mm (Mitteldeutsche Baustoffe GmbH, Sennewitz, Germany). To exclude AMF, the substrate was heated for 48 h at 200°C. Part of the sterilized substrate was ground in a mill for chemical analyses. Total carbon and nitrogen contents were measured by high temperature combustion with subsequent gas analysis with an Elementar Vario EL element analyser (Elementar Analysengeräte GmbH, Hanau, Germany), and plant available phosphorus was extracted in double lactate and detected by inductively coupled plasma-atomic emission spectrometry using a Spectro Ciros CCD analyser (SPECTRO Analytical Instruments GmbH, Kleve, Germany). Analyses prior to the experiments showed that the sterilized substrate was

generally low in nutrients with a pH (H_2O) of 6.58, 0.48% organic C, 0.10% N and 36.85 $\mu g\ g^{-1}$ plant available P.

Seven perennial grassland species common at the field site were selected for the experiments (Table 2). Seeds of two grasses (*Festuca rubra*; *Holcus lanatus*), two legumes (*Trifolium pratense*; *Vicia craca*), two mycorrhizal forbs (*Plantago lanceolata*; *Veronica chamaedrys*) and one non-mycorrhizal forb (*Rumex acetosa*) were collected from populations at the field site. For plant species of which not enough seeds could be collected in the field, commercially available material was used (Rieger Hofmann GmbH, Germany). All seeds were sown into trays containing heat sterilized substrate and germinated. Seedlings were then grown in a greenhouse for 4 weeks with a 14 h/10 h day/night cycle at 18°C/13°C.

As hemiparasitic plant *Rhinanthus minor* L. (Orobanchaceae) was chosen, a facultative annual root hemiparasite which grows in natural and semi-natural grasslands throughout Europe and North America (Westbury 2004). It has a wide host range of at least 50 plant species from 18 different families, preferably fast-growing grasses and legumes (Gibson and Watkinson 1989; 1991). *R. minor* is very abundant at the field site where seeds were collected. To break dormancy, *R. minor* seeds were placed on moist filter paper in Petri-dishes and stratified at 5°C for three months in darkness until germination (Gibson and Watkinson 1991).

Two species of AMF were selected, *Gigaspora margarita* (isolate Isol UPLB/PH), and *Glomus intraradices* (isolate BEG 140), both species occur naturally in the study area (Börstler et al. 2006; Hempel et al. 2007; see also chapter 2). These fungi were bought from a commercial supplier (SYMbio-M®, Lanškroun, Czech Republic). In addition, a field-sampled inoculum, consisting of roots including all plant species used in the experiment was collected. For this purpose, roots from approximately 10 L soil collected at the field site were washed free of soil, cut into pieces of approximately 1 cm length and added as inoculum. This field inoculum contained AMF, but presumably also saprophytes, pathogens and their antagonists.

3.2.2 Experiment 1: Multi-species experiment

3.2.2.1 Experimental set-up and design

To test the interactive effect of AMF and parasitic plants on plant communities, a fully factorial experiment was set up as a randomized block design. The plant community comprised seven species (Table 2) which were planted together in mesocosms. The fungal treatments contained five levels: plant communities either inoculated with each of the commercial AM strains (*Gi. margarita* or *G. intraradices*) alone, a mixture of both strains, the

field-sampled mycorrhiza or left uninoculated as non-mycorrhizal control treatment. The parasite treatment consisted of two levels, either with or without eleven individuals of *R. minor* per mesocosm. Each treatment combination was replicated 10 times, resulting in 100 mesocosm. The mesocosms were grouped into ten randomized blocks, each of them containing one replicate of each treatment, and placed in a greenhouse chamber.

Each mesocosm (27 cm x 17 cm x 21.5 cm deep) was filled with a 3 cm layer of expanded slate for drainage, a 3 cm layer of washed sand and a 14 cm layer of the substrate. All materials were heat sterilized at 200°C for 2 days prior to use. For each fungal species of the commercially available AM inoculum 160 g were placed 4 cm below the soil surface (80 g per species in the mixture). For the field-sampled treatment the respective pots received 32 g cut roots. To establish a natural microbial community, all mesocosms were irrigated with 35 ml suspensions of the field soil filtered through a filter paper (No. 4, Whatman International Ltd., Kent, UK) to exclude AM propagules.

Two seedlings of each plant species (4 weeks old) were randomly planted into each mesocosm. During the first two weeks dead seedlings were replaced by seedlings of the same age that were grown in sterile substrate. The communities were allowed to establish during six weeks with temperatures between 15°C at daytime and 10°C at night at ambient light conditions. Afterwards temperatures ranged between 20°C (14 h day) and 13°C (night) and additional light was provided by 400 W lamps. The plants were watered three times a week but did not receive any fertilizer. After 15 weeks, all plants were cut 3 cm above the surface and eleven *R. minor* seedlings with established cotyledons and a 1-2 cm long root were transplanted to half of the mesocosms. This procedure was carried out in order to satisfy the importance of well developed host roots for a successful parasitism by *R. minor* (Saona 2002 cit. in Westbury 2004). During the first week, dead hemiparasite seedlings were replaced. After this transplanting event, the experiment continued for eight weeks until final harvest.

3.2.2.2 Data collection

At the final harvest, all plants were cut above the soil surface. Aboveground biomass was sorted by species and dried for two days at 60°C before weighing. Total biomass (not including the first cut after 15 weeks) was used as an estimate of total aboveground net productivity per mesocosm, but biomass of *R. minor* was excluded.

To quantify plant species diversity, expressed as evenness, the Shannon index was calculated using the function: $HE' = \frac{-\sum(p_i)(\ln p_i)}{\ln S}$,

where $p_i = m_i/M$, m_i is the aboveground biomass for species i, M is the total aboveground biomass of the community, and S is the number of species of the community. Again *R. minor* was excluded from the calculation.

Mycorrhizal dependency of each plant species was calculated by (1-[$bn/\sum a$]) x 100%, when a > b, and by (-1+[$\sum a/bn$]) x 100%, when a < b,

where a is the mean dry mass of a treatment containing AMF, b is the mean dry mass of the non-mycorrhizal treatment, n is the number of treatments containing AMF (van der Heijden 2002). Mycorrhizal dependency was calculated over all AMF treatments.

Dried aboveground biomass of each plant species from five randomly selected mesocosms per treatment that included the hemiparasite was ground in a ball mill. The phosphorus concentrations were determined by the molybdate blue ascorbic acid method (Watanabe and Olsen 1965) after combusting the samples at 550°C and dissolving the ash with 4 N nitric acid. Total nitrogen content was measured with an Elementar Vario EL element analyser (Elementar Analysengeräte GmbH, Hanau, Germany). The sum of individual P and N content of each plant species per mesocosm was calculated and gave the total P and N content per mesocosm.

It was not possible to separate the roots of the different species and therefore root biomass as well as the amount of mycorrhizal colonization was not quantified. Instead, a mixed root sample from three soil cores (diameter: 2 cm, depth: 12 cm) was taken per mesocosm, washed free of soil and stored in formaldehyde-acetic acid (FAA, 6.0% formaldehyde, 2.3% glacial acetic acid, 45.8% ethanol, 45.9% H_2O (v/v)). These root samples were used to record presence or absence of arbuscules and vesicles.

3.2.3 Experiment 2: Single-species experiment

3.2.3.1 Experimental set-up and design

The single-species experiment was set up in order to distinguish direct effects of AMF on the plants from indirect effects via plant competition. To investigate the response of the plant species grown without competition, a split-plot experiment was set up consisting of the seven plant species, five fungal treatments and two parasite treatments. For each plant species, single individuals were grown with the same five fungal treatments as described for the multi-species experiment. The parasite treatment consisted of two levels, either with or without one

individual of *R. minor*. Additionally, *R. minor* was grown without host plant but with the same fungal treatments. Each treatment combination was replicated ten times resulting in a total of 750 pots. Fifteen pots with the same fungal treatment were placed together in a tray, resulting in ten trays per fungal treatment. Thus, the fungal treatment represented the plot-level treatment whereas plant species and the parasite treatment represented the within-plot treatments. The trays were randomly positioned in ten blocks.

The pots (9 cm diameter, 10 cm height) were filled with slate and sand for drainage as well as soil accordingly to the mesocosms. The pots received 20 g per fungal species of the commercially available AM inoculum, 10 g per AMF species in the mixture, and 6 g cut roots for the field treatment. The experiment was proceeded according to the multi-species experiment concerning growing and transplanting the plant species as well as the hemiparasite.

Ten weeks after transplantation of hemiparasite seedlings, all plants were harvested and separated in above- and belowground biomass.

3.2.3.2 Data collection

Aboveground biomass was dried for two days at 60°C and weighed. To quantify belowground biomass, roots were washed free of soil, dried for three days at 60°C and weighed. To quantify the mycorrhizal status, a subsample of 2 g fresh mass of roots per species was stored in FAA. Root samples were dyed using the staining procedure of Vierheilig et al. (1998). Mycorrhizal colonization of host roots was determined for each species ($n = 4$). Percent colonization of root length was determined with a Zeiss Axioplan light microscope (Zeiss, Oberkochen, Germany) using the line intersect method based on at least 300 segments per root sample (Ambler and Young 1977; modified after Schmitz et al. 1991).

3.2.4 Statistical analysis

The statistical software SAS version 9.1 (SAS Institute, Cary, NC, USA) was used for all analyses. Biomass data were log-transformed prior to analysis. To test the effects of AMF treatment and parasitic plants on aboveground biomass and on evenness of the plant communities a three-way analysis of variance (ANOVA) was used, with block, parasite and AMF treatment as main factors (PROC GLM). Additionally, the effect of AMF treatment on *R. minor* biomass and on nutrient contents of the plant communities was analyzed accordingly in a two-way ANOVA with block and AMF treatment as factors. Data of the single species experiment were analyzed using a three-way, split-plot ANOVA, with fungal treatment as

plot factor tested against variation among trays, and host plant and parasite as within-plot factors. Two host plant individuals (*T. pratense*) and 19 individuals of the parasite (*R. minor*) died during the experiment. Because this plant mortality caused the data to be unbalanced, type III sum of squares were used (Shaw and Mitchell-Olds 1993). Differences in arcsine-transformed mycorrhizal colonization rates within each plant species were analyzed by two-way ANOVA and posthoc pairwise comparison of AMF treatments (Tukey´s HSD). In both experiments, separate ANOVAs were performed for each plant species to explicitly characterize fungal effects and to investigate interaction effects in detail. Using orthogonal contrasts, the following four a priori hypothesis were tested: (1) "non-AMF vs. fungi": Plants inoculated with AMF should perform better than plants grown in non-mycorrhizal (non-AMF) soil. (2) "field vs. commercial": Due to niche complementarity and resource partitioning, a more diverse soil microbe community should provide more host benefit compared with the commercially available treatment. (3) "both vs. single": Same as (2), the mixture of *Gi. margarita* and *G. intraradices* should provide more host benefit compared with each single AMF treatment. (4) "giga vs. glom": The two different AMF species should provide different benefits to host plants. The Benjamini-Hochberg method was applied (Verhoeven et al. 2005) to account for multiple comparisons. In general, belowground biomass and aboveground biomass of host plants grown in the single-species experiment responded similarly to AMF treatments. Thus, only data for aboveground biomass of host plants are presented.

To investigate to what extent the response of individual plant species to AMF was mediated by indirect effects, e.g. competition, the species' response in the mesocosms with their response in the single-species experiment was compared. For this purpose, the log response ratios (Hedges et al. 1999) were calculated for plant species in the two experiments separately as $\ln RR = \ln(B_{fungi}/B_{non\text{-}AMF})$, where B_{fungi} represents aboveground biomass of a plant species grown in the respective fungal treatment and $B_{non\text{-}AMF}$ represents aboveground biomass of a plant species grown in the non-AMF treatment. This index has recently been used to quantify competitive interactions between plants (Goldberg et al. 1999; Weigelt et al. 2005). Linear regressions of the lnRR of plants in the multi-species experiment versus the lnRR of singly grown plants were calculated, and the coefficient of determination was used to characterize the relative importance of indirect effects of AMF on plant species grown in mixture. If indirect effects are weak, species' response in mixture should be highly predictable by their response when grown singly, as indicated by a large R^2. If indirect effects are strong, their response should be less predictable and R^2 should be low.

3.3 Results

3.3.1 Mycorrhizal colonization

At the end of both experiments, no AMF structures were found in the non-AMF treatments, whereas all plants growing in the different AMF treatments were infected by AMF, indicating successful inoculation and absence of contamination.

The degree of total mycorrhizal colonization determined in the single-species experiment varied between the seven plant species ($F_{6, 84} = 53.99$, $P < 0.001$) and the different AMF treatments ($F_{3, 84} = 26.35$, $P < 0.001$, Table 2). Highest degree of colonization by arbuscules was found in *V. chamaedrys* inoculated with *G. intraradices* (23.5%). *R. acetosa* was confirmed as a non-mycorrhizal forb with less than 1% mycorrhizal colonization. No mycorrhizal structures were found in the roots of *R. minor*. The only structures found were external hyphae attached to the root surface of *R. minor*.

3.3.2 Effect of AMF on plant – hemiparasite interactions

The effects of AMF and the hemiparasite on total community biomass were dependent on each other, as indicated by a significant AMF x parasite interaction (Table 3). More specifically, *R. minor* did not suppress total community biomass in the non-AMF treatment. In contrast, infection with *R. minor* significantly reduced the total aboveground biomass of AMF inoculated plant communities. The parasitized plant communities produced 22.3% (inoculated with *Gi. margarita*), 21.9% (inoculated with *G. intraradices*), 5.4% (inoculated with both commercial fungi) and 10.3% (inoculated with field-sampled mycorrhiza) less biomass than the respective non-parasitized plant communities (Fig. 9a). This reduction was obviously due to a significantly negative impact of *R. minor* on the grass *H. lanatus*, as the biomass of other plant species was not significantly reduced by parasitism of *R. minor* (Tab. 3). Averaged over all AMF treatments, *H. lanatus* parasitized by *R. minor* produced 20.1% less biomass than unparasitized plants.

The diversity of the communities, expressed as evenness, was not affected by the *R. minor*, but it was positively affected by AMF inoculation (Table3). Evenness in the non-AMF treatment was significantly lower compared to the AMF treatments and it was highest in the treatment with both commercial AM strains (Fig. 9b). In contrast to biomass, this effect was independent of the hemiparasite treatment.

Table 2 Investigated plant species and their degree of mycorrhizal colonization. Arbuscules (A) and total colonization rates (T) including colonization by hyphae, vesicles and arbuscules in the roots of plants grown in the single species experiment were recorded (mean ± se, n = 4). Within each plant species, AMF treatments with different letters are significantly different in percent colonization of (A) or (T) by Tukey's post-hoc test results at $P < 0.05$. The non-AMF treatment was excluded from the comparisons as no mycorrhizal structures were observed. Mycorrhizal dependency was calculated for each plant species grown in the multi-species experiment not including the hemiparasite treatment (see statistical analysis in paragraph 3.2 Materials and Methods). For calculation of mycorrhizal dependency of *R. minor* mesocosms without the hemiparasite were excluded.

Plant species (Family)	Origin of seeds	Percent colonization of root length depending on mycorrhizal treatment								Mycorrhizal dependency
		Gigaspora margarita		*Glomus intraradices*		both		field sampled		
		A	T	A	T	A	T	A	T	
Rumex acetosa (Polygonaceae)	purchased	0.8±0.1^{a}	4.4±2.7^{a}	0.6±0.2^{a}	4.5±1.4^{a}	0.7±0.2^{a}	8.3±1.6^{a}	0.0±0.0^{a}	1.6±0.3^{a}	0.00
Veronica chamaedrys (Plantaginaceae)	purchased	13.5±2.3^{a}	37.2±9.0^{a}	23.5±2.3^{a}	60.8±5.3^{a}	22.8±1.9^{a}	51.7±9.6^{a}	20.4±2.0^{a}	44.4±2.4^{a}	0.72
Plantago lanceolata (Plantaginaceae)	field sampled	8.0±0.7^{a}	37.0±2.5^{a}	22.0±2.1^{b}	63.1±6.0^{b}	21.8±1.6^{b}	51.8±5.1^{ab}	10.3±1.1^{ab}	43.8±5.1^{a}	-0.19
Vicia cracca (Fabaceae)	purchased	2.7±0.2^{a}	26.5±2.2^{a}	5.2±0.7^{a}	48.0±1.2^{b}	20.2±2.9^{b}	50.8±7.2^{b}	5.6±0.6^{a}	17.7±6.2^{a}	0.93
Trifolium pratense (Fabaceae)	field sampled	11.7±0.6^{a}	53.7±3.0^{a}	5.1±0.4^{b}	55.5±6.5^{a}	12.7±1.3^{a}	46.0±10.1^{a}	3.2±0.5^{b}	21.3±2.9^{b}	0.94
Festuca rubra (Poaceae)	field sampled	9.6±1.0^{a}	28.5±2.5^{a}	4.0±0.3^{ab}	28.6±2.9^{a}	6.2±1.0^{ab}	29.7±1.8^{a}	2.3±0.4^{b}	10.4±3.3^{b}	0.25
Holcus lanatus (Poaceae)	purchased	6.4±0.9^{a}	20.9±2.9^{a}	3.6±0.7^{a}	16.8±4.4^{a}	5.1±0.6^{a}	15.4±0.6^{a}	3.3±0.6^{a}	5.1±1.7^{a}	-0.27
Rhinanthus minor (Orobanchaceae)	field-sampled	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.82

Table 3 Results of ANOVAs for the multi-species experiment – Plant community diversity and productivity as well as biomass of each plant species responding to different mycorrhizal treatments and parasitism by *R. minor*. Evenness was calculated as Shannon Index. Biomass data are log-transformed prior to analysis. Orthogonal contrasts are calculated for fungal treatments which are described in detail in the material & methods section. F values and significance levels are given after accounting for multiple comparisons with the Benjamini-Hochberg method (line-by-line): * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Source of variation	d.f.	*Evenness*	Total biomass	*R.acetosa*	*V.chamaedrys*	*P. lanceolata*	*V.cracca*	*T. pratense*	*F.rubra*	*H.lanatus*
Block	9	0.88	0.83	1.66	0.26	1.83	0.54	0.88	0.59	2.05
Parasite	1	0.49	12.65 **	0.84	0.02	2.32	0.03	1.31	4.48	6.57 *
Fungi	4	11.73 ***	8.48 ***	1.88	6.85 ***	11.21 ***	18.35 ***	8.75 ***	1.13	9.56 ***
non-AMF vs. fungi	1	34.30***	29.46***	0.14	9.16**	14.41 ***	65.92***	8.90**	2.15	23.27***
field vs. commercial	1	6.22*	4.17*	5.15	7.62*	25.25***	3.89	2.23	0.01	0.24
both vs. single	1	6.27*	0.01	2.00	1.74	5.13	3.03	10.85*	1.83	2.75
giga vs. glom	1	0.13	0.28	0.21	8.87*	0.05	0.57	5.02	0.55	0.16
Fungi*parasite	4	0.45	2.95 *	0.50	0.74	0.62	1.27	1.91	1.07	1.32
Residuals (MS)	81	(0.005)	(0.03)	(0.21)	(0.07)	(0.38)	(0.83)	(0.22)	(0.37)	(0.13)

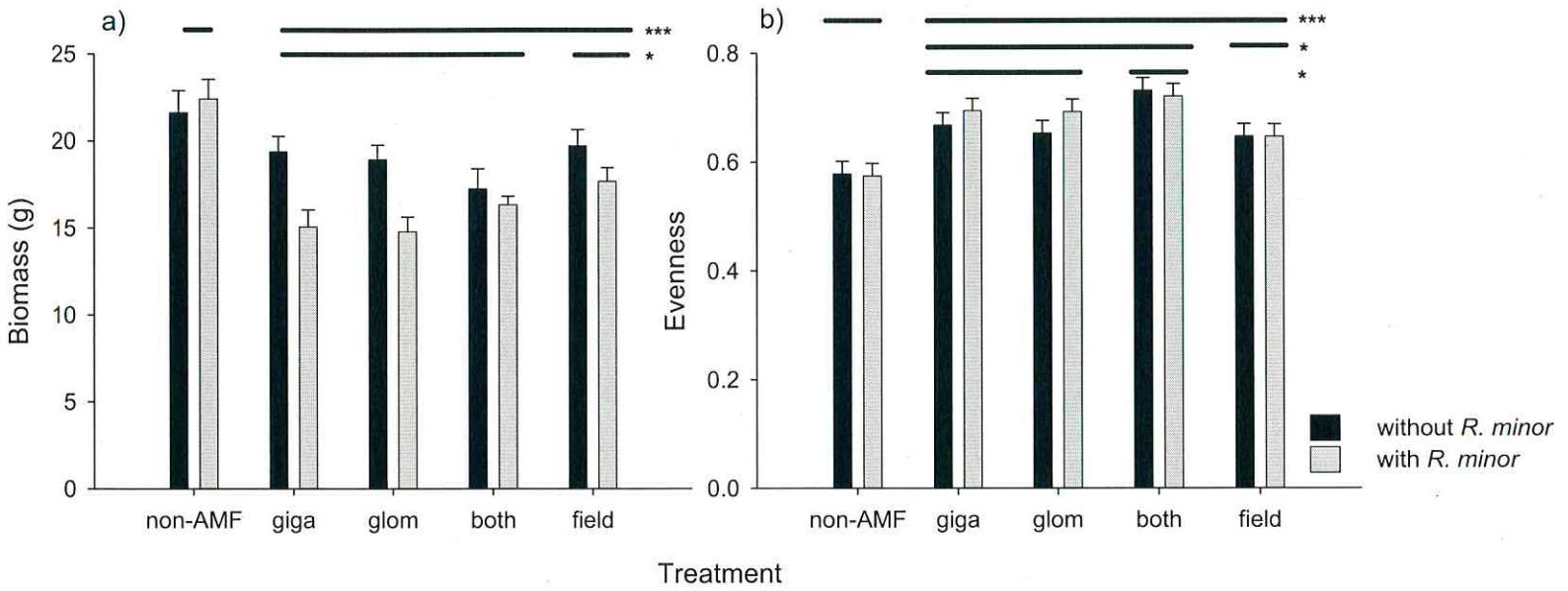

Figure 9 Biomass (a) and evenness (b) of plant communities (mean + se) responding to different mycorrhizal treatments and parasitism by *R. minor*. Community grown without AMF (non-AMF), or inoculated either with *Gi. margarita* (giga), *G. intraradices* (glom), a mixture of *Gi. margarita* and *G. intraradices* (both), field sampled mycorrhiza (field). Horizontal lines above bars indicate a significant difference between AMF treatments (averaged over both hemiparasite treatments), according to linear contrasts and accounting for multiple comparisons with the Benjamini-Hochberg method. ***: $P < 0.001$; *: $P < 0.05$.

3.3.3 Response of the grassland community to different AMF treatments

Even though changes in plant community biomass were influenced by interactive effects of AMF and *R. minor*, the direction of the response to AMF inoculation was the same (Fig. 9a): Total community biomass decreased in the AMF inoculation treatments, whereby the negative effect of the two commercial AM strains was stronger than the one of the field-sampled AMF (Fig. 9a). The significant biomass increase in the non-AMF treatment was related to the high biomass of *H. lanatus* and *P. lanceolata* (Fig. 10). In all treatments, these two species and *R. acetosa* were the dominant ones in the communities, producing more than 85% of the total biomass. The three species were not dependent on AMF as shown by their negative or low or negative mycorrhizal dependency (Table 2), i.e. their growth was not or even negatively influenced by AMF (Fig. 10). Concomitantly, AMF inoculation promoted the growth of the subordinate species *V. chamaedrys, T. pratense* and *V. cracca* which were highly mycorrhizal dependent (Table 2). Contrasts showed that *V. chamaedrys* benefited most from inoculation with *Gi. margarita* and the legume *T. pratense* from inoculation with a mixture of *Gi. margarita* and *G. intraradices*, whereas *V. cracca* benefited similarly from all types of AMF inoculations (Fig. 10, Table 3). In general, association with AMF stimulated mean plant biomass of these three subordinate species across the four AMF treatments compared with the non-AMF treatment. Hence, their contribution to the total biomass of the community increased from 1% to 8% due to AMF inoculation.

Total P content of the community was positively affected by AMF (Fig. 11a). Compared with the non-AMF treatment ($P = 10.13$ mg g^{-1}) the P content increased significantly due to AMF inoculation ($F_{4, 20} = 7.84$, $P < 0.011$). Noteworthy, no significant differences were found between the different AMF treatments. Total N content significantly decreased due to AMF inoculation (Fig. 11b, $F_{4, 20} = 14.95$, $P < 0.001$), with again no significant differences between the different AMF inoculates.

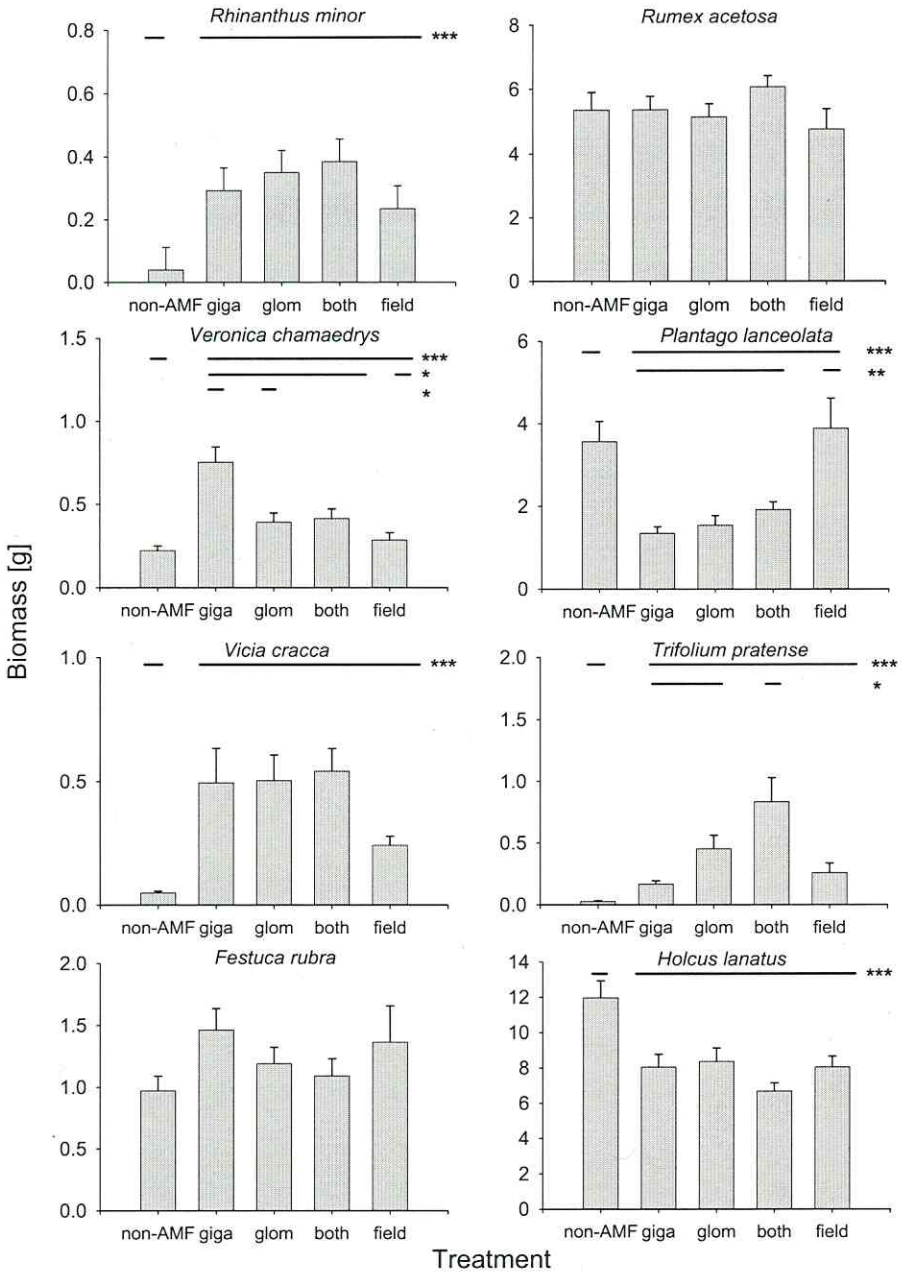

Figure 10 Responses of *Rhinanthus minor* and host plants grown in the mesocosms (mean + se) responding to different mycorrhizal treatments (multi-species experiment). Results are averaged over both hemiparasite treatments. Plants grown without AMF (non-AMF), or inoculated either with *Gi. margarita* (giga), *G. intraradices* (glom), a mixture of *Gi. margarita* and *G. intraradices* (both), field sampled mycorrhiza (field). Horizontal lines above bars indicate a significant difference among AMF treatments, according to linear contrasts and accounting for multiple comparisons with the Benjamini-Hochberg method. ***: $P < 0.001$; **: $P < 0.01$; *: $P < 0.05$.

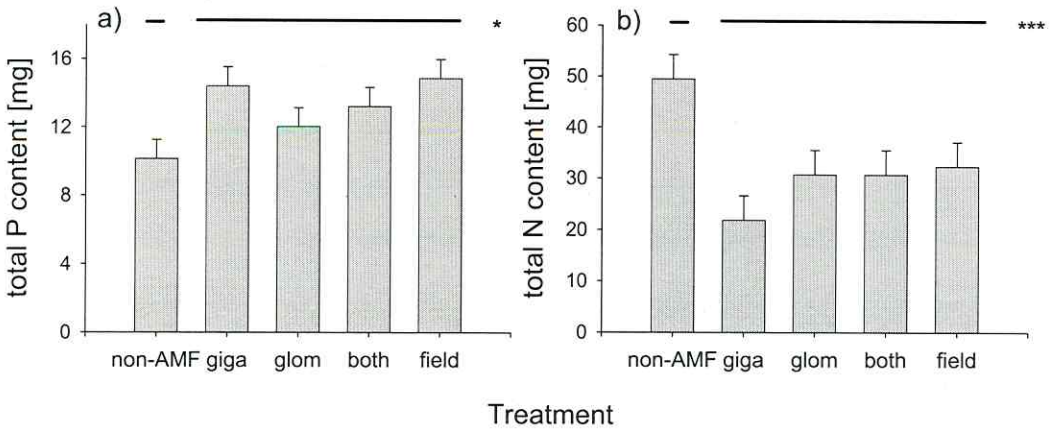

Figure 11 Nutrient contents of plant communities (mean + se) responding to different mycorrhizal treatments. Results are based upon four replicates including the hemiparasite. Plants grown without AMF (non-AMF), or inoculated either with *Gi. margarita* (giga), *G. intraradices* (glom), a mixture of *Gi. margarita* and *G. intraradices* (both), field sampled mycorrhiza. Horizontal lines above bars indicate a significant difference among AMF treatments, according to linear contrasts and accounting for multiple comparisons with the Benjamini-Hochberg method. ***: $P < 0.001$; *: $P < 0.05$.

3.3.4 Response of the hemiparasite to different AMF treatments

Rhinanthus minor was significantly influenced by the AMF treatments (Table 4). When grown in the mesocosms, *R. minor* produced five to ten times more aboveground biomass in the AMF treatments compared with the non-AMF treatment (mycorrhizal dependency of 0.82), but no differences were found between the different AMF treatments (Fig. 10). Also total P content of the hemiparasite was influenced by mycorrhizal inoculation ($F_{4,18} = 3.35$, $P < 0.05$), but it showed a distinct pattern compared with the aboveground biomass of *R. minor*. P content was highest when the parasitized plant communities were inoculated with *Gi. margarita* (4.05 ± 0.29 mg g^{-1}), but it was similar among all other treatments – including the non-AMF treatment – and ranged from of 2.80 mg g^{-1} in the *G. intraradices* treatment to 3.25 mg g^{-1} in the mixture of *Gi. margarita* and *G. intraradices*.

When *R. minor* was grown with host plants in the single-species experiment, significant differences between the AMF types were found (Table 4). The *R. minor* plants attached to host plants inoculated with AMF produced more biomass than those attached to hosts without inoculation, and the highest biomass was produced in the treatment with both commercial AM strains (Appendix Fig. I). Furthermore, *R. minor* benefited from AMF even without host plants, having a mycorrhizal dependency of 0.66 when grown alone.

Only the design of the single-species experiment allowed comparisons between the effects of the different host plant species. The results showed that the growth of *R. minor* was significantly influenced by host plant species (Table 4). *R. minor* grew better on its own than

with *P. lanceolata* and *R. acetosa* as host plants and it grew better when associated to grasses and legumes as host plants (data not shown).

Table 4 Results of ANOVAs on the aboveground biomass of *Rhinanthus minor* (data log transformed prior to analysis) grown in the single-species and in the multi-species experiment. Orthogonal contrasts are calculated for fungal treatments which are described in detail in the material & methods section. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Single-species experiment

Source of variation		d.f.	MS	F	P	
Fungi		4	3.06	12.35	<0.001	***
	non-AMF vs. fungi	1	6.32	25.54	<0.001	***
	field vs. commercial	1	0.87	3.52	0.07	
	both vs. single	1	4.11	16.59	<0.001	***
	giga vs. glom	1	0.91	3.68	0.06	
Tray [fungi]		45	1.33	1.51	0.03	*
Host plant		7	31.57	35.96	<0.001	***
Fungi x Host plant		28	1.78	2.02	0.002	**
Residuals		294	0.88			

Multi-species experiment

Source of variation		d.f.	MS	F	P	
Block		9	0.38	0.34	0.95	
Fungi		4	7.75	7.34	<0.001	***
	non-AMF vs. fungi	1	30.18	30.34	<0.001	***
	field vs. commercial	1	0.08	0.01	0.75	
	both vs. single	1	0.68	0.67	0.42	
	giga vs. glom	1	0.05	0.04	0.83	
Residuals		35	1.06			

3.3.5 Responses of single grown plant species to AMF and importance of indirect effects

The results of the single-species experiment – when the plants were grown without competition – showed that the AMF treatments had a significant effect on plant biomass ($F_{4, 45} = 5.46$, $P = 0.001$). A significant fungi x host plant interaction ($F_{24, 583} = 8.4$, $P < 0.001$) indicated that different plant species showed distinct responses to the five AMF treatments. Detailed responses of plants grown in the single-species experiment to the different AMF treatments are shown in the Appendix (Fig. I, for statistics see Table II).

The single-species experiment was set up in order to distinguish the direct mycorrhizal effects on the plants from indirect effects via plant competition in the community experiment. Using the log response ratio (lnRR) the responses to the different AMF treatments of each plant species in the single-species and in the multi-species experiment were compared. High coefficients of determination between the lnRR of the two experiments showed that plant growth responses without and with competition were roughly similar for three AMF treatments: *G. intraradices* ($R^2 = 0.70$, $P < 0.02$), the mixture of both AMF strains ($R^2 = 0.68$, $P < 0.02$) and the field-sampled AMF treatment ($R^2 = 0.82$, $P < 0.005$). In contrast, when inoculated with *Gi. margarita*, plant responses in mixtures were poorly predictable by their responses when grown without competition ($R^2 = 0.49$, $P > 0.08$), highlighting the importance of indirect effects in plant community responses to inoculation with this AMF type.

The magnitude of indirect effects was also dependent on plant species. For example *V. cracca* responded stronger to AMF in the multi-species experiment compared with non-competitive conditions (Fig. 12). In contrast, response of *P. lanceolata* was weaker in the multi-species experiment when inoculated with *Gi. margarita* or *G. intraradices* compared with its responses without competition.

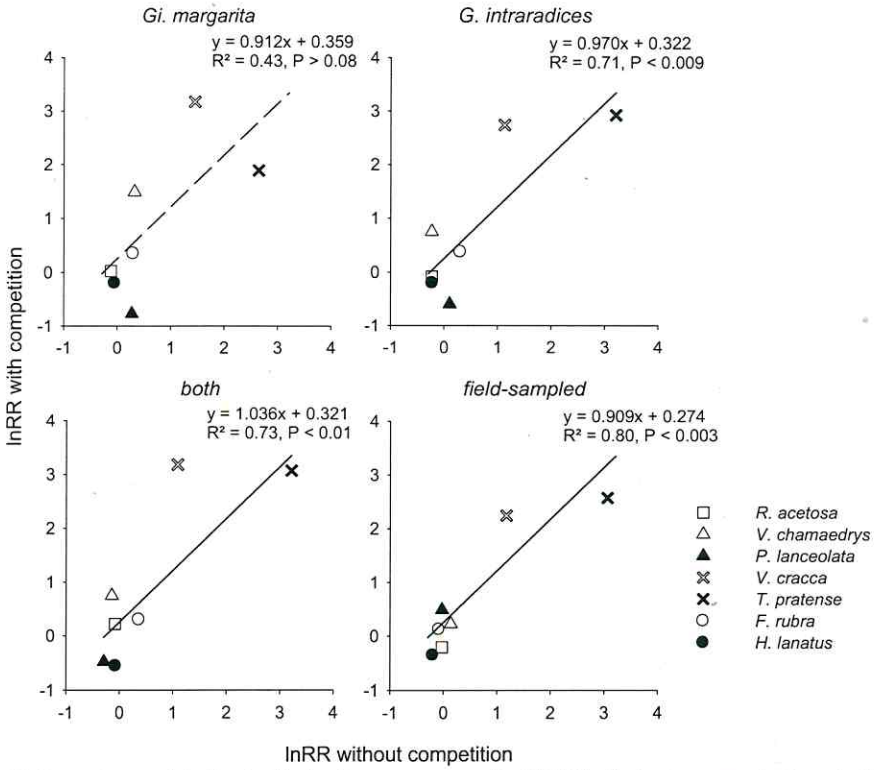

Figure 12 Importance of indirect effects. Log response ratio (lnRR) of plants grown in the single-species experiment and lnRR of plants grown in the multi-species experiment when inoculated with different AMF. Positive residuals are indicating positive indirect effects.

3.4 Discussion

3.4.1 Mycorrhizal fungi induce a negative effect of the hemiparasite on plant community

The most important result was that the negative impact of the hemiparasite on plant community biomass was strongly dependent on the presence or absence of AMF. Infection with the hemiparasite *R. minor* did not have a negative effect on total biomass of the non-mycorrhizal plant communities. When plant communities were grown with AMF, *R. minor* reduced total biomass production of the plant communities, irrespective of the different AMF treatments (Fig. 9a). This reduction was due to a significant growth suppression of the dominant species *H. lanatus*. This confirms results from other studies in which *R. minor* has been shown to parasitize and to suppress the competitively superior grasses, and therefore reduce total biomass of the plant communities (Davies et al. 1997; Bardgett et al. 2006). However, legumes or other dicotyle forbs have been reported not to be negatively affected by *R. minor* (Cameron et al. 2006), which is consistent with our results. In contrast to our initial hypothesis, AMF generated rather than mitigated the negative impact of the hemiparasite on total aboveground biomass of the plant communities.. These findings are in contrast to results obtained in another pot experiment in which the grass *Lolium perenne* L. was infected both with AMF and *R. minor*, and where host responses were not affected by an interaction between AMF and the parasite (Davies and Graves 1998).

Nevertheless, the hemiparasite did not influence the diversity of the plant community. This result is in contrast to previous studies (Bullock and Pywell 2005; Bardgett et al. 2006) showing that parasitism by *R. minor* increases the diversity of grasslands in field and also in mesocosm experiments. This suggests that in this study the AMF are the main driving forces of diversity.

3.4.2 Mycorrhizal dependency of dominant species determines community responses

The investigated plant community was dominated by *R. acetosa* a non-mycorrhizal forb, and *H. lanatus* and *P. lanceolata*, two species which were negatively affected by AMF inoculation. This may explain that the community structure was significantly affected by AMF inoculation. Total community productivity decreased and concomitantly, diversity increased because AMF promoted the growth of subordinate species with a high mycorrhizal dependency. This response is consistent with AMF effects on plant communities dominated by weakly mycotrophic plants (Grime et al. 1987; van der Heijden et al. 1998a), in which plant diversity increases with little effect on community productivity. Especially dominant species in a community and their mycorrhizal dependency or responsiveness are supposed to

play a key role in determining plant community structure (Bergelson and Crawley 1988; van der Heijden 2002). In communities dominated by high mycorrhizal dependent species, suppression of AMF led to an increase in diversity owing to a release of subordinate species from competition (Hartnett and Wilson 1999; O'Connor et al. 2002). The results demonstrate that even in communities dominated by plant species which are independent or negatively affected by mycorrhiza, AMF can lead to an increase in diversity when subordinate or competitive inferior species show a high mycorrhizal dependency.

3.4.3 Hemiparasitic plants profit from mycorrhizal fungi

AMF have been shown to impact parasitic plants both positively (Sanders et al. 1993b; Salonen et al. 2001) or negatively (Lendzemo and Kuyper 2001; Gworgwor and Weber 2003). In the experiments the hemiparasite *R. minor* was positively affected by AMF inoculation. On the one hand, *R. minor* had an indirect relationship to AMF by having increased growth and reproductive output (data not shown) when host plants were inoculated with AMF. Those indirect positive effects were also reported by Davies and Graves (1998), who found that *R. minor* was non-mycorrhizal itself, but profited when its host plants (*Lolium perenne* L.) were mycorrhizal. According to these authors, the stimulation of hemiparasite growth by AMF can be attributed to increases in the availability of carbon or mineral nutrients in the host plants. An increased phosphorus content in host plant tissues due to AMF inoculation was detected (Fig. 11a) but a decreased nitrogen content (Fig. 11b) and no differences in carbon content (data not shown). This suggests that if there is an indirect promoting effect of AMF on hemiparasites, it could be via improved P allocation to the host plants. However, P content of *R. minor* was significantly increased in the *Gi. margarita* treatment only.

On the other hand, direct effects of AMF on the hemiparasite were also found: *R. minor,* grown without host plants in the single-species experiment, was significantly affected by the different types of AMF inoculates, profiting most from the mixture of both commercial fungi and least from the non-mycorrhizal treatment. Notwithstanding these direct effects of AMF on *R. minor*, the hemiparasite has been listed as a non-mycorrhizal plant (Harley and Harley 1987). Recently, *R. minor* has been listed as weakly mycorrhizal (Wang and Qiu 2006). This was based on a survey of three semi-natural grasslands in Norway (Eriksen et al. 2002), where only a few samples of *R. minor* roots were infected with less than 1% mycorrhizal structures (internal hyphae and vesicles). The only fungal structures found in this study were external hyphae attached to the root surface of *R. minor*, which are not a

definite sign for AMF colonization (McGonigle et al. 1990) but can be considered to facilitate nutrient and especially phosphate uptake (Jakobsen et al. 2005). Phosphorus is known to increase the photosynthetic rates of unattached *R. minor* plants (Seel et al. 1993), which could explain the increased biomass. However, the experiments were not designed to elucidate these particular mechanisms.

3.4.4 AMF treatments differ in their effects on the plant community

The hypothesis that a more diverse AMF community leads to higher plant productivity and diversity could not generally be confirmed. Plant community biomass was highest in the non-mycorrhizal control (Fig. 9a). At least, the field-sampled AMF provided significantly more benefit to the plant community than the commercial AMF treatments which might indicate functional complementarity within a diverse field-derived AMF community (Maherali and Klironomos 2007; Jansa et al. 2008). However, diversity was highest for communities inoculated with a combination of both commercial AMF strains, but lower in the field-sampled inoculum compared with any single AMF treatment (Fig. 9b). The variability in these result indicate that functional complementarity among AMF species may become evident in one particular ecosystem trait (productivity) but not in the other (plant diversity).

In the present study, the effects of the different AMF treatments were larger at the level of individual plant species compared with the effects on the whole community. Each plant species was affected in a different way by the respective AMF treatments (Fig. 10). Therefore, it was confirmed that the direction and magnitude of plant growth responses to mycorrhizal colonization is strongly dependent on the specific combination of AMF species and plant species (Klironomos 2003) and the suggestion that a more diverse or complex AMF community should provide more host benefit could not generally be verified. *P. lanceolata* was the only plant species producing the highest biomass in the field-sampled treatment. This result might be interpreted as hint for local adaptation because both, seeds of *P. lanceolata* and AMF, originated from the same grassland site.

Significant differences in plant growth responses to the two AMF species were only detected for *V. chamaedris* which profited most from the single species inoculation with *Gi. margarita* in both experiments. *P. lanceolata* exhibited the same pattern but only in the single-species experiment. Our study does not elucidate the mechanisms responsible for differences in plant growth responses. One explanation might be that different AMF species display different strategies in their association with host plants (Hart and Reader 2002b) and provide different benefits (Johnson 1993). For example, AMF of the family Gigasporaceae

were shown to form large extraradical mycelia whereas members of the genus *Glomus* do obviously form a large intraradical mycelium (Hart and Reader 2002b). These differences in mycelia size and location are likely to affect host plant responses (Hart and Reader 2002a) as a large extraradical mycelium might be better able to mobilize nutrients over long distances for its host. However, the finding that *Glomus* spp. have a smaller extraradical mycelium has to be viewed with some caution because of the marker system used by Hart and Reader (2002b) (Hart and Reader 2003; Olsson et al. 2003).

3.4.5 Importance of indirect effects varies among fungal treatments

Many studies showed that AMF can alter competitive relationships between plants (Fitter 1977; Moora and Zobel 1996; West 1996; Koide and Dickie 2002). A pattern that has been repeatedly observed is that the effects of mycorrhizal fungi on a plant community are not simply the sum of their effects on the individual plant species (Koide and Dickie 2002). Therefore, indirect effects, e.g. mediation of competition between plant species, should be taken into account when analyzing responses of a plant community to AMF inoculation. The design of the experiments gave the opportunity to appraise the magnitude of those indirect effects but gave no clear evidence that differences between plant growth responses to AMF are only due to competition between plants. A confounding effect in this study is the utilization pots of different sizes for the experiments, therefore plants did not grow in the same volume of soil in both experiments.. However, as the lnRR values to compare multi and single species experiments were calculated within the respective datasets, this confounding effect is probably small. Nevertheless, the results showed that the response of plant species in a community can differ to some extent from their responses when grown without competitors (Fig. 12). Hence, indirect effects are obviously involved in the responses of the plant community to AMF inoculation. Additionally, the importance of these indirect effects varies with the AMF species used for inoculation, suggesting that not only the presence of AMF but also their identity influences species interactions in a community. These findings are in line with the results of Scheublin et al. (2007) who have shown that not only the presence but also the identity of AMF can influence, for instance, competitive interactions between plants.

3.5 Conclusions

The results highlight the importance of interactions between aboveground and belowground organisms determining plant community structure. Mycorrhizal fungi changed the structure of the grassland community by influencing the various plant species differently: the presence of

AMF decreased community productivity but promoted plant diversity (expressed as evenness), disregarding which mycorrhizal species was introduced. Mycorrhizal fungi also promoted the growth of *R. minor*, thereby generating a negative impact of the hemiparasite on host plant biomass. Furthermore, it became apparent that the effect of mycorrhizal fungi on a plant community is not simply the sum of their effects on the individual plant species. Besides direct effects of AMF on plants, the experiments revealed great indirect effects of AMF due to the strong impact on interactions between plants and their antagonists, and due to a change in competitive relations among plant species. The results show that mutualistic interactions with mycorrhizal fungi, but not antagonistic interactions with a root hemiparasite promote plant diversity in this grassland community.

Chapter 4: Bottom-up effects of arbuscular mycorrhizal fungi across a plant-herbivore-parasitoid system

4.1 Introduction

Multitrophic interactions between above- and belowground organisms are powerful forces shaping the structure and diversity of natural communities (van der Putten et al. 2001). For example belowground herbivores can influence aboveground herbivores *via* a shared host plant and *vice versa* (van Dam et al. 2003; Wurst and Van der Putten 2007). One interaction that has been found to affect the performance of both above- and belowground organisms is the symbiosis between plants and arbuscular mycorrhizal fungi (AMF) (Gehring et al. 2002; Bezemer and van Dam 2005; Bennett et al. 2006). AMF infection of plants affects plant interactions with root pathogenic fungi (Newsham et al. 1995), Collembola (Gange 2000), saprotrophic fungi (Tiunov and Scheu 2005), above- and belowground herbivores (Goverde et al. 2000; Gange 2001) as well as parasitic plants (Davies and Graves 1998; Chapter 3).

Aphids, as one guild of herbivores directly feeding on plant phloem, can be influenced by AMF colonizing the roots of their host plants (e.g. Gange et al. 1999; Guerrieri et al. 2004; Wurst et al. 2004), but the direction of the effect on aphids varied between different experiments. While Gange and West (1994) and Gange et al. (1999) found a positive influence of AMF on weight and fecundity of two *Myzus* species reared on *Plantago lanceolata*, negative AMF effects were reported with *Chaitophorus populicola* reared on *Populus angustifolia* x *P. fremontii* (Gehring and Whitham 2002) and *Macrosiphum euphorbiae* reared on *Lycopersicon esculentum* (Guerrieri et al. 2004). One possible explanation for this inconsistency in results may be the variability of arbuscular mycorrhizal symbiosis itself, ranging from mutualism to parasitism, depending on various biotic and abiotic factors (Johnson 1993; Klironomos 2003). In addition, infection by different AMF species can have different effects on several plant traits e.g. biomass or nutrient capture (van der Heijden et al. 1998b). There are also indications that AMF infection of plants can have cascading effects in the food chain up to higher trophic levels (Gange et al. 2003). For example, there is evidence that AMF symbioses with plants can affect both, the rate of aphid parasitism by parasitoid wasps (Gange et al. 2003) and parasitoid preference, where aphid infested non-mycorrhizal plants are as attractive to parasitoid wasps as uninfested mycorrhizal plants (Guerrieri et al. 2004). However, both studies did not assess parasitoid performance directly, although it is likely that the strong effects of AMF reported on primary producers (plants) and primary consumers (herbivores) cascade upwards in the food chain and thus also

affect several traits in predator or parasitoid performance of, e.g. food consumption or reproductive output (Bezemer et al. 2005).

In this chapter it was intended to test AMF species effects on the tri-trophic interaction of a typical grassland plant species (*Phleum pratense*), its' insect herbivore *Rhopalosiphum padi* and the parasitoid *Aphidius rhopalosiphi.* In a greenhouse experiment, the grass species was inoculated with either one of the two AMF species *Glomus intraradices* or *G. mosseae* to be compared with a non-mycorrhizal control. These three treatments were combined with three insect treatments: (1) plants only (no insects), (2) plant + aphid, and (3) plant + aphid + parasitoid.

It was proposed that (1) the association with AMF improves plant biomass and nutrient capture, (2) there is an increase in food quality which benefits aphid reproduction and supports larger aphid populations on mycorrhizal plants, (3) larger aphid populations allow female parasitoids to chose more suitable aphids for parasitization, which leads to an increase in parasitoid weight and a decrease in parasitoid development time and that (4) the two AMF species have different effects on the tri-trophic interaction

4.2 Materials and methods

4.2.1 Plant, aphid and parasitoid material

Plant seeds and soil were collected from a hay meadow in the Franconian Forest in Central Germany (11°26'44''E/50°23'04''N). Seeds from *Phleum pratense* L. (timothy grass) were collected in summer and autumn 2006. *P. pratense* was chosen as this species is commonly found in European grasslands beeing important in hay production. The substrate used in the experiment consisted of 50% sieved soil (1 cm) collected from the top 10 cm of the field site and, and 50% washed silica sand. The substrate was heated for 48 h at 200°C to kill soil organisms, including AMF. Pre-experimental soil analyses prior to the experiment showed soil nutrient contents of 0.48% organic carbon, 0.1% nitrogen and 36.85 µg g^{-1} plant available phosphor at a pH of 6.6.

Inoculum of two AMF species *Glomus intraradices* N.C. Schenck & G.S. Sm. isolate BEG140 and *Glomus mosseae* (T.H. Nicolson & Gerd.) Gerd. & Trappe isolate BEG25 were purchased as two separate mixtures of spores and mycorrhizal roots from a commercial supplier (SYMbio-M®, Lanškroun, Czech Republic). Both isolates have been widely used in greenhouse experiments and both species are commonly found in grasslands (e.g. Rosendahl and Stukenbrock 2004; Hempel et al. 2007; Chapter 2).

Aphids (*Rhopalosiphum padi* L., cherry oat aphid) were purchased from Katz Biotech AG (Bayreuth, Germany) and propagated on wheat (*Triticum aestivum* L.). *R. padi* was previously shown to be compatible with *P. pratense* (Orlob 1961) and is widely used in greenhouse experiments (e.g. Ponder et al. 2000; Vestergård et al. 2004). The parasitoid wasp species *Aphidius rhopalosiphi* (DeStefani-Perez) was chosen, this wasp is a natural enemy of *R. padi* (e.g. Gonzáles et al. 1999) and commonly occurring throughout Northern Europe (Muratori et al. 2004). Wasps were bought as mummies (Katz Biotech AG) and allowed to hatch and mate. After two days wasps were anaesthetized with CO_2 and sorted according to sex. Single female wasps between two and four days old were then introduced into the parasitoid treatments.

4.2.2 Experimental set-up

The experiment was set up in ten blocks in the greenhouse in a full randomized block design. Three mycorrhizal treatments (non-mycorrhizal control, inoculation with *G. intraradices* or *G. mosseae*) were combined with the three insect treatments (no insects added, aphids added or aphids and female parasitoid added). These nine treatments were replicated 20 times resulting in 180 pots in total. Two plants from each of the nine treatments were randomly assigned to each block (see Fig. 13).

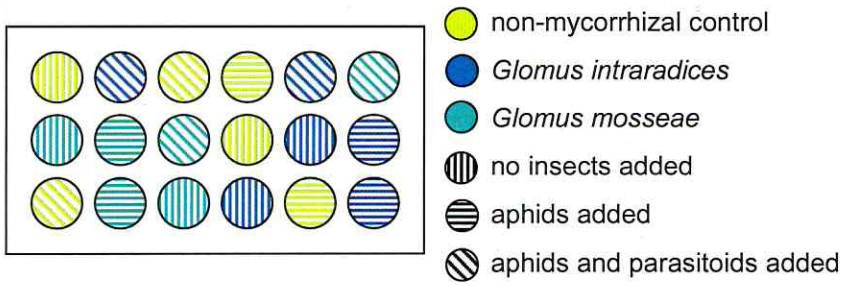

Figure 13: Experimental setup of one block. The whole experiment consisted of ten blocks in total

Each one litre pot (height = 13.5 cm, diameter = ca. 10 cm) was filled with 2 cm expanded slate + 1 cm washed sand for drainage. Pots were then filled with sterile substrate with the mycorrhizal inoculum placed 1 cm below the surface. One third of the pots received either 15 g inoculum of *G. intraradices*, *G. mosseae* or an autoclaved mixture of both for the non-mycorrhizal control, respectively. To establish a natural microbial community, all pots were

irrigated with 10 ml soil suspensions from the field soil filtered through a Whatman filter paper No. 4 with pore sizes of 20-25 µm (Whatman International Ltd., Kent, UK) to exclude AM propagules from the suspension (Schroeder and Janos 2004). A bulk seed collection of *P. pratense* was germinated in sterile substrate. After two weeks one seedling was planted into each pot and its height recorded as initial plant size. Temperatures in the greenhouse ranged from 18°C (14 h day) to 13°C (night) with additional light provided by 400 W lamps. Plants were watered three times a week with tap water.

Plants were cut 2 cm above the soil surface 15 and 21 weeks after planting to mimic mowing as the plants originated from regularly mown grasslands. Furthermore, it was intended to give the plants time for establishment of the mycorrhizal symbiosis. One week after the second cut, five *R. padi* instars (three to five days old) were added to the respective treatments (120 pots) using a fine brush. All pots were encaged in air-permeable cellophane bags (width 185 mm and length 390 mm). Twenty-five days after aphid introduction single females of *A. rhopalosiphi* were introduced into the parasitoid treatments (60 pots) and allowed to parasitize aphids for 12 hours during daytime after which they were removed from the cellophane bags. Plants was harvested two weeks after introduction of the parasitoids (i.e. 39 days after aphid introduction), when visible mummies had developed. Shoots were cut at the soil surface and aphids and mummies were carefully separated from plant material.

4.2.3 Plant measurements

Plant roots were washed free from soil and an aliquot of 2 g from each pot was stored in formaldehyde-acetic acid (FAA = aquatic solution of 6.0% formaldehyde, 2.3% glacial acetic acid, 45.8% ethanol (all v/v)). Root subsamples stored in FAA from five pots of each mycorrhizal treatment were dyed in lactophenol blue solution according to Phillips and Hayman (1970) with modifications after Schmitz et al. (1991). Observing 300 stained root segments under a light microscope at 200x magnification using the line intersect method (Brundrett et al. 1996) mean mycorrhizal colonization rates of 42% and 21% in the *G. intraradices* and *G. mosseae* treatments were detected, respectively. However, these values represent a snapshot only, as mycorrhizal colonization varies within time (Jansa et al. 2008). Observations also confirmed the absence of mycorrhizal structures in the control treatment.

Above- and belowground plant material was dried at 60°C for 48 hours and then weighed. In addition, phosphorus concentrations (P) and total nitrogen (N) and carbon (C) content from five plants in the mycorrhizal and control treatments were determined using plant material ground in a ball mill. The P concentrations were analysed with a CIROS ICP

spectrometer (SPECTRO Analytical Instruments GmbH & Co. KG, Kleve, Germany) after combusting subsamples at 550°C and dissolving the ash with 4 N nitric acid. Total N and C contents were measured with an Elementar Vario EL element analyser (Elementar Analysengeräte GmbH, Hanau, Germany).

4.2.4 Aphid and parasitoid measurements

The numbers of aphids per plant were determined eleven days after adding to monitor aphid population establishment. No second counting was performed during to experiment to avoid aphid disturbance (Godfray 1994). Aphids, winged aphids and mummies were counted at the end of the experiment (day 39). Mummies were placed singly into gelatine capsules in a growth chamber (16h light with 22:12°C day:night temperatures, 50% relative humidity) until emergence. Capsules were checked three times per day and freshly hatched wasps were immediately frozen and their development time recorded until all wasps had emerged one week after the end of the experiment (day 46). Wasps were sexed, dried at 60°C for 24 hours and weighed. For each mummy it was determined whether the aphids were adult at mummification using the shape of the cauda as a criterion (Minks and Harrewijn 1987).

4.2.5 Data analysis

All calculations were carried out with JMP version 7 (SAS Institute, Cary, NC, USA), exceptions are given below. One plant inoculated with *G. mosseae* and one control plant died during the experiment; those two were excluded from the analysis. As mortality caused the data to be unbalanced, type III sum of squares were used (Shaw and Mitchell-Olds 1993). Data on initial plant size, plant dry weights and numbers of aphids were log transformed, proportion data (sexratio and proportion of adults among mummies) were arcsine–square root transformed to achieve normal distribution. The combined effects of mycorrhizal treatments and aphids on shoot and root biomass, were analysed in separate analyses of co-variance (ANCOVA). Initial plant size was used as covariate, block, aphid presence, AMF and the interaction of the latter two as main effects in both analyses. Additionally, using orthogonal contrasts, the following the two initial hypotheses were tested: (a) plants perform better with AMF than without - "control vs. AMF" and (d) plants perform better with one of the AMF isolates used - "*G. intraradices* vs. *G. mosseae*". These contrasts were also calculated on the level of aphids and parasitoids (see below). In addition, root and shoot biomass of aphid infested and un-infested plants within each mycorrhizal treatment were compared using

orthogonal contrasts. Plant C, N and P content data were compared in a one-way analysis of variance (ANOVA) between the fungal and control treatments.

In order to test for possible effects of mycorrhizal treatments on aphid population establishment (i.e. the number of aphids detected eleven days after adding) an ANCOVA with initial plant size as covariate and block and AMF treatment as main effects were used. According to this analysis, initial aphid population establishment was independent of mycorrhizal treatments ($F_{2, 6.09} = 2.33$; $P = 0.10$). It is conceivable that some of the released aphids were not able to localize adequate feeding sites on the plants in time and thus died due to starvation. Therefore the counting eleven days after adding was used as starting point and excluded all pots in the aphid treatments showing no aphids at this time point from further analysis. Subsequently, aphid population growth rates per day were calculated between day eleven and 39 (harvest) for each pot. The impact of AMF treatments on aphid population growth rates was analysed using an ANCOVA. Initial plant size was included as covariate; block, parasitoid presence and AMF were used as main effects. As the population growth rates were negative in one third of all pots, the AMF treatment effect on the proportion of pots with this negative growth pattern were analysed using an analysis of deviance with quasi F-statistics, binomial error distribution and logit link function with the same main effects as for aphid population growth rates. This model fits the data reasonably well as indicated by the goodness-of-fit statistics (model deviance = 85.0, DF = 75, $P = 0.2$).

For parasitoid data the average dry mass and the average development time for each pot were calculated. The impact of AMF inoculation on parasitoid dry mass and development time was then assessed using ANCOVA. To account for the highly variable number of mummies in each pot, this number was used as weighting factor in the ANCOVA. Blocks were poorly replicated due to the extinction of aphid populations on some plants and therefore excluded from the analyses. The number of aphids present on the respective plant at harvest was used as covariate and parasitoid sex ratio together with AMF as main factors in the analysis. Aphid numbers and parasitoid sex ratio are very likely to have an influence on dry weight and development time of parasitoids, as in larger aphid populations, ovipositions can be made in more suitable aphid stages and male parasitoids are usually smaller and develop faster (Sequeira and Mackauer 1992). ANCOVAs were also used to separately analyse the impact of the different mycorrhizal inoculations on sex ratio and the number of adults among parasitized aphids, using the number of mummies as weighing factor, the number of mummies as covariate and the AMF treatment as main effect.

In order to test for the impact of AMF inoculation on rates of parasitism, major axis (MA) regression was used (SMATR version 2.0; Falster et al. 2006). MA regression is an appropriate method for evaluating the association between variables that have been measured with error, and where error variances are unknown, but expected to be within similar dimensions (Sokal and Rohlf 2003). With the algorithms given in SMATR intercept and slope between the MA regression of each mycorrhiza treatment were compared to test for changes in rates of parasitism and aphid density dependent reactions of parasitoids, respectively.

4.3 Results

4.3.1 Plant responses

Shoot biomass at harvest was increased by AMF inoculation (Fig. 14a), with contrasts showing significant differences compared to control plants but no differences between the two AMF species (Table 5). A similar pattern was found for root biomass (Fig. 14b) with an even stronger mycorrhizal effect (Table 5).

Aphids had a significant negative impact on shoot biomass with a reduction by 14.2, 10.3 and 5.2% in the control, *G. intraradices* and *G. mosseae* treatments, respectively (Fig. 14a). ANCOVA showed a significant effect of the interaction between AMF and aphids on root biomass. This reflects the increase in root biomass (+12.8%) in the *G. mosseae* treatment under aphid presence (Fig. 14b), whereas aphids strongly reduced root biomass in the control and *G. intraradices* treatments by 32.2 and 38.3%, respectively.

Plant C, N and P values varied between the fungal treatments, but ANOVA showed no significant differences (Table 6).

Table 5. Results of ANCOVA on shoot and root biomass of *Phleum pratense* at harvest. Orthogonal contrasts are calculated for AMF treatments which are described in detail in the material & methods section. Significant *P* values below 0.05 are given in bold.

		Shoot biomass			Root biomass		
Source of variation	d.f.	MS	F	P	MS	F	P
Initial plant size	1	0.09	3.21	0.08	0.63	4.13	**0.04**
Block	9	0.10	3.34	**<0.001**	0.92	6.06	**<0.001**
Aphid presence	1	0.43	14.57	**<0.001**	0.61	3.99	**0.05**
AMF	2	0.12	3.91	**0.02**	1.04	6.84	**0.001**
AMF vs. control	1	0.21	6.94	**0.009**	2.07	13.60	**<0.001**
intra vs. moss	1	0.03	0.86	0.36	0.01	0.10	0.76
Aphid presence*AMF	2	0.01	0.47	0.62	0.53	3.48	**0.03**
Residuals	162	0.03			0.15		

Table 6. Mean foliar nutrient concentrations (±SE) and element ratios of five randomly chosen plants. There were no significant differences due to AMF inoculation.

	Control	*Glomus intraradices*	*Glomus mosseae*	F	P
C (mg g^{-1})	417 ± 2.6	410 ± 1.0	414 ± 1.0	3.78	0.06
N (mg g^{-1})	23.5 ± 1.9	23.4 ± 1.6	21.4 ± 1.1	0.53	0.60
P (mg g^{-1})	4.15 ± 0.27	4.25 ± 0.07	3.73 ± 0.15	1.79	0.21
C/N ratio	18.3 ± 1.49	17.8 ± 1.13	19.6 ± 1.05	0.87	0.44
N/P ratio	5.6 ± 0.2	5.5 ± 0.3	5.7 ± 0.2	0.25	0.78

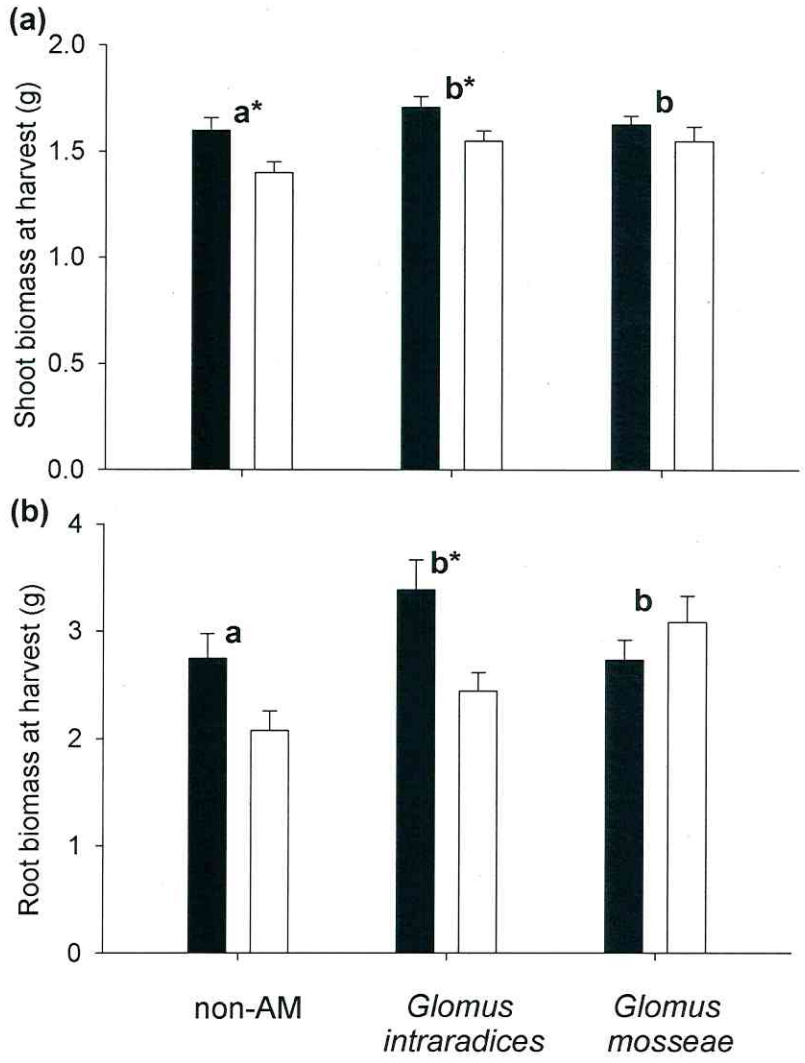

Figure 14 (a) Mean shoot biomass and (b) mean root biomass (+SE) at harvest (closed bars – without aphids, open bars – with aphids). Different letters above bars indicate a significant difference between AMF treatments; asterisks indicate a significant difference between aphid infested and uninfested plants within the AMF treatments ($P < 0.05$) according to orthogonal contrasts.

4.3.2 Aphid population development

Aphid population growth rates were significantly smaller in the AMF inoculated plants (growth rate per day ± SE: 2.5 ± 0.8 and 3.5 ± 1.2 for *G. intraradices* and *G. mosseae*, respectively) compared to control plants (6.2 ± 1.6, Fig. 15a, Table 7). As in case of plant biomass, contrasts showed no differences between the two AMF species. According to the analysis of deviance, populations with negative growth rates were also more frequent on mycorrhizal plants (42% and 38% for *G. intraradices* and *G. mosseae*, respectively) than on non-mycorrhizal plants (24%; Fig 15b, Table 7). Parasitoid presence had no effect on aphid numbers at harvest.

Winged aphids were detected on two pots in the non-mycorrhizal control only, with four and one winged aphid, respectively.

Table 7. Results of ANCOVA on aphid population growth rates and of the analysis of deviance on the proportion of aphid populations with negative growth rates. Orthogonal contrasts are calculated for AMF treatments which are described in detail in the material & methods section. Significant *P* values below 0.05 are given in bold.

		Aphid population growth rates			Populations with negative growth rates	
Source of variation	d.f.	MS	F	P	log likely-hood ratio	P
Initial plant size	1	23.00	0.27	0.61	3.12	0.07
Block	9	154.11	1.78	0.09	23.46	**0.005**
Parasitoid presence	1	71.35	0.82	0.37	0.16	0.69
AMF	2	280.84	3.24	**0.04**	6.01	**0.05**
AMF vs. control	1	491.36	5.67	**0.02**	45.47	**0.01**
intra vs. moss	1	60.53	0.70	0.41	42.50	0.90
Residuals	75	86.59				

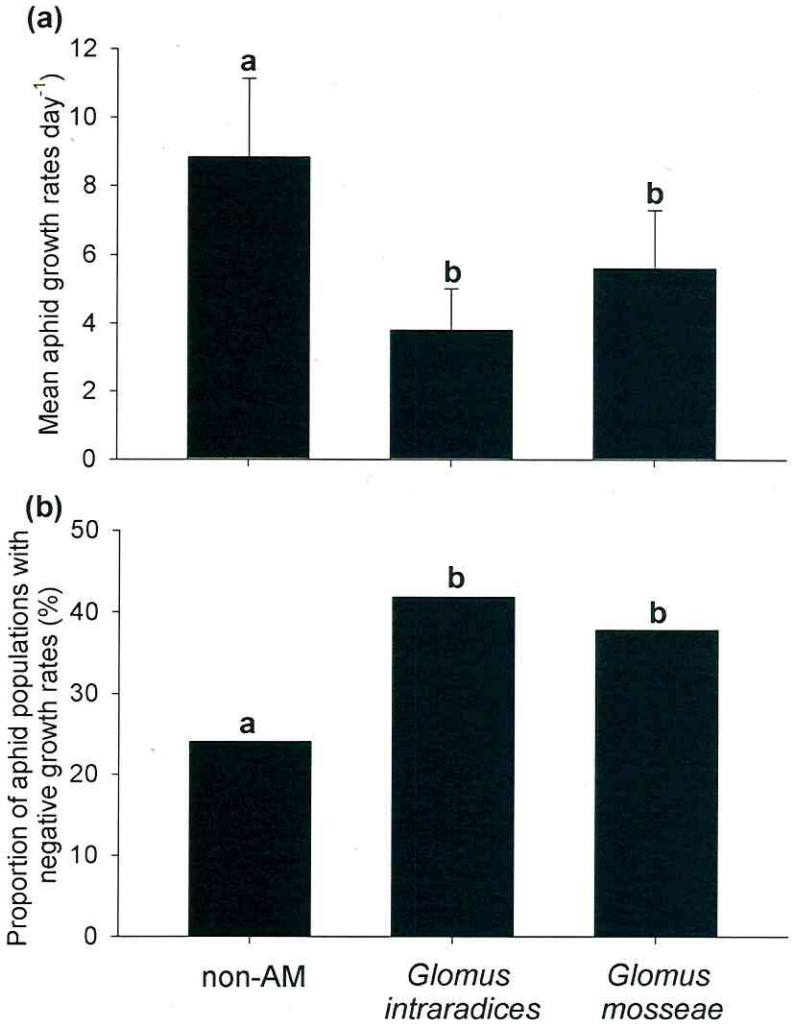

Figure 15 (a) Mean aphid population growth rates per day. (b) Proportion of aphid populations with negative growth rates. Different letters above bars indicate a significant difference between AMF treatments ($P < 0.05$) according to orthogonal contrasts.

4.3.3 Parasitoid responses

Emerging parasitoid wasps were significantly heavier when they developed on plants in the two AMF treatments (mean dry weight ± SE: 53.7 ± 3.9 µg and 54.0 ± 2.6 µg for *G. intraradices* and *G. mosseae*, respectively) than on non-mycorrhizal plants (43.5 ± 4.1 µg, Fig. 16, Table 8). Additionally, the development of parasitoids was significantly faster in the AMF treatments (mean development time ± SE: 17.0 ± 0.2 and 16.8 ± 0.2 days for *G. intraradices* and *G. mosseae*, respectively) compared to the control (17.7 ± 0.2 days, Table 8). There was no significant difference in parasitoid dry weight and developmental time between the two AMF species. Sex ratio had a significant effect on dry weight with females being heavier than males but there was no difference between the sexes concerning development time. The opposite was true for the number of aphids, which had a significant impact on development time of emerging wasps but not on parasitoid dry weight (Table 8).

A comparison of sex specific mean values for dry weight at eclosion and development time in the control and the two AMF treatments showed rather uniform responses of males and females in the control and *G. intraradices* treatments, with a better performance in the latter (Fig. 16). In contrast, *A. rhopalosiphi* expressed a clear sex specific reaction in the *G. mosseae* treatment with males developing faster and females getting heavier. Nevertheless, there was no significant difference in parasitoid sex ratio (mean ± SE 49±15, 37±10 and 51±13% females in control, *G. intraradices* and *G. mosseae* treatments, respectively, $F_{2, 1.36} = 0.47$, $P = 0.63$). The proportion of adult aphids among mummies was also not significantly different between the AMF treatments (mean ± SE 27±8, 51±8 and 50±12% in control, *G. intraradices* and *G. mosseae* treatments, respectively, $F_{2, 0.14} = 0.22$, $P = 0.80$).

Table 8. Results of ANCOVA on mean parasitoid dry mass and development time as means per pot. Orthogonal contrasts are calculated for AMF treatments which are described in detail in the material & methods section. Significant *P* values below 0.05 are given in bold.

		Dry weight			Development time		
Source of variation	d.f.	MS	F	P	MS	F	P
Number of aphids	1	357.3	0.44	0.52	28.84	9.14	**0.007**
Sexratio	1	4316.9	5.27	**0.03**	1.99	0.63	0.44
AMF	2	2726.6	3.33	**0.05**	19.64	6.22	**0.008**
AMF vs. control	1	5401.8	6.59	**0.02**	33.78	10.70	**0.004**
intra vs. moss	1	28.4	0.03	0.85	4.84	1.53	0.23
Residuals	21	819.8			3.16		

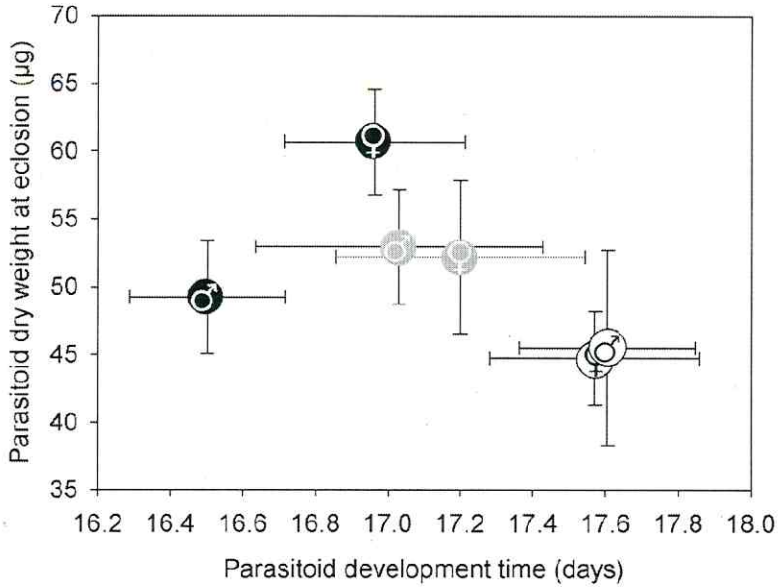

Figure 16 Mean parasitoid dry weight (+SE) plotted against mean parasitoid development time (+SE) for both sexes separately. White symbols – non-mycorrhizal control, grey symbols – plants inoculated with *Glomus intraradices*, black symbols – plants inoculated with *Glomus mosseae*.

Rate of parasitism (expressed as intercept in Fig. 17) was significantly different between the *G. intraradices* (15.9 ± 3.7%) and the two other treatments (6.6 ± 1.6% and 6.3 ± 1.0% in control and *G. mosseae* treatments, respectively, $P = 0.007$). However, MA regression showed no significant difference in aphid density dependent reactions of parasitoids between the fungal treatments (expressed as slope, $P = 0.64$). Rates of parasitism decreased with increasing number of aphids (slope<1).

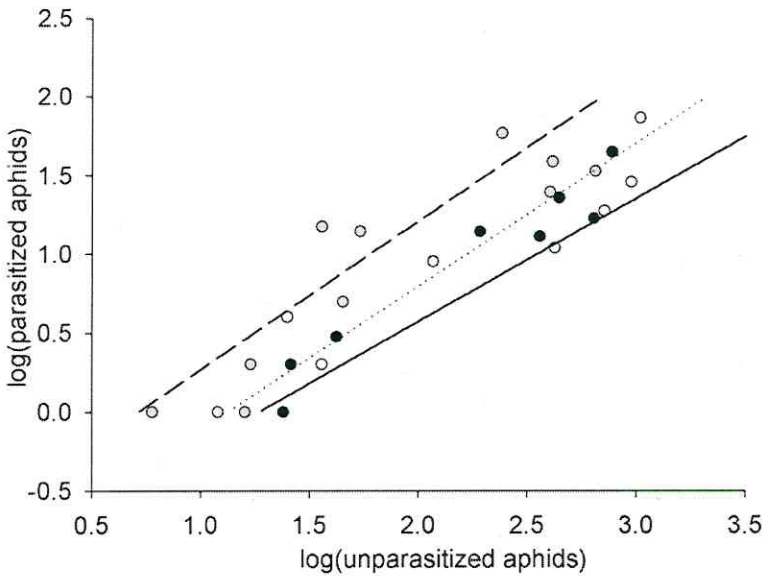

Figure 17 Scatterplot of unparasitized against parasitized aphids for control (white dots), *G. intraradices* (grey dots) and *G. mosseae* (black dots) treatments. Major axis regression: control (solid line, $y = 0.785x-0.996$, $R^2 = 0.89$); *G. intraradices* (dashed line, $y = 0.942x-0.674$, $R^2 = 0.82$); *G. mosseae* (dotted line, $y = 0.913x-1.027$, $R^2 = 0.93$).

4.4 Discussion

In contrast to the initial hypotheses, positive effects of AMF inoculation on performance could only be observed in two trophic levels, namely the primary producers (*Phleum pratense*) which benefit from the association in terms of an increase in biomass and parasitoids (*Aphidius rhopalosiphi*) which showed increased weight at eclosion and shorter development time on mycorrhizal plants (Figs. 14, 15, 16). The population growth rates of aphids (*Rhopalosiphum padi*), primary consumers decreased on plants inoculated with AMF. The hypothesized significant differences between the two AMF species could be detected in plant biomass under aphid attack and in the rates of parasitism but not in aphid population growth rates.

The fact that the AMF isolates and the plant seeds used in this study share no common ecological background (AMF inoculum was a common cultivar) and might therefore not be adapted to each other, has to be considered (Fitter et al. 2005). Klironomos (2003) showed that the combination of non-adapted AMF and plants can narrow the span of host plant responses. In this context, the study demonstrates potential effects of different AMF species on plants and higher trophic levels, rather than revealing the outcome of these interactions under natural conditions. However, a positive impact of the two AMF isolates on plant biomass was present and highly adapted AMF can be expected to provide even more benefits to their host plants (Helgason et al. 2007). Therefore comparable or even stronger effects on higher trophic levels, as seen in this chapter, might be expected.

4.4.1 AMF and aphid effects on plants

The positive effect of AMF inoculation on plant biomass was also present at the two interim clippings (data not shown). In case of *G. mosseae* this positive effect was clearly not reflected in foliar N and P contents (Table 6), as these values tended to be lower than in the control. Such species specific effects of AMF on several plant variables are also in accordance with previous studies (e.g. van der Heijden et al. 1998b; Maherali and Klironomos 2007; Jansa et al. 2008), which showed that biomass and nutrient capture of a plant community varied independently with the identity of the inoculated AMF species.

Although aphid presence had a consistently negative impact on shoot biomass this reduction was only significant in the control and *G. intraradices* inoculated plants, indicating a higher tolerance to aphid feeding in the *G. mosseae* inoculated plants (Fig. 14a). In contrast, an inconsistent pattern was present in root biomass. Plants inoculated with *G. mosseae* showed an increase in root biomass under aphid presence, in contrast to decreasing values in

control and *G. intraradices* inoculated plants under aphid herbivory (Fig. 14b). Such interactive effects of AMF species and aphids were previously reported (Gange and West 1994) and might reflect differences in nutrient allocation within plants under aphid attack (Vestergård et al. 2004).

4.4.2 AMF Effects on aphids

The negative effect of AMF inoculation on aphid population growth rates (Fig. 15) is in contrast to some publications on AMF-aphid interactions (e.g. Gange and West 1994; Gange et al. 1999). However, negative interactions have also been reported (Gehring and Whitham 2002; Guerrieri et al. 2004). Three potential mechanisms limiting aphid growth in the experiment are likely and have to be discussed; aphid crowding, nutrient limitation and plant defence compounds. Aphid crowding is unlikely, as winged morphs, a good indicator for aphid crowding (Hodgson 2001), were rarely detected. A nutrient limitation as limiting factor is also not likely, as the comparison between aphid populations growth rates (Fig 15a) and plant nutrient contents at harvest (Table 6) shows no clear relations. Inoculation with *G. intraradices* induced the highest decrease in aphid population growth rates but the respective plants contained as much N as control plants and tended to contain even more P than plants of the two other fungal treatments. This lack of correlation between N contents and aphid performance is in accordance with a field study by Gange and West (1994), who hypothesized that changes in aphid numbers were more related to a changed leaf morphology (phloem location and size) in mycorrhizal plants than to differences in N content. In contrast, previous studies (Ponder et al. 2000; Bezemer et al. 2005) reported decreased aphid population sizes in parallel with decreased foliar N concentrations using the same aphid species (*R. padi*) as in this study. Another explanation for the high proportion of aphid populations with negative growth rates on mycorrhizal plants might be the presence of defence compounds in the phloem of *P. pratense,* indicating increased plant resistance against aphids induced by AMF (Pozo and Azcón-Aguilar 2007). Bezemer et al. (2005) have recently shown that *R. padi* might be sensitive to phenolic compounds, and it has been shown that mycorrhizal inoculation may induce a higher content of phenols in the phloem (Zhu and Yao 2004). However, an induction of defence compounds by the repeated cutting of *P. pratense* is not likely, as the mechanisms involved in plant responses to wounding and to the attack of phloem feeding insects (i.e. aphids) follow different signalling pathways (Pozo and Azcón-Aguilar 2007).

The missing effect of parasitoid presence on aphid numbers is not surprising as the proportion of parasitized aphids was generally low, not exceeding 16% and aphid populations were only parasitized once for 12 h.

4.4.3 AMF effects on parasitoids

First studies concerning the effects of AMF on parasitoid wasps concentrated on parasitoid preferences. Gange et al. (2003) provided data on AMF species depending variations in rates of parasitism of a leaf mining fly (*Chromatomyia syngenesiae*) by the ichneumon *Diglyphus isaea*. Guerrieri et al. (2004) showed that non-mycorrhizal tomato plants infested with aphids were as attractive to *Aphidius ervi* as mycorrhizal, non-infested plants. Within this chapter, it was revealed that the performance of parasitoid wasps is also influenced by the presence and identity of AMF, as *A. rhopalosiphi* got heavier and developed faster when their host *R. padi* was reared on mycorrhizal plants (Fig. 16). Changes in weight at eclosion and development time are highly correlated to several fitness traits such as longevity, number of hosts attacked (in case of females) and number of matings achieved (in case of males; Godfray 1994). These changes were rather uniform for male and female parasitoids in case of the *G. intraradices* treatment, but varied substantially between sexes in the *G. mosseae* treatment. While the underlying reasons for the sex-specific difference cannot be discerned, one possibility is that females adjusted their behaviour when plants were infested by *G. mosseae* such that fertilized eggs resulting in females were laid in different-sized hosts compared to unfertilized eggs. On the other hand the observed sex specific pattern in the *G. mosseae* treatment might reflect the fact that larger female parasitoids enjoy a proportionally greater increase in fitness than larger males (Godfray 1994). Therefore female parasitoids might have invested additional resources in an increased weight rather than decreased development time, which is also reflected by the significant influence of the ratio between sexes within pots on parasitoid dry weight (Table 8). Despite this sex-specific difference in the *G. mosseae* treatment, the observed differences in parasitoid dry weight and development time between mycorrhizal and control treatments were not due to changes in sex ratio, as this variable was relatively constant in all AMF treatments (proportion of females 44.8% ± 7.1%). Alternatively, it might be the case that male and female parasitoids can use the resources provided by *G. mosseae* infection in different ways. This hypothesis needs however to be investigated in further studies.

Parasitoid developmental time was correlated with aphid density, i.e. parasitoids developed faster when more aphids were available for oviposition. One possible explanation for this relationship is that at higher aphid densities, the parasitoids encounter more aphids of

different larval instars and more ovipositions are made in more suitable aphid stages. In the pea aphid (*Acyrthosiphon pisum)*, oviposition in intermediate instars reduced developmental time of *Aphidius ervi* compared to ovipositions in younger instars or adults (Sequeira and Mackauer 1992). While parasitoid oviposition was not directly studied, such selection behaviour is conceivable. More generally, there is little information about density-dependence in parasitoid host selection behaviour and the consequences for offspring fitness.

The values of weight and development time were shown to covary in the study by Sequeira and Mackauer (1992) and were furthermore highly dependent on the age of the parasitized aphids. That connection was not present in this study, as the proportion of aphids that died as adults was the same in the two AMF inoculation treatments and the control, indicating aphids were parasitized at comparable larval stages in all fungal treatments.

In accordance to the study by Gange et al. (2003) changes in rates of parasitism, expressed as a significant higher proportion of parasitized aphids in the *G. intraradices* treatment, were found (Fig. 17). Gange et al. (2003) partially attributed their observed mycorrhizal effects on rates of parasitism to a decreased parasitoid searching efficiency due to changes in plant architecture. However, the limited space under the cellophane bags in the experiment surely interfered with this effect. Additional effects like the induction of volatiles influencing parasitoid activity are also likely, as these can be AMF species specific (Bezemer and van Dam 2005).

Interactions of belowground organisms with plant roots resulting in contrasting reactions on aboveground aphids and parasitoids were also reported by Bezemer et al. (2005). They attributed increasing parasitoid performance to a visually observed increase of aphid size, although they did not explicitly measure this parameter. Another possible explanation for the observed effects on aphid and parasitoid level would be a decrease in aphid immune answer against parasitoid eggs on mycorrhizal plants, which could have led to an increase in parasitoid performance (W. Völkl - personal communication, see also Godfray 1994). However, all these hypothetical mechanisms do not seem to follow a linear relation, as aphid population growth rates were highest on control plants, intermediate on plants from the *G. mosseae* treatment and worst on *G. intraradices* inoculated plants. In contrast, parasitoid weight and development time were best on *G. mosseae* inoculated plants, worst on control plants and intermediate with *G. intraradices*.

4.5 Conclusions

The results show that the performance of three interacting trophic levels is significantly affected by both by the presence of AMF and the species identity of the plants fungal symbionts. However, the data do not allow drawing clear conclusions on the underlying mechanisms driving the observed performance alterations. Food choice experiments (Prince et al. 2004) and stable isotope probing (Langellotto et al. 2006) would be useful tools to monitor changes in preferences and nutrient fluxes. The observed changes in the trophic interactions due to AMF inoculation emphasise that belowground interactions can have strong implications for aboveground food webs (van der Putten et al. 2001). As such interactions are e.g. used in modelling approaches (Hoover and Newman 2004; van der Putten et al. 2004) the impact of a symbiosis as widespread as arbuscular mycorrhiza (Treseder and Cross 2006) should be included in such models.

Chapter 5: General discussion

In the last two phases of the BIOLOG - DIVA Jena project the relationship between plant diversity and functions within ecosystems was studied along the plant diversity and productivity gradient represented by the meadows in the Thüringer Schiefergebirge and the Franconian Forest. Kahmen et al. (2005a) could show that the concrete plant species composition instead of plant species diversity is the main driver for plant productivity. In addition, arthropod abundance was also found to be mainly determined by plant species composition (Perner et al. 2005). In a study on the impact of climate change, i.e. early summer drought, plant communities with a higher diversity showed a higher increase in belowground productivity than communities with a low diversity, indicating a lower resilience to drought in the latter (Kahmen et al. 2005b). Unsicker et al. (2006) detected a clear correlation between plant community composition and insect herbivory levels, with more diverse communities suffering less herbivore damage. The meadows were also shown to be dispersal limited, as plant species diversity and productivity increased after the addition of seeds from regional species (Stein et al. in press). A missing group in those studies is the one of the Glomeromycota, although their AM symbiosis with plants is an important driver of plant diversity (e.g. van der Heijden et al. 1998a; van der Heijden et al. 1998b) and can influence plant communities in various ways, e.g. by increased plant nutrient acquisition, which is an important trait in nutrient poor grasslands (van der Heijden et al. 2008). A first study within the BIOLOG - DIVA Jena project comparing two meadows representing the two ends of the plant diversity gradient detected strong differences in the AMF species composition at both sites (Börstler et al. 2006). A complete survey of the AMF diversity on all 19 sites studied within the project would be extremely laborious and expensive with the techniques currently used in such studies (see Chapter 1: Introduction). However, a recent publication tested a new method which seems promising to reduce time and cost efforts. Gamper et al. (2008) presented a real-time PCR assay with specific Taq-Man® probes which could be used to quickly detected and even quantify AMF species in environmental samples.

Therefore, the results from Chapter 2 gave important clues for future molecular studies on AMF diversity in the field, as they detected clear differences in AMF diversity at the level of familiy/species group between extra- and intra-radical mycelium and spores. The highest diversity was detected in the extra-radical mycelium, indicating that this compartment is the most adequate for exhaustive diversity assessments. Investigating this part of the mycorrhizal network avoids the limitations of diversity assessments using spores or mycorrhizal roots, since the presence and abundance of AMF spores does not necessarily reflect the AMF

community composition (Clapp et al. 1995; see also Sanders 2004) and several studies (e.g. Vandenkoornhuyse et al. 2003) found indications for a certain host plant preference of AMF species. The latter fact would necessitate the assessment of all potential mycotrophic plants at a given site in order to study the AMF diversity. Though the sampling of extraradical mycelium clearly circumvents these limitations, Mummey and Rillig (2008) showed that AMF species in the soil can be spatially clustered at a very small scale. This emphasizes the need for an intensive sampling design in future AMF diversity surveys. The results from Chapter 2 outline the huge gap of knowledge concerning the different life strategies of AMF species or families, as a clear dissimilarity of AMF taxa detected as extra- and intra-radical mycelium was detected. The potential explanation given in Chapter 2, that some AMF might follow a non-mutualistic lifecycle, is just one possible explanation. It is clear that further studies on the lifecycle strategy, as done e.g. in Hart and Reader (2002b) or Hildebrandt et al. (2006) are desperately needed.

It is however to note, that the molecular approaches as e.g. used in Chapter 2 can only describe the number and identity of AMF species present in a certain environment. The next step of linking these diversity data to the functional significance of AMF in these environments is difficult due to the missing knowledge on functional diversity of AMF (van der Heijden and Scheublin 2007). Therefore, experimental approaches are needed to elucidate this field of AMF ecology. As the manipulation of AMF diversity in nature is a hardly manageable task (see Chapter 1: Introduction) and the large number of factors influencing a field experiment are difficult to monitor, greenhouse experiments allow the controlled observation of organisms and interactions potentially influenced by AMF (Grime et al. 1987). Van der Heijden and Scheublin (2007) identified several ecosystem traits potentially influenced by AMF. For example the presence of AMF species with different host plant preferences or compatibilities can impact the structure of a plant community and the degree of root colonization can influence the degree of protection against fungal root pathogens. Another important aspect is the function AMF exert directly on their host plants in terms of nutrient acquisition or plant growth stimulation and indirectly via the stimulation or inhibition of other mutualists, parasites or herbivores which feeds back to the plants (van der Heijden et al. 2008). The knowledge on such specific AMF effects will help to understand the role of these fungi in important ecosystem services, such as resistance against invasive plants (Fitter 2005) or the storage of carbon in soils (Rillig 2004).

The two greenhouse experiments in Chapters 3 and 4 clearly show the variability in the outcomes of plant - AMF interactions. The single species and community experiments in Chapter 3 (Fig. 9, Fig. II) confirm the results of previous studies, that plant benefits derived from mycorrhizal interactions depend on the identity of both the plant and fungal partners (e.g. van der Heijden et al. 1998b). Although a general positive trend in plant reactions to mycorrhizal inoculation for the mycorrhiza dependent plants could be observed, the reactions to inoculation with *Gigaspora margarita*, *Glomus intraradices*, a combination of both or field sampled mycorrhiza were highly variable between plants, probably due to differential effects of specific plant-fungus combinations (Finlay 2008). In addition, the different AMF treatments modified the negative impact of the hemiparasite *Rhinathus minor* on the plant communities specifically, with the highest reduction in plant biomass in the *Gi. margarita* inoculated treatment and the lowest reduction in the treatment inoculated with a combination of *Gi. margarita* and *G. intraradices*. A comparable pattern, although only one plant species was regarded, could be seen in the mutitrophic experiment (Chapter 4). Again, there was a general mycorrhizal effect cascading up in the food chain, i.e. increased plant biomass, decreased aphid numbers and an increased parasitoid performance, but there were also substantial differences between the AMF species *G. intraradices* and *G. mosseae* (Figs. 14, 15 and 16). Inoculation with the first had the highest negative impact on aphid population growth rates but the latter had the highest positive effect on parasitoids. Although AMF within an ecosystem might exert a certain functional redundancy (van der Heijden et al. 2008), the results of the green house experiments clearly point to the importance of distinct AMF species, indicating functional complementarity between them (Maherali and Klironomos 2007; Jansa et al. 2008). The concept of functional redundancy is also strongly challenged by Fitter (2005), who argues that although species may possess overlap in some metabolic pathways, they most certainly differ in their reaction to environmental conditions, e.g. drought. This view is also supported by the fact that the plant communities under investigation in chapter 3 produced significantly more biomass when inoculated with a field sampled mixture of AMF compared to inoculations with one or two species (Fig. 8), indicating stronger positive effects of AMF, when their species richness is higher.

Applied to the aims of BIOLOG, i.e. the maintenance of biodiversity and ecosystem functions, the results highlight the importance of preserving of a diverse AMF species community. This view is also supported by a study by Helgason et al. (2007), who argued that rare AMF species with a narrow host range provide highest benefits to their host plants, as

both partners might share a co-evolutionary response (Fitter et al. 2005). In contrast, two widespread "generalist" AMF species, which were less vulnerable to perturbations by fungicides than specialist fungi, provided low benefits to their host plants (Helgason et al. 2002; Helgason et al. 2007). Similar results concerning the negative effect of disturbance on AMF diversity were obtained in studies by Jansa et al. (2002) and Hijri et al. (2006), both detecting generalist members of the *Glomus* group A to be most resilient to high input farming.

According to these results, an extensive management strategy currently applied to most of the meadows in the Thüringer Schiefergebirge and the Franconian Forest is appropriate to preserve a diverse AMF community, which in turn can support a diverse plant community and buffer its reactions to single environmental events.

There is still a lot of room and need for future research in mycorrhizal ecology. The present work shows different directions in which attempts could be made. On the one hand, it would be interesting to understand mechanisms involved in the various AM effects on either different plant species (Chapter 3) or different trophic levels (Chapter 4). Interesting insights could be gained by plant gene expression patters, as they were shown to differ in accordance to the AMF species inoculated (Massoumou et al. 2007; Feddermann et al. 2008). Such mechanistic approaches, as e.g. reviewed in Pozo and Azcon-Aguilar (2007) concerning mycorrhiza-induced resistance of plants against pathogens, bear interesting insights in the underlying processes of the mutual interactions between plants and AMF and might help to develop new strategies for the protection of plants against pathogenic organisms.

On the other hand, it would be valuable to validate the results in Chapters 3 and 4 by combining plant species native to the study area with local AMF isolates. Klironomos (2003) could show that such combinations express the widest span of plant responses due to local adaptation. Highly adapted AMF – plant combinations share a common ecological background of co-evolution (Fitter et al. 2005) and can be expected to provide host plants with the highest benefits (Helgason et al. 2007).

A third interesting aspect in future AMF research are the different functional traits or life strategies of AMF species, as pointed out by van der Heijden and Scheublin (2007). A true understanding of the attributes, apart from spore morphology and ribosomal sequence divergence, that are really different between AMF species, is a crucial step in understanding AMF ecology. The work by Hart and Reader (2002b) proofed that the AMF taxonomy has a functional basis in terms of colonization strategies. One potential tool to extend this

knowledge would be the assessment of the diversity of functional genes in different habitats, as widely done for prokaryotes (Sharma et al. 2007; Kellner et al. 2008) and recently adapted for several soil fungi (Luis et al. 2004; Kellner et al. 2007). Such experiments would help to translate the temporal (e.g. Husband et al. 2002) and spatial (Mummey and Rillig 2008) variability in AMF community composition detected within different habitats and between habitats (Öpik et al. 2006) into potential effects on plant performance and ecosystem processes.

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Summary

To gain insight into mechanisms influencing and maintaining biodiversity and ecosystem functioning in grasslands, 19 as well extensively or intensively managed meadows in the Thüringer Schiefergebirge and in the Franconian Forest in Central Germany were investigated by six closely cooperating subprojects within an interdisciplinary research project 'Biodiversity and Global Change' (BIOLOG) as described in Chapter 1. Within this dissertation I present the results of the subproject MYKO. The focus of this project was on the diversity and functioning of arbuscular mycorrhiza (AM), one of the most important symbiotic interactions in grasslands.

In Chapter 2 it was therefore intended to test which compartment, i.e. spores, intra-radical or extra-radical mycelium, should be investigated in order to assess the AM fungal (AMF) diversity present at one site. The data from molecular analyses revealed remarkable differences in the composition of AMF taxa both between the spores and the mycelia, and between the two types of mycelia. *Glomus* group Ab was dominant in roots and spores, in accordance with previous research. However, members of this group were rarely detected as extra-radical mycelium, in which Paraglomeraceae were dominant, although no evidence was found for the presence of Paraglomeraceae in roots or spores, even when a specific primer set was used. However, the highest AMF diversity was detected in the extra-radical mycelium indicating that this compartment is the most promising for diversity assessments.

In the following two chapters, the focus was on the functioning of AM within the grassland ecosystems. In Chapter 3 the interactive effects of AMF and a hemiparasitic plant *Rhinanthus minor* on the productivity and diversity of plant communities were investigated in a greenhouse study. AMF changed plant community structure by influencing the plant species differently. Generally, AMF decreased plant productivity because the two dominant plant species showed no or even a negative mycorrhizal dependency. Concomitantly, plant diversity increased as subordinate plant species were released from competition. AMF also affected the hemiparasite positively, and thereby amplified its negative impact on host plant biomass. The results highlight the importance of soil microbe interactions with plants shaping their community structure. It could be shown that these indirect effects vary with different AMF species combinations inoculated. It could be concluded that mutualistic rather than parasitic interactions promote plant diversity of this grassland community.

In order to study the influence of AMF on complex trophic chains, the impact of two AMF species, *Glomus intraradices* and *Glomus mosseae* on the interactions between the grass *Phleum pratense*, the aphid *Rhopalosiphum padi* and the parasitoid wasp *Aphidius*

rhopalosiphi were investigated in another greenhouse experiment in chapter 4. AMF inoculation benefited plants by increasing plant biomass and decreasing aphid population growth, irrespective of the AMF species inoculated. Aphid – wasp interactions were modified such that the rate of parasitism was increased compared to the *G. mosseae* and control treatments when plants were inoculated with *G. intraradices*. The developmental time of the wasps decreased and weight at eclosion increased when plants were infected by AMF. Analysis of foliar nitrogen and phosphorus concentrations in plants revealed no clear effect of AMF infection showing that mycorrhizal effects on the trophic chain go beyond a simple increase in nutrient availability. The results demonstrate that the effects of AMF cascade up the food chain and impact performance parameters of plants, aphids and their parasitoids in an AMF species-specific way.

The results of both green house experiments clearly point to the importance of distinct AMF species, as substantial differences between the AMF species inoculated could be detected. Applied to the aims of BIOLOG, i.e. the maintenance of biodiversity and ecosystem functioning, the results appeal to the preservation of a diverse AMF species community. According to this, an extensive management strategy currently applied to most of the meadows in the Thüringer Schiefergebirge and Franconian Forest is appropriate to preserve a diverse AMF community, which in turn can support a diverse plant community and buffer its reactions to single environmental events.

Zusammenfassung

Im Rahmen eines interdisziplinären Forschungsprojektes wurden im Thüringer Schiefergebirge und im Frankenwald (Grenzgebiet Thüringen / Bayern) 19 extensiv genutzte Mähwiesen von sechs eng kooperierenden Forschergruppen im Rahmen des Forschungsprogramms „Biodiversität und Globaler Wandel" (BIOLOG) untersucht. Wie in Kapitel 1 beschrieben, war es Ziel dieser Kooperation, Einblicke in die Mechanismen zu erlangen, die Biodiversität und Ökosystemfunktionen in Grasländern beeinflussen und erhalten. Im Rahmen dieser Dissertation präsentiere ich die aktuellen Ergebnisse des Teilprojekts MYKO, das sich insbesondere mit der Diversität und der Funktion von arbuskulärer Mykorrhiza (AM), einer der wichtigsten symbiotischen Interaktion in Grasländern, beschäftigt.

Kapitel 2 umfasst eine Analyse der AM Pilze (AMF) bezogen auf unterschiedliche Kompartimente: AM-Sporen, intra-radikales Myzel und extra-radikales Myzel. Hierbei stand die Frage im Vordergrund welche dieser Strukturen sich besonders gut eignet um die Diversität von AMF auf einer Fläche zu erfassen. Die Ergebnisse der molekularbiologischen Analysen zeigen hierbei erstaunliche Unterschiede in der Zusammensetzung der AMF-Taxa sowohl zwischen Sporen und den Myzelien als auch zwischen den beiden Arten von Myzelien. Die *Glomus*-Gruppe Ab dominierte hierbei entsprechend früheren Studien in den intraradikalen Myzelien und bei den Sporen. Im Gegensatz dazu wurde diese Gruppe deutlich seltener im extra-radikalen Myzel nachgewiesen. Letzteres wurde von Arten der Paraglomeraceae dominiert, obwohl diese Gruppe weder in den Wurzeln noch als Sporen nachgewiesen werden konnte, auch nicht bei Anwendung spezifischer Primer. Die größte Diversität wurde innerhalb des extra-radikalen Myzels nachgewiesen. Dieses Kompartiment eignet sich somit am besten für die Erfassung der AMF Diversität.

Die folgenden beiden Kapitel konzentrieren sich auf die Funktion der AM in Grasland-Ökosystemen. In Kapitel 3 wurden im Rahmen eines Gewächshausexperiments die interaktiven Effekte zwischen AMF und einem Hemiparasiten *Rhinanthus minor* auf die Produktivität und Diversität von Pflanzengemeinschaften untersucht. Dabei zeigte sich, dass AMF die Struktur der Pflanzengemeinschaft änderten, da sie jede der beteiligten Pflanzenarten unterschiedlich beeinflussten. Im Allgemeinen verringerten die AMF die pflanzliche Produktivität, da die zwei dominierenden Pflanzenarten nicht bzw. negativ auf AMF-Inokulation reagierten. Gleichzeitig erhöhten sie die pflanzliche Diversität, da sich weniger konkurrenzstarke Arten besser durchsetzen konnten. AMF hatten ebenfalls einen positiven Effekt auf den Hemiparasiten und verstärkten damit dessen negativen Einfluss auf

die Biomasse der Wirtspflanzen. Diese Ergebnisse zeigen, wie wichtig Interaktionen zwischen Pflanzen und Bodenmikroorganismen für die Struktur von Lebensgemeinschaften sind. Außerdem konnte gezeigt werden, dass die Effekte in Abhängigkeit von den inokulierten AMF-Arten variieren können. Daraus kann geschlussfolgert werden, dass die Diversität von Grasländern eher von mutualistischen und weniger von parasitischen Interaktionen gefördert wird.

In Kapitel 4 wurde der Einfluss von AMF auf komplexe trophische Interaktionen untersucht. Hierzu wurde in einem Gewächshausexperiment getestet, welche Effekte die zwei AMF Arten *Glomus intraradices* und *Glomus mosseae* auf die Interaktion des Grases *Phleum pratense*, der Blattlaus *Rhopalosiphum padi* und der parasitoiden Wespe *Aphidius rhopalosiphi* haben. Dabei zeigte sich, dass unabhängig von der inokulierten AMF-Art, die Pilze einen positiv Einfluss auf die Pflanzen haben, da sich die pflanzliche Biomasse erhöhte, aber das Wachstum der Blattläuse verringert wurde. Die Parasitierungsraten auf Pflanzen mit *G. intraradices* verdoppelten sich im Vergleich zur *G. mosseae*- und der nicht-mykorrhizalen Kontrollbehandlung, was auf eine Veränderung in der Interaktion zwischen Blattläusen und Wespen schließen lässt. Wenn die Pflanzen mit AMF inokuliert waren, kam es zu einer Verkürzung der Entwicklungszeit der Wespen bei gleichzeitiger Erhöhung des Schlupfgewichtes. Eine Analyse der Stickstoff- und Phosphatkonzentration in den Blättern der Pflanzen zeigte keine deutlichen mykorrhizalen Effekte, was darauf hindeutet, dass die Effekte der AMF auf die Nahrungskette über eine einfache Verbesserung der pflanzlichen Versorgung mit Nährstoffen hinausgehen. Die Ergebnisse zeigen, dass sich AM-Effekte in Abhängigkeit von der AMF-Art innerhalb der Nahrungskette fortsetzen und wichtige Parameter im Lebenszyklus von Pflanzen, Blattläusen und Parasitoiden beeinflussen können.

Die Ergebnisse aus beiden Gewächshausexperimenten heben deutlich die Rolle einzelner AMF-Arten hervor, da jeweils klare Unterschiede zwischen den inokulierten Arten gefunden wurden. In Bezug auf die Ziele von BIOLOG, d. h. die Bewahrung von Biodiversität und Ökosystemfunktionen, unterstreichen die Ergebnisse dieser Arbeit die Wichtigkeit der Erhaltung diverser AMF-Artengemeinschaften. Diesbezüglich ist die aktuell auf vielen Projektflächen praktizierte extensive Nutzung im Thüringer Schiefergebirge und Frankenwald die beste Möglichkeit eine solche Artengemeinschaft zu erhalten. Diese Pilze können dann wiederum eine diverse Pflanzengemeinschaft stützen.

Appendix

Table I. Host plants of Paraglomeraceae species as extracted from literature. We considered references which detected Paraglomeraceae with molecular methods directly on roots or studies (mainly in the greenhouse) where Paraglomeraceae was grown in monocultures and identified based on spore samples (continued on the next page).

Paraglomeraceae species	Host plant	Plant family	Reference
P. brasilianum (sub *Glomus brasilianum*)	*Allium porrum* L.	Alliaceae	Spain & de Miranda (1996)
brasilianum (sub *G. brasilianum*)	*Stylosanthes guyanensis* (Aubl.) Sw.	Fabaceae	Spain & de Miranda (1996)
brasilianum (sub *G. brasilianum*)	*Sorghum bicolor* (L.) Moench [Syn.: *Sorghum sudanense* (Piper) Staph.; *Sorghum vulgare* Pers.]	Poaceae	Spain & de Miranda (1996); Morton & Redecker (2001)
brasilianum (sub *G. brasilianum*)	*Zea mays* L.	Poaceae	Millner et al. (2001); Morton & Redecker (2001)
occultum (sub *G. occultum*)	*Acer pseudoplatanus* L.	Aceraceae	Walker (1982)
occultum	*Pistacea lentiscus* L.	Anacardiaceae	Ferrol et al. (2004)
occultum (sub *G. occultum*)	*Asparagus officinalis* L.	Asparagaceae	Walker (1982)
occultum (sub *G. occultum*)	*Cirsium arvense* (L.) Scop.	Asteraceae	Walker (1982)
occultum (sub *G. occultum*)	*Lactuca sativa* L.	Asteraceae	Ruiz-Lozano et al. (1995)
occultum (sub *G. occultum*)	*Leontodon hispidus* L.	Asteraceae	Tadych & Błaszkowski (2000)
occultum (sub *G. occultum*)	*Sonchus arvensis* L.	Asteraceae	Walker (1982)
occultum (sub *G. occultum*)	*Alnus glutinosa* (L.) Gaertn.	Betulaceae	Walker (1982)
occultum (sub *G. occultum*)	*Equisetum arvense* L.	Equisetaceae	Walker (1982)
occultum (sub *G. occultum*)	*Acacia mangium* Willd.	Fabaceae	Ghosh & Verma (2006)
occultum (sub *G. occultum*)	*Glycine max* (L.) Merr.	Fabaceae	Walker (1982)
occultum (sub *G. occultum*)	*Trifolium repens* L.	Fabaceae	Marulanda et al. (2002)
occultum (sub *G. occultum*)	*Juglans nigra* L.	Juglandaceae	Walker (1982)
occultum (sub *G. occultum*)	*Plantago lanceolata* L.	Plantaginaceae	Smith & Read (1997)
occultum (sub *G. occultum*)	*Agrostis canina* L.	Poaceae	Koske et al. (1997)
occultum (sub *G. occultum*)	*Agrostis palustris* Huds.	Poaceae	Koske et al. (1997)
occultum (sub *G. occultum*)	*Bromus inermis* Leyss.	Poaceae	Walker (1982)
occultum	*Paspalum notatum* Flügge	Poaceae	da Silva et al. (2005)
occultum (sub *G. occultum*)	*Phragmites australis* (Cav.) Trin. ex Steud. [Syn.: *Phragmites communis* Trin.]	Poaceae	Walker (1982)
occultum (sub *G. occultum*)	*Poa annua* L.	Poaceae	Koske et al. (1997)
occultum (sub *G. occultum*)	*Setaria* sp.	Poaceae	Walker (1982)
occultum (sub *G. occultum*)	*Sorghum bicolour* (L.) Moench [Syn.: *Sorghum sudanense* (Piper) Staph.; *Sorghum vulgare* Pers.]	Poaceae	Smith & Read (1997); Morton & Redecker (2001); Walker (1982)
occultum	*Sporobolus wrightii* Munro ex Scribn	Poaceae	Kennedy et al. (2002)

Table I. (continued from the previous page).

Paraglomeraceae species	Host plant	Plant family	Reference
P. occultum (sub *G. occultum*)	*Zea mays* L.	Poaceae	Millner et al. (2001); Morton & Redecker (2001); Walker (1982)
occultum (sub *G. occultum*)	*Persicaria maculosa* Gray [Syn.: *Polygonum persicaria* L.]	Polygonaceae	Walker (1982)
occultum (sub *G. occultum*)	*Potentilla erecta* (L.) Räuschel	Rosaceae	Smith & Read (1997)
occultum (sub *G. occultum*)	*Citrus volkameriana* V.Ten. et Pasq.	Rutaceae	Fidelibus et al. (2000)
occultum (sub *G. occultum*)	*Populus* x *euramericana* (Dode) Guinier	Salicaceae	Smith & Read (1997); Walker (1982)
occultum (sub *G. occultum*)	*Lycopersicon esculentum* Mill.	Solanaceae	Smith & Read (1997); Walker (1982)
sp.	*Plantago major* L.	Plantaginaceae	Börstler et al. (2006)
sp.	*Dactylis glomerata* L.	Poaceae	Wirsel (2004)
sp.	*Lolium multiflorum* Lam.	Poaceae	Börstler et al. (2006)
sp. (sub *G.* sp. HM-CL5)	*Fragaria vesca* L.	Rosaceae	Turnau et al. (2001)
sp.	*Zea mays* L.	Poaceae	Hirji et al. (2006)
sp.	*Triticum aestivum* L.	Poaceae	Hirji et al. (2006)

References Table I

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Table II Results of ANOVAs for the single-species experiment. Biomass data are log-transformed prior to analysis. Orthogonal contrasts are calculated for fungal treatments which are described in detail in the material & methods section. F values and significance levels are given after accounting for multiple comparisons with the Benjamini-Hochberg method (line-by-line): * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Source of variation	d.f.	*R. acetosa*	*V. chamaedrys*	*P. lanceolata*	*V. cracca*	*T. pratense*	*F. rubra*	*H. lanatus*
Fungi	4	4.66 **	8.43 ***	11.92 ***	6.50 ***	12.54 ***	8.20 ***	2.89 *
non-AMF vs. fungi	1	4.20	2.19	1.92	21.95*	47.66	5.13	3.80
field vs. commercial	1	11.00**	1.26	18.58***	0.44	0.58	27.18***	1.14
both vs. single	1	2.84	6.66*	18.29***	3.56	1.19	0.39	2.08
giga vs. glom	1	0.59	23.62***	8.89*	0.04	0.62	0.10	4.55
Tray [fungi]	45	1.72	0.73	1.30	1.36	0.86	1.91	1.46
Parasite	1	0.10	0.67	1.61	3.34	0.11	0.003	1.13
Fungi x parasite	4	1.53	0.65	1.70	1.84	1.44	0.95	0.32
Residuals (MS)	45	(0.27)	(0.62)	(0.39)	(0.33)	43 (0.33)	(0.35)	(0.85)

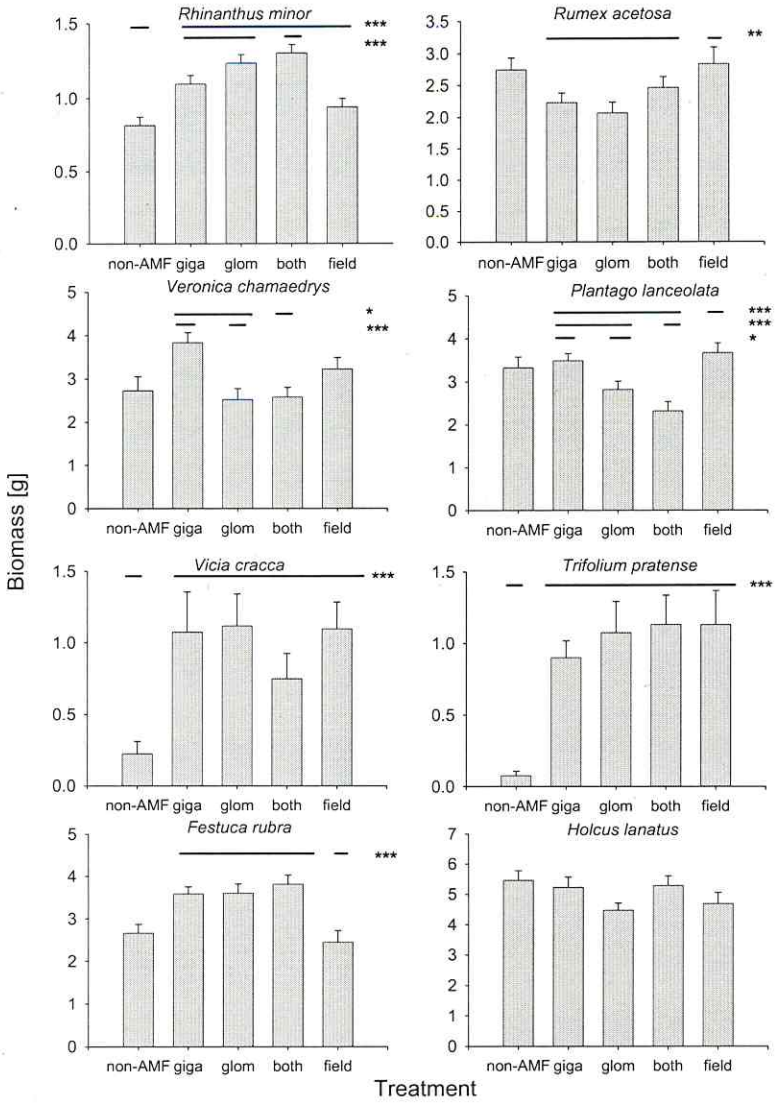

Figure 1 Responses of *Rhinanthus minor* and host plants (mean + se) grown in the single-species experiment responding to different mycorrhizal treatments. Biomass of host plant species was averaged across the two hemiparasite treatments. Plants grown without AMF (non-AMF), or inoculated either with *Gi. margarita* (giga), *G. intraradices* (glom), a mixture of *Gi. margarita* and *G. intraradices* (both), field sampled mycorrhiza. Horizontal lines above bars indicate a significant difference among AMF treatments, according to linear contrasts and accounting for multiple comparisons with the Benjamini-Hochberg method. *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

Cooperations

The present work was conducted at the University of Leipzig, Institute of Botany and at the Helmholtz Centre for Environmental Research in Halle (UFZ), Department Soil Ecology in the group of Prof. F. Buscot.

The following cooperations were important for the completion of this work:

Chapter 2 is based on a publication which was written in close cooperation with Dr. Carsten Renker (University of Leipzig, UFZ Halle, present address: Naturhistorisches Museum Mainz).

The greenhouse experiment in chapter 3 was carried out in cooperation with Claudia Stein (UFZ Halle, Department of Community Ecology) and Cornelia Rißmann (UFZ Halle, Department of Community Ecology, present address: TU Cottbus).

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Element analyses (chapters 3 and 4) were done by Antje Thondorf (UFZ Halle, Department of Community Ecology) and Dr. Hans-Joachim Stärk (UFZ Halle, Department of Analytical Chemistry).

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Ehrenwörtliche Erklärung

Die geltende Promotionsordnung der Biologisch-Pharmazeutischen Fakultät der Friedrich-Schiller-Universität Jena ist mir bekannt.

Ich habe die Dissertation selbst angefertigt. Sämtliche von mir benutzten Hilfsmittel, persönliche Mitteilungen order Quellen sind in der vorliegenden Arbeit angegeben.

Personen, die mich bei der Auswahl und Auswertung des Materials und bei der Herstellung des Manuskripts unterstützt haben, sind unter „Cooperations" und in der Danksagung (Acknowledgements) genannt.

Die Hilfe eines Promotionsberaters wurde nicht in Anspruch genommen. Dritte haben weder unmittelbar noch mittelbar geldwerte Leistungen von mir für Arbeiten erhalten, die im Zusammenhang mit dem Inhalt der vorgelegten Dissertation stehen.

Die vorgelegte Dissertation habe ich noch nicht als Prüfungsarbeit für eine staatliche oder andere wissenschaftliche Prüfung eingereicht. Ebenso habe ich nicht die gleiche, eine in wesentlichen Teilen ähnliche oder eine andere Abhandlung bei einer anderen Hochschule als Dissertation eingereicht.

Halle, den 2. Juli 2008

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Publications

Hempel S., Renker C., Buscot F. (2007)
Differences in the species composition of arbuscular mycorrhizal fungi in spore, root and soil communities in a grassland ecosystem.
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