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Diversity and structure of AMF communities as affected by tillage in a temperate soil

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Abstract Arbuscular mycorrhizal fungi (AMF) were studied in differently tilled soils from a long-term field experiment in Switzerland. Diversity and structure of AMF communities were surveyed either directly on spores isolated from the field soil or on spores isolated from trap cultures, planted with different host plants. Single-spore cultures were established from the AMF spores obtained from trap cultures. Identification of the AMF was made by observation of spore morphology and confirmed by sequencing of ITS rDNA. At least 17 recognised AMF species were identified in samples from field and/or trap cultures, belonging to five genera of AMF – *Glomus*, *Gigaspora*, *Scutellospora*, *Acaulospora*, and *Entrophospora*. Tillage had a significant influence on the sporulation of some species and non-*Glomus* AMF tended to be more abundant in the no-tilled soil. The community structure of AMF in the field soil was significantly affected by tillage treatment. However, no significant differences in AMF diversity were detected among different soil tillage treatments. AMF community composition in trap cultures was affected much more by the species of the trap plant than by the original tillage treatment of the field soil. The use of trap cultures for fungal diversity estimation in comparison with direct observation of field samples is discussed. Electronic supplementary material to this paper can be obtained by using the Springer Link server located at http://dx.doi.org/10.1007/s00572-002-0163-z.

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Introduction

The importance of arbuscular mycorrhizal fungal (AMF) diversity for plant diversity, productivity and ecosystem processes has been recognised recently (van der Heijden et al. 1998). This work and other studies (Bever et al. 1996; Streitwolf-Engel et al. 1997) have shown that different AMF species originating from the same soil can have different effects on plant growth. As a consequence, it appears important to assess the effect of soil management practices on AMF community structure and its diversity.

Mostly, the diversity of AMF in field soils has been studied based on their spore populations. It has been suggested that the diversity of AMF in a temperate grassland soil is limited to ten species and in cultivated soils to even fewer (Johnson 1993). However, a detailed survey of a grassland ecosystem (including also study of AMF assemblages in pots planted with different plants) has shown that 23 species of AMF co-existed in a single field site (Bever et al. 1996) associated with 25 plant species. This high diversity of AMF communities observed under field conditions might not be a site-specific observation, but could just be a result of the analysis of multiple soil samples (Morton et al. 1995). However, observation of spore populations alone may not provide adequate information about AMF community structure, because of the differences in growth and sporulation among AMF species (Land and Schönbeck 1991).

Higher diversity of AMF communities in the woodland ecosystem than on arable land was suggested from analysis of AMF sequences amplified from roots sampled from those two ecosystems (Helgason et al. 1998). The difference in diversity observed between woodland and arable ecosystems was assigned to a complex selective pressure of agricultural practices, such as ploughing, fertilisation and fungicide application, on the AMF com-

munities. Abbott and Robson (1977), Blaszkowski (1993) and Talukdar and Germida (1993) reported prevalence of *Glomus* spp. in agriculturally used soils, in contrast to rich AMF communities containing *Gigaspora* spp., *Scutellospora* spp. and *Acaulospora* spp. in uncultivated soils (Blaszkowski 1993). Johnson (1993) observed a decrease in AMF community diversity due to application of mineral fertilisers, associated with a loss of non-*Glomus* fungi and increase in population of *Glomus intraradices*. Low infection potential together with lower incidence of some AMF in high-input agricultural soils was also reported by Douds et al. (1993). This indicates that agricultural use of the soil may be an important factor affecting AMF diversity.

It was suggested that no-tillage conditions stimulate mycorrhizal activity in soil and in that way also nutrient uptake by the plants (Miller et al. 1995; Dodd 2000; Mozafar et al. 2000). Soil disturbance has been shown to reduce the density of AMF spores, species richness and the length of extraradical mycelium of AMF relative to undisturbed soil (Boddington and Dodd 2000). The amount of immunoreactive glycoprotein glomalin, which is an exudation product of AMF hyphae and which affects soil aggregation, was 1.5 times higher in no-tilled than tilled soils (Wright et al. 1999). However, the effect of soil tillage on the diversity and structure of AMF communities has been studied rarely for a single field tillage experiment.

The aim of this present study was to investigate the effect of tillage intensity on the AMF diversity and community structure in a Swiss agricultural soil.

Materials and methods

Study site

The AMF communities were studied in a long-term field tillage experiment established in 1987 at Hausweid, Tänikon, Switzerland (N 47°29′10.0″, E 8°55′10.1″, altitude 540 m). Average climatic conditions are as follows: annual precipitation 1,179 mm, annual air temperature 8.2°C, July temperature 17.4°C, January temperature 0.6°C. The experiment was established in a completely randomised block design with four replicates. Each experimental plot had a rectangular shape of 6×12 m. Four-year crop rotation consisted of a rapeseed (*Brassica napus* L.), winter wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), and winter wheat, with one harvest every year. The soil is a dystric Gleysol (FAO Classification System, Fitzpatrick 1980). The surface horizon (0–25 cm) has the following characteristics: sand 49%, silt 32%, clay 16%, organic matter 2.9%, pH (H_2O) 5.8, available P content (Olsen) 52 mg P kg^{-1} soil. For a detailed description of the field site, see Anken et al. (1997). The study was restricted to only three tillage treatments: conventional tillage (ploughed to the depth of 25 cm), chisel treatment (loosening soil with a wing share chisel to the depth of 25 cm without turning soil upside-down), and no tillage.

Spore isolation from field

Soil cores were sampled always under winter wheat crops from the uppermost 10 cm horizon using a soil borer (3 cm diameter) in January 1999 and in January 2001, following rapeseed and maize cropping seasons, respectively. Thirty individual soil cores were taken randomly from each experimental plot, pooled and mixed to obtain a representative sample. Subsamples (10 g fresh weight) were mixed with 1% Deconex (Borer-Chemie, Zuchwil, Switzerland) in the ratio 1:8 and shaken on a linear shaker for 1 h. The suspension was decanted five times through analytical sieves (500 and 40 µm) and the material collected on the 40-µm sieve was subjected to step gradient centrifugation (5 min, 1,000 *g*) in 2.5 M sucrose-water gradient. The centrifugation was repeated three times. Soil humidity was estimated after drying soil samples at 105°C for 24 h.

Culture establishment

Trap cultures were established from fresh field soil sampled in January 1999 from tilled and no-tilled soils. The soil from each plot was mixed with autoclaved quartz sand (grain size 0.7–1.2 mm) in a ratio of 1:4 (v: v) and filled in 300-ml pots. Pots were planted with one of the five host plants: (1) sugar maize (*Zea mays* L.) cv. “Tasty Sweet”, (2) leeks (*Allium porrum* L.) cv. “Zefa Plus”, (3) *Plantago lanceolata* L. (collected in Eschikon, Switzerland), (4) sunflower (*Helianthus annuus* (L.)Merill.) cv. “Gelber Knirps” or (5) soybean (*Glycine max* L.) cv. “Paradies”. The factorial combinations of soils and plants were replicated 3 times. Traps from the chisel treatment were established only 3 months later and thus were not included in most of the statistical analyses.

Plants were grown in the greenhouse for 5 months, automatically drip-irrigated with deionised water and fertilised once a month with 20 ml of eightfold concentrated Hoagland nutrient solution (Sylvia and Hubbell 1986) containing 1/100 of the original phosphorus concentration. The conditions in the greenhouse were as follows: day/night photoperiod of 16/8 h, 25/20°C, respectively, aerial humidity 40–50%. Illumination was provided at a minimum intensity of 400 µmol photons m^{-2} s^{-1}.

Spores of AMF were isolated from the substrate by wet sieving and decanting (Daniels and Skipper 1982) and by step-gradient centrifugation as described above. Monosporic cultures were established by placing healthy and fresh single spores of AMF on the germination root of a sterile *P. lanceolata* seedling and growing the plants for 3 months in the greenhouse in sterilised substrate (soil-quartz sand mixture, 1:4 v: v). Both components were first separately autoclaved and the soil was treated afterwards with soil bacterial filtrate and incubated for 4 weeks. The cultures were irrigated with a tensiometer-type controlled watering system (Blumat, Austria) maintaining the substrate humidity at 50–60% of its water holding capacity.

Identification of AMF

Soil samples from the field

Spores isolated from the field soils were examined microscopically and identified according to the taxonomic system proposed by Morton (1988) and Walker (1992). Original descriptions were consulted (Schenck and Perez 1990) and spore morphology was compared with an internet-published reference culture database established by Morton (http://invam.caf.wvu.edu/Myc_Info/Taxonomy/species.htm). Spores were compared also with freshly formed AMF spores from trap cultures originating from the same field site. Spores were observed and photographed with either a stereomicroscope (Olympus SZX12), using combined through- and reflected-light illumination provided by fibre optics, or on a compound microscope (Olympus AX70) using Nomarski contrast. The spores were mounted in water (for stereomicroscopy only) or in either polyvinyl-lacto-glycerol (PVLG) or PVLG + Melzer reagent (1:1 v:v). Spores mounted in Melzer reagent containing media were crushed in order to observe staining of different spore wall layers. At least five spores of each AMF species were mounted in both PVLG and PVLG + Melzer media to observe their morphology. Only apparently viable spores were used for the identification with the exception of non-*Glomus* AMF, from which only damaged spores were

recovered from the field soil. In this case, identification could be performed only to the genus level based on the spores from the field soil. The particular AMF were then identified using the AMF spores from the trap cultures (as described below).

Spores from soil sampled after a rapeseed cropping season (sampled 1999) were to a great extent damaged or dead and could not be identified easily by microscopy. Therefore, we only recorded numbers of spores in different size classes (spore diameter 0–100 μm, 100–150 μm, 150–200 μm, and spores larger than 200 μm). Spores from soil after a maize cropping season (sampled 2001) could be identified to the level of genera or species. Therefore, spore numbers per taxa were recorded for the samples collected in 2001 per soil weight aliquot. The abundances of separate classes within each season were compared between the different soil treatments.

AMF from trap and pure cultures

The morphology of the spores was examined under the microscope as described above. Additionally, the internal transcribed spacer (ITS) region of rDNA from selected spores was sequenced to cross-check the visual identification. DNA from single spores (three separate spores from each AMF species recovered from the trap pots or three spores from each AMF monosporic isolate) was extracted, amplified, and sequenced according to Sanders et al. (1995) with the following modifications:

1. A heating step (3 min, 95°C) was included in the DNA extraction procedure to improve efficiency of extraction.
2. PCR Purification Kit (Qiagen) was used to purify the PCR product, which was subsequently inserted into pGEM-T Easy vector (Promega).
3. Cycle sequencing (using BigDye Terminator) was performed on a Perkin-Elmer ABI 310 Capillary Sequencer. One sequence clone was sequenced for each of the monosporic AMF isolates (because only minor sequence variation was encountered while sequencing multiple sequence clones). One to five sequence clones were sequenced from each PCR reaction with the spores recovered from the trap cultures.

Sequences obtained in this study were submitted to GenBank (NIH genetic sequence database http://www.ncbi.nlm.nih.gov) under accession numbers AY035639-AY035666.

Presence/absence of spores of different AMF species was recorded in the substrate of individual trap pots. The sporulation rate of each AMF was estimated according to a semi-quantitative scale from 0 to 3 (0: absence, 1: sporadic, up to 1 spore per g of substrate, 2: abundant, up to 5 spores per g, 3: many spores, over 5 spores per g of substrate).

Statistical analysis

Shannon-Wiener and Simpson's indices of diversity (Begon et al. 1998) were calculated using the spore counts in the field soil samples. One-way ANOVAs were used to compare the AMF diversity indices among different soil tillage treatments. The effect of tillage treatment on the abundance of different AMF species (different spore size classes) was assessed by univariate ANOVA tests following multivariate analysis of variance (MANOVA) analysis. The effect of tillage intensity on the community structure of AMF in the field soil was estimated by redundancy analysis and discriminant analysis. The effects of different tillage practices and the species of the host plant in trap cultures on the abundance of AMF species were assessed by univariate ANOVAs following MANOVA analysis, and by two-way ANOVA. Redundancy analysis was employed to assess the contribution of the tillage treatment of the soil and the identity of the host plant on the community structure of AMF in the trap cultures.

MANOVAs and discriminant analysis were calculated employing Systat software version 9.0 (SPSS 1999), redundancy analysis was computed using CANOCO software version 4.0 (TerBraak 1988). ANOVAs were calculated using Statgraphics software version 3.1 (Manugistics 1997), and the *P*-values were calculated using an LSD-based *F* test. In the redundancy analysis, the *P*-values reported stand for the significance of results as tested by the Monte-Carlo permutation test (Jongman et al. 1987). In MANOVA, the *P*-value was derived from an *F*-ratio calculated from Hotelling-Lawley trace statistics. For purposes of redundancy analysis, abundance data of all AMF species in the field soil were classified along the tillage gradient scale (0: no till, 1: chisel, 2: conventional tillage). To test the effect of host plant species on AMF community composition in trap pots using redundancy analysis, host plant data were organised in five independent columns with value 1 indicating presence and 0 indicating absence of a particular plant species.

Phylogenetic analysis

The 5.8S sequences excised from the full-length ITS-rDNA sequences (ITS1–5.8S-ITS2) were used for comparison of different AMF species within the group of Eumycota. Reference sequences were obtained from the internet-located dataset of Redecker et al. (1999). For more detailed intraspecific analysis, variable ITS2 region was used. Reference sequences of ITS2 region were obtained from the GenBank. Multiple alignment of sequences was processed using ClustalW software (Thompson et al. 1994). Phylogenetic relationships were inferred using the Phylip Program Package (Felsenstein 1993), employing neighbour joining. The Kimura distance model was used, in which the value ratio of transition/transversion was set to 2. Delete-half-jack-knife (obtained by 500 times repeated sampling) was used as a criterion for support of phylogenetic tree branching. The phylograms were rooted on an outgroup species (*Phaseolus vulgaris* L. for analysis of all fungal sequences, or *Glomus claroideum* and *Gigaspora margarita* for analysis of separate glomalean subgroups (*Gigasporaceae* and *Glomaceae*, respectively).

Results and discussion

Isolation and identification of AMF

Morphological identification of AMF from the field soil samples (where 14 species were detected) was confirmed by observing the morphology of freshly formed AMF spores in trap cultures (where 16 species were detected). The identification was also supported by sequencing of rDNA from the spores recovered from the trap cultures and performing phylogenetic analyses (Fig. 1, Fig. 2, Electronic Supplementary Material Fig. S2). A list of all AMF species found within the experimental field is given in Table 1. Seventeen AMF species (belonging to 5 genera) were detected in the no-till plots (see Electronic Supplementary Material Fig. S1). Almost all of the AMF detected in the no-tilled soils were also recorded in the tilled treatment, with the exception of *Scutellospora*, *Entrophospora*, and *Acaulospora*, which were missing. However, the identification of some AMF, especially if based on the spores obtained from the field soil only (as was the case for *G. aggregatum*-like AMF and *G. microaggregatum* in this study), is prone to error and will need confirmation in subsequently established pot cultures, where the particular AMF grow. The AMF species richness observed in the field soil in this study was similar to the AMF species richness reported by Franke-Snyder et al. (2001), who detected 15 AMF species in a field ex-

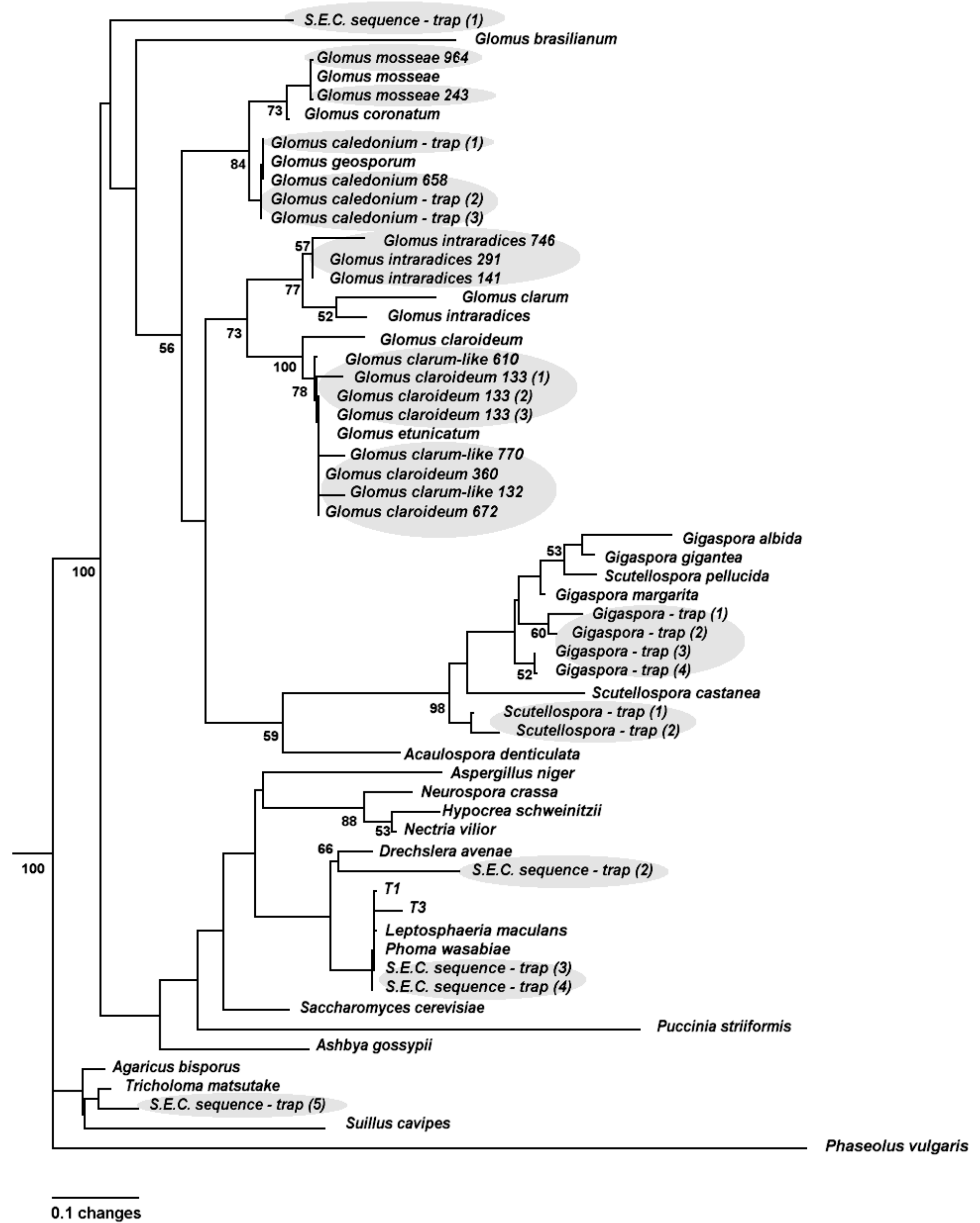

Fig. 1 Molecular identification of AMF based on phylogenetical inference analysis of 5.8S rDNA sequence data. Neighbour-joining phylogram with delete-half jack-knife values higher than 50 (500 times resampled) is shown. DNA was obtained from AMF spores isolated from trap cultures (labelled with "*trap*" and replicate number in parentheses), or from pure isolate cultures (labelled with isolate number and replicate number in parentheses). *Phaseolus vulgaris* sequence was used as outgroup. All newly reported sequences are contrasted by grey background. Several different sequences (mainly of ascomycetous and basidiomycetous origin) were found in *Entrophospora* spores (labelled "*S.E.C. sequence*") from trap cultures. Fungal DNA sequences used for comparison were obtained from the dataset of Redecker et al. (1999)

periment under different farming managements in the United States. Our results are, however, contradictory to the findings of Helgason et al. (1998), who suggested low richness of AMF in arable soils, reaching down to a single species (*Glomus mosseae*).

Trap cultures allowed detection of *Acaulospora* sp. and confirmation of the morphological identification of *Gigaspora* and *Scutellospora* by DNA sequencing. *Gigaspora*, *Scutellospora*, *Acaulospora*, and *Entrophospora* could not be subcultivated to single-spore cultures (20 pot cultures were established for each of these AMF genera), but *Gigaspora* sp. and *Scutellospora* sp. were subcultivated recently using multisporic cultures in which 10–15 spores were used as starting inoculum. In contrast to our findings, Brundrett et al. (1999a) compared different isolation techniques to obtain pure AMF cultures and reported low recovery of *Scutellospora*, *Gigaspora* and *Acaulospora* by trap culturing. They also proposed starting pure cultures of these AMF genera directly from spores collected in the field in order to obtain the highest isolation efficiency. This was not applicable in our case, as we established our trap cultures from field soil after the rapeseed season, when most of the AMF spores were damaged or dead.

In the time frame of our study, only five species of AMF could be cultivated in single spore cultures, all belonging to *Glomus* spp. (altogether over 1,000 single-spore cultures were established and 96 of them were successful). Twelve single-spore cultures belonging to these five *Glomus* species (*G. intraradices*, *G. mosseae*, *G. clarum*-like, *G. claroideum*, and *G. caledonium*) were deposited in INVAM (International Culture Collection of

Table 1 AMF species detected (based on the spore morphology) in different tillage treatments of the long-term field tillage experiment at Tänikon (Switzerland). *F* and *T* denote detection of the spores in the field soil samples and in trap cultures, respectively

AMF species	Detected in soil tillage treatment		
	Conventional tillage	Chisel	No-till
Acaulospora paulinae (?) Blaszkowski			T
Entrophospora infrequens Ames & Schneider		F	F, T
Gigaspora margarita Becker & Hall	F	F	F, T
Glomus aggregatum[a] Schenck & Smith emend. Koske	F	F	F
Glomus caledonium Trappe & Gerdemann	F, T	F, T	F, T
Glomus claroideum Schenck & Smith	F, T	F, T	F, T
Glomus clarum-like[b] Nicolson & Schenck	F, T	F, T	F, T
Glomus constrictum Trappe	F, T	F, T	F, T
Glomus diaphanum Morton & Walker	F, T	F, T	F, T
Glomus etunicatum Becker & Gerdemann	F, T	F, T	F, T
Glomus fasciculatum Gerd. & Trappe emend. Walker&Koske			T
Glomus geosporum Walker	F	F	F, T
Glomus intraradices Schenck & Smith	F, T	F, T	F, T
Glomus invermaium Hall	F, T	F, T	F, T
Glomus microaggregatum Koske, Gemma & Olexia	F	F	F
Glomus mosseae Gerdemann & Trappe	F, T	F, T	F, T
Scutellospora calospora Walker & Sanders			T
Scutellospora pellucida Walker & Sanders			F, T

[a] The morphology of this AMF was distinct from *G. intraradices* in the field soil samples (the spores formed more compact clusters than *G. intraradices* and the outer wall layer was significantly thicker than the inner wall layer). Both *G. aggregatum* and *G. intraradices*, however, probably belong to the same species, as a continuum of morphologies was recognised between these two species (J.C. Dodd, personal communication). *G. aggregatum* morphotype was not confirmed in the trap cultures

[b] The morphology of these AMF closely resembled *G. clarum*, although some of the monosporic isolates identified as *G. clarum*-like were later assigned to *G. luteum*. However, all the monosporic isolates, which were assigned to these two species (*G. clarum*-like and *G. luteum*) based on their spore morphology, were all closely related and probably represented a single AMF species (see isoenzyme data in Electronic Supplementary Material Fig. S3) with transient morphology between *G. clarum* and *G. claroideum*-*G. luteum*

Vesicular-Arbuscular Mycorrhizal Fungi, Morgantown, Fla., USA, http://invam.caf.wvu.edu) as accession numbers SW201-SW212 and also submitted for registration to La Banque Européenne des Glomales (BEG).

The results of molecular analysis based on the 5.8S rDNA (Fig. 1) show clearly that the group of AMF is delimited from other Asco- and Basidiomycetes. Several groups were identified among the AMF (Fig. 1). The distinct phylogenetic lineages of AMF were further analysed using sequences of the highly variable ITS2 region (Fig. 2, Electronic Supplementary Material Fig. S2). Using this approach, *Gigaspora* sp. and *Scutellospora* sp. from trap cultures were found to be similar to *Gigaspora margarita* and *Scutellospora pellucida*, respectively (Fig. 2). Our observation of *Gigaspora margarita* from Switzerland is the first confirmed report of the presence of this AMF species in Europe. Previously this genus was thought to be absent from the European continent (Walker 1992). *Gigaspora gigantea* and some unrecognised *Gigaspora* sp. have been reported already from Poland (Blaszkowski 1993), but neither photographic records nor DNA analyses of those fungi have been reported and they were not isolated into culture.

Our sequences from *Glomus mosseae* fitted well into the highly variable group of *G. mosseae*, *G. coronatum*, and *G. dimorphicum.* The sequences of *G. caledonium* from both trap and pure cultures clustered into a separated branch from *G. mosseae*, together with *G. geosporum*. Unfortunately, there is as yet no ITS2 sequence of *G. caledon-*

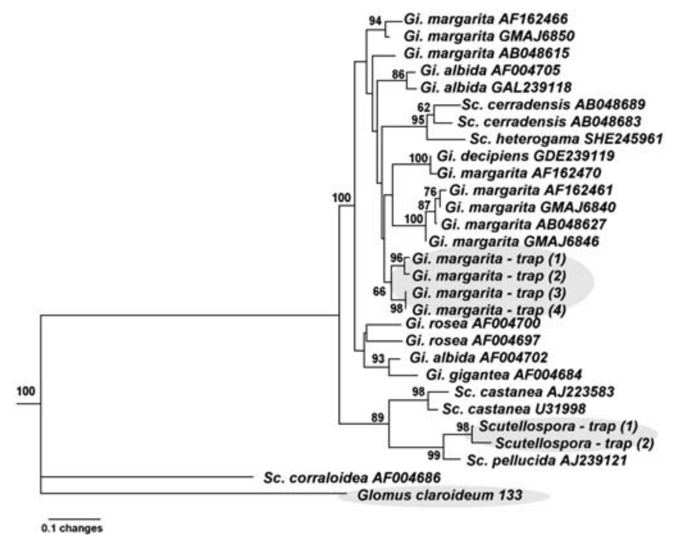

Fig. 2 Molecular identification of *Gigaspora* sp. and *Scutellospora* sp. from trap pots based on phylogenetical inference analysis of sequence data of ITS2-region of rDNA. Neighbour-joining phylogram with delete-half jack-knife values higher than 50 (500 times re-sampled) is shown. DNA was obtained from AMF spores isolated from trap cultures (replicate number are given in parentheses). *Glomus claroideum* sequence was used as outgroup. All newly reported sequences are contrasted by grey background. Sequences are compared with previously published fungal sequences (obtained from GenBank), which are marked with their accession numbers (combination of letter and number coding)

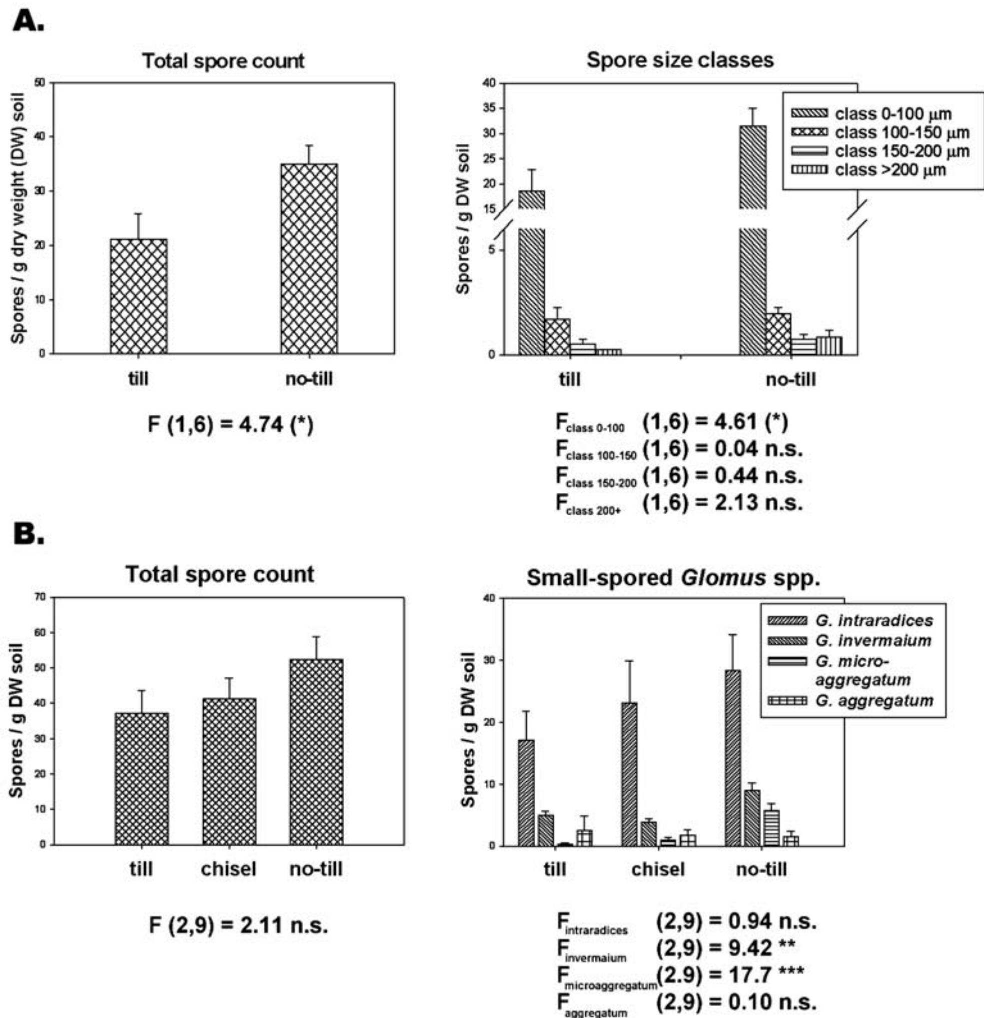

Fig. 3 Spore counts in field soils after a rapeseed season (1999), when only two tillage treatments were compared (**A**), and spore counts in soils following maize season (2001), where three tillage treatments were compared (**B**). *F*-values following ANOVAs are given. Statistical significance of results is shown (*n.s.* not significant (P=0.1); (*) P<0.1; * P<0.05; ** P<0.01)

ium in the GenBank for comparison. In addition, *Glomus caledonium* and *G. mosseae* could be well matched with their reference cultures by means of isoenzyme analysis (Electronic Supplementary Material Fig. S3).

A large phylogenetic distance was observed between the groups of *Glomus intraradices* and *G. claroideum* in the phylogenetic analysis based on the ITS2 region. The AMF isolates identified as *Glomus clarum*-like (identification kindly confirmed by Dr. J. Morton) could not be separated from *G. claroideum* based on the variable ITS2 sequences (Electronic Supplementary Material Fig. S2), but the isoenzyme banding pattern supported the separation of *G. clarum*-like and *G. claroideum* (Electronic Supplementary Material Fig. S3). It was shown previously that different isolates of *G. clarum* had different phylogenetic affinities, although they were all morphologically similar to *G. clarum* (Schüssler et al. 2001).

Most of our sequences obtained from *Entrophospora* sp. spores clustered with asco- or basidiomycetous fungi; one of them was linked distantly to the group *Glomus occultum* – *G. brasilianum* (Fig. 1). Therefore, it is probable that most of the amplified DNA did not originate from AMF but probably from intrasporal contaminations. Similar findings have been reported by Redecker et al. (1999), who noted that the sequences T1 and T3 (shown in Fig. 1) originating from *Scutellospora* spores clustered together with the Ascomycete fungus *Leptosphaeria* sp. Some of our sequences from *Entrophospora* spores also clustered very close to the *Leptosphaeria* – *Phoma* complex. PCR amplification of rDNA from some other AMF (e.g. *G. fasciculatum*, *G. diaphanum*) from the trap cultures was not successful and, therefore, the sequences could not be obtained. In the context of molecular identification of AMF, it has to be mentioned that a large degree of molecular variability exists even within a single individual AMF (a single spore), as discussed, for example, by Kuhn et al. (2001). This points to the danger of identification of AMF from one or a few sequences and, thus, the results presented here must be interpreted with caution.

Effect of soil tillage on the AMF community in the field

Significantly more AMF spores were observed in soil from no-tilled wheat field than from the tilled field following the rapeseed season (P=0.05, Fig. 3A). However, the total spore count was not significantly affected by

soil tillage following the maize season (Fig. 3B). Higher spore counts in the no-tilled soils after rapeseed may be due to increased presence of mycorrhizal weed plants (e.g. from the family *Asteraceae*) in the no-tilled plots (Streit et al. 2000). These weeds may have supported AMF development in their roots and also caused some spore formation under the rapeseed crop, which is a non-mycorrhizal plant. In the tilled plots, ploughing eliminated the majority of the weed plants (Streit et al. 2000) and, therefore, AMF development during the rapeseed season would be negligible. Generally, higher numbers of AMF spores were observed in the soil following the maize season than the rapeseed season. This is because maize is a very good AMF host and supports AMF development and spore formation, which in turn leads to increased AMF infectivity of the soils (Gavito and Varela 1993). We observed high absolute spore counts in the field soils than those reported from a range of different environments (Blaszkowski 1993; Allen et al. 1998). This could be due to an improved method for recovery of spores from soil, as well as to the fact that we studied agricultural soils, where the development of at least certain types of AMF is greatly enhanced. Up to 300 spores per gram of soil in fields used intensively for agriculture have been reported in the United States (Kurle and Pfleger 1996). The high spore counts reported in this present study do not, however, represent the actual numbers of infective propagules in the soil, which were about three times lower than the actual spore counts (data not shown). This is probably because some of the spores included in the counts were not viable, or were present in clusters which would function as one (unseparable) infective propagule.

The differences among total numbers of AMF spores in the tilled and no-tilled soils were mostly due to spores in the smallest size class (under 100 µm). Both the small-spore fraction and the total number of spores were affected by different tillage treatments ($P<0.1$, Fig. 3A). The differences in other size fractions of spores were not significant. Spore counts in season 2001 were not significantly different between the soil treatments, because the largest contributor to spore counts (*G. intraradices*) did not show significant differences with respect to soil tillage treatments (Fig. 3B). However, a significant effect of tillage on abundance of *G. invermaium* and *G. microaggregatum* was observed. An apparent increase in the incidence of *Gigaspora*, *Scutellospora*, and *Entrophospora* was noted in the no-tilled soil, but the results were not significant, probably because very few spores of the non-*Glomus* AMF were sampled. The increased presence of non-*Glomus* fungi (*Gigaspora* and *Scutellospora*) in uncultivated soils was shown previously based on both spore observation (Blaszkowski 1993) and root analyses (Helgason et al. 1998). However, the occurrence of *Entrophospora infrequens* was not influenced by agricultural use of the soil in a previous study (Blaszkowski 1993). The low sporulation rate of some AMF may be an inherent character (Bever et al. 1996). Thus, the spore counts might not give representative values for the actual abundance of the AMF in the soil (Clapp et al. 1995). To approach a reliable quantification of the AMF in the rhizosphere by targeting soil mycelium network or intraradical colonisation structures, other methods, such as specific primer quantitative PCR or specific primer PCR coupled with multiple sampling, are needed (Edwards et al. 1997; Jacquot-Plumey et al. 2001).

Abundance of some large-spored *Glomus* spp. in the field in 2001 was slightly increased in conventionally tilled soil (*G. mosseae*, $P=0.12$), which points to the possible adaptation of different AMF species to different levels of soil disturbance. Similar observations were already reported by Abbott and Robson (1977) and Blaszkowski (1993), who observed *G. deserticola* and *G. mosseae*, respectively, to be more or less associated with cultivated soils. Prevalence of *Glomus* spp., especially *G. mosseae* and *G. clarum*, over *Gigaspora* spp. and *Acaulospora* spp. was also reported from cropped soils in Canada (Talukdar and Germida 1993).

Comparison of spore size classes and species of AMF may be difficult because sizes of different AMF species could overlap (Schenck and Perez 1990). Thus, only a very broad comparison was made. The small size fraction (<100 µm) in 1999 could be attributed to *G. intraradices* and other small-spores cluster forming AMF. The less numerous larger spore fractions (>100 µm) determined in the soils in 1999 would represent the whole range of AMF species, which could only be identified based on the apparently viable spores in 2001.

Soil tillage had no significant effect on the diversity of the AMF community, as assessed by the diversity indices ($P=0.62$ and $P=0.74$ for comparison of Simpson and Shannon-Wiener diversity indices, respectively). However, these indices may not reflect properly the ecosystem structure, especially if the differences in abundance between different species are too large (for example, if there is pronounced dominance by one or a few

Table 2 Results of multivariate analysis of variance (MANOVA) on abundance of 12 different AMF species in trap pots, which were established from the field soil sampled from the plots subjected to two different levels of tillage intensity (conventional and zero tillage). The trap cultures were planted with five different host plants. Hotelling-Lawley (HTL) trace statistic was used to calculate an approximate *F* ratio with accompanying numerator (*num*) and denominator (*den*) degrees of freedom

Source of variation	*df*	HTL	*F*-ratio	Num *df*	Den *df*	*P*
Tillage	1	0.187	1.543	12	99	0.122
Host plant	4	1.055	2.143	48	390	0.000
Tillage × Host plants	4	0.397	0.807	48	390	0.818

Table 3 Effect of the tillage treatment and trap host plant species on abundance of different AMF in trap cultures (only data for conventional and no-tillage treatments are shown). *F*-values from univariate *F*-tests (df, nummerator, residual df as denominator) following MANOVA are given. Statistical significance of results is shown

(*n.s.* not significant (P=0.1); (*)P<0.1; *P<0.05; **P<0.01

Factor:	Tillage (A) $F_{1,110}$=	Host plant (B) $F_{4,110}$=	Interaction A×B $F_{4,110}$=
Fungus			
Gigaspora sp.	3.194 (*)	1.419 n.s.	1.419 n.s.
Scutellospora sp.	1.000 n.s.	1.000 n.s.	1.000 n.s.
Entrophospora sp.	1.000 n.s.	1.000 n.s.	1.000 n.s.
Acaulospora sp.	2.000 n.s.	0.750 n.s.	0.750 n.s.
Glomus fasciculatum	1.960 n.s.	0.760 n.s.	0.760 n.s.
Glomus mosseae	0.831 n.s	2.424 (*)	1.928 n.s.
Glomus caledonium	4.851 *	0.742 n.s.	0.317 n.s.
Glomus clarum-like	0.640 n.s.	0.355 n.s.	0.088 n.s.
Glomus claroideum	0.776 n.s.	3.057 *	0.561 n.s.
Glomus etunicatum	0.046 n.s.	4.730 **	0.634 n.s.
Glomus intraradices	0.221 n.s.	0.939 n.s.	1.222 n.s.
Glomus diaphanum	0.824 n.s.	4.888 **	0.394 n.s.

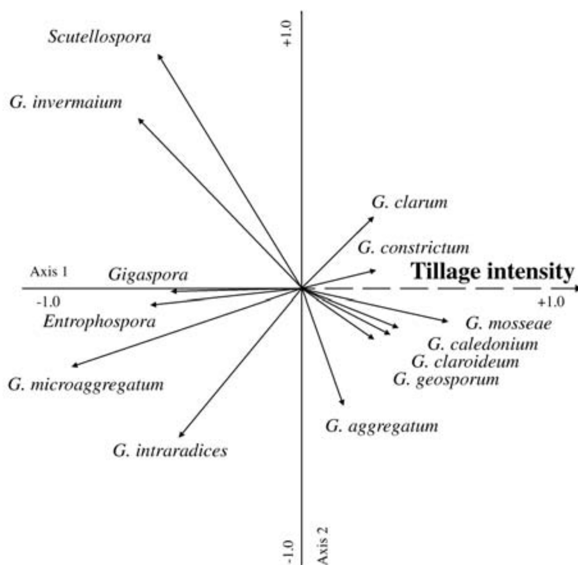

Fig. 4 Effect of soil tillage intensity on the community structure of AMF. Results of redundancy analysis are shown using the spore abundance data of the AMF species in the field soils in 2001, following the maize season. Size and orientation of the vectors represents correlation among them and with the axes. The smaller the angle between the vectors (or a vector and an axis) and the longer the vectors, the more correlated are the variables represented by the vectors. Axis 1 represent the effect of the tillage intensity, axis 2 is the most explanatory axis pooling all other environmental effects on population structure of AMF. Abundance data of the AMF species in the field soil after maize crop (2001) were used

species; Jongman et al. 1987), as was the case in this study (Fig. 3).

MANOVA confirmed significant differences in abundance of some AMF with respect to soil tillage treatment (Hotelling-Lawley trace statistics P=0.018). This also means that the effects of tillage on the abundance of the different AMF species were not the same. A subsequently applied discriminant analysis identified five AMF whose abundance explained 79.3% of the variability of the community structure shift along the tillage intensity gradient: *Gigaspora* sp., *Scutellospora* sp., *G. invermaium*, *G. mosseae*, and *G. microaggregatum*. Redundancy analysis was then used to assess how the community composition was affected by different tillage treatments (Fig. 4). The first canonical axis (corresponding to the tillage intensity gradient) explained 23.9% variability of the dataset. A Monte Carlo permutation test confirmed the community structure of the AMF to be significantly affected by different tillage management (P=0.005). Contrary to our results, Franke-Snyder et al. (2001) observed no significant change in composition of AMF community after 15 consecutive years of low-input, as compared with a conventional farming system.

Effect of soil origin and host plant species on the AMF community in trap cultures

Analysis of the composition of spore assemblages in trap pots by MANOVA (Table 2) revealed a large effect of host plant species on the composition of AMF communities in the pots, while the effect of tillage treatment of the original soil was not significant. The abundance of three *Glomus* spp. in trap pots was significantly affected by the presence of a specific host plant (Table 3), while the host influence on another fungus (*G. mosseae*) was only marginally significant (ANOVA, *F*-test, P=0.05). On the other hand, the abundance of only a single AMF species (*G. caledonium*) was significantly affected (P=0.03) by the tillage treatment of the original soil. All non-*Glomus* AMF (*Gigaspora*, *Scutellospora*, *Acaulospora*, *Entrophospora*) and *G. fasciculatum* were detected exclusively in trap cultures from no-tilled soils (Table 1), but usually only in very low numbers; this might also be due to the disturbance of the original soil samples while establishing the trap cultures. ANOVAs for abundance data of these AMF species were not significant.

Redundancy analysis quantified the effect of the two factors (tillage, host plant species) on AMF community composition. Tillage treatment of the original soil used for establishment of trap cultures accounted for 1.2% of the variability of AMF community composition and was not significant, as assessed by the Monte-Carlo permutation test (Fig. 5A, P=0.18). On the other hand, the effect of the host plant species accounted for 7.7% of the variability and was highly significant (Fig. 5B, P=0.01). This is consistent with previous reports showing a strong influence of the host plant species on fungal develop-

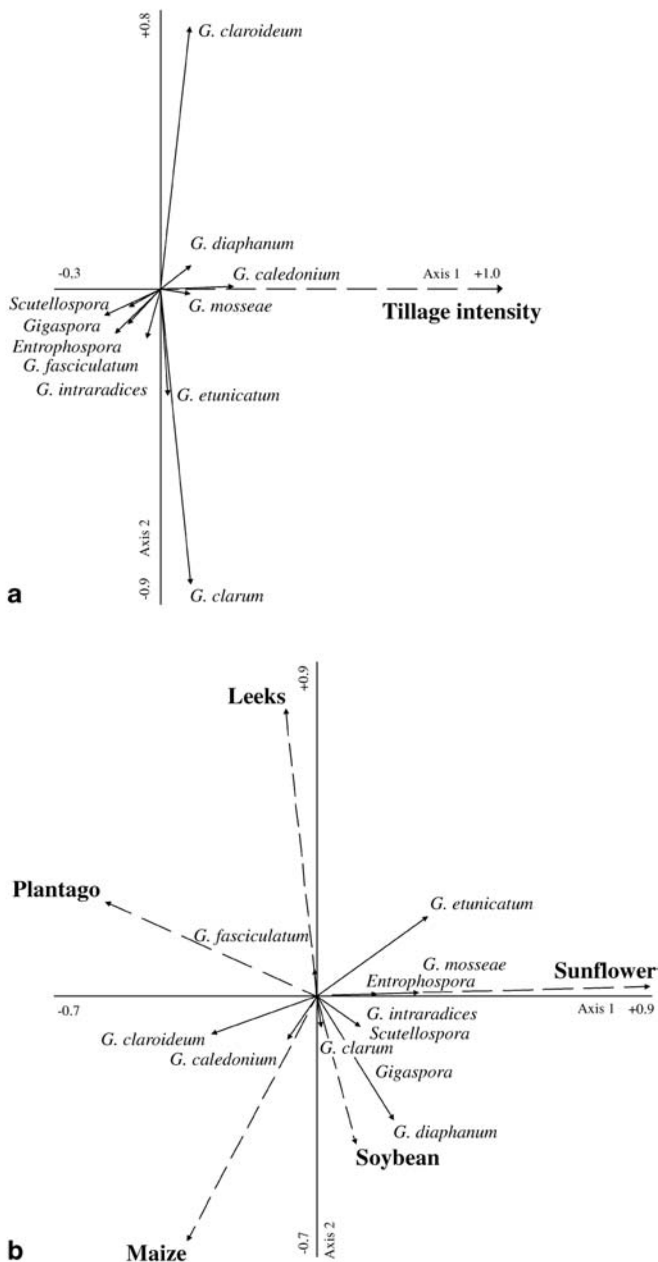

Fig. 5 Effect of tillage treatment of the original soil used for trap culture establishment, and the plant species, on the composition of AMF communities growing in the trap cultures. Redundancy analysis of mycorrhizal spore abundance data along a tillage intensity gradient (**A**), and redundancy analysis of AMF species abundance as affected by the species of a host plant (**B**), are shown. Vectors, which represent factors (tillage intensity or plant species), are dashed; vectors representing fungal species abundance are shown as full lines. Size and orientation of the vectors represents correlation among them and with the axes

ment in trap cultures (Brundrett et al. 1999b). It seems that the most important factor limiting growth of certain AMF taxa is the degree of their host specificity, as proposed by Bever et al. (1996). The highest level of diversity in the AMF community in trap pots was found when soybean (or sunflower) was used as a host plant (Fig. 5B). On the other hand, *P. lanceolata* supported development of *Glomus* spp. only. This is in contradiction to Bever et al. (1996), who found high sporulation of *Scutellospora calospora* on *P. lanceolata*, but it is in agreement with the results of Sanders and Fitter (1992), who reported that *Scutellospora* sp. did not sporulate well in association with *P. lanceolata*.

Conclusions

We found a trend of increase in the incidence of certain AMF, especially those not belonging to *Glomus* spp., in intensively used agricultural soil under long-term (13 years) reduced tillage management (chisel, no till). In conventionally tilled soils, almost exclusively AMF belonging to *Glomus* spp. were present (*G. mosseae*, *G. claroideum*, *G. caledonium*, *G. constrictum*, *G. clarum*-like). Five genera (*Glomus*, *Gigaspora*, *Scutellospora*, *Acaulospora*, and *Entrophospora*), and about 17 AMF species were recorded from a relatively small-scale field experiment (0.6 ha). It seems that the most efficient approach to observing the influence of environmental factors on AMF communities is to study the fungi directly from field samples (despite the difficulties with their identification) and simultaneously to cultivate them in pot cultures, where the spores can be identified reasonably well. To eliminate selective effects of trap plant species on the AMF community structure, it is recommended to use several host plant species for establishment of trap cultures. Although morphological identification of AMF is the most widespread method and in many cases still unavoidable (it is fast, relatively cheap and easy to perform, though rather subjective), it should be carried out concomitantly with other (e.g. molecular) methods for mutual confirmation.

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