Biol Fertil Soils (2006) 42: 286–298
DOI 10.1007/s00374-005-0026-9

ORIGINAL PAPER

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Species composition of arbuscular mycorrhizal fungi in two mountain meadows with differing management types and levels of plant biodiversity

Received: 20 August 2004 / Revised: 7 June 2005 / Accepted: 8 June 2005 / Published online: 22 July 2005

Abstract Species composition of arbuscular mycorrhizal fungi (AMF) was analysed in two differently managed mountain grasslands in Thuringia (Germany). Arbuscular mycorrhizal fungi were studied in the roots of 18 dominant plant species from a total of 56 (32%). Additionally, spores of AMF were isolated from soil samples. Arbuscular mycorrhizal fungi species composition was analysed based on 96 sequences of the internal transcribed spacer of the nuclear ribosomal DNA, 72 originated from mycorrhizal roots, and 24 originated from AMF spores. Phylogenetic analyses revealed a total of 19 AMF species representing all genera of the Glomeromycota except *Scutellospora* and *Pacispora*. Despite a different farming intensity, resulting in remarkable differences concerning their plant species diversity (27 against 43 plant species), the diversity of AMF was found to be similar with 11 species on the intensively farmed meadow and ten species on the extensively farmed one. Nevertheless, species composition between both sites was clearly different. It thus seems likely that the AMF species composition, but not necessarily the species number, is related to above ground plant biodiversity in the system under study.

Keywords Arbuscular mycorrhizal fungi · Farming intensity · Internal transcribed spacer · Plant species diversity · Species composition

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Introduction

Arbuscular mycorrhizal fungi (AMF) form mutualistic symbioses with the majority of vascular plants below ground. For plants, AMF enhance nutrient availability (e.g. phosphate) and, in turn, receive carbohydrates from plants (Smith and Read 1997). AMF can also play a crucial role for protecting plants against heavy metals and pathogens and stabilising their water supply (Hardie 1985; Galli et al. 1994; Filion et al. 1999; Augé et al. 2001). It is assumed that AMF influenced the evolution of vascular plants (Simon et al. 1993), and that today's flora would probably not exist without AMF. Despite the ecological significance, little is known about factors driving biodiversity and species composition of AMF in natural habitats. Several studies investigating AMF have analysed AMF spore communities (Gerdemann and Trappe 1974). However, spores may not mirror the actual functional active AMF associated with plants (Clapp et al. 1995; Merryweather and Fitter 1998; Turnau et al. 2001; Renker et al. 2005) due to taxon-specific differences between sporulation and root colonisation rates. Molecular methods have now been established, which allow a direct characterisation of AMF species in plant roots (Sanders et al. 1996).

In general, experiments in micro- and macrocosms have led to the hypotheses that high AMF species richness determines high plant species richness, and that AMF composition in the soil determines the community structure of plants (Grime et al. 1987; van der Heijden et al. 1998a,b). Recent results from studies in natural and semi-natural ecosystems have shown that AMF diversity and species composition might be affected by environmental factors such as soil nutrient content and land use intensity. Compared to non-fertilised field sites with high plant diversity, agriculturally used sites showed a reduced AMF diversity (Helgason et al. 1998; Daniell et al. 2001).

Biodiversity is discussed as an essential fundament of ecosystem functioning. Tilman (2000) points out that greater diversity may lead to greater ecosystem stability. For

this reason, a project (BIOLOG–DIVA-Jena) was set-up to investigate the relationship between biodiversity and ecosystem functioning in grasslands of the "Thüringer Schiefergebirge" (Thuringia, Germany), representing a range of differently farmed mountain meadows. The first results of this study indicate that habitat characteristics might influence the genetic diversity of plant species (Odat et al. 2004). Based on these results we hypothesised that habitat characteristics might also influence the AMF species composition and diversity. Therefore, we investigated the species composition of AMF in an intensively farmed meadow (IFM) with low plant diversity and an extensively farmed meadow (EFM) with high plant diversity. We assumed a higher AMF species number at the EFM because of its higher plant diversity, lower phosphate content and lower farming intensity compared to the IFM.

Materials and methods

Field sites

Two different mountain meadows in the Thüringer Schiefergebirge (Thuringia, Germany) were investigated. This area is characterised by flat soils over presilurian and Devonian schists (Hattenbach 1959). The mountain range reaches altitudes of 792 m above sea level (Wetzstein, south of Lehesten). The climate is temperate with an oceanic influence, a 950- to 1,099-mm annual precipitation and an average temperature of 6.0–7.0°C (average temperature in January −3.0°C, average temperature in July 15°C, absolute minimum temperature −29°C, absolute maximum temperature 35°C and 190 days per year with temperatures above 5°C).

Table 1 Soil data of the IFM and the EFM in the Thüringer Schiefergebirge (Thuringia, Germany)

Soil data	IFM	EFM
K (mg/kg)	119.37	40.40
Mg (mg/kg)	310.33	391.99
Na (mg/kg)	120.38	147.57
P (mg/kg)	76.28	Nd
S (mg/kg)	10.39	13.89
C (mg/g)	44.96	51.41
C_{org}(wt.-%)	5.0	6.3
N (mg/g)	4.21	4.54
N_{min} (mg/kg)	5.49	6.71
C/N	12.46	13.21
pH	6.20	5.78
Soil moisture (%)	28.7	34.1
Soil texture (wt.-%)		
Sand	30.4	22.8
Silt	44.8	50.6
Clay	24.8	26.6

Nd Not detectable

Plots of 19×13 m were analysed for their AMF species composition at each meadow. While one site, located near Schlegel (11°37′31″E/50°24′32″N) at an elevation of 640 m above sea level, represents an IFM, the second one, located near Grumbach (11°30′48″E/50°25′22″N) at an elevation of 705 m above sea level, is an EFM. The IFM was mown twice a year in summer and autumn, and the EFM was mown only once a year in summer. Properties of soils from both sites are given in Table 1, showing slightly more acidic conditions and phosphorus under the detection limit of 9 mg/kg soil at the EFM, indicating nutrient-poor conditions in comparison to the IFM site. The most pronounced contrast between both sites was plant diversity and species composition, with only 27 plant species at the IFM vs 43 at the EFM. Plant biomass production was nearly twice as high at the IFM (640 g dry weight/m^2) at the beginning of June due to the dominance of highly productive grasses compared to the EFM (370 g dry weight/m^2), where the dominance of herbs resulted in a lower productivity (Table 2; for details, see Kahmen et al. 2005).

Root and spore sampling from the field

The investigated arbuscular mycorrhizal (AM) roots or spores all originated from fresh soil samples. To get an insight into the species composition of AMF *in planta*, roots of the seven most common plant species at the IFM and of 13 plant species at the EMF were successfully examined. This represents 26 and 30%, respectively, of the occurring plants at the sites and a total of 18 species (Tables 2 and 3). The total root systems of these plants were removed, and fine roots were chosen randomly for DNA extraction (see below). In addition, AM spores were isolated from soils following the protocol of Esch et al. (1994).

DNA extraction and nested PCR with restriction digest

DNA extractions from single 2-cm-long root fragments from 98 plant root systems (Table 4) were performed as described by Redecker (2000). To amplify DNA from 64 single spores, these were separated in a drop of sterile water. The water was removed before each spore was crushed, pipetted with 20 µl of the PCR Mix and used directly for PCR.

The technique used for the nested PCR has been described by Renker et al. (2003). The first PCR step was performed with the primer pair small subunit (SSU)-Glom1 (ATT ACG TCC CTG CCC TTT GTA CA) and large subunit (LSU)-Glom1 (CTT CAA TCG TTT CCC TTT CA). While most Glomeromycota do not show any *Alu*I restriction site within their internal transcribed spacer (ITS) region, it was also shown that most amplicons containing this restriction site belong to fungi other than AMF (Renker et al. 2003). Therefore, 5 µl of the products of the

Table 2 Plant species and plant species coverage found on plots of 247 m^2 at an IFM and an EFM in the Thüringer Schiefergebirge (Thuringia, Germany)

Plant species	IFM	EFM
Apiaceae		
Anthriscus sylvestris (NM)	2 (+)	12 (+)
Daucus carota (M)	–	1 (+)
Meum athamanticum (?)	–	36 (+)
Asteraceae		
Achillea millefolium (M)	1 (+)	1 [+]
Centaurea pseudophrygia (?)	–	1 (+)
Leontodon hispidus (M)	–	1 (+)
Leucanthemum vulgare (M)	–	2 (+)
Tanacetum vulgare (M)	1 [+]	–
Taraxacum officinale (M)	30+	1 [+]
Boraginaceae		
Myosotis arvensis (NM)	1 (+)	–
Brassicaceae		
Cardamine pratensis (NM)	–	1 +
Campanulaceae		
Campanula rotundifolia (M)	–	9 (+)
Phyteuma spicatum [M(-NM)]	–	9 (+)
Caryophyllaceae		
Cerastium holosteoides [(M−)NM]	1 (+)	1 [+]
Lychnis flos-cuculi [(M−)NM]	–	2 +
Stellaria graminea (NM)	4 [+]	2 [+]
Cyperaceae		
Carex pilulifera (NM)	–	1 [+]
Dipsacaceae		
Knautia arvensis (M)	–	4 (+)
Fabaceae		
Lathyrus linifolius (M)	–	4 (+)
Trifolium pratense (M)	8 +	8 +
Trifolium repens (M)	34 +	5 (+)
Hypericaceae		
Hypericum maculatum (M)	1 (+)	29 (+)
Juncaceae		
Luzula campestris [(M−)NM]	–	10 (+)
Lamiaceae		
Ajuga reptans (M)	–	1 +
Lamium purpureum (M)	1 (+)	–
Thymus pulegioides (?)	–	1 (+)
Orchidaceae		
Listera ovata (M)	–	1 [+]
Plantaginaceae		
Plantago lanceolata (M)	–	13 +
Plantago major (M)	2 +	–
Poaceae		
Agrostis capillaris (M)	–	11 (+)
Anthoxanthum odoratum (M)	–	9 +
Arrhenatherum elatius [M(−NM)]	–	10 +
Briza media (M)	–	1 +
Bromus hordeaceus [M(−NM)]	1 [+]	–
Dactylis glomerata (M)	70 +	5 +
Festuca pratensis (M)	20 [+]	–
Festuca rubra (M)	–	39 [+]
Helictotrichon pubescens (M)	–	2 (+)
Lolium multiflorum (M)	25 +	–
Lolium perenne (M)	10 (+)	–
Nardus stricta (M)	–	2 [+]
Phleum pratense (M)	2 (+)	–
Poa pratensis [M(−NM)]	4 [+]	7 [+]
Poa trivialis [M(−NM)]	14 (+)	–
Trisetum flavescens (M)	–	9 +
Polygonaceae		
Rumex acetosa [(M−)NM]	3 (+)	4 (+)
Ranunculaceae		
Ranunculus acris (M)	1 (+)	12 (+)
Ranunculus repens (M)	1 [+]	–
Rosaceae		
Alchemilla monticola (M)	1 (+)	24 (+)
Potentilla erecta [M(−NM)]	–	13 +
Rubiaceae		
Galium album (M)	1 +	–
Galium uliginosum (NM)	–	1 +
Santalaceae		
Thesium pyrenaicum (?)	–	1 [+]
Scrophulariaceae		
Veronica arvensis (M)	1 [+]	–
Veronica chamaedrys [M(−NM)]	4 (+)	11 +
Veronica officinalis (M-NM)	–	2 [+]

Behind the name of the plant species their mycorrhizal status is given according to Harley and Harley (1987) (*M* Mycorrhizal; *NM* non-mycorrhizal; ? mycorrhizal status unknown). Numbers given in the columns concern per cent coverage of each plant species. Numbers were averaged out of four subplots. Species marked with [+] were detected in the plot but were not considered in this study. Species marked with (+) were chosen for DNA extraction but PCR was not successful. Sequences from AM fungi were revealed on roots of species marked with +

first PCR were digested with *Alu*I for 1 h in a total volume of 20 µl with 5 U of *Alu*I (MBI Fermentas, St. Leon-Rot, Germany), excluding all amplicons cut within their ITS region from the second step of the nested PCR. After this digestion, the DNA was precipitated with 50 µl of 100% ethanol and incubated on ice for at least 1 h. The samples were centrifuged at 20,000×*g* for 10 min, and after discarding the supernatant, the pellets were dried for 30 min at 50°C and redissolved in 10 µl demineralised water. For the second reaction of the nested PCR performed with the primers ITS5/ITS4 (White et al. 1990), 1 µl of this DNA template was used.

Cloning

Polymerase chain reaction products with the expected length for AMF (400–650 bp), that is, 58 products from roots and 21 from spores, were cloned into the pCR4-TOPO Vector following the manufacturer's protocol of

Table 3 Arbuscular mycorrhizal fungi diversity and community structure of the IFM and EFM in the Thüringer Schiefergebirge (Thuringia, Germany) in comparison with the number of the respective plant and spore samples (S)

Molecular species and number of sequences from all samples of both meadows	IFM (number of sequences in comparison to the number of plant and spore samples)	EFM (number of sequences in comparison to the number of plant and spore samples)
Glomus sp. A, *Glomus* group Ab (3)		3 (1 Cp, 1 Pe, 1 Pl)
Glomus sp. B, *Glomus* group Ab (8)	8 (2 Pm, 3 Tp, 1 Tr)	
Glomus sp. C, *Glomus* group Ab (15)	15 (1 Dg, 2 Pm, 1 To, 5 Tp, 4 Tr)	
Glomus sp. D, *Glomus* group Ab (4)		4 (1 Dg, 1 Lf)
Glomus sp. I, *Glomus* group Ab (1)	1 (1 Tp)	
Glomus sp. II, *Glomus* group Ab (1)	1 (1 To)	
Glomus sp. III, *Glomus* group Ab (1)		1 (1 Ae)
Glomus sp. IV, *Glomus* group Ab (1)		1 (1 Bm)
Glomus sp. V, *Glomus* group Ab (1)		1 (1 Pe)
Glomus intraradices (21)	8 (5 Pm)	13 (1 Ar, 1 Gu, 2 Pl, 1 Tp, 2 Tf, 1 S)
Glomus sp. "Bad Sachsa" (7)		7 (1 S)
Glomus versiforme (1)	1 (1 Tr)	
Paraglomus sp. (3)	3 (1 Lm, 1 Pm, 1 S)	
Acaulospora sp. (3)		3 (1 Ao, 1 Pl)
Gigaspora sp. (3)	3 (1 S)	
Archaeospora leptoticha (1)		1 (1 Pl)
Glomus sp. E, *Glomus* group B (4)	4 (3 S)	
Glomus sp. VI, *Glomus* group B (1)	1 (1 Pm)	
Glomus claroideum (17)	9 (1 Ga, 1 Pm, 3 S)	8 (1 Gu, 3 Tp, 1 Vc, 1 S)
96 sequences	54 sequences (41 sequences from 23 plants[a], 13 sequences from 8 spores)	42 sequences (31 sequences from 19 plants[b], 11 sequences from 3 spores)
19 molecular species	11 molecular species (four families, two groups of Glomeraceae)	Ten molecular species (three families, three groups of Glomeraceae)

Ae Arrhenatherum elatius, *Ao Anthoxanthum odoratum*, *Ar Ajuga reptans*, *Bm Briza media*, *Cp Cardamine pratensis*, *Dg Dactylis glomerata*, *Ga Galium album*, *Gu Galium uliginosum*, *Lf Lychnis flos-cuculi*, *Lm Lolium multiflorum*, *Pe Potentilla erecta*, *Pl Plantago lanceolata*, *Pm Plantago major*, *Tf Trisetum flavescens*, *To Taraxacum officinale* agg., *Tp Trifolium pratense*, *Tr Trifolium repens*, *Vc Veronica chamaedrys*

[a]Four plants with, in each case, two sequences of the same molecular species; six plants with, in each case, two sequences of different molecular species; one plant with nine sequences of four different molecular species; and 12 plants with, in each case, one molecular species

[b]Two plants with, in each case, two sequences of the same molecular species; two plants with, in each case, three sequences of the same molecular species; two plants with, in each case, two sequences of the same molecular species and one other sequence type; two plants with, in each case, two sequences of different molecular species; and 11 plants with, in each case, one molecular species

the TOPO TA Cloning Kit (Invitrogen Life Technologies, Karlsruhe, Germany) and transformed into TOP10 Chemically Competent *Escherichia coli* (Table 4). At least four clones per cloning reaction were tested for presence of an ITS insert, and according to the material (roots/spore) and site of origin, 25–50% of the clones were found to contain fragments of the expected size (number of positive tested clones in Table 4).

Sequencing and sequence analyses

One hundred and thirty-five of the 165 clones were sequenced (see Table 4). Sequencing was done using a LI-COR DNA Sequencer Long Reader 4200 and the Thermo Sequenase fluorescent labelled primer cycle sequencing kit with 7-deaza-dGTP (Amersham Pharmacia Biotech, Little Chalfont, UK).

DNA sequences of the complete ITS were submitted to the European Molecular Biology Laboratories (EMBL) database under the accession numbers AJ504636–AJ504638, AJ504640, AJ504641, AJ504644, AJ504645, AJ504649, AJ504650, AJ517782, AJ534718, AJ534719, AJ567728–AJ567752 and AJ567761–AJ567819. In addition to these sequences, sequences from four other field sites (Renker et al. 2003, 2005) were included to interpret the results in a regional context. Reference sequences to analyse the systematic position of the new sequences were taken from

Table 4 Molecular processing of the field samples in total numbers

Site	Root samples		Spore samples		Σ
	IFM	EFM	IFM	EFM	
Sample numbers	48	50	32	32	162
DNA extracts	53 (43×1+5×2)	55 (45×1+5×2)	Nd	Nd	108
PCR products after nested PCR (%)	38 (71)	27 (49)	19 (59)	21 (66)	105 (65)
Clonings	29	29	12	9	79
Total number of tested clones	124	129	64	82	399
Positive tested clones	63	55	27	20	165
Vector isolation	53	45	23	19	140
Sequencing	53	43	22	17	135
Non-mycorrhizal sequences (%)	10 (19)	12 (28)	7 (32)	4 (24)	33 (24)
AMF sequences (%)	41 (77)	31 (72)	13 (59)	11 (65)	96 (71)

Percent values in the line "PCR products" are calculated using the number of DNA extracts from roots or the number of sampled spores of the respective site as 100%, respectively (sample numbers were used for the overall calculation). DNA extraction from roots was normally done once (×1). To check for spatial heterogeneity, pieces from root systems of five plants were extracted twice (5×2). DNA extraction from spores was not done (Nd), as spores were used directly in PCR

GenBank. A direct alignment of the full-length ITS sequences was not possible due to high variations within the whole data set. Therefore, in a first step according to Cullings and Vogler (1998) and Redecker et al. (1999), only the 5.8S subunit genes embedded between the ITS1 and ITS2 regions were aligned by hand and used to separate the main AMF taxa (genera or species groups) present in the field. Species resolution was obtained in a second step by aligning the full-length ITS sequences within each sequence cluster separated by the 5.8S alignment.

Phylogenetic trees (Figs. 1, 2, 3) were inferred using distance criteria as implemented in Phylogenetic Analysis Using Parsimony (* and other Methods) (PAUP*) 4.0b10 (Swofford 2003). The confidence of branching was assessed using 1,000 bootstrap resamplings in distance analysis (neighbor-joining method) based on the Kimura-2-Parameter model.

Classification of the sequences was performed based on analyses of the whole ITS region, including reference sequences of identified AMF from GenBank. Sequences falling into a clade, including such reference sequences and showing sequence identities of at least 92% (which was, in most cases for clades, supported by bootstrap values higher than 90%), were attributed and named according to these reference taxa. Sequences forming distinct clades within the Glomeromycota, but without high analogies to reference sequences from GenBank, were termed unknown species (sp.) of the respective genus. In the case of the genus *Glomus*, these sequences were either differentiated by letters (*Glomus* sp. A to *Glomus* sp. E) when forming clades, or numbers (*Glomus* sp. I to *Glomus* sp. VI) when representing single lineages (see Figs. 2 and 3, and also Discussion). This nomenclature, developed on the basis of the full-length ITS sequences, was also used afterwards in the phylogram of selected 5.8S ribosomal DNA (rDNA) sequences, giving an overview of the AMF diversity at the field sites (Fig. 1).

Results

Altogether, we generated 96 sequences, 72 from roots (41 IFM and 31 EFM) and 24 from spores (13 IFM and 11 EFM) (Tables 3 and 4). A first phylogenetic analysis, based on the approximately 159-bp 5.8S region, allowed us to affiliate the sequences to genera within the Glomeromycota (Fig. 1). Sequences matching all families of the Glomeromycota except Pacisporaceae were obtained and, except *Scutellospora* and *Pacispora*, all AMF genera described so far were detected.

Whole ITS alignments were performed within each main clade separated in the 5.8S analysis (Fig. 1) to get better inter- and intraspecific resolution. Figures 2 and 3 give the most representative examples of such phylogenetic analyses within *Glomus* group Ab and *Glomus* group B (*sensu* Schüßler et al. 2001), respectively. Based on these analyses, 19 molecular species were separated.

The numbers of species found at both sites were almost identical, as the IFM and EFM contained 11 and 10 AMF molecular species, respectively. However, the AMF species composition appeared to differ between both sites. Apart from *Glomus intraradices* and *Glomus claroideum* that were frequently found at both meadows, and which accounted for approximately 40% of the detected sequences (Figs. 2 and 3), most molecular species were only detected either at the IFM or at the EFM (Figs. 1, 2, 3 and Table 3). However, as they were rarely detected and sometimes only represented by one single spore or one plant root sample, it cannot be concluded that these differing molecular species were really site-specific.

Besides the identification of *G. intraradices* and *G. claroideum*, the following molecular species were assigned to known species due to high identities with reference sequences from GenBank: *Glomus* sp. "Bad Sachsa", a member of *Glomus* group Aa (*sensu* Schüßler et al. 2001), was found at the EFM (see also Błaszkowski et al. 2004),

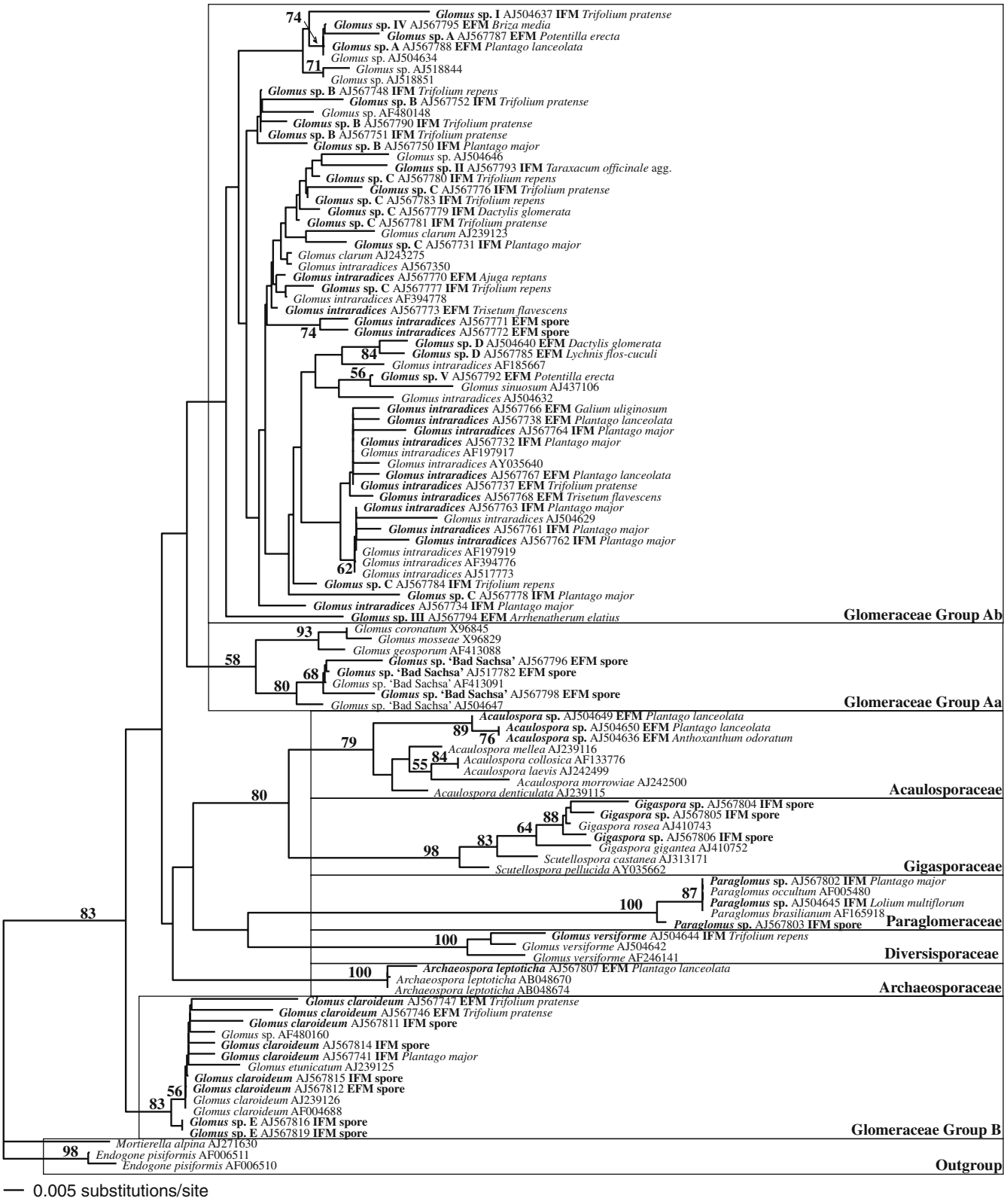

Fig. 1 Rooted neighbor-joining phylogram of selected sequences from the IFM and EFM in the Thüringer Schiefergebirge (Thuringia, Germany) based on distance analyses (Kimura-2-Parameter) of the 5.8S rDNA. For comparison, identified sequence types from GenBank were included in the analyses. Source and sample type is just added for sequences gathered in this study. Bootstrap supports above 50% are given on the branches (1,000 resamplings)

Glomus versiforme was detected at the IFM and *Archaeospora leptoticha* was detected at the EFM (Fig. 1). Altogether, only five from 19 molecular species (i.e. 26%) were assigned to known species, leaving a bulk of sequences with an unknown identity.

Glomus group Ab was found to be the most dominant (58% of all sequences) and diverse group at both sites (Fig. 2). In this group we separated five species (*Glomus* sp. A to *Glomus* sp. D and *G. intraradices*), represented by at least three sequences, building distinct clades, and five further species, termed *Glomus* sp. I to *Glomus* sp. V, that were represented by single sequences. Members of this group dominated on roots (75% of all sequences from roots) but were rarely found as spores (8% of all sequences from spores).

The three species belonging to *Glomus* group B (i.e. *G. claroideum*, *Glomus* sp. E and *Glomus* sp. VI; 23% of the overall sequences) were found evenly distributed on roots and spores. They made up 15% of the sequences

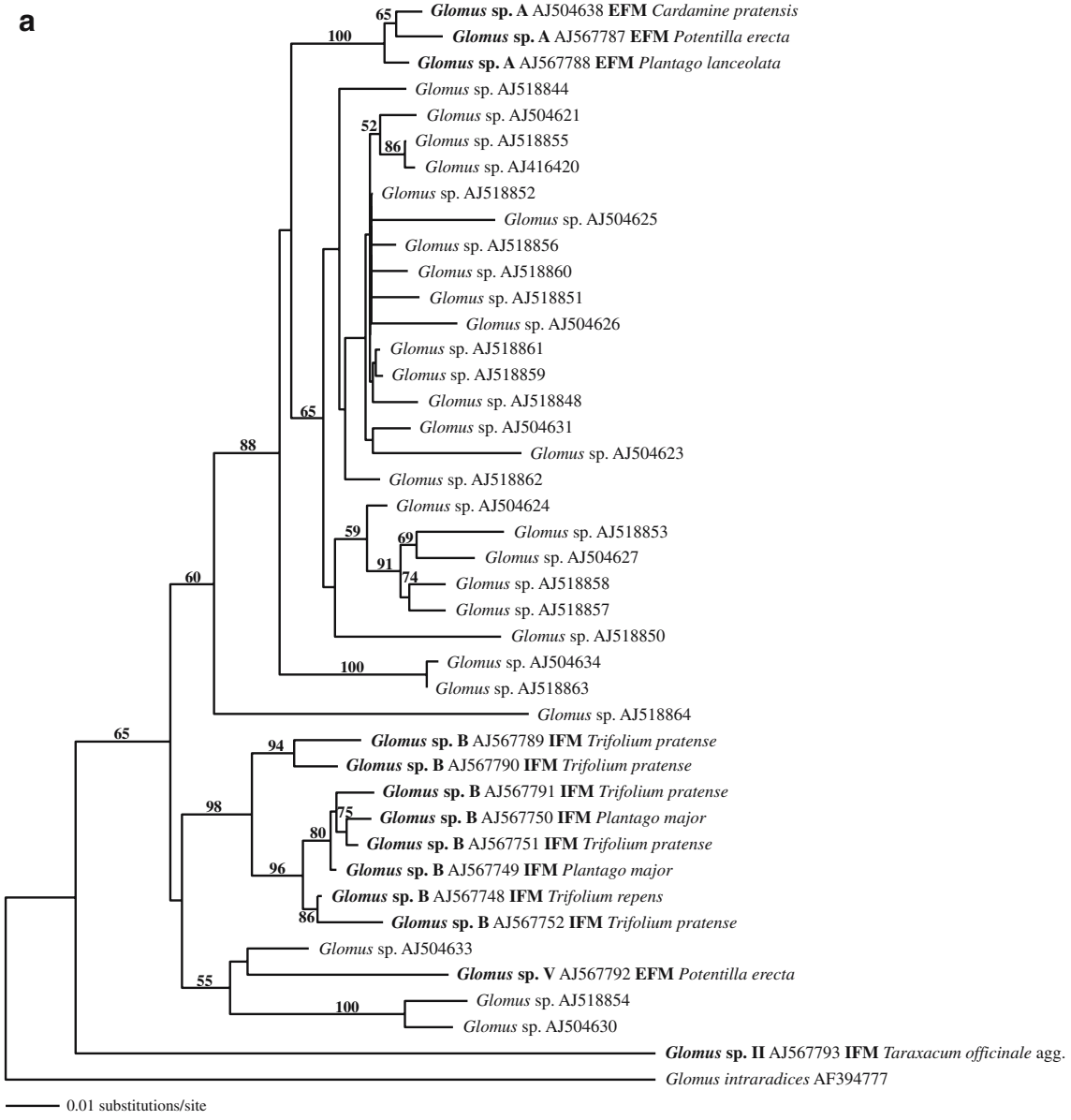

Fig. 2 Rooted neighbor-joining phylogram of sequences belonging to *Glomus* group Ab (*sensu* Schüßler et al. 2001) from the IFM and EFM in the Thüringer Schiefergebirge (Thuringia, Germany) based on distance analyses (Kimura-2-Parameter) of the ITS rDNA. For comparison, identified sequence types from GenBank were included in the analyses. Source and sample type is just added for sequences gathered in this study. Bootstrap supports above 50% are given on the branches (1,000 resamplings). Since an alignment of all sequences within this group was impossible due to sequence variation, **a** is based on an alignment containing all sequences of *Glomus* sp. A and B, while **b** is based on an alignemt containing all sequences of *G. intraradices*, *Glomus* sp. C and D within *Glomus* group Ab. Per cent values given in the right part of **b** refer to sequence identities between the two sequences at both ends of the bracket

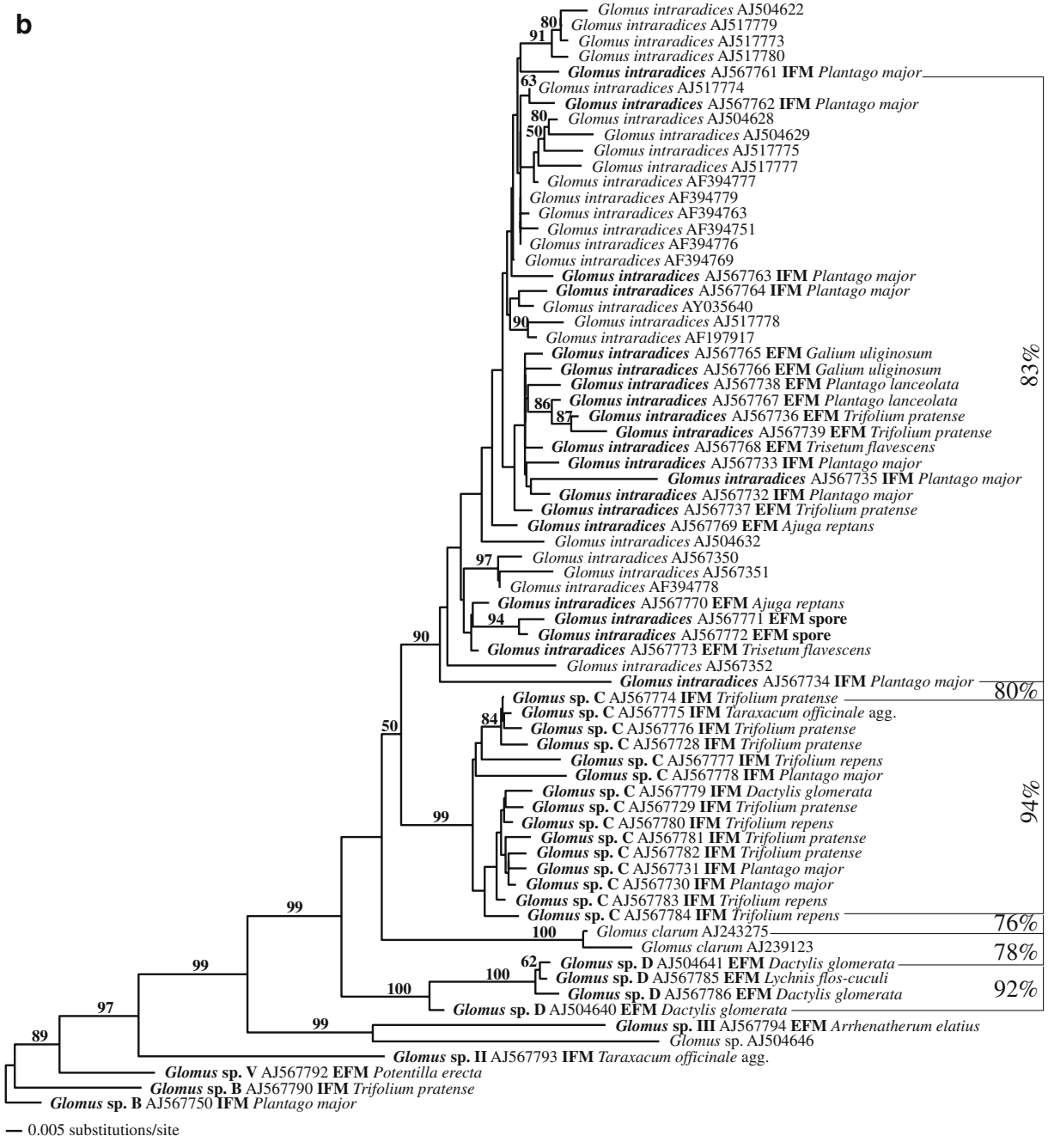

Fig. 2 (continued)

from roots and 46% of the sequences from spores, respectively (Table 3 and Fig. 3).

The detected sequences assigned to the Paraglomeraceae (IFM), Acaulosporaceae (EFM) and Gigasporaceae (IFM) must be considered as belonging to, so far, non-sequenced species because of major differences to any reference sequence in GenBank. This separation was apparent at the 5.8S level for *Acaulospora* (Fig. 1), but becomes also clear when considering the whole ITS for *Paraglomus* and *Gigaspora* (data not shown).

Apart from differences in the AMF species composition at both meadows, a clear discrepancy between AMF detected on roots and on spores was found. *G. intraradices*, *G. claroideum* and *Paraglomus* sp. were the only species detected on both roots and spores, while all other species were only detected on roots (i.e. *Acaulospora* sp., *Glomus* sp. A to *Glomus* sp. D and *Glomus* sp. I to *Glomus* sp. VI) or spores (i.e. *Glomus* sp. "Bad Sachsa", *Gigaspora* sp. and *Glomus* sp. E; see Table 3). It should be emphasised that the spore investigations took place only once in autumn.

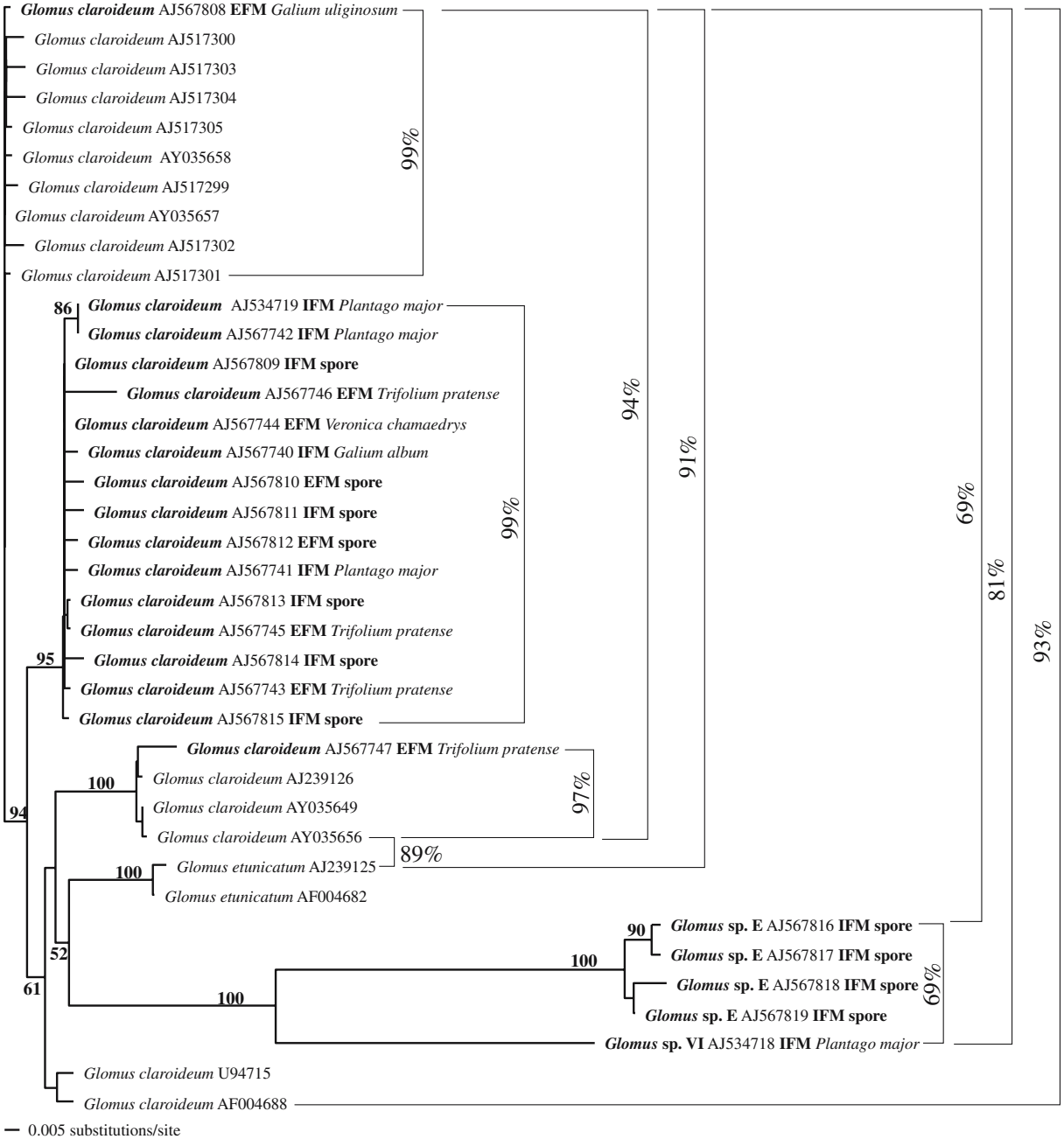

Fig. 3 Unrooted neighbor-joining phylogram of sequences belonging to *Glomus* group B (*sensu* Schüßler et al. 2001) from the IFM and EFM in the Thüringer Schiefergebirge (Thuringia, Germany) based on distance analyses (Kimura-2-Parameter) of the ITS rDNA. For comparison, identified sequence types from GenBank were included in the analyses. Source and sample type is only added for sequences gathered in this study. Bootstrap supports above 50% are given on the branches (1,000 resamplings). Per cent values given in the right part of the figure refer to sequence identities between the two sequences at both ends of the bracket

Discussion

The species concept applied in this study

Taxonomical concepts based on spore morphology proposed 150–200 AMF species worldwide (Walker and Trappe 1993). However, AM roots themselves provide poor morphological criteria for the determination of their mycobionts to the species level. Therefore, molecular methods have to be used to characterise these fungi. Initial works based on field-collected roots and/or spores were focused on the small subunit (SSU) of the rDNA (e.g. Helgason et al. 1998; Johnson et al. 2003; Vandenkoornhuyse et al. 2002, 2003). Later on, results were published regarding the ITS region (e.g. Haug et al. 2004; Hildebrandt et al. 2001; Landwehr et al. 2002; Renker et al. 2005; Wubet et al. 2003a,b). Many recent studies are based on the large subunit (LSU) of the rDNA (e.g. Gollotte et al. 2004;

Haug et al. 2004; Munkvold et al. 2004; Nielsen et al. 2004; Rosendahl and Stukenbrock 2004). Within the rDNA, the non-coding ITS displays a higher polymorphism than the SSU and LSU (White et al. 1990). It is routinely used to identify ectomycorrhizas (Buscot et al. 2000) and was demonstrated to be also adequate for AMF identification (Lloyd-MacGilp et al. 1996; Redecker 2000). In a recent study, Wubet et al. (2003a) have analysed the ITS region of AMF and grouped sequences displaying at least 93% sequence identity in common species. However, in the work presented here, we separated putative species at sequence identities below 92%. In most assessable cases, this separation was consistent with the nomenclature used for the sequence data of GenBank. For example, in *Glomus* group B, our ITS analyses separated four distinct clades in *G. claroideum* (Fig. 3), with three of them containing reference sequences from GenBank that are assigned to *G. claroideum*. Variability within each clade was low (97–99% sequence identity), and variability between clades did not exceed 7% (Fig. 3). In contrast, the sequence identities between the respective clades of *G. claroideum*, *Glomus* sp. E and *Glomus* sp. VI were clearly below 92% (see Fig. 3). The situation is more complex in *Glomus* group Ab, especially for *G. intraradices*. The large clade containing our own sequences assigned to *G. intraradices*, based on sequence comparison with data from GenBank, represents a continuum without clear clade separation. However, both ends of this broad cluster displayed only 83% identity. For *G. intraradices*, a species separation based on at least 92 or 93% sequence identity of the whole ITS, as used in this work and by Wubet et al. (2003a), respectively, is difficult to handle.

Species diversity in AMF

Based on the criterion "92% sequence identity for the whole ITS", we separated 19 taxa, of which 14 had no matches with references in GenBank. This high proportion (74%) of putative new species is in line with the findings of Husband et al. (2002), Wubet et al. (2003a) and Haug et al. (2004) that suggest the number of approximately 200 AMF species recently given in Allen et al. (2002) might be largely underestimated. Assuming a worldwide proportion of "unknown species" similar to the one found in this study, this would lead to a theoretical estimate of at least 1,250 species in AMF. This estimate is low, considering that AMF biodiversity in Europe is reduced in comparison to the tropics (Wubet et al. 2003a), suggesting that AMF diversity remains largely unexplored. Thus, coordinated studies integrating spore morphotyping, the entrapment of AMF and molecular analyses are urgently needed.

Studying the biodiversity of AMF in the field

The bottleneck of using molecular techniques is the complex mixture of DNA molecules in soil extracts, which might lead to competitive inhibition in the PCR between the diverse DNA species at different concentrations. Therefore, some AMF taxa might not be detected. Performing highly sensitive nested PCR on minute target DNA quantities, with reduced diversity contained in extracts from small portions of single roots, reduces this risk. Coupling this PCR strategy with systematic sequencing allows detection of non-target species and more exhaustive and precise analyses of AMF species composition. This approach, used by Wubet et al. (2003a) and this paper is, however, too time-consuming to allow quantitative work. The weakness of this approach is the limited amount of root material that can be studied. In this context, the results must be interpreted cautiously. Due to the high proportion of molecular species detected in only one root, we must assume that our analysis of AMF species composition was not exhaustive. As we considered a broad range of host plants that, according to Helgason et al. (2002), may display a preference for specific AMF, some of these rarely detected species might be rare due to certain host specificity. Otherwise, species detected more often, especially *G. intraradices* and *G. claroideum*, showed no host specificity.

We took the precaution of sequencing a number of clones for some cloning reactions. This revealed some sequence variability which was at 2–3% (data not shown) and does not justify the distinction of additional molecular species (see also a discussion on intraspecific variability in Pawlowska and Taylor 2004). Thus, even if not exhaustive, our approach gives a picture of the AMF species composition at the sites and should not have failed to detect differences in the species number between the EFM and the IFM. Similarly, if we cannot definitively conclude that a given molecular species is only found at one site and is not present at the other one, the produced sequence amount allows us to suppose that the species composition between the sites was different.

Possible explanations for different AMF communities at both field sites

It was shown in macrocosm experiments that an enhanced AMF diversity promotes high plant biodiversity and ecosystem productivity (van der Heijden et al. 1998a), but also that a specific composition of AMF in soil determines plant community structure (van der Heijden et al. 1998b). However, AMF have also been shown to be detrimental to some non-mycotrophic plants (Francis and Read 1995; Renker et al. 2004), and, besides the positive effects of AMF on plant diversity just mentioned, some studies have described the opposite effect (Hartnett and Wilson 2002; Newsham et al. 1995).

Accordingly, the relationship between diversity of plants and diversity or species composition of AMF is not simple. In addition, there is evidence that, apart from the plant/fungus interaction itself, other ecological factors rule the fate of AMF biodiversity in ecosystems. Boddington and Dodd (2000) have clearly shown that there is a potential to alter the formation of mycorrhizas through agricultural

practices. Johnson (1993) found fertilisation of soils to decrease the abundance of AMF species such as *Scutellospora calospora*, while the one of *G. intraradices* was increased. Lastly, Jansa et al. (2002) showed that the community structure of AMF is affected by tillage but could not observe significant variations in AMF diversity of differently tilled soils.

We propose that, within the diverse ecological factors influencing AMF diversity (i.e. plant species composition, nutrient context, and land use intensity), some may be of higher hierarchical importance. However, the fact that AMF species numbers at both sites were quite similar pleads for constraining influences other than plant species diversity. Analysing known autecological traits of some of the AMF species found in our study may help to determine which ecological factor has ruled their presence at one or at both sites.

Ecological potential of the observed species

The majority of the detected taxa belonged to *Glomus* group Ab. In this group, *G. intraradices* was the only species found at both sites. *G. intraradices* by itself seems to be a widespread AMF species with a high tolerance against a multitude of environmental factors. Other studies showed its high abundance in fertilised soils (Johnson 1993; Jansa et al. 2002). To interpret cautiously, because some were only detected three times, the finding of the other four members of the *Glomus* group Ab (*Glomus* sp. A–D) at only one site (either at the IFM or EFM) might indicate that the broad ecological tolerance of *G. intraradices* does not apply to other members of this *Glomus* group.

A broad ecological amplitude can also apply to a member of *Glomus* group B, *G. claroideum*, which was present in high abundance on roots, and as spores at both meadow types. This species was also found by Turnau et al. (2001) to be one of the dominating fungi at zinc waste sites in Poland, suggesting a high tolerance towards different ecological conditions.

For some members of the other families, more restricted preferences to environmental conditions are known. Jansa et al. (2002), for example, observed a decreasing abundance of *Acaulospora* caused by tillage. The more intensive farming at the IFM could therefore be one reason why *Acaulospora* was only detected at the EFM. However, *Acaulospora* is thought to be much more diverse in the tropics (Allen et al. 1995), which might explain the reduced number of records in our study. Nevertheless, there exist some other records from Europe (Helgason et al. 1998; Jansa et al. 2002; Vandenkoornhuyse et al. 2002).

Gigaspora is also thought to be rare in Europe (Allen et al. 1995). Prior to our own record, findings in Europe were mentioned from Switzerland and Scotland (Jansa et al. 2002; Vandenkoornhuyse et al. 2002). Interestingly, Treseder and Allen (2002) could not find significant variations in the abundance of this genus among sites with different fertilisation treatments in Hawaii, while Johnson (1993) observed a decrease in the abundance of *Gigaspora margarita* (which is closely related to our *Gigaspora* sp.) with an increasing nutrient content in experimental studies. Our detection of *Gigaspora* sp. in the IFM does not match this result, but as it is only based on a single spore, this apparent discrepancy should not be overestimated.

Differences in AMF species detected as spores or in roots

The discrepancy between species detected as spores and on roots must be discussed cautiously, because we only gathered spores at one sampling date. However, even the comparison with the AMF we detected on roots at the same time shows major differences. Furthermore, species only detected as spores in autumn should have been revealed in the exhaustive root sampling during the year if they were present as active mycorrhizas on roots.

Except for a single spore record, *G. intraradices* and other members of *Glomus* group Ab were exclusively represented by root samples. *G. claroideum* was found to a larger extent on roots and on spores. Some species, i.e. *Glomus* sp. “Bad Sachsa”, *Glomus* sp. E and *Gigaspora* sp., accounting for 16% of the observed species diversity, were never revealed as active mycorrhizas but only as spores. This finding shows the necessity to directly detect AMF in plant roots to be sure which species form active mycorrhizas but also to gather all AMF species at one site.

Acknowledgements The study is part of BIOLOG-Europe, a biodiversity scientific programme funded by the German Federal Ministry of Education and Research (BMBF), grant no. 01LC0013. We wish to thank Thomas Scholten for providing soil data and Herbert Boyle who improved the English of the manuscript.

References

Allen EB, Allen MF, Helm DJ, Trappe JM, Molina R, Rincon E (1995) Patterns and regulation of mycorrhizal plant and fungal diversity. Plant Soil 170:47–62

Allen MF, Lansing J, Allen EB (2002) The role of mycorrhizal fungi in the composition and dynamics of plant communities: a scaling issue. Prog Bot 63:344–367

Augé RM, Stodola AJW, Tims JE, Saxton AM (2001) Moisture retention properties of a mycorrhizal soil. Plant Soil 230:87–97

Błaszkowski J, Blanke V, Renker C, Buscot F (2004) *Glomus aurantium* and *G. xanthium*, new species in Glomeromycota. Mycotaxon 90:447–467

Boddington CL, Dodd JC (2000) The effect of agricultural practices on the development of indigenous arbuscular mycorrhizal fungi. II. Studies in experimental microcosms. Plant Soil 218:145–157

Buscot F, Munch JC, Charcosset JY, Gardes M, Nehls U, Hampp R (2000) Recent advances in exploring physiology and biodiversity of ectomycorrhizas highlight the functioning of these symbioses in ecosystems. FEMS Microbiol Rev 24:601–614

Clapp JP, Young JPW, Merryweather JW, Fitter AH (1995) Diversity of fungal symbionts in arbuscular mycorrhizas from a natural community. New Phytol 130:259–265

Cullings KW, Vogler DR (1998) A 5.8S nuclear ribosomal RNA gene sequence database: applications to ecology and evolution. Mol Ecol 7:919–923

Daniell TJ, Husband R, Fitter AH, Young JPW (2001) Molecular diversity of arbuscular mycorrhizal fungi colonising arable crops. FEMS Microbiol Ecol 36:203–209

Esch H, Hundeshagen B, Schneider-Poetsch H, Bothe H (1994) Demonstration of abscisic acid in spores and hyphae of the arbuscular–mycorrhizal fungus *Glomus* and in the N_2-fixing cyanobacterium *Anabaena variabilis*. Plant Sci 99:9–16

Filion M, St-Arnaud M, Fortin JA (1999) Direct interaction between the arbuscular mycorrhizal fungus *Glomus intraradices* and different rhizosphere microorganisms. New Phytol 141:525–533

Francis R, Read DJ (1995) Mutualism and antagonism in the mycorrhizal symbiosis with special reference to impacts on plant community structure. Can J Bot 73:1301–1309

Galli U, Schüepp H, Brunold C (1994) Heavy metal binding by mycorrhizal fungi. Physiol Plant 92:364–368

Gerdemann JW, Trappe JM (1974) The Endogonaceae in the Pacific Northwest. Mycol Mem 5:1–76

Gollotte A, van Tuinen D, Atkinson D (2004) Diversity of arbuscular mycorrhizal fungi colonising roots of the grass species *Agrostis capillaris* and *Lolium perenne* in a field experiment. Mycorrhiza 14:111–117

Grime JP, Mackey JML, Hillier SH, Read DJ (1987) Floristic diversity in a model system using experimental microcosms. Nature 328:420–422

Hardie K (1985) The effect of removal of extraradical hyphae on water uptake by vesicular–arbuscular mycorrhizal plants. New Phytol 101:677–684

Harley JL, Harley EL (1987) A check-list of mycorrhiza in the British Flora. New Phytol (Suppl.) 105:1–102

Hartnett DC, Wilson GWT (2002) Mycorrhizae influence plant community structure and diversity in tallgrass prairie. Ecology 80:1187–1195

Hattenbach K (1959) Thüringer Schiefergebirge. In: Meynen E, Schmidthüsen J, Gellert J, Neef E, Müller-Miny H, Schultze HJ (eds) Handbuch der naturräumlichen Gliederung Deutschlands, Band II. Bundesanstalt für Landeskunde und Raumforschung, Bad Godesberg, pp 615–616

Haug I, Lempe J, Homeier J, Weiß M, Setaro S, Oberwinkler F, Kottke I (2004) *Graffenrieda emarginata* (Melastomataceae) forms mycorrhizas with Glomeromycota and with a member of the *Hymenoscyphus ericae* aggregate in the organic soil of a neotropical mountain rain forest. Can J Bot 82:340–356

Helgason T, Daniell TJ, Husband R, Fitter AH, Young JPW (1998) Ploughing up the wood-wide web? Nature 394:431

Helgason T, Merryweather JW, Denison J, Wilson P, Young JPW, Fitter AH (2002) Selectivity and functional diversity in arbuscular mycorrhizas of co-occurring fungi and plants from a temperate deciduous woodland. J Ecol 90:371–384

Hildebrandt U, Janetta K, Ouziad F, Renne B, Nawrath K, Bothe H (2001) Arbuscular mycorrhizal colonization of halophytes in Central European salt marshes. Mycorrhiza 10:175–183

Husband R, Herre EA, Turner SL, Gallery R, Young JPW (2002) Molecular diversity of arbuscular mycorrhizal fungi and patterns of host association over time and space in a tropical forest. Mol Ecol 11:2669–2678

Jansa J, Mozafar A, Banke S, McDonald BA, Frossard E (2002) Intra- and intersporal diversity of ITS rDNA sequences in *Glomus intraradices* assessed by cloning and sequencing, and by SSCP analysis. Mycol Res 106:670–681

Johnson NC (1993) Can fertilization of soil select less mutualistic mycorrhizae? Ecol Appl 3:749–757

Johnson D, Vandenkoornhuyse PJ, Leake JR, Gilbert L, Both RE, Grime JP, Young JPW, Read DJ (2003) Plant communities affect arbuscular mycorrhizal fungal diversity and community composition in grassland microcosms. New Phytol 161:503–515

Kahmen A, Perner J, Audorff V, Weisser W, Buchmann N (2005) Effects of plant diversity, community composition and environmental parameters on productivity in montane European grasslands. Oecologia 142:606–615

Landwehr M, Hildebrandt U, Wilde P, Nawrath K, Tóth T, Biró B, Bothe H (2002) The arbuscular mycorrhizal fungus *Glomus geosporum* in European saline, sodic and gypsum soils. Mycorrhiza 12:199–211

Lloyd-MacGilp SA, Chambers SM, Dodd JC, Fitter AH, Walker C, Young JPW (1996) Diversity of the ribosomal internal transcribed spacers within and among isolates of *Glomus mosseae* and related mycorrhizal fungi. New Phytol 133:103–111

Merryweather J, Fitter A (1998) The arbuscular mycorrhizal fungi of *Hyacinthoides non-scripta*. I. Diversity of fungal taxa. New Phytol 138:117–129

Munkvold L, Kjøller R, Vestberg M, Rosendahl S, Jakobsen I (2004) High functional diversity within species of arbuscular mycorrhizal fungi. New Phytol 164:357–364

Newsham KK, Watkinson AR, West HM, Fitter AH (1995) Symbiotic fungi determine plant community structure: changes in a lichen-rich community induced by fungicide application. Funct Ecol 9:442–447

Nielsen KB, Kjøller R, Olsson PA, Schweiger PF, Andersen FO, Rosendahl S (2004) Colonisation and molecular diversity of arbuscular mycorrhizal fungi in the aquatic plants *Littorella uniflora* and *Lobelia dortmanna* in southern Sweden. Mycol Res 108:616–625

Odat N, Jetschke G, Hellwig FH (2004) Genetic diversity of *Ranunculus acris* L. (Ranunculaceae) populations in relation to species diversity and habitat type in grassland communities. Mol Ecol 13:1251–1257

Pawlowska TE, Taylor JW (2004) Organization of genetic variation in individuals of arbuscular mycorrhizal fungi. Nature 427: 733–737

Redecker D (2000) Specific PCR primers to identify arbuscular mycorrhizal fungi within colonized roots. Mycorrhiza 10:73–80

Redecker D, Hijri M, Dulieu H, Sanders IR (1999) Phylogenetic analysis of a dataset of fungal 5.8S rDNA sequences shows that highly divergent copies of internal transcribed spacers reported from *Scutellospora castanea* are of ascomycete origin. Fungal Genet Biol 28:238–244

Renker C, Heinrichs J, Kaldorf M, Buscot F (2003) Combining nested PCR and restriction digest of the internal transcribed spacer region to characterize arbuscular mycorrhizal fungi on roots from the field. Mycorrhiza 13:191–198

Renker C, Zobel M, Öpik M, Allen MF, Allen EB, Vosátka M, Rydlová J, Buscot F (2004) Structure, dynamics, and restoration of plant communities: do arbuscular mycorrhizae matter? In: Temperton VM, Hobbs RJ, Nuttle T, Halle S (eds) Assembly rules and restoration ecology—bridging the gap between theory and practice. Island, Washington DC, pp 189–229

Renker C, Blanke V, Buscot F (2005) Diversity of arbuscular mycorrhizal fungi in grassland spontaneously developed on area polluted by a fertilizer plant. Environ Pollut 135:255–266

Rosendahl S, Stukenbrock E (2004) Community structure of arbuscular mycorrhizal fungi in undisturbed vegetation revealed by analyses of LSU rDNA sequences. Mol Ecol 13:3179–3186

Sanders IR, Clapp JP, Wiemken A (1996) The genetic diversity of arbuscular mycorrhizal fungi in natural ecosystems—a key to understanding the ecology and functioning of the mycorrhizal symbiosis. New Phytol 133:123–134

Schüßler A, Schwarzott D, Walker C (2001) A new fungal phylum, the *Glomeromycota*: phylogeny and evolution. Mycol Res 105:1413–1421

Simon L, Bousquet J, Lévesque RC, Lalonde M (1993) Origin and diversification of endomycorrhizal fungi and coincidence with vascular land plants. Nature 363:67–69

Smith SE, Read DJ (1997) Mycorrhizal symbiosis, 2nd edn. Academic, London

Swofford DL (2003) Paup*. Phylogenetic analysis using parsimony (* and other Methods), Ver. 4.0b10. Sinauer, Sunderland, MA

Tilman D (2000) Causes, consequences and ethics of biodiversity. Nature 405:208–211

Treseder KK, Allen MF (2002) Direct nitrogen and phosphorus limitation of arbuscular mycorrhizal fungi: a model and field test. New Phytol 155:507–515

Turnau K, Ryszka P, Gianinazzi-Pearson V, van Tuinen D (2001) Identification of arbuscular mycorrhizal fungi in soils and roots of plants colonizing zinc wastes in Southern Poland. Mycorrhiza 10:169–174

van der Heijden MGA, Boller T, Wiemken A, Sanders IR (1998a) Different arbuscular mycorrhizal fungal species are potential determinants of plant community structure. Ecology 79:2082–2091

van der Heijden MGA, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders IR (1998b) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396:69–72

Vandenkoornhuyse P, Husband R, Daniell TJ, Watson IJ, Duck JM, Fitter AH, Young JPW (2002) Arbuscular mycorrhizal community composition associated with two plant species in a grassland ecosystem. Mol Ecol 11:1555–1564

Vandenkoornhuyse P, Ridgway KP, Watson IJ, Fitter AH, Young JPW (2003) Co-existing grass species have distinctive arbuscular mycorrhizal communities. Mol Ecol 12:3085–3095

Walker C, Trappe JM (1993) Names and epithets in the Glomales and Endogonales. Mycol Res 97:339–344

White TJ, Bruns T, Lee S, Taylor J (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications. Academic, San Diego, pp 315–322

Wubet T, Weiß M, Kottke I, Teketay D, Oberwinkler F (2003a) Molecular diversity of arbuscular mycorrhizal fungi in *Prunus africana*, an endangered medicinal tree species in dry Afromontane forests of Ethiopia. New Phytol 161:517–528

Wubet T, Weiß M, Kottke I, Oberwinkler F (2003b) Morphology and molecular diversity of arbuscular mycorrhizal fungi in wild and cultivated yew (*Taxus baccata*). Can J Bot 81:255–266