Glomus intraradices dominates arbuscular mycorrhizal communities in a heavy textured agricultural soil

Mycorrhiza (2005) 16: 61–66
DOI 10.1007/s00572-005-0014-9

SHORT NOTE

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Received: 27 May 2005 / Accepted: 13 July 2005 / Published online: 23 August 2005

Abstract Arbuscular mycorrhizal fungal (AMF) spore communities were surveyed in a long-term field fertilization experiment in Switzerland, where different amounts of phosphorus (P) were applied to soil. Plots receiving no P as well as plots systematically fertilized in excess to plant needs for 31 years were used to test the hypothesis that application of P fertilizer changes the composition and diversity of AMF communities. AMF spores were isolated from the field soil, identified, and counted so as to quantify the effect of P fertilization on AMF spore density, composition, and diversity. Trap cultures were established from field soil with four host plants (sunflower, leek, maize, and *Crotalaria grahamiana*), and the spore communities were then analyzed in substrate samples from the pots. Altogether, nine AMF species were detected in the soil. No evidence has been acquired for effect of P fertilization on spore density, composition, and diversity of AMF in both the field soil and in trap cultures. On the other hand, we observed strong effect of crop plant species on spore densities in the soil, the values being lowest under rapeseed and highest under *Phacelia tanacetifolia* covercrop. The identity of plant species in trap pots also significantly affected composition and diversity of associated AMF communities, probably due to preferential establishment of symbiosis between certain plant and AMF species. AMF spore communities under mycorrhizal host plants (wheat and *Phacelia* in the fields and four host plant species in trap pots) were dominated by a single AMF species, *Glomus intraradices*. This resulted in exceptionally low AMF spore diversity that seems to be linked to high clay content of the soil.

Keywords Identification · Arbuscular mycorrhizal fungi (AMF) · Field experiment · Phosphorus (P) fertilization · *Glomus intraradices*

Electronic supplementary material Supplementary material is available for this article at http://dx.doi.org/10.1007/s00572-005-0014-9 and accessible for authorised users.

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Introduction

Phosphorus (P) is indispensable for plant growth. To sustain crop yields, P is supplied to the soil as fertilizer, for example, in mineral, water-soluble form such as triple superphosphate. Long-term application of P fertilizers at rates exceeding those of crop removal has resulted in accumulation of P in the surface horizons of most European soils (Gallet et al. 2003). Consequently, this leads to P losses to water bodies through leaching, runoff, and erosion, causing their eutrophication (Schärer et al. 2005)

Increase in soil P availability negatively affects colonization of plant roots as well as mycelium development of arbuscular mycorrhizal fungi (AMF) (Douds and Millner 1999). Mycorrhizal benefits are usually reduced under conditions of sufficient soil fertility (Allison and Goldberg 2002). This may ultimately lead to selection by soil fertilization of AMF that are less beneficial for plant growth and nutrient uptake (Johnson 1993). Yet, it is not clear whether P fertilization has any consistent effect on the diversity and species composition of AMF communities in the soil. Although the network of interactions and dependencies in a soil–plant system is still too complex to be fully understood (Allen et al. 2003), it is probably wise to be concerned about agricultural practices causing loss of diversity and/or functions that may prove necessary for recovery of sustainable agroecosystems (Hooker and Black 1995). Although the importance of AMF for crop nutrition in production agriculture has recently been questioned, it

was admitted that some nonnutritional benefits of AMF such as soil stabilization may still need to be considered (Ryan and Graham 2002).

In this study, we aimed at characterization of AMF community in the oldest running P fertilization experiment in Switzerland. We hypothesized that the fertilization would affect AMF community composition and decrease its diversity. The soil in the field experiment has been either fertilized in excess or according to plant needs, or not fertilized since 1971. Here, we employ observation of AMF spores directly isolated from the field soil as well as trap culturing to multiply native AMF communities under controlled conditions.

Materials and methods

Study site and soil sampling

This study was performed in a long-term field fertilization experiment in Changins, Canton Vaud, Switzerland (6°14′ 26″E, 46°23′55″N, elevation 438 m). This field experiment was started in 1971 to observe long-term effects of different rates of application of phosphorus (P) and potassium (K) into the soil on crop yields. P was applied as triple superphosphate and K as potassium chloride. The experiment was set up in randomized block design with four replicate blocks and with plots of size 15×8 m. Soil was annually tilled to the depth of 15–20 cm and cropped under a simple rotation consisting of wheat, maize, wheat, and rapeseed. The soil type at the site is Gleyic Cambisol containing 54% clay and 16% sand, pH_{H2O} being 6.7. Further details about soil physico-chemical properties can be found elsewhere (Gallet et al. 2003). We studied AMF communities in three fertilization treatments: (1) no P and K application, P0; (2) P and K inputs equivalent to crop removal, P1; (3) P and K inputs exceeding crop removal by 26.2 and 166 kg ha^{-1} $year^{-1}$, respectively, P2. Nitrogen fertilization and other inputs (according to integrated production guidelines) were the same for all experimental plots. Soil was sampled in March 2002 [rapeseed, 205 days after sowing (DAS)], April 2003 (wheat, 171 DAS), and October 2003 (99 days after wheat harvest, *Phacelia tanacetifolia* Benth. covercrop being grown for 6 weeks and then treated with Roundup herbicide 1 month before the sampling). Thirty soil cores (3 cm diameter) were randomly collected from depths of 0–15 cm from each experimental plot and pooled together to obtain one composite sample per plot and sampling time. The samples were then sieved (4 mm) to remove coarse debris and stored at 4°C until further study.

AMF in field soil

The spores were isolated from 50 g (fresh weight) of each of the soil samples using wet sieving and sucrose density gradient centrifugation (Jansa et al. 2002). Spores were observed under stereomicroscope (Olympus SZX12), mounted in PVLG or PVLG-Melzer reagent, and identified under compound microscope (Olympus AX70) as described elsewhere (Jansa et al. 2002). Numbers of spores for each AMF species were recorded. Soil humidity was estimated after drying soil samples at 105°C for 24 h.

Trap cultures

Field soil sampled in March 2002 from P0 and P2 treatments was used as inoculum for trap cultures, separately for each of the eight field plots. Soil was mixed with quartz sand and expanded Montmorillonite clay (Oil Dri Chem-Sorb WR24/18, Brenntag, Vitrolles, France) in a ratio of 1:2:2 (v:v:v) and filled in 800-ml pots. Each pot was planted with one of the following plant species: *Crotalaria grahamiana* Wight & Arn., leek (*Allium porrum* L.), maize (*Zea mays* L.), and sunflower (*Helianthus annuus* L. Merill.). These plant species (especially the *Crotalaria* sp.) were selected to allow comparability of results with another study on tropical soil from Western Kenya. Four replicate pots were established for each soil sample and host plant combination, yielding a total of 128 pots. The plants were grown for 5 months in a climate chamber (Conviron PGV36, Winnipeg, Canada) under following conditions: photoperiod 16/8 h, 24/18°C, 55/70% relative aerial humidity (day/night, respectively), photosynthetically active radiation flux during daytime 400 μmol photons m^{-2} s^{-1}. Plants were irrigated using tensiometer units (Blumat, Austria) and fertilized once in 2 weeks with 25 ml of eightfold concentrated Hoagland nutrient solution (Sylvia and Hubbell 1986) containing 80 μM P (which is 1% of the original recipe).

AMF in trap cultures

The spores were isolated from 20 g (fresh weight) of substrate from each trap pot following the same procedure as for the field soil samples. The spores were observed and identified under a compound microscope as above. Abundances of spores of each AMF species were estimated on the following semiquantitative scale: 1, up to 5 spores; 2, 6–20 spores; 3, 21–50 spores; 4, 50–200 spores; 5, more than 200 spores per sample. AMF spores from trap pots were further used to establish monospecific cultures according to Jansa et al. (2002) using leek as host plant.

Molecular identification

Spores of *Glomus intraradices* (the only species successfully subcultured in pure monospecific cultures) were used for sequencing of part of the large ribosomal subunit (LSU). Four spores were collected from one monosporic culture isolated from P2 treatment; DNA was extracted, amplified, and sequenced as described elsewhere (Jansa et al. 2003). The sequences were manually edited and blasted against GenBank sequence database to ensure affinities with glomeralean sequences. Then, they were aligned with

other glomeralean sequences from GenBank using Clustal X (version 1.83), and phylogenetic tree was constructed. The sequences were deposited in the GenBank under accession numbers AY970832–AY970836.

Calculations and statistical analysis

Spore numbers estimated on fresh weight basis of field soil were adjusted to dry weight of soil using soil humidity values. This could not have been done for spore abundances in trap cultures because only ranks of abundances were recorded there. Shannon–Weaver diversity index (H') was calculated from spore abundances as described previously (Oehl et al. 2004), with all values transformed by adding 0.0000001 to handle zeros. Multivariate analysis of variance (MANOVA) with Hotelling–Lawley Trace Statistics for calculation of approximate F value was performed in SYSTAT (version 10) for spore abundances from both field soil and trap pots to identify factors significantly affecting composition of AMF communities. For those factors, two- and one-way analyses of variance (ANOVAs) were calculated in Statgraphics (version 3.1) to analyze effects of P fertilization and trap plant species identity on AMF community composition and diversity. Following significant ANOVA, differences between treatment means were analyzed by multiple range least significant difference (LSD) test. Phylogenetic tree was constructed using Treecon software for Windows (version 1.3b) (Vandepeer and Dewachter 1994), considering distance estimation by Jukes and Cantor (1969) and running bootstrap analysis with 1,000 times resampling, taking into account insertions and deletions. Tree topology was inferred by neighbor joining, and tree was rooted on *Sinapis alba* LSU sequence, considered as outgroup.

Results

AMF in field soil

Two-way ANOVA revealed marginally significant differences (p=0.06) in the diversity of AMF communities (mean value H'=0.86±0.04) among the three P fertilization treatments, whereas the diversity was unaffected by the sampling time of the soil (p=0.35). The diversity of AMF spores in P1 soil tended to be higher than in P0 soil, whereas the diversity in P2 soil was similar to both P1 and P0 soils. Spores of seven AMF species were recorded directly in the field soil in March 2002, all belonging to the genus *Glomus* (*Glomus caledonium*, *Glomus claroideum*, *Glomus constrictum*, *Glomus dimorphicum*, *Glomus geosporum*, *Glomus microaggregatum*, *Glomus mosseae*). Neither the composition of AMF spore community (MANOVA p=0.75) nor its diversity (ANOVA p=0.17) was affected by P fertilization of the soil at this time. At second and third sampling times, spores of four *Glomus* spp. were recorded in the soil (*G. caledonium*, *G. constrictum*, *G. intraradices*, *G. microaggregatum*). Neither composition nor diversity of AMF communities was affected by P fertilization at second and third sampling times (analyses not shown), but in contrast to the first sampling time, only four AMF species were detected in the soil. AMF communities at these times were dominated by small-spored *Glomus* spp., forming either soil-borne spores (identified as *G. intraradices*) or sporulating inside dead spores (identified as *G. microaggregatum*). The numbers of spores assigned to *G. microaggregatum* and *G. intraradices* made 58±3 and 34±4% of all recorded spores at the second sampling date and 48±3 and 48±4% of the spores at third sampling date, respectively. AMF spore density (Fig. 1) was strongly affected by the time of sampling (p<0.001), whereas P fertilization had no effect (p=0.36), as revealed by two-way ANOVA, the interaction between the two factors not being significant (p=0.80).

AMF in trap cultures

AMF communities in trap cultures were dominated by *G. intraradices*, whose spore numbers exceeded other AMF species by two orders of magnitude (data not shown). The identification of this AMF species was confirmed by LSU sequencing. In total, five AMF species (*G. caledonium*, *G. claroideum*, *G. intraradices*, *G. mosseae*, *Paraglomus occultum*) were observed in trap cultures. *P. occultum* was not detected in field soil previously. Four AMF species previously observed in the field soil (*G. geosporum*, *G. constrictum*, *G. dimorphicum*, and *G. microaggregatum*) were not detected in traps. Altogether, spores of nine AMF species were observed either in field soil or in trap pots. The composition of AMF spore community in trap pots was significantly affected by host plant species identity (Table 1). Host plant was an important determinant of spore abundance of two AMF species (*G. caledonium* and *G. intraradices*, showing preferences for leek and sunflower, respectively). Diversity of AMF spore communities in trap pots was not affected by P fertilization of the

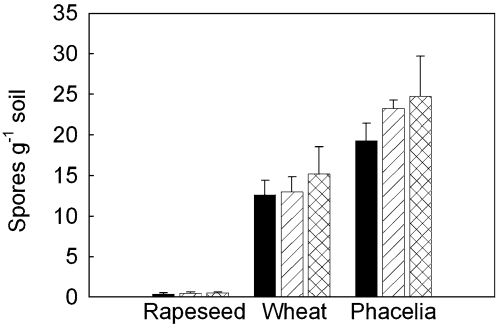

Fig. 1 AMF spore densities in soil subjected to three levels of P fertilization for more than 30 years, either nonfertilized (P0, *black bars*), fertilized to the level of crop export (P1, *hatched bars*), and fertilized in excess of crop needs (P2, *crossed bars*). AMF spores were observed in March 2002 under rapeseed [205 days after sowing (DAS)], in April 2003 under wheat (171 DAS), and in October 2003, following wheat harvest and *Phacelia tanacetifolia* covercropping. Reported data are per unit of soil dry weight. Mean values of four replicates ±1SE of means are shown

Table 1 MANOVA results for the effects of host plant species identity and P fertilization of field soil on spore abundances of AMF species in trap pots

Factor	*df*	HTL	*F* ratio	Num *df*	Den *df*	*p*
Host plant	3	1.661	10.487	18	341	<0.001
P fertilization	1	0.031	0.592	6	115	0.736
Host plant×P fertilization	3	0.246	1.552	18	341	0.071

Hotelling–Lawley Trace statistics (HTL) was used to calculate an approximate *F* ratio with accompanying numerator (Num) and denominator (Den) degrees of freedom

field soil (p=0.25), but by host plant species identity (p=0.01). Interaction between host plant species and P fertilization also proved to be significant (p<0.01) due to higher diversity in trap pots planted with leek from P2 than P0 soil. Generally, the diversity of AMF communities in trap pots planted with leek (H'=0.19) and maize (H'=0.21) was greater than in pots with *Crotalaria* (H'=0.13) and sunflower (H'=0.02).

Discussion

Only nine AMF species were recorded in the studied field site, all of them belonging to the genus *Glomus*. Moreover, AMF communities were clearly dominated by *G. intraradices* and *G. microaggregatum* and by *G. intraradices* in the field soil and in trap pots, respectively. Because it is difficult to properly identify field-collected spores by their morphology (Sanders 2004), because of the similarity of spores of *G. intraradices* and *G. microaggregatum* (Koske et al. 1986; Schenck and Smith 1982), because of lack of evidence for *G. microaggregatum* in trap pots, and because of the fact that AMF similar to *G. intraradices* abundantly sporulate within seed shells (Rydlová et al. 2004), we suspect that at least some of the spores identified as *G. microaggregatum* in field samples were actually spores of *G. intraradices*. This means that AMF communities were dominated by a single AMF species, *G. intraradices*. However, an ultimate proof of AMF identity will have to be performed by another method such as using molecular markers (Jansa et al. 2003). When the total species number recorded in this site is compared to other field sites in Switzerland with similar environmental conditions (Jansa et al. 2002; Oehl et al. 2003, 2004), this site represents the lowest recorded AMF species richness in Swiss agricultural soil (Table 2). Although the genus *Glomus* is usually dominating AMF communities in agricultural soils in Europe (Blaszkowski 1989; Jansa et al. 2002; Sjoberg et al. 2004), it was the only genus detected in the studied field. This result is contrasting to another study reporting other AMF genera such as *Gigaspora*, *Scutellospora*, *Entrophospora*, and *Acaulospora* in addition to *Glomus* in Swiss agricultural soil under identical crop rotation as in this study (Jansa et al. 2002). Because the environmental conditions and most soil properties of the studied site were not much different from other studies (Table 2), we can speculate that the relative paucity of AMF communities in

Table 2 Comparison of climatic conditions and soil properties at the study site with other three conventionally managed fields (fertilized with mineral fertilizers) in Switzerland, where AMF communities were studied

	Field site			
	P fertilization experiment, Changins (this study)	Tillage experiment, Tänikon	Agricultural field, Binningen (site L)	Production system experiment, Therwil (treatment MIN)
Information source	(Gallet et al. 2003)	(Jansa et al. 2002)	(Oehl et al. 2003)	(Oehl et al. 2004)
Mean rainfall year^{-1} (mm)	940	1,179	750	785
Mean temperature year^{-1} (°C)	9.5	8.2	9.5	9.5
Soil type	Gleyic Cambisol	Dystric Gleysol	Haplic Luvisol	Haplic Luvisol
Available P (mg P kg^{-1} soil)				
P_{Olsen}	11.2(P0); 28.6(P1); 52.7(P2)[a]	52	62[b]	NA
$E1_{min}$ (IEK)	2.5(P0); 8.3(P1); 21.3(P2)	15.4	NA	5.8
pH_{H2O}	6.7	5.8	7.1	6.0
Crop rotation	Wheat–maize–wheat–rapeseed	Wheat–maize–wheat–rapeseed	7 years rotation[c]	7 years rotation[d]
Clay content (%)	54	16	15 (approximately)[e]	15
No. of AMF species	9	17	18	26

Total numbers of AMF species recorded at each of the sites are shown
[a]Paolo Demaria, personal communication
[b]Double-lactate extraction
[c]Wheat–rapeseed–various legumes
[d]Potato–rapeseed–wheat/rye–beetroot–wheat–barley–meadow
[e]Fritz Oehl, personal communication
IEK Isotope exchange kinetics (Fardeau 1996), *NA* information not available

the studied field was due to heavy texture of the soil. Its clay content reached 54% compared to markedly lower values (15–16%) in the other sites. Small size of pores in clay soil may hamper the growth of AMF hyphae in soil (Nadian et al. 1996) either mechanically by imposing a penetration barrier to AMF hyphae (Drew et al. 2003) or by affecting oxygen concentration in the soil (Saif 1981). Such conditions may exert strong selective pressure on AMF, resulting in changes in their community composition. Indeed, the experiment conducted by Nadian et al.(1998) shows *G. intraradices* to be less sensitive to increase in soil bulk density (accompanied by lower oxygen concentration in soil and changes in pore size distribution) compared to *Glomus etunicatum* and *G. mosseae*. Whether the genotypes of *G. intraradices* encountered at this study are indeed more tolerant to unfavorable soil conditions (lack of oxygen, small pores) than other AMF species remains unresolved until direct experimental proof is available. It is also not possible to completely dismiss other possible factors shaping AMF communities and possibly contributing to differences between studies listed in Table 2. Among them, availability of spores and composition of AMF communities in adjacent fields could play an important role.

Here, we observed no significant effect of P (and K, which is very likely to be less important with respect to AMF) fertilization on AMF spore density or diversity. This was in contrast to the observation made by others (Ezawa et al. 2000; Kahiluoto et al. 2001) who reported P fertilization to decrease AMF spore density without significantly changing the species composition. However, even under conditions of very high P fertility (60.3 mg kg^{-1}, Bray P), roots of soybean were extensively (89%) colonized by AMF (Khalil et al. 1992). Unlike P fertilization, the identity of associated plants in this study did significantly affect AMF spore density and diversity in the field soil and in the traps, respectively. Such effects have been described previously, and it is now broadly accepted that these effects are due to mycorrhizal status (host vs nonhost) and dependency of each specific plant species and due to preferential association among certain plant and fungal species (Arihara and Karasawa 2000; Jansa et al. 2002). Higher AMF diversities were found in trap pots planted with leek or maize here, unlike in a similarly designed study with Kenyan soil where higher diversities were associated with maize and *Crotalaria*, which were the two plant species grown in the field there (Mathimaran et al., unpublished observations). Higher diversity of AMF associated with maize or leek (monocots) in this study compared to *Crotalaria* and sunflower (dicots) could also be due to higher capacity of monocots to transfer oxygen to the roots (Cornwell et al. 2001).

In conclusion, our study shows low diversity of AMF in intensively managed heavy textured soil in Switzerland. It is most likely that high clay content was the key determinant of the AMF community composition by selecting species such as *G. intraradices* tolerant to these soil conditions.

Acknowledgements We thank Dr. Marcel Bucher (ETH Zürich) for providing lab space to perform the molecular identification part of this study. Financial support of the Swiss Federal Institute of Technology (ETH) Zürich (project No. TH-5/01-2/00447) is gratefully acknowledged.

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