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Arbuscular mycorrhizal fungal diversity and species dominance in a temperate soil with long-term conventional and low-input cropping systems

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Abstract The aim of this work was to study the effect of long-term contrasting cropping systems on the indigenous arbuscular mycorrhizal fungal (AMF) spore populations in the soil of a field experiment located in western Finland. Conventional and low-input cropping systems were compared, each with two nutrient management regimes. The conventional cropping system with a non-leguminous 6-year crop rotation (barley–barley–rye–oat–potato–oat) was fertilized at either full (rotation A) or half (rotation B) the recommended rate. In the low-input cropping system, plant residues were returned to the plots either as such (rotation C) or composted (rotation D). In the rotation of this system, 1 year with barley was replaced by clover, and oat was cultivated mixed with pea. Thus, the 6-year rotation was barley–red clover–rye–oat + pea–potato–oat + pea. Each rotation was replicated three times, starting the 6-year rotation in three different years, these being designated point 1, point 2, and point 3, respectively. In the low-input system, biotite and rock phosphate were used to compensate for K and P in the harvested yield, while animal manure was applied at the start only. After 13 years, rotation points 1 and 3 were studied. Barley was the standing crop in all plots of rotation point 1, while oat and oat + pea were grown in rotations C and D, respectively. AMF spores were studied by direct extraction and by trapping, sampled on 15 June and 15 August. In addition, a special assay was designed for isolation of fast colonizing, dominating AMF. The cropping system did not significantly affect AMF spore densities, although the low-input cropping system with composted plant residues had the highest density with 44 spores on average and the conventional system with full fertilization 24 spores per 100 cm^3 soil in the autumn samples. Species richness was low in the experimental area. Five *Glomus* spp., one *Acaulospora*, and one *Scutellospora* were identified at the species level. In addition to these, three unidentified *Glomus* spp. were found. Species richness was not affected by cropping system, rotation point, or their interactions. The Shannon–Wiener index of AMF spore distributions was significantly higher in the fully fertilized than in the half-fertilized conventional plots. *Glomus claroideum* was the most commonly identified single species in the experimental area. It occurred in all the cropping systems and their various rotation points, representing about 30% of the total number of identified spores. In August, *G. claroideum* accounted for as much as 45–55% of the total numbers of spores identified in the conventional system with halved fertilization. In contrast, *Glomus mosseae* occurred more commonly in June (26%) than in August (9%). A bioassay using roots as inoculum for isolation and culture of dominating AMF was successfully developed and yielded only *G. claroideum*. This indicates a high probability of being able to more generally identify, isolate, and culture fast colonizing generalist AMF for use as inoculants in agriculture and horticulture.

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Introduction

Anthropogenic activities have caused a significant decline in agricultural soil quality worldwide through adverse changes not only in the biological but also in the chemical and physical properties of soil (Arshad and Martin 2002). To be able to protect the soil from degradation and to manage it on a sustainable basis, methods of studying the biological quality of soil are needed (Stenberg 1999). In recent years, low-input agricultural systems have gained increasing importance in many industrialized countries for conservation of natural resources and reduction of environmental degradation (Mäder et al. 2002). Long-term field experiments are invaluable sites for studying changes in soil quality due to different management regimes such as fertilizer application, crop rotation, or farming system (Vestberg et al. 2009). We have taken advantage of a long-term cropping system experiment located in western Finland to study the impact of agricultural practices on various functional and diversity traits of the indigenous arbuscular mycorrhizal fungi (AMF) of the area.

The AM fungal symbiosis is the most widespread symbiosis between a plant and a microorganism. As obligate symbionts, AMF are dependent on the host plant for fixed carbon. AMF have been shown to produce a wide range of benefits for their hosts. These benefits include improved uptake of essential nutrients like phosphorus (P) and zinc (Zn) (Smith and Read 1997), stabilization of soil aggregates (Miller and Jastrow 1990) by producing the glycoprotein glomalin (Wright and Upadhyaya 1996), alleviation of abiotic (Goicoechea et al. 1997) and biotic stress (Jaizme-Vega et al. 1997), and modification of root morphology (Berta et al. 1990).

Many agricultural practices used in modern farming, e.g., high levels of fertilization, frequent soil disturbance, or monoculture, may affect indigenous AMF negatively (Douds and Millner 1999). In several studies, AMF communities were found to have deteriorated in species composition in intensively managed agricultural systems compared with low-input systems (Sieverding 1989; Galvez et al. 2001; Oehl et al. 2004). Other studies, however, show only small or no differences between contrasting systems (Franke-Snyder et al. 2001; Vestberg et al. 2009). The incorporation of individual crops with various degrees of mycotrophic status in the crop rotation has clearly affected the amount and function of indigenous AMF. Non-host crops and long fallow periods may reduce AMF in soil (Arihara and Karasawa 2000; Troeh and Loynachan 2003). Vestberg et al. (2005), studying the impact of 3 years of cultivation of eight crops with different degrees of mycotrophic status, found highly elevated levels of AMF spores in soil after strawberry. In a study comprising 17 different field sites, Karasawa et al. (2001) showed that the mycotrophic status of the preceding crop (non-mycorrhizal mustard vs. mycorrhizal sunflower) was the most important factor influencing the growth and yield of successive maize.

Temperate arable fields are usually dominated by *Glomus* while spores of the genera *Acaulospora* and *Scutellospora* are found at low numbers at these sites. It is generally believed that *Glomus* has a highly infective extra-radical mycelium, whereas members of the Gigasporaceae (*Gigaspora* and *Scutellospora*) most frequently regenerate from spores (Biermann and Linderman 1983; Hart and Reader 2002). This might explain why *Glomus* is favored in frequently tilled agricultural soil and *Scutellospora* is rare in this situation. In a natural ecosystem, the ratio between these two genera is often the reverse. A small number of mainly *Glomus* species occur in most agricultural soils. Oehl et al. (2003) call such species "generalists". In their study of eight sites situated at the "three-country corner" of Switzerland, Germany, and France, such generalists were *Glomus mosseae*, *Glomus geosporum*, *Glomus albidum*, *Glomus etunicatum*, *Glomus diaphanum*, *Glomus constrictum*, and *Scutellospora calospora*. Probably other species like *Glomus intraradices* can also be characterized as generalists. In a large Finnish study based on identification of AMF from trap cultures of soil from 266 agricultural sites, *Glomus hoi*, *Glomus claroideum* (named *Glomus fistulosum* in that study), and *G. mosseae* were the most frequently identified species and can be classified as generalists in temperate Finnish arable land (Vestberg 1995).

The aim of this work was to study the effect of long-term contrasting cropping systems, conventional vs. low-input, both with two nutrient management regimes, on the indigenous AMF spore populations in a field experiment located in western Finland. The AM contribution to crop nutrition and growth was reported by Kahiluoto et al. (2009) from the same experiment. They found, among other things, that the low-input cropping system with recycled organic matter composted before incorporation into the soil favored the AM contribution to crop performance in the long term compared with a conventional cropping system and with a low-input system with no composting. It remained unclear, however, what was the role of different underlying mechanisms, such as the potential change in the

AMF community, in the overall impact of the cropping systems.

We ask:

1. Has a conventional vs. low-input cropping system or different nutrient management regimes in the long term resulted in differences in AMF spore communities?
2. Are there AMF species with a widespread distribution in the experimental area so that they can be classified as AMF generalists?
3. Do the AMF species differ in their rapidity of colonization and consequently in their dominance in crop roots?
4. If there are dominating AMF species, can these AMF be isolated by a bioassay?

Materials and methods

Field experiment

A long-term field experiment with contrasting cropping systems established in 1983 at MTT Agrifood Research Finland/Ylistaro, western Finland (63°55′N, 22°33′E), was studied. The experiment was established on a loamy soil. The initial soil properties (0–20 cm depth) were pH_{H2O} 5.8, organic carbon 2.3%, total nitrogen (N) 0.19%, ammonium-acetate-extractable calcium (Ca) 986 mg kg^{-1}, potassium (K) 112 mg kg^{-1}, magnesium (Mg) 125 mg kg^{-1}, phosphorus (P) 5.2 mg kg^{-1} (Vuorinen and Mäkitie, 1955), hot-water-extracted boron 0.5 mg kg^{-1}, and HAAc–EDTA-extractable copper 4.6 mg kg^{-1} and zinc 2.2 mg kg^{-1}.

The experiment consisted of 36 plots arranged in three complete blocks with a plot size of 50 m^2 (5 m×10 m; width × length). Four management systems (A, B, C, and D) were compared. The conventional (CONV) cropping system with a non-leguminous crop rotation (barley–barley–rye–oat–potato) was fertilized at either full (A) or half (B) the recommended rate. Fertilizers were applied at recommended rates, or at half those rates. For oat and barley, N, P, and K were applied before sowing at rates of 102–108, 30–36, and 60–72 kg ha^{-1} a^{-1}, depending on the concentrations of commercial fertilizers available, for winter rye at 50, 35, and 85 kg ha^{-1} a^{-1}, and for potato at 70–80, 100, and 120 kg ha^{-1} a^{-1}, respectively, or at half those rates. For winter rye, 83 kg N or half that rate was applied in the spring of the year of harvesting.

In the low-input (LOW-INPUT) cropping system, 1 year with barley was replaced by red clover and oat was cultivated mixed with pea (barley–red clover–rye–oat + pea–potato–oat + pea). Plant residues from these plots were returned to the same plot either as such (C) or composted (D). In LOW-INPUT, plant nutrition was based on biological N fixation and replacing K and P in harvested yields using biotite and apatite. In C, straw and other crop residues were directly incorporated into the soil in the autumn similarly to the conventional system, including clover green manure with straw of the preceding barley. Biotite (K 5%, Mg 10%, Ca 7%) at 2 t ha^{-1} and magmatic Kola apatite (17% P; 0.01% soluble in water, 1.7% soluble in 2% citric acid) at 500 kg ha^{-1} were for the first rotation incorporated in soil before sowing as divided for all the crops, while for the following rotations biotite was applied at 3 t ha^{-1} and apatite was applied at 300 kg ha^{-1} in potato plots only. In D, they were mixed with crop residues and clover green manure, which were composted in a separate heap per each block.

Within the blocks, each rotation was replicated three times by starting the 6-year rotation from three different years, these being designated point 1, point 2, and point 3, respectively. Further details concerning the experimental history prior to 1995 can be found in Kahiluoto et al. (2009).

The impact of long-term contrasting cropping systems on AMF traits in soil was studied after more than two full rotations in 1995. Standing crops of this year and the two previous years are reported in Table 1. Soil samples for determination of various AMF traits were collected on 15 June and 15 August 1995, from the uppermost 15 cm soil layer of all blocks and cropping systems A–D, but only from rotation points 1 and 3. Barley was grown in all plots of rotation point 1, while oat and oat + pea were grown in A and B, and C and D, respectively, of rotation point 3. Ten sub-samples were collected per plot which were pooled and mixed to form one composite sample which was stored at +6°C until use. From these soil samples, AMF spore numbers and species diversity were studied in three different ways: (1) by extraction directly from soil, (2) by trap culture assay in an AM-susceptible host, and (3) by isolation of rapidly colonizing, dominating AMF species.

Analyses

AMF spore extraction from soil

AMF spores were extracted from soil by wet sieving and decanting (Gerdemann and Nicolson 1963) followed by centrifuging in water and in a 50% sucrose solution (Walker et al. 1982). A 500-μm and a 50-μm sieve were used for wet sieving. After centrifuging, the spores were transferred to a dish of water for examination under a dissecting microscope at magnifications up to 50 times with illumination by incident

Table 1 Standing crops in the Ylistaro cropping system experiment in 1995 and in two previous years

Cropping system	Fertilization regime	Rotation point	Standing crops		
			1995	1994	1993
CONV_A	Full mineral fertilization	1	Barley	Oat	Potato
CONV_B	Half mineral fertilization	1	Barley	Oat	Potato
LOW-INPUT_C	Plant residues added as such	1	Barley[a]	Oat + pea	Potato
LOW-INPUT_D	Plant residues added composted	1	Barley[a]	Oat + pea	Potato
CONV_A	Full mineral fertilization	3	Oat	Potato	Oat
CONV_B	Half mineral fertilization	3	Oat	Potato	Oat
LOW-INPUT_C	Plant residues added as such	3	Oat + pea	Potato	Oat + pea
LOW-INPUT_D	Plant residues added composted	3	Oat + pea	Potato	Oat + pea

CONV_A and *CONV_B* refer to a conventional cropping system with full or half fertilization, respectively. *LOW-INPUT_C* and *LOW-INPUT_D* refer to a low-input cropping system where plant residues were added to the plots without or after composting, respectively

[a] With undersown red clover

light from a fiber-optic, quartz-halogen light source with a color temperature of 3,200 K (Walker et al. 1993). Spores were characterized and, whenever possible, identified to species using a high-power microscope.

The Shannon–Wiener index (Krebs 1985) was calculated as a measure of AM spore diversity. The index combines two components of diversity, i.e., species richness and evenness. It is calculated using the formula $H' = -\Sigma\, p_i \ln p_i$ where p_i is the relative spore abundance of the ith species compared to all species identified in a sample.

Trapping and culturing of AMF

The composite soil samples were also used for establishing trap cultures and further establishment of single-species AMF pot cultures. For trap culturing, the soil was diluted with sterilized sand (1:1) and sown with *Trifolium pratense* 'Bjursele'. The pots were kept in a greenhouse (22/18°C day/night, photoperiod 16/8 h day/night). When necessary, artificial light (Philips High-Pressure Mercury Vapor HPI 400 W) was supplied. Trapping was done in late 1995 and early 1996. The pots were not fertilized during trapping. After 6–12 months, AMF sporulation was checked in trap culture pots using the technique described in "AMF spore extraction from soil" section.

Dominating AMF assay

To isolate dominating AMF, field soil was mixed with steam-sterilized sand (1:1; v/v) and 150 ml of the mixture was placed in 200-ml plastic pots measuring 75 mm × 55 mm × 65 mm (height × base diameter × top diameter). Three seeds of each test plant were sown per pot. After emergence, the seedlings were thinned to leave one per pot. Pots were adjusted to 70% of field capacity. Watering was done three times a week on a weight basis. No extra fertilizers were given to the pots during the experiment. The pots were arranged in a completely randomized manner with three replicates (pot = replicate) for each plant. The experiment was conducted in a growth chamber under warm white artificial light of approximately 100–120 μmol m^{-2} s^{-1} at a temperature of 20/17°C (16/8 h day/night). Five weeks after onset, the trap plants were harvested and their roots cut into 1-cm pieces. From each root system, five root pieces were chosen at random and placed on the root system of 5-week-old *Plantago lanceolata* seedlings which had been raised on a sterile P-deficient sandy substrate. The resulting AM culture attempts were treated in a manner similar to that explained in "Trapping and culturing of AMF" section. A representative sample of cut roots was also stained with methyl blue (Grace and Stribley 1991) and the percentage of colonized root length was determined by the gridline intersect method (Giovannetti and Mosse 1980). AMF sporulation in pots was checked after 6–10 months.

Two experiments were carried out. First, a preliminary study was conducted to determine the feasibility of the method described above. The plant species compared in the preliminary study were leek (*Allium porrum* 'Titan'), barley (*Hordeum vulgare* 'Arra'), flax (*Linum usitatissimum* 'Linetta'), alfalfa (*Medicago sativa*, unknown cultivar), red clover (*Trifolium pratense* 'Björn'), white clover (*Trifolium repens* 'Huia'), and subclover (*Trifolium subterraneum*, unknown cultivar). For the preliminary study, a sandy moraine field soil was chosen that had a pH of 6.2 [analyzed from a soil–water suspension, 1:2.5 (v/v)] and acid ammonium-acetate-extractable (Vuorinen and Mäkitie 1955) P 26.6 mg kg^{-1} soil. The AMF species diversity and the number of AMF spores per volume of test soil were also determined from the preliminary study soil as described in "AMF spore extraction from soil" section.

Based on the level of root colonization and the occurrence of sporulation in trap plants, two plant species were selected to trap the dominating AMF from soil collected at Ylistaro in June and August 1995.

Statistical methods

In the field experiment and final dominating AMF experiment, the experimental design for the two factors (rotation point and cropping system) was a split-plot design where the whole-plot treatments (two rotation points) were in a randomized complete-block design and the split-plot treatments (two cropping systems) were completely randomized within each whole plot. There was also an additional factor, fertilization, the levels of which were nested to the cropping system and completely randomized.

AMF spore density, species richness and Shannon–Wiener index in the field experiment: AMF colonization in the dominating AMF experiment

For both the field experiment and the final dominating AMF experiment (plants flax and barley), the data from June and August were analyzed separately. In the final dominating AMF experiment, also flax and barley were analyzed separately as there was greater variation in the observations of flax than of barley. The data were analyzed using general linear mixed models. The data from the dominating AMF preliminary study were also analyzed using a general linear mixed model.

Analysis of species composition in the field experiment

Of the ten observed AMF species, the most commonly occurring morphological types, i.e., *G. mosseae* (Gmoss), *G. claroideum* (Gclaroi), *Glomus* sp. 'small white' (Smallwh), and *Glomus* sp. 'pigmented' (Gpigm), were chosen for statistical analysis. The species composition was described by a three-part composition (x_1, x_2, x_3) where x_1 is the proportion of Gmoss (some zero proportions in the data were converted to small positive values), x_2 is the proportion of the sum of Smallwh and Gpigm, and $x_3=1-(x_1+x_2)$ the proportion of Gclaroi. Consequently, the three proportions sum up to 1, and one proportion is dependent on the others. This was taken into account through logratio analysis of the data (Aitchison 1986) where the compositions were transformed to the logratios $y_1=\ln(x_1/x_3)$ and $y_2=\ln(x_2/x_3)$. In addition, in order to carry out statistical analyses, a new factor (Part) was created, which assumed the value 1 if y_1 was concerned and value 2 in the case of y_2.

The data on species composition were analyzed using a general linear mixed model. The appropriate covariance structure relating to the factor Part was tested with a likelihood ratio test (Littell et al. 2006). The resulting covariance structure was compound symmetry in the analysis of June and unstructured (see Littell et al. 2006) in the analysis of August. In the unstructured covariance structure, the variances and covariances of levels of a factor are allowed to vary freely, whereas in compound symmetry there is only one variance and covariance.

Results

Spore densities

The spore data for the soil collected in June and August were treated separately. Comparisons between fertilizer levels (full vs. half for CONV and uncomposted vs. composted for LOW-INPUT) were performed at the cropping system level separately for each rotation point.

Similar total numbers of AMF spores were extracted from soil samples collected in June (31.8 spores 100 cm^{-1} soil) and in August (33.8 spores 100 cm^{-1} soil). In June, small differences in estimated spore numbers were observed between cropping systems A–D. When comparing all four cropping systems, and not taking rotation point into account, the lowest estimated mean was found in CONV A, 27.0 spores (95% CI=9.0 to 45.0), and the highest in CONV B, 35.5 spores (95% CI=17.5 to 53.5). In August, more pronounced differences were observed with the smallest spore numbers found in CONV A, 24.3 spores (95% CI=3.8 to 44.8), and the highest in LOW-INPUT D, 44.2 spores (95% CI=23.7 to 64.7). However, the cropping system had no significant overall effect on spore numbers, either in June or in August. Similarly, the rotation point had no effect on spore numbers, either in June or in August. However, a statistically significant interaction (Pr>F=0.004) between cropping system and rotation point was observed in June. Slightly higher numbers of AMF spores were observed in rotation D1 as compared with C1 (P=0.083) and in CONV rotation A3 as compared with CONV rotation B3 (P=0.057) (Fig. 1).

AMF species composition in soil

Five *Glomus* spp., one *Acaulospora*, and one *Scutellospora* were identified at the species level (Fig. 2). In addition to these, three unidentified *Glomus* morphotypes were found (Fig. 2). Negligible differences in the relative abundance of AMF species occurred between CONV and LOW-INPUT. *G. claroideum* was the most commonly identified single species; it occurred in all cropping systems and their various rotation points, making up about 30% of the total number of identified spores. The sampling time had some

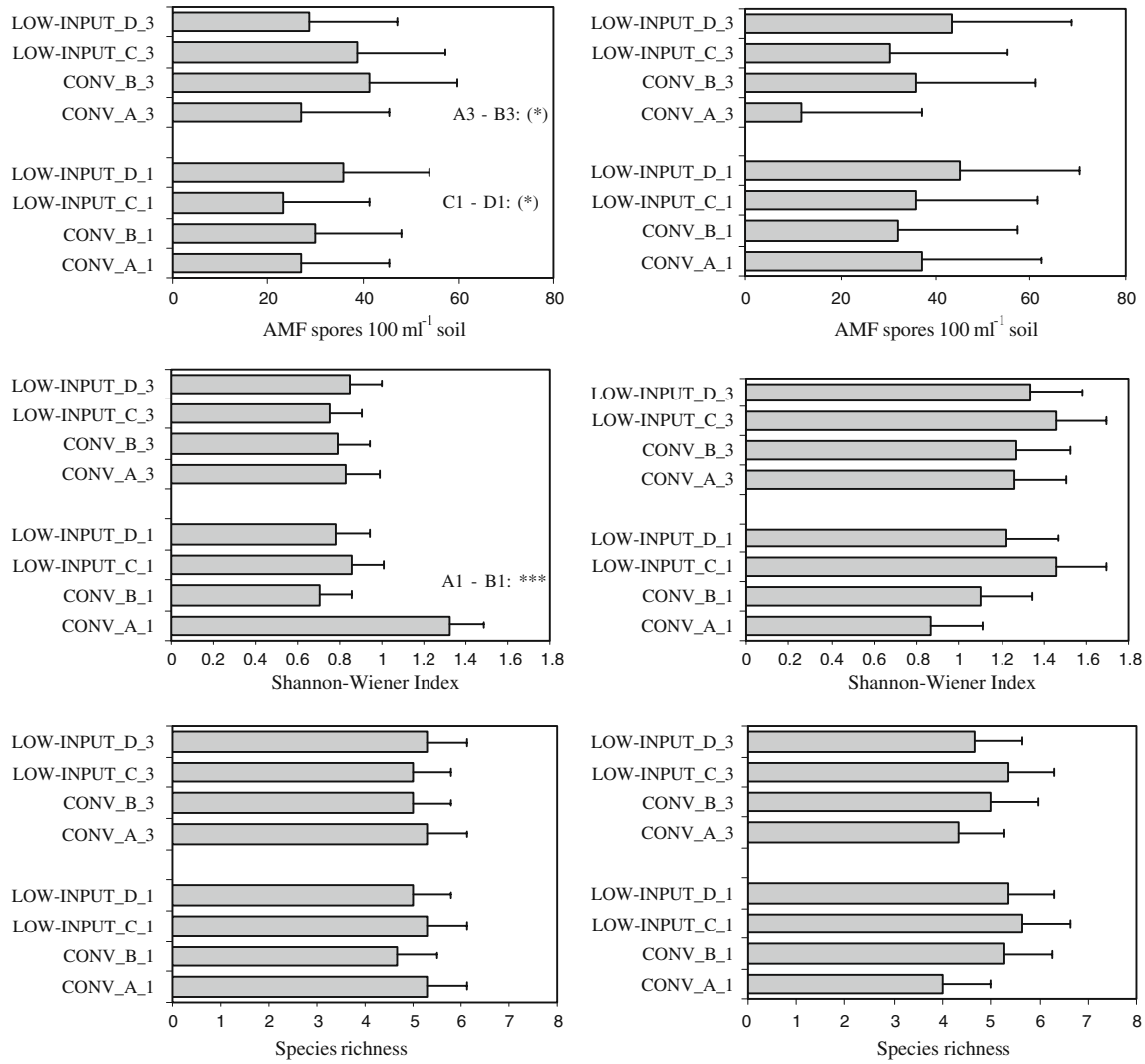

Fig. 1 Effect of cropping system (CONV, LOW-INPUT), fertilization/use of compost (A, B, C, D), and rotation point (1, 3) on estimated means of numbers of AMF spores, Shannon–Wiener Index and AMF species richness in soil collected in June and August, 1995, from the Ylistaro long-term cropping system experiment. $N=3$. Effects marked with (*), *, **, or *** are significant at $P \leq 0.1$, 0.05, 0.01, and 0.001, respectively. *Bars* indicate upper 95% confidence limits of the means. *CONV* conventional cropping system, *LOW-INPUT* low-input cropping system, *A* full mineral fertilization, *B* half mineral fertilization, *C* plant residues added without composting, *D* plant residues added after composting, *1*= rotation point 1, *3* rotation point 3

effect on the distribution of spores. In August in CONV B, *G. claroideum* made up as much as 45–55% of the total number of spores identified. *G. mosseae* occurred more commonly in June (26%) than in August (9%). In contrast to the occurrence of *G. mosseae*, a type of unidentified *Glomus*, here called 'small white', occurred less in June (10%) than in August (31%). A *Glomus* sp. called 'dark pigmented' was also identified frequently, but its occurrence varied little with sampling time, cropping system, or rotation point (Fig. 2). The same was true for *Acaulospora scrobiculata*.

The most commonly occurring *Glomus* species (*G. claroideum*, *G. mosseae*, *Glomus* sp. 'small white', and *Glomus* sp. 'dark pigmented') were selected for testing the statistical significance of differences in their relative occurrence. For this purpose, a new class level entitled "ratio" with two variables (V1 and V2), both ratios to *G. claroideum*, was created: V1=log (proportion of *G. mosseae*/proportion of *G. claroideum*), V2=log (proportion of 'small white' + 'dark pigmented'/proportion of *G. claroideum*). In June, no factor influenced the variables V1 and V2. In August, however, there was a significant ratio × fertilization interaction effect (Pr>F=0.045). Ratio V2 was significantly higher in fertilization A (rotation) than in fertilization B ($P=0.037$). Ratio V1 was significantly smaller than V2 at all fertilization levels (A, B, C, D).

Species richness was not affected by the cropping system, rotation point, or their interactions. In June, the

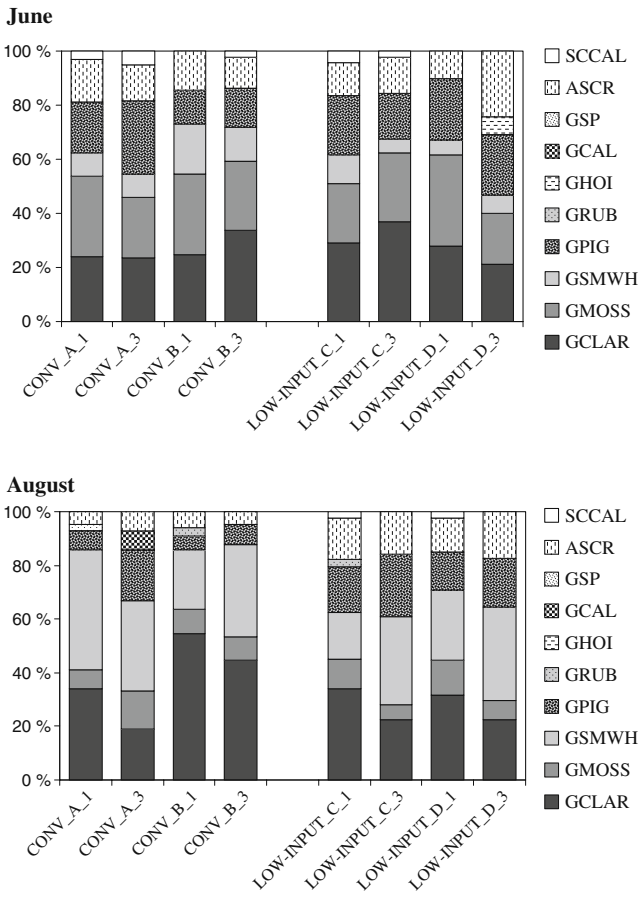

Fig. 2 Effect of cropping system (CONV, LOW-INPUT), fertilization/use of compost (A, B, C, D), and rotation point (1, 3) on relative AMF spore abundance in soil collected in June and August, 1995, from the Ylistaro cropping system experiment. *N*=3. *CONV* conventional cropping system, *LOW-INPUT* low-input cropping system, *A* full mineral fertilization, *B* half mineral fertilization, *C* plant residues added without composting, *D* plant residues added after composting, *1* rotation point 1, *3* rotation point 3, *GCLAR Glomus claroideum*, *GMOSS G. mosseae*, *GSMWH Glomus* sp. 'small white', *GPIG Glomus* sp. 'dark pigmented', *GRUB G. rubiforme*, *GHOI G. hoi*, *GCAL G. caledonium*, *GSP Glomus* sp., *ASCR Acaulospora scrobiculata*, *SCCAL Scutellospora calospora*

numbers of species found in CONV varied between 4.7 and 5.3 and in LOW-INPUT between 5.0 and 5.3. The corresponding values for August were 4–5.3 and 4.7–5.7, respectively. As a consequence of the low species richness, the Shannon–Wiener Index was also low, varying in June between 0.7 and 1.3 in CONV and between 0.8 and 0.9 in LOW-INPUT (Fig. 1). The corresponding values for August were 0.9–1.3 and 1.2–1.4, respectively. In June, both cropping system (Pr>F=0.069) and rotation point (Pr>F=0.050) had a nearly significant impact on SWI. A significant interaction between the cropping system and the rotation point also occurred (Pr>F=0.004). In rotation point 1, with barley as the standing crop, SWI was significantly higher in fully fertilized CONV A plots than in half-fertilized CONV B ($P<0.0001$) and also higher than the values in LOW-INPUT plots C ($P=0.0004$) and D ($P=0.0001$). No such effects occurred in rotation point 3 with oat + pea as standing crops.

AMF species composition in trap cultures

The AMF species *G. claroideum*, *G. mosseae*, *Glomus caledonium*, *G. hoi*, *S. calospora*, and *Glomus* sp. 'small white', detected in direct counting, were also sporadically detected in trap cultures irrespective of the cropping system, fertilization regime, rotation point, or time of collection of the soil sample (results not shown). An *Acaulospora* sp., with very tiny spores that was not detected in the direct extraction, was observed in LOW-INPUT C in the autumn samples. On the other hand, the *Glomus* sp. 'dark pigmented' and *Ac. scrobiculata*, frequently found by direct counting, were not observed in the trap cultures (Table 2).

Dominating AMF

Preliminary study

The roots of seven trap plant species used in the preliminary testing for the dominating AMF assay were well colonized after 5 weeks with the highest mean levels in flax (73.3%) and the lowest in subclover (46.7%). However, no significant differences between plant species were found. The success of transferring colonized root pieces to *P. lanceolata* was dependent on the host. After 9 weeks in *P. lanceolata*, little or no colonization was observed when the root inocula originated from white clover (0%) and alfalfa (5%). *Plantago* roots inoculated with root pieces from the other five trap plants were colonized well: leek (50%), subclover (53%), barley (57%), flax (67%), and red clover (75%). AMF sporulation occurred only in pots inoculated with root pieces originating from flax, barley, and red clover. The spores all belonged to *G. claroideum*. The largest numbers of spores were observed in the pots with root inocula from barley. After 20 weeks, sporulation of *G. claroideum* was detected also in pots inoculated with root pieces of subclover. Based on these results, barley and flax were chosen for studying the soil of the Ylistaro experiment.

Cropping system experiment at Ylistaro

Both barley and flax were well colonized in the bioassay with soil from the long-term field experiment (Fig. 3). However, statistical differences occurred only in the June samples trapped with barley where the mean colonization levels were significantly ($P=0.0001$) lower in cropping

Table 2 AMF species detected by different methods in the long-term cropping system experiment at Ylistaro, Finland

AMF species	Direct spore extraction				Trapping				Dominating AMF assay			
	A	B	C	D	A	B	C	D	A	B	C	D
Acaulospora scrobiculata	x	x	x	x								
Acaulospora sp.							x					
Glomus caledonium	x					x		x				
Glomus claroideum	x	x	x	x	x	x	x	x	x	x	x	x
Glomus hoi				x			x					
Glomus mosseae	x	x	x	x	x	x	x	x				
Glomus rubiforme		x	x									
Glomus sp., 'dark pigmented'	x	x	x	x								
Glomus sp. 'small white'	x	x	x	x	x	x	x	x				
Glomus spp.						x	x	x				
Scutellospora calospora	x		x	x		x						

A and *B* refer to a conventional cropping system given full or half fertilization, respectively. *C* and *D* refer to a low-input cropping system where plant residues were added to the plots without or after composting, respectively

system CONV (25.0%) than in LOW-INPUT (36.6%) in rotation point 1 but not in rotation point 3. Nutrient regime also affected colonization levels significantly ($P<0.001$) with the highest levels occurring in LOW-INPUT treatment C (41.7%) and the lowest in CONV treatment A (25.0%). Weakly significant interactions between rotation point and cropping system (Pr>F=0.01) and between rotation point and management system/nutrient regime (Pr>F=0.013) also occurred. In rotation point 1, higher levels of colonization were observed in LOW-INPUT C than in LOW-INPUT D ($P<0.0001$) and CONV A ($P<0.0001$). In rotation point 3, higher colonization was observed in CONV B than in CONV A ($P=0.031$) and also higher in LOW-INPUT C than in LOW-INPUT D ($P=0.031$) (Fig. 3).

In June, barley trapped AMF better in LOW-INPUT than in CON, but with flax the reverse was true (Table 3). The AM culturing was 100% successful or close to 100% in samples collected in autumn both for barley and flax. All the successful cultures were *G. claroideum*. These cultures remained species-pure with no AMF other than *G. claroideum* being observed 10 months after initiation.

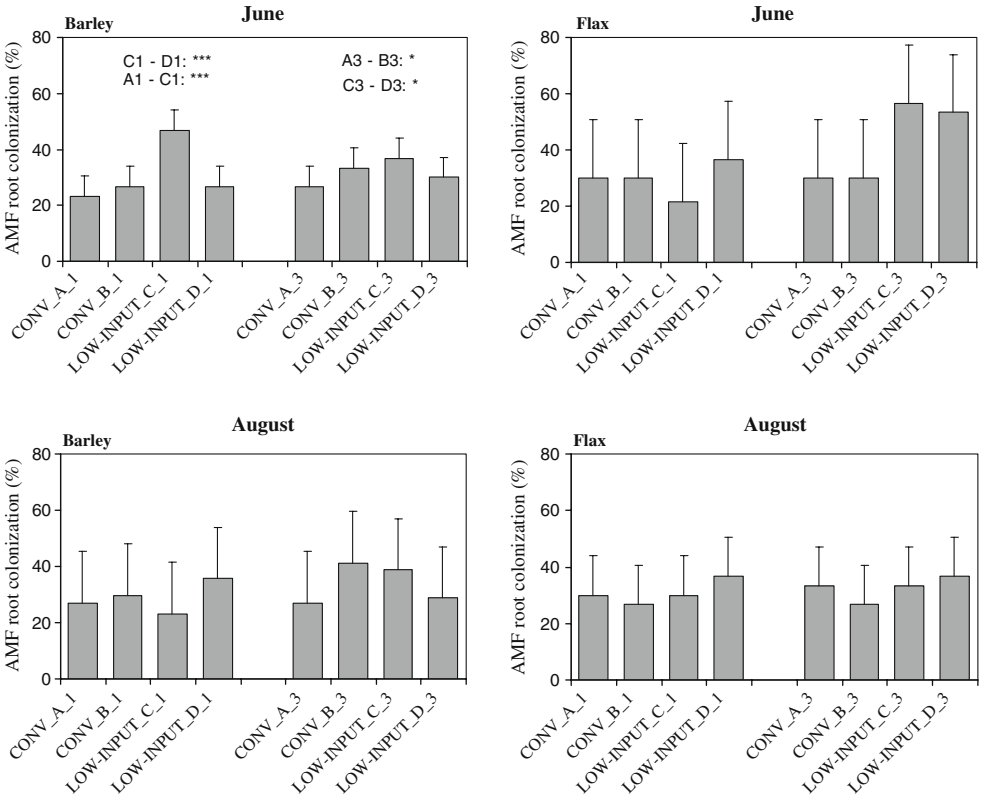

Fig. 3 Effect of cropping system (CONV, LOW-INPUT), fertilization/use of compost (A, B, C, D), and rotation point (1, 3) on AMF root colonization in barley and flax in soil collected in June and August, 1995, from the Ylistaro cropping system experiment. Pot experiment, $N=3$. Effects marked with (*), *, **, or *** are significant at $P\leq0.1$, 0.05, 0.01, and 0.001, respectively. *Bars* indicate upper 95% confidence limits of the means. *CONV* conventional cropping system, *LOW-INPUT* low-input cropping system, *A* full mineral fertilization, *B* half mineral fertilization, *C* plant residues added without composting, *D* plant residues added after composting, *1* rotation point 1, *3* rotation point 3

Table 3 Effect of cropping system (CONV vs. LOW-INPUT), fertilization/use of compost (full or half fertilization in CONV and residues used uncomposted or composted in LOW-INPUT), and collection time of soil sample on the number of positive AM culture attempts in a dominating AM fungus assay

Cropping system	Positive AM cultures out of 6 attempts			
	Barley as trap plant		Flax as trap plant	
	June	August	June	August
CONV_A	3	6	4	5
CONV_B	4	6	2	6
LOW-INPUT_C	6	6	1	6
LOW-INPUT_D	5	6	1	6

Pot experiment. N=6. *CONV_A* and *CONV_B* refer to a conventional cropping system with full or half fertilization, respectively. *LOW-INPUT_C* and *LOW-INPUT_D* refer to a low-input cropping system where plant residues were added to the plots without or after composting, respectively

Discussion

The cropping system did not significantly affect AMF spore densities, although the low-input cropping system showed higher mean spore numbers than the conventional cropping system. This result is similar to the studies of Franke-Snyder et al. (2001) who did not find any differences in AMF sporulation, species composition, or AMF biodiversity indices between conventional and low-input farming systems that had been going on for 15 years. Franke-Snyder et al. (2001) study was performed on soil with high P and in both farming systems the soil was tilled annually. In contrast to our study, AMF spore numbers have been higher in low-input or organic systems than in conventional systems in the majority of studies (for example Douds et al. 1995; Galvez et al. 2001; Oehl et al. 2004).

The spore density of the experimental area was low, varying from 27 to 44 spores per 100 cm^3 soil. This is less than one AMF spore per gram of soil which is a very low number compared with studies on arable land in other temperate regions, for example Sweden (4–44 spores g^{-1} soil; Sjöberg et al. 2004), Switzerland (about 10 spores g^{-1} soil; Oehl et al. 2004), and North America (about 270 spores g^{-1} soil; Kurle and Pfleger 1996). Relatively low AMF spore densities have, however, also been recorded in arable soil (Hendrix et al. 1995; Douds et al. 1997). In our study, the low AMF spore densities may be due to a combination of high tillage frequency and the low mycotrophy of standing crops in the study year. In the long-term experiment, the conventional plots had been tilled yearly, but also the low-input rotations C and D had included only one no-till year out of six. This no-till year was 5 years before the study in rotation point 1 and 4 years before the study in rotation point 3. Frequent soil disturbance has been shown to decrease the density of AMF spores and species richness in soil compared with undisturbed soil (Boddington and Dodd 2000). Jansa et al. (2002) recorded AMF species belonging to only two genera in conventionally tilled soil, while five AMF genera were present in no-till soil.

The low AMF spore density might also be a result of standing crops of low mycotrophy. Both cropping systems had a cereal as standing crop in the study year; barley in both systems of rotation point 1, oat in the conventional system of rotation point 3, and oat + pea in the low-input system of rotation point 3. Cereals like oat and wheat are reported to have little dependence on mycorrhiza compared with, for example, maize, legumes, and potato (Plenchette et al. 1983). Since cereals have low field mycorrhizal dependence, it is also expected that they will not increase AMF sporulation in soil. This was in fact found in another study of ours (Vestberg et al. 2005) in which the AMF spore numbers in the soil remained low after 3-year cultivation of rye, while the more mycotrophic crops strawberry and caraway strongly increased the spore numbers in the soil. Host dependence on the sporulation rate of AMF fungi has also been verified in pot experiments (Hetrick and Bloom 1986; Bever et al. 1996).

The conventional and low-input cropping systems did not differ much from each other regarding the abundance of spores of certain AMF species. Instead, the species *G. claroideum* was largely spread over the whole study area, occurring relatively evenly in all cropping systems and their various rotation points, making up about 30% of the total number of identified spores. The frequency of this species was pronounced in August when it represented about 50% of the number of identified spores. In contrast to *G. claroideum*, another commonly found AMF species of agricultural soil, *G. mosseae*, occurred more commonly in samples collected in June than in August. These results are in conflict with most other studies in which the cropping systems have clearly affected the relative densities of spores of AMF species in the soil. Douds et al. (1995) found that soil in low-input agriculture had greater populations of *Glomus occultum* type spores and other *Glomus* spp., whereas the conventionally farmed soil had greater populations of *G. etunicatum* type spores. In a Swiss study, Oehl et al. (2004) found that soil in conventional treatments harbored significantly higher proportions of *G. caledonium* and *G. diaphanum*, while the spores of *G. mosseae* tended to be more abundant in the organic treatments. Franke-Snyder et al. (2001) found that the sporulation of particular fungal species differed among farming systems and/or among hosts, but the general structure of AM fungal communities was similar for all treatments.

Not only the total numbers of spores but also the species richness was low in the experimental area. In total, seven identified and three unidentified spore types were found. Species richness was not affected by cropping system, rotation point, or their interactions. Relatively low AM species richness in temperate arable land has been observed also in several other studies (Land and Schönbeck 1991; Johnson 1993; Daniell et al. 2001; Sjöberg et al. 2004). However, in some studies carried out in agricultural soils, the species diversity has been clearly higher (Ellis et al. 1992; Bever et al. 1996; Oehl et al. 2004), even exceeding 20 AMF species per site and being comparable with the numbers of species often found in natural ecosystems (Douds and Millner 1999). At one single site in a long-term field experiment in Switzerland, Oehl et al. (2004) recorded as many as 35 AMF species. Latitude is a factor that may have an impact on AMF species richness. Latitude also, however, strongly correlates with temperature and plant species. Koske (1987) found that AM fungal species richness in barrier dunes from northern New Jersey to Virginia correlated positively with distance south along a transect and with temperature parameters. Few comparative studies have, however, been conducted on agroecosystems, although the general rule is that AM fungal species richness decreases from the tropics towards temperate and arctic regions.

The method of investigation is another factor that certainly affects the results of AM fungal diversity in field studies. We used only morphological methods. These methods are essential when the main purpose of a study is to isolate and culture AMF fungal strains from a certain soil. However, like any other single method, the morphological methods do not give a full picture of the prevailing AM fungal diversity in soil. Recent molecular studies have shown that several unknown and uncultured AMF types are abundant in field soil (van der Heijden and Scheublin 2007). Helgason et al. (2002) reported that sequences of about 60% of AMF types found in the roots of woodland plants had no cultured counterpart. On the other hand, Clapp et al. (2002) reported that some spore types detected in soil are never found in roots. Thus, morphological and genetic diversity studies should be combined in order to get as full a picture as possible of the AMF diversity in a certain situation.

The bioassay used for isolation and culture of dominating AMF yielded only *G. claroideum*, despite the relatively abundant occurrence also of *G. mosseae*, two unidentified *Glomus* sp., and *Ac. scrobiculata* in the experimental area. An explanation for this result may be found in the very different root colonization speeds of different AMF species. This phenomenon was clearly shown by Hart and Reader (2002) who studied 21 AMF isolates from the five genera in three Glomeromycotan families. They found that the Glomaceae (*Glomus* and *Acaulospora*) had the fastest root colonization. In fact, *G. claroideum* together with *G. constrictum*, *Glomus aggregatum*, and *G. intraradices* started to colonize roots 1 week after inoculation. The initiation of root colonization by *G. mosseae* took 1 week longer and some species of the Gigasporaceae family started to colonize roots even as late as 4 to 7 weeks after inoculation. From this point of view, it is not surprising that the bioassay yielded cultures of only *G. claroideum*. Thus, the bioassay was biased against fungi in the Gigasporaceae. The cultures of *G. claroideum* remained species-pure and no other AMF had sporulated in these cultures 10 months after initiation. The success of the assay opens up possibilities for a large-scale search for effective fast colonizing generalist *Glomus* AMF with potential for use as inoculants in agriculture and horticulture. Such dominating AMF seem to compete successfully against other indigenous AMF species and would therefore ensure a predictable impact of AMF inoculation in field conditions. However, the method may also have some biases—choice of the most appropriate plant species, time of transfer of root fragments and tolerance of the method to disturbance, etc. The impact of these factors needs to be optimized in further studies. In addition, the efficacy of these rapid colonizers needs to be tested in various crops before they are used in inoculum production strategies.

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