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Infectivity and community composition of arbuscular mycorrhizal fungi from different soil depths in intensively managed agricultural ecosystems

Jingping Gai · Wenjun Gao · Lei Liu · Qing Chen · Gu Feng · Junling Zhang · Peter Christie · Xiaolin Li

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Abstract
Purpose Arbuscular mycorrhizal (AM) fungi represent a functionally important component of soil microbial communities. It is critical to achieve an improved understanding of the community structure of the indigenous AM fungi if we are to use this group of fungi either as indicators of ecosystem health or to enhance the sustainability of agricultural systems. In the present study, we assessed the impact of land use and soil depth on the number and community composition of infective AM fungal propagules in high-input agricultural ecosystems.
Materials and methods Three different agricultural land use systems with high management intensity were selected, i.e., vegetable greenhouses, arable land, and open vegetable fields. Soil samples were collected from different soil depths (0–30, 30–60, and 60–90 cm) of three replicated (triplicate) land-use types at two sites in north China. The modified mean infection percentage (MIP) method was used to determine the inoculum potential of each soil sample. The community composition of AM fungi was analyzed using PCR, cloning, and sequencing techniques.
Results and discussion Land use and soil depth greatly influenced the development of root-associated AM fungi and also edaphic properties. MIPs and species richness were lower in the top (0–30 cm) of soil profile in the vegetable greenhouses than in the nearby open vegetable fields or arable fields. Moreover, AM fungal distribution in the soil profile varied with land use. The MIPs and species richness decreased with increasing soil depth in the arable land and open vegetable fields. By contrast, in the greenhouse vegetable soils, the highest richness was found in the deepest soil depth (60–90 cm). The presence and prevalence of AM fungi also varied with land-use type and soil depth. Of the 13 total phylotypes investigated, Glo 6, Div 1, and Glo 1 accounted for 82.8 % of AM fungal clones analyzed and were present in each land-use type and soil depth. There were some observed phylotypes that were only found below 30 cm depth (Glo 4, Glo 5, Div 3, and Div 4).
Conclusions AM fungal infectivity and richness were not always lower in the deeper soil profiles in the greenhouse than in the agricultural fields. High land-use intensity was correlated with a preferential persist of some AM fungi in the deeper soil layers to escape from the adverse conditions caused by intensive farming practices.

Keywords Arbuscular mycorrhiza · Farming systems · Greenhouse vegetable soils · Molecular diversity · Soil depth

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J. Gai
Department of Environmental Sciences and Technology, College of Resources and Environmental Sciences, China Agricultural University, Beijing 100193, China

W. Gao · L. Liu · Q. Chen · G. Feng · J. Zhang · P. Christie · X. Li (✉)
Key Laboratory of Plant-Soil Interactions, Ministry of Education, College of Resources and Environmental Sciences, China Agricultural University, Beijing 100193, China
e-mail: lixl@cau.edu.cn

1 Introduction

Arbuscular mycorrhizal (AM) fungi are a main component of the soil microbiota in most agroecosystems and they form symbiotic associations with a majority of plant species

including nearly all crop species (Newman and Reddell 1987; Simon et al. 1993; Jansa et al. 2006). AM fungi are known to increase plant growth by facilitating nutrient uptake from the soil via an extensive extraradical mycelium (Li et al. 1991a,b) and increase the tolerance of plants to a range of biotic and abiotic stresses (Meharg and Cairney 2000; Augé 2001; Borowicz 2001; Pozo and Azcón-Aguilar 2007). It is evident from the beneficial effects that AMF are crucial for the functioning of terrestrial ecosystems (Smith and Read 2008).

However, modern intensive farming practices such as tillage, chemical pesticide, and fertilizer use may reduce the abundance and diversity of AM fungi (Abbott and Robson 1991; Helgason et al. 1998; Oehl et al. 2003, 2004). Such management practices can result in a certain reduced subset of AM fungal taxa and other soil organisms (Johnson et al. 1992; 1993; Kiers et al. 2002). Nevertheless, some studies still suggested this decrease can be avoided to maintain the diversity of AM fungi in arable soils as a possible agricultural resource of the future (Franke-Snyder et al. 2001; Hijri et al. 2006; García et al. 2007; Lekberg et al. 2008; Martinez and Johnson 2010).

Although there have been numerous studies on AM fungal diversity in intensively managed agricultural ecosystems (Oehl et al. 2004, 2005, 2010; Köhl et al. 2014), only a few studies have described AM fungal diversity in intensively managed systems such as greenhouse vegetable production systems (Jiao et al. 2011). In China, the total area devoted to vegetable production has increased to over 18.2 million ha over the last two decades and accounted for 11.6 % of the total agricultural area in 2007 (China Ministry of Agriculture 2008). The high market value of vegetables has encouraged farmers to use large amounts of organic manures and mineral fertilizers. Nutrient application rates are considerably higher in vegetable production systems than in arable systems. For example, in Shouguang County, Shandong Province, more than 65 % of the arable land is used for intensive greenhouse production. This area is supplied with 2220 kg fertilizer N ha^{-1} and 1,800 mm irrigation water per year (Zhu et al. 2005; He et al. 2007). Furthermore, in north China, specific vegetables are grown in continuous cropping in some areas. Whether AM fungi can persist in such high-input and intensively cultivated systems and re-inoculate their host plants successfully is not well characterized.

Previous studies on AM fungal abundance and AM fungal community structure are generally restricted to the main rooting zone (10–30 cm soil depth; Redecker 2002; Vandenkoornhuyse et al. 2002; Jansa et al. 2003; Johnson et al. 2003). However, work by Oehl et al. (2005) indicates that an AM fungi "gene bank" might persist in the subsoil, which may facilitate agro-ecological restoration when a high-input farming system is switched to a low-input system since it must rely more on internal biotic and abiotic resources. Therefore, in the present study, we sought to test the infectivity and composition of the indigenous AM fungi to understand how they are distributed in the soil profile in high-input agricultural ecosystems.

In general, studies characterizing AM fungal communities in field soils are based on the analysis of spores, which can sometimes be complemented by trap culturing (Douds et al. 1993; Oehl et al. 2004, 2010). Spore-based surveys are considered to be the baseline for assessing the impact of agricultural practices on AM fungal communities (Douds and Millner 1999). However, morphological characterization of AM fungal spores and their diversity might not reflect the actual functional symbiosis that facilitates the formation of active fungal structures within and outside roots (Clapp et al. 1995; Jansa et al. 2003). These active structures, such as fungal hyphae in the roots and soil as well as arbuscules within roots, can only be identified accurately using molecular or immunological approaches (Redecker et al. 2003; Sanders 2004). The present study investigated the molecular diversity of AM fungi at three soil depths from the surface soil to the subsoil in areas with three types of high-input land use.

2 Materials and methods

2.1 Sites and agricultural practices

The study sites, namely, Taiyuan (T) and Shouguang (S), are located at the middle and lower reaches, respectively, of the Yellow River Basin, one of the most important agricultural zones in China, which accounts for 12.4 % of the cultivated land area and 7.7 % of total grain production. Although these percentages are low the area is an important ecological barrier in north China. T and S have yearly precipitation of 456 and 594 mm, annual average temperatures of 9.5 and 12.7 °C, and yearly sunshine of 2808 and 2549 h, respectively. At site T, three typical intensive cropping systems were studied, i.e., vegetable greenhouses, open vegetable fields, and arable land. At site S, only vegetable greenhouses and arable land were studied because typical open vegetable land was absent from this area. The three selected land-use types are common at the target area and the land management practices at these plots reflect current practices used in the region. The plot details are shown in Table 1. Rooting depths of crop roots can extend to 90 cm depth, while tilling only penetrates the soil to a depth of 30 cm. Then, the current soil sampling depths extended to 90 cm and samples were sectioned into 0–30, 30–60, and 60–90 cm depth categories according to the local tillage regimes and plant root distribution range.

2.2 Soil sampling, preparation, and chemical analysis

Soil samples collected from arable land and open vegetable fields system at sites T and S were obtained from March to

Table 1 Principal agricultural management practices, crop rotations, and standing crop on date of sampling at the field sites

Land use	Site	Farming system, rotation, and/or crop/age	Soil type	Plant protection	Standing crop in March 2010	Latitude and longitude
Greenhouse vegetable soils	Taiyuan (T)	One cropping season, monocropping, tomato, 3-year-old	Eutric Luvisol	Chemical	Tomato	37°37′ N, 112°48′ E
	Shouguang (S)	Double cropping seasons, monocropping, tomato, 6-year-old	Haplic Luvisol	Chemical	Tomato	36°55′ N, 118°44′ E
Open vegetable fields	T	Conventional, long-term rotation, mainly tomato and Chinese cabbage	Eutric Luvisol	Chemical	Tomato	37°37′ N, 112°48′ E
Arable land	T	Conventional, double cropping seasons, wheat and maize	Eutric Luvisol	Chemical	Wheat	37°37′ N, 112°48′ E
	S	Conventional, monocropping spring maize	Haplic Luvisol	Chemical	Maize	36°55′ N, 118°44′ E

April 2010 and in the vegetable greenhouse systems at harvest time of the winter–spring season (May–June 2010). Three replicate plots of open vegetable fields and arable lands were selected in villages that had all three (or two) land uses for sampling (plot size 102–120 m^2). Three typical commercial greenhouses at each site (100 m×8 m at site T and 84×8.5 m at site S), constructed of clay walls and covered with polyethylene film, were randomly selected in each selected village.

We used a stratified random sampling approach to collect individual soil cores. In each of the three replicate plots (or greenhouse) per field site, six soil cores were drilled to a depth of 90 cm using an 8-cm-diameter soil corer and the cores were separated in sections corresponding to 0–30, 30–60, and 60–90 cm soil depth. Soils were sieved on-site through a 4-mm mesh, and then all six of the cores from a given plot (or greenhouse) that corresponded to a certain soil depth were homogenized by hand into a single composite sample per plot (or greenhouse). A subsample of 200 g soil was carefully ground by hand from each of the 45 pooled composite samples, thoroughly mixed, and air-dried. They were stored at 4 °C until analysis for pH, organic carbon, and available P. Additional subsamples of 500 g soil were stored in self-sealing plastic bags at 4 °C and used for the bioassay of inoculum potential.

Soil physical and chemical properties were measured according to standard methods, i.e., pH (1:2.5 soil/water), soil total N (Kjeldahl method), soil total P (H_2SO_4–$HClO_4$ digestion; Mo–Sb colorimetric method), available P (Olsen and Sommers 1982), and organic matter content (Cambardella et al. 2001).

2.3 Bioassay for inoculum potential

The bioassay for inoculum potential was performed using a modified mean infection percentage (MIP) method (Richter et al. 2002). Soil samples were sieved to 2 mm (large root fragments were cut to 1 cm and returned to the soil) and diluted in a 1:3 ratio (*v*/*v*) with autoclaved no. 20 silica sand (121 °C for 1 h). Each soil dilution was used to fill six containers (3.8×14 cm, 104 ml), giving a total of 270 containers. Cucumber (*Cucumis sativus* L.) seeds were surface sterilized in 10 % bleach for 3 min, and two seeds were sown in each container and covered with a 1-cm layer of autoclaved no. 12 sand. The containers were placed in a greenhouse in a randomized design under natural light conditions and day/night temperatures of 31/22 °C. After emergence, the seedlings were thinned to one per container.

The plants were harvested at 30 days. Roots were washed free of soil and cut into 1-cm segments. One half of the roots were stored at −20 °C for molecular analysis, and the remainders were stored at 4 °C for MIP determination. Approximately 0.5 g fresh roots per sample was stained with Trypan blue. Root segments (100 of each replicate) were mounted on glass microscope slides and examined under a light microscope for infection. A segment was considered infected if it contained hyphae plus arbuscules, coils, or vesicles. Percentage infection was recorded as the number of infected segments per 100 root segments (Moorman and Reeves 1979).

2.4 DNA extraction from roots and nested-PCR for AM fungi

The present study focused on the AMF with vital AMF propagules in the field. AM fungal community was therefore analyzed using root samples from the MIP bioassay. A subsample (~0.5 g) of the stored roots was cut into 0.5-cm pieces and immediately frozen in liquid nitrogen. Genomic DNA was extracted by a modification of the CTAB method (Li 2000; Li et al. 2014). Volumes of 2–5 μL of each DNA extract were used as polymerase chain reaction (PCR) templates.

A two-step procedure (nested PCR) was conducted using the primer pairs SSUmAf–LSUmAr and SSUmCf–LSUmBr (Krüger et al. 2009). The target region for PCR included the partial sequences of LSU rDNA and SSU rDNA and the whole ITS rDNA. The first amplification was performed using Ar/Af. The 25-μL reaction volume contained 2 μL 10× PCR buffer, 0.2 mM dNTPs, 0.5 mM of each primer, 1 U Taq polymerase (Trans-easy taq), and 1 μL DNA template. The PCR program was as follows: initial denaturation at 94 °C for 5 min, 30 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C for 1 min, and extension at 72 °C for 1.5 min, followed

by extension at 72 °C for 10 min. The quantity of PCR products was estimated by conducting 1 % agarose gel electrophoresis using 5 μL PCR products. Next, 1 μL of PCR products was used directly as a template for the second PCR that was conducted using Cf/Br under the reaction conditions described above. Nested PCR products were separated by 1 % agarose gel electrophoresis and purified using an Extraction Kit (BioTeke, Beijing, China) according to the manufacturer's instructions.

2.5 Cloning, sequencing, and construction of clone libraries

The PCR products from roots of the replicated six containers were pooled (totally 45 samples) and cloned into pGEM-T Easy Vector System (Promega) and transformed into competent cells of *Escherichia coli* DH-5α strain following the manufacturer's protocol. Three replicates of each land use were grouped into one clone library, and 15 clone libraries for AM fungi were constructed from all soil depths of each land use. Clones containing the converted DNA fragments were selected by blue/white screening, and eventually, 80 white colonies were used and grown overnight at 37 °C in liquid Luria–Bertani medium (5 g yeast extract, 10 g tryptone, 10 g NaCl, and deionized water added to a final volume of 1 L, pH 7.0) containing ampicillin (50 μg mL^{-1}). Thereafter, the clones were identified by PCR with primer pair T7/SP6.

2.6 Restriction analysis, sequencing, and taxonomic classification

To roughly detect intrasporal and intersporal sequence variability in the clones, PCR products of 64 positive clones in each library were digested with the restriction enzymes Hinf and MboI (Fermentas, USA; Krüger et al. 2009) for 5 h to produce restriction fragment length polymorphism profiles according to the manufacturer's protocols. Restriction fragments were separated at 100 V for 1.5 h in 1× TAE buffer in 2 % (*w/v*) agarose gel, stained with ethidium bromide (0.5 μg ml^{-1}), visualized under 300 nm UV light, and photographed. Representatives of each RFLP type from each sample were sequenced (Liu et al. 2001) by Beijing ZhongKeXiLin Biotechnology Company (Beijing, China).

The sequence data obtained were checked with the BLAST tool at GenBank to determine whether sequences were derived from Glomeromycota (based on the origin of the best scoring hit in the GenBank).

2.7 Sequence analysis

DNA sequences were edited with the SeqMan program of the Lasergene Package (DNAStar Inc., Madison, WI, USA). Before processing the sequences for phylogenetic analysis, representative sequences were targeted to define the divergent sequences from the same species using the DOTUR program (Schloss and Handelsman 2005). A dataset containing these representative sequences and reference sequences obtained from GenBank broadly representing the Glomeromycota was constructed. These sequences were aligned using Megalign program of the Lasergene Package, and the alignment was used to generate tree topologies. Phylogenetic trees were constructed by distance analysis using the neighbor-joining (NJ) algorithm in Paup 4.0 using the Kimura two-parameter model. Confidence in specific clades from the resulting topology was tested by bootstrap analysis with 1000 replicates. Branches corresponding to partitions reproduced in <70 % bootstrap replicates were collapsed. Sequence phylotypes were therefore defined in a conservative manner as consistently separated monophyletic groups in the phylogenetic tree. All the gene sequences in this phylogenetic tree have been deposited in GenBank under accession numbers JX683733 to JX683763.

2.8 Statistical data analysis

The sampling effort in each library was evaluated by calculating the Good coverage (*C*) according to the equation $C=1-(n/N)$, where *n* is the number of representative sequences and *N* is the total number of clones analyzed in the corresponding library (Good 1953). AM fungal richness was assessed as the number of different phylotypes detected in a sample.

Significance of differences in soil properties, species richness, and MIP between the samples from different soil depths and land uses at a given site was tested using Fisher's least significant difference (LSD) at $P<0.05$ after a one-way analysis of variance (ANOVA). The relationships between AM fungi and soil chemical parameters were examined with Pearson's correlation coefficients. Dependency of MIP and fungal richness on selected soil parameters was confirmed using stepwise linear regression analysis. The SPSS 13.0 software package was used to conduct all statistical analysis.

To explore the relationship between environmental variables and AMF community composition, the environmental variables (including soil depths and soil properties) were fitted as vectors onto the nonmetric multidimensional scaling (NMDS) plot using function "envfit" in the R version (R Development Core Team 2011). To test whether different soil types from sites T and S harbor different community composition, soil types were fitted as ellipse onto NMDS plot using function "ordiellipse" from "vegan" library in the R.

The present study assessed the AM fungal community within the roots of the MIP assay and not directly from the roots or bulk soil collected from the field. The MIP assay is often used to assess the inoculum potential of AM fungi in the field. Thus, some highly mycorrhiza-dependent plant species are used as the host plant, such as maize and clover. When Richter et al. (2002) assessed MIP from abandoned

agricultural fields and semi-arid grasslands in riparian floodplains, they selected corn (*Zea mays* L.) as the host plant. Cucumber is a highly mycorrhiza-dependent vegetable (Jakobsen and Rosendahl 1990), and we therefore selected it as the host plant in the present study. We also tried to extract DNA directly from the roots and bulk soil in a vegetable greenhouse at site S. Most of samples from 60 to 90 cm soil depth failed (not published data). The low MIP values also provide convincing evidence that there were very few propagules there (Fig. 1). Compared to the bias from cultures, we consider that the bias involved in extracting DNA directly from a small sample of roots and bulk soil is very high. This method might be helpful in the evaluation of the AM fungal community under adverse conditions.

3 Results

3.1 Edaphic properties

Soil properties were found to vary with land use and soil depth (Table 2, Table S2). Vegetable greenhouse soils (0–30 cm) had approximately three times the total P of arable fields at site S and about twice of arable land at site T. Likewise, Olsen P in vegetable greenhouses (0–30 cm) was more than ten times greater than in arable soil at site S and eight times greater than at site T. Both total and Olsen P in open vegetable fields was at an intermediate level for all the land-use types (Table 2). In most cases, nutrients (organic matter, total N, P, and Olsen P) in the topsoil (0–30 cm) were considerably higher than in the intermediate and/or the deepest layer but pH was often lowest in the topsoil (Table 2).

3.2 MIP

Bioassay results indicate that the number of infective propagules differed according to the land-use type and soil depth (Fig. 1, Table S2). The highest MIP value was noted in the top soil obtained from arable fields at both sites (Fig. 1a, b). In the vegetable greenhouses, there was no significant difference of propagules among soil depths at both sites. In contrast, the topsoil contained considerably more propagules and showed

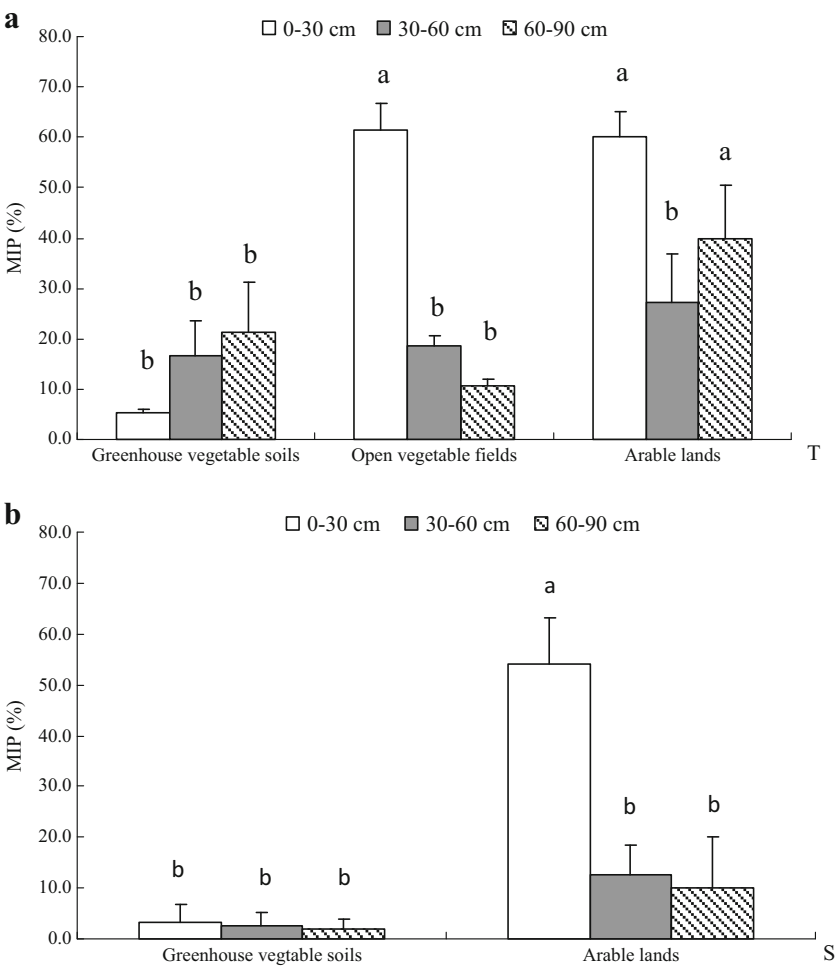

Fig. 1 Mycorrhizal inoculum potential (*MIP*) under different land-use types and soil depth categories at the three sites studied. Values are mean±1 SE. *Lowercase letters* represent differences within soil depth. *Uppercase letters* represent differences among land-use types. All differences are at the significance level $P<0.05$

Table 2 Soil chemical and physical properties at different soil depths at the field sites

Site abbreviation	Land use	Soil depth (cm)	pH	Organic matter (g kg^{-1})	Total N (g kg^{-1})	Total P (g kg^{-1})	Olsen P (mg kg^{-1})
T	Greenhouse vegetable soil	0–30	7.6 (0.1)d[a]	11.09 (2.23)b	0.720 (0.082)a	1.055 (0.097)a	100.9 (11.69)a
		30–60	7.9 (0.0)bc	9.64 (1.76)bc	0.351 (0.066)cd	0.505 (0.058)cd	19.2 (11.67)bc
		60–90	8.0 (0.0)abc	5.43 (1.03)d	0.259 (0.024)d	0.445 (0.014)cd	4.2 (0.54)g
	Open vegetable fields	0–30	7.9 (0.0)c	15.00 (0.61)a	0.611 (0.062)ab	0.656 (0.024)b	21.9 (4.20)b
		30–60	8.0 (0.1)ab	6.24 (0.08)cd	0.237 (0.026)d	0.477 (0.008)cd	8.2 (0.24)e
		60–90	8.1 (0.0)a	6.48 (0.06)cd	0.229 (0.014)d	0.390 (0.005)d	5.8 (0.19)f
	Arable land	0–30	7.9 (0.1)bc	11.30 (0.47)b	0.492 (0.060)bc	0.591 (0.070)bc	13.1 (1.34)d
		30–60	8.0 (0.0)ab	6.33 (0.53)cd	0.309 (0.023)d	0.492 (0.002)cd	5.9 (0.70)f
		60–90	8.0 (0.0)abc	6.37 (0.32)cd	0.286 (0.042)d	0.427 (0.033)d	3.7 (0.16)h
S	Greenhouse vegetable soil	0–30	7.1 (0.2)a	13.25 (0.93)a	0.696 (0.091)a	1.539 (0.170)a	260.2 (23.3)a
		30–60	7.1 (0.2)a	7.20 (0.26)b	0.365 (0.028)d	0.614 (0.058)b	106.7 (9.09)b
		60–90	7.3 (0.2)a	4.45 (0.24)c	0.197 (0.025)e	0.393 (0.045)b	31.9 (3.22)c
	Arable land	0–30	7.3 (0.2)a	10.98 (0.49)a	0.597 (0.016)b	0.528 (0.006)b	22.2 (4.56)c
		30–60	7.6 (0.3)a	7.36 (0.05)b	0.405 (0.034)c	0.309 (0.069)b	11.4 (2.09)c
		60–90	7.8 (0.1)a	6.54 (0.92)b	0.300 (0.032)d	0.454 (0.132)b	7.9 (0.78)c

[a] Means within the data from each site and within each column sharing the same letter are significantly equal. Standard errors of the three replicates (SEM) are indicated in parentheses

a decreasing trend with increasing soil depth under the other two land uses (Fig. 1a, b).

3.3 Molecular analysis of AM fungal sequences

Target rDNA sequences from all root samples of the MIP assay were successfully amplified by nested PCR. A total of 2880 clones from 15 libraries were screened by RFLP and 492 clones were subsequently sequenced (two clones of each RFLP type). Preliminary BLAST searches revealed 481 sequences (2767 clones) belonging to AM fungi, excluding non-AM fungi (mostly were uncultured Zygomycetes, uncultured Ascomycete clones and Zygomycete spp.) and chimeric sequences (113 clones). These non-AM fungi and chimeric sequences were excluded from sequence analyses. Each library provided good coverage above 97.5 %, with an integrated value of 98.9 %, which suggests that the majority of fungal species in the 15 libraries were selected. Partial LSU rDNA sequences were grouped into 31 different OTUs on the basis of sequence similarities (95–100 %) using the DOTUR program (Table S1).

The topology of the phylogenetic tree and the results of BLAST indicate that all the AM fungal sequences were clustered into 13 discrete groups or phylotypes with an NJ bootstrap support of ≥75 %. The 13 sequence phylotypes were grouped into three families: Glomeraceae, Diversisporaceae, and Paraglomeraceae (Fig. 2). Most sequences appeared to represent *Glomus*, *Funneliformis*, *Rhizophagus*, *Paraglomus*, and *Diversispora*. Five clusters (Glo 4, 5, 7, and Div 3, 4) were likely not related to any sequences of AM fungi in culture or previously reported in the database. Clone numbers indicate the overall dominance of Glo 6 (31.4 % of all clones isolated), Div 1 (30.5 % of all clones isolated), and Glo 1 (20.9 % of all clones isolated) sequence groups. No other sequence phylotypes contributed to more than 10 % of clones (Fig. 2).

3.4 AM fungal composition and diversity

AM fungal community composition varied with land use and soil depth. NMDS ordination yielded sample groupings according to land uses at specific sites (Fig. 3). Of the 13 total phylotypes, the most common types (Glo 6, Div 1, and Glo 1) accounted for 82.8 % of AM fungal clones analyzed and were present in all the soil depths and land-use types examined (Fig. 2, Table 3).

Almost 40 % (5 out of 13) phylotypes were unique to a specific land-use type or soil depth. For example, Glo 4 was found only in the deepest layer of greenhouse vegetable soils at both sites, and Div 3 was found only in the middle soil depth of arable land. Glo 4, Glo 5, Div 3, and Div 4 were found only below the plough layer (below 30 cm; Table 3).

Richness of AM fungal phylotypes varied with land-use type and soil depth (Fig. 4, Table S2). At both sites, richness was much higher in the 0–30 and 30–60 soil depth of arable land than in the greenhouse vegetable soils. AM fungi were most numerous in the deepest layer of the greenhouse vegetable soils, while the topsoil contained considerably more AM fungi in the other two land-use types (Fig. 4a, b).

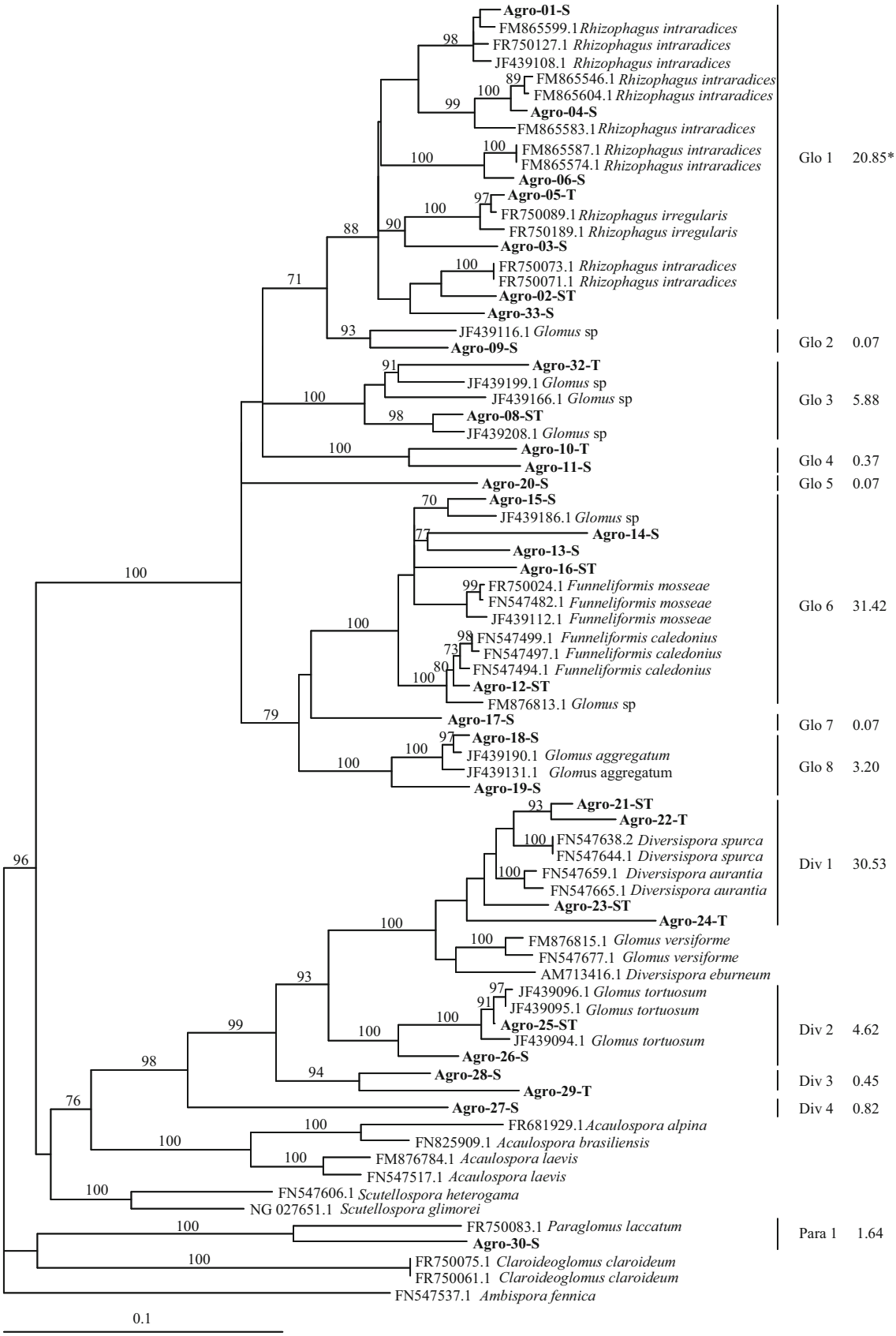

Fig. 2 Neighbor-joining phylogram of AMF sequences obtained from plant roots in the MIP bioassay. *Number above the nodes* indicates the bootstrap support in neighbor-joining analysis. *Asterisk* Relative abundance of the particular phylotype in the investigation

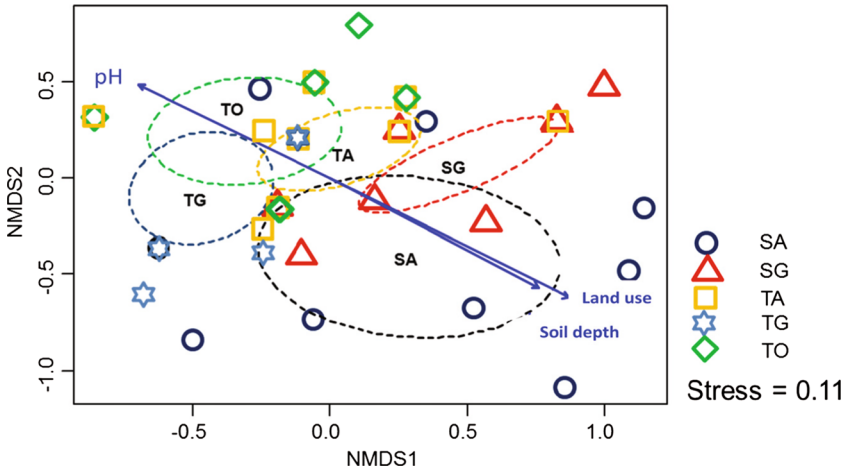

Fig. 3 Joint plot of NMDS ordination of AMF communities in soils from sites T and S and the vectors of significant ($P<0.05$, except elevation, $p=0.58$) environmental variables across sites. The stress value reflects how well the ordination summarizes the observed distances among samples. The *length of the arrow* is proportional to the strength of correlation between environmental variable and community composition dissimilarities. *Blue circles* Samples from arable field at S (SA), *red triangles* samples from greenhouse vegetable soil at S (SG), *yellow squares* samples from arable land at T (TA), *blue stars* samples from greenhouse vegetable soil at T (TG), *green diagonal squares* samples from open vegetable field at T

3.5 Effects of soil properties on MIP, species richness, and composition

Correlation analysis shows that all soil property indices were strongly related to each other. Using multiple stepwise regression analysis, the infectivity of AM fungi was strongly correlated with the changes in soil Olsen P (negative effect) and total N content (positive effect) across the landscape investigated ($R^2=0.387$, $p<0.001$). Only soil pH was negatively correlated with richness ($R^2=0.371$, $p=0.016$).

Seven environmental factors fitted as vectors onto the NMDS plot showed that three factors, i.e., land-use type ($R^2=0.426$, $p<0.001$), soil depth ($R^2=0.334$, $p<0.001$), and soil pH ($R^2=0.270$, $p<0.001$) were significantly correlated with AM fungal community composition (Fig. 3).

4 Discussion

We know that land-use change can have significant and long-lasting effects on soil carbon and nutrient contents, soil texture, and pH (Post and Mann 1990; Murty et al. 2002) that arise largely from changes in plant species composition and management practices. Here, we have observed significant differences in nutrient concentrations (total P and Olsen P)

Table 3 AM fungal phylotypes detected from different land-use types at 0–90 soil depth

Site	Land use	Soil depth (cm)	Glo 1	Glo 2	Glo 3	Glo 4	Glo 5	Glo 6	Glo 7	Glo 8	Div 1	Div 2	Div 3	Div 4	Para 1
T	Greenhouse vegetable soil	0–30	■								■				
		30–60						■			■				
		60–90	■			■		■			■				
	Open vegetable fields	0–30	■					■			■				
		30–60			■			■			■	■			
		60–90									■				
	Arable land	0–30			■			■			■	■			
		30–60	■					■			■		■		
		60–90	■					■			■				
S	Greenhouse vegetable soil	0–30	■		■			■			■				
		30–60			■		■	■							
		60–90	■		■	■		■			■	■			
	Arable land	0–30	■	■	■				■	■	■				■
		30–60	■		■			■		■	■		■		
		60–90	■		■			■			■			■	

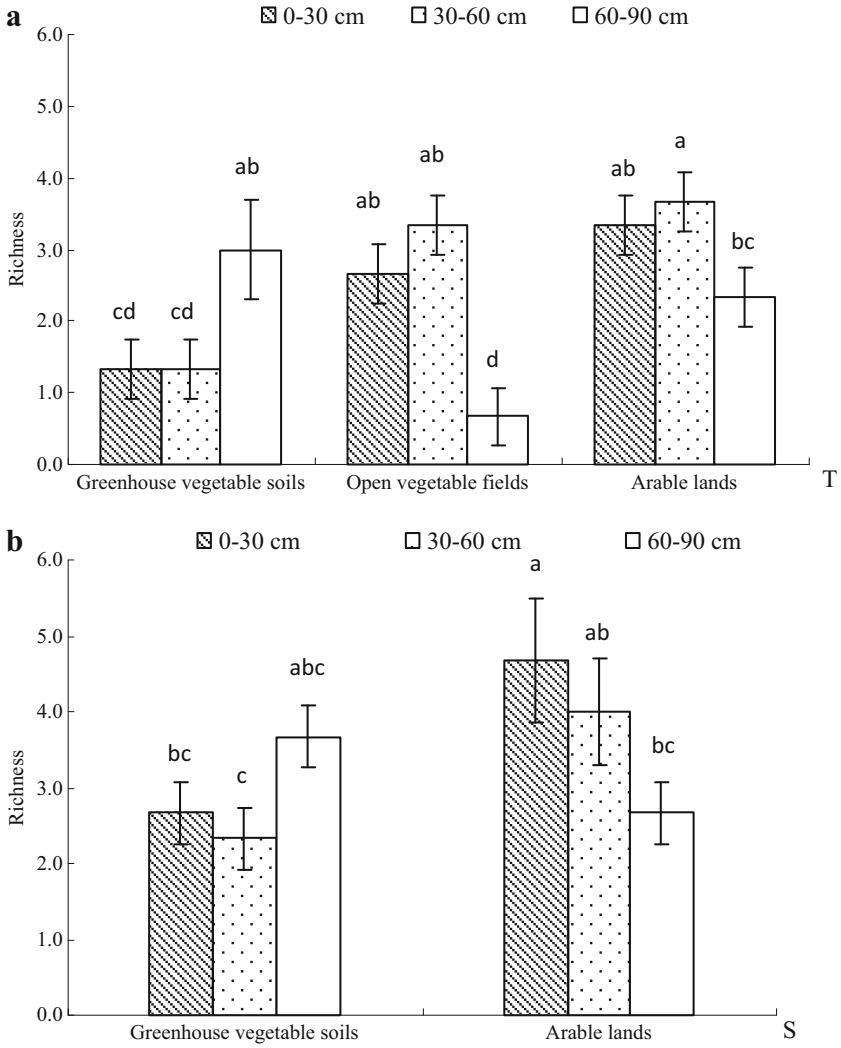

Fig. 4 Richness detected for each AMF phylotype at different soil depths and land-use categories. Values are mean±1 SE. *Lowercase letters* represent differences within soil depth. *Uppercase letters* represent differences among land-use types. All differences are at the significance level $P<0.05$

between land-use types and soil depths. However, a lack of consistent land-use effects on edaphic factors such as soil pH, soil organic matter, and total N content was also found. Lauber et al. (2008) also demonstrated that some soil properties (soil texture and nutrient status) differed significantly across land-use types, but other edaphic factors (e.g., pH) did not vary consistently with land use. The higher N and P contents and low pH of the greenhouse vegetable soils may be attributable to very high inputs of fertilizers and manures incorporated into the soil to maintain high vegetable yields (Qiu et al. 2010). With the sole exception of pH at site S, soil depth consistently influenced soil edaphic factors (soil pH, C, N, and P) at the two sites, which is in agreement with the results of Ju et al. (2007).

Land use and soil depth also greatly influence the development of root-associated AM fungi. The MIPs in topsoils of open, greenhouse vegetable and arable lands ranged from 30 to 70 %, which was similar to the top 20 cm layer of paired grassland and abandoned agricultural fields in southeastern Arizona (Richter et al. 2002). The MIPs in greenhouse vegetable soils varied greatly among the sites (Fig. 1), ranging from 0 to 21 %, and the explanation for this is not clear. The different climatic conditions, soil types, and crop rotation history of the sites would have been expected to make some impact on the variance of MIPs. Nevertheless, the results demonstrate the persistence of a certain number of AM fungi in the highly intensified land-use types.

High inputs of fertilizer have often been found to be correlated with a decrease in AM fungal species richness and infectivity (Douds et al. 1993; Douds and Johnson 2004; Kurle and Pfleger 1994; Oehl et al. 2004; Verbruggen et al. 2010). Results of the present investigation also show that AM fungal propagule abundance and phylotype richness were lowest in the greenhouse vegetable soils (Figs. 1 and 4). One of the possible explanations for this decline is that greenhouse soils experience a lack of diversity of host plant species. The application of fungicides and pesticides, together with the application of large quantities of fertilizers and especially phosphorus also can inhibit the growth, development, and functioning of AM fungi (Tawaraya et al. 1994; Oehl et al. 2003, 2004,

2005). Stepwise regression analysis showed that AM fungal infectivity was strongly negatively correlated with soil Olsen P content across the landscapes investigated. Some other factors such as low temperature and low light levels inside the vegetable greenhouses are also disadvantageous to AM fungal development (Smith and Read 2008).

Some studies have shown that niche partitioning can maintain AM fungal communities in intensively managed agricultural ecosystems (Jakobsen and Nielsen 1983; Kabir et al. 1998; Neville et al. 2002). We investigated AM fungi in the soil over a large depth range (0–90 cm) and found the result: The presence and prevalence of AM fungi varied with soil depth. This was particularly striking in the greenhouse vegetable soils where AM fungal richness was much higher at the deepest soil depth studied than the top. The distribution of AM fungi may have been driven by comprehensive forces such as soil physical and chemical properties and pressures of oxygen and root distribution (Anderson et al. 1987; Brady and Weil 1996; Entry et al. 2002), which were favorable to AM fungi living in surface soil or deeper in the profile.

Management practices associated with conventional agriculture might favor few AM fungal taxa tolerating the adverse conditions (Entry et al. 2002; Hijri et al. 2006; Chagnon et al. 2013). Studies have shown that frequently tilled agricultural soils tend to be dominated by species belonging to the Glomeraceae (e.g., Helgason et al. 1998; Jansa et al. 2002; Maherali and Klironomos 2012). These species are sometimes called "typical AM fungi of arable lands" or AM fungal "generalists" (Oehl et al. 2003) or even AM fungal "weed" species. In the present study, Glomeraceae accounted for 61.9 % of AM fungal clones in the soils investigated. Phylogenetic analysis suggests that *Glomus mosseae* (Glo 6), which is related to *Glomus caledonium*, was the most frequently isolated species in our study, followed by a *Diverspora* phylotype (Div 1) and *Glomus intraradices* (Glo 1). Daniell et al. (2001) also found that *G. mosseae* was the most frequently found phylotype in the roots of arable crops.

Some AM fungal species were only found in a specific land-use type or soil depth. For example, at both sites, Glo 4 was found only at 60–90 cm depth in greenhouse vegetable soils, and Div 3 was found only in the middle soil depth (30–60 cm) of arable land. The possibility of the survival of sensitive AM fungal species in the subsoil has important implications. They might facilitate agro-ecological restoration when switching from a high- to a low-input farming system.

5 Conclusions

The results presented here indicate that AM fungal diversity and community composition strongly depended on land use and soil depth. The vertical distribution pattern of AM fungal infectivity and richness in greenhouse vegetable soils were quite different from agricultural soils. These findings may be useful in understanding how to control intensively managed agricultural ecosystems to make them both sustainable and productive.

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