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Abstract Previous work has shown considerably enhanced soil fertility in agroecosystems managed by organic farming as compared to conventional farming. Arbuscular mycorrhizal fungi (AMF) play a crucial role in nutrient acquisition and soil fertility. The objective of this study was to investigate the diversity of AMF in the context of a long-term study in which replicated field plots, at a single site in Central Europe, had been cultivated for 22 years according to two "organic" and two "conventional" farming systems. In the 23rd year, the field plots, carrying an 18-month-old grass-clover stand, were examined in two ways with respect to AMF diversity. Firstly, AMF spores were isolated and morphologically identified from soil samples. The study revealed that the AMF spore abundance and species diversity was significantly higher in the organic than in the conventional systems. Furthermore, the AMF community differed in the conventional and organic systems: *Glomus* species were similarly abundant in all systems but spores of *Acaulospora* and *Scutellospora* species were more abundant in the organic systems. Secondly, the soils were used to establish AMF-trap cultures using a consortium of *Plantago lanceolata*, *Trifolium pratense* and *Lolium perenne* as host plants. The AMF spore community developing in the trap cultures differed: after 12 months, two species of the Acaulosporaceae (*A. paulinae* and *A. longula*) were consistently found to account for a large part of the spore community in the trap cultures from the organic systems but were found rarely in the ones from the conventional systems. The findings show that some AMF species present in natural ecosystems are maintained under organic farming but severely depressed under conventional farming, indicating a potentially severe loss of ecosystem function under conventional farming.

Keywords Sustainable agriculture · Soil microbiology · Biodiversity · Symbiosis · Plant nutrition

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Introduction

Arbuscular mycorrhizal fungi (AMF) are believed to support plant growth by increasing the supply of immobile soil nutrients, notably P, enhancing tolerance or resistance to soil pathogens and abiotic stresses, and by improving the soil structure (Smith and Read 1997). These beneficial effects of AMF are important in natural ecosystems, although they may be unimportant in high-input agriculture (Barea and Jeffries 1995; Galvez et al. 2001). Clearly, agricultural management factors such as the intensity of cultivation, the quality and quantity of fertilizers applied and the plant protection strategies used may have severe impacts on the AMF community structure (Sieverding 1989; Douds and Millner 1999; Oehl et al. 2003a).

In recent years, low-input agricultural systems have gained increasing importance in many industrialized countries for the conservation of natural resources and reduction of environmental degradation (Mäder et al. 2002). Conventional farming systems with reduced input of fertilizers and pesticides have been developed, sometimes called "integrated systems", but more and more

farmers have also changed to low-input, organic farming systems where fertilization is mainly or exclusively based on animal manure produced on-farm, in addition to the nutrient input received by legume-based crop rotations. This management practice often leads to negative nutrient balances and a reduced nutrient availability, especially with respect to P (Oehl et al. 2002). It is under these conditions that plants are expected to be particularly dependent on an effective AMF symbiosis (Smith and Read 1997; Scullion et al. 1998).

There have been some recent reports comparing the AMF community structures within farming systems differing in management practices such as tillage, fertilizer input, pesticide use and crop rotation (reviewed by Douds and Millner 1999). In some cases only small differences were detected (Land and Schönbeck 1991; Kurle and Pfleger 1996; Franke-Snyder et al. 2001) but when the differences found were more striking, the AMF community was generally found to be impoverished in species composition in more intensively managed agricultural land use systems (Sieverding 1989; Douds et al. 1993; Johnson and Pfleger 1992; An et al. 1993; Galvez et al. 2001; Jansa et al. 2002; Oehl et al. 2003a). The present study takes advantage of a field trial established in Switzerland which has been running for >22 years, designed to compare long-term effects of "conventional" versus "organic" farming systems (Mäder et al. 2002). In this trial, crop yields were found to be reduced by only about 20% in the organic as compared to the conventional systems although the input of fertilizer and energy was reduced as much as 34–53% and the pesticide input by 97%. It was suggested that enhanced soil fertility associated with a higher microbial activity and biodiversity in the organic fields might have rendered these farming systems less dependent on an input of external resources. Previously we reported that in this field trial about 40% more roots were colonized by AMF in the organic systems than in the conventional ones (Mäder et al. 2000). However, it is well known that different AMF species differ in functional characteristics such as spore production and plant growth promotion (Bever et al. 1996; Van der Heijden et al. 1998). Moreover, less efficient AMF species might be selected by high-input farming (Johnson 1993; Scullion et al. 1998). It is important, therefore, to assess the impact of farming systems also on AMF species diversity and community structure.

All methods designed to study the diversity of AMF in field soils have constraints. Only a few Glomalean genera can readily be distinguished by observing the morphology of mycorrhizal root colonization (Merrywheather and Fitter 1998). Furthermore, mycorrhizal root colonization, as well as spore numbers (Clapp et al. 1995) do not necessarily reflect the AMF populations in the soils. One reason for this is that freshly formed spores can often not be readily distinguished from spores formed earlier in the season (Lee and Koske 1994). An advantage is that spore numbers are indicators over a longer time period, i.e. over months. AMF community structures can be readily described based on spore morphology (Douds and Millner, 1999; Oehl et al. 2003a) with the exception of putative non-sporulating AMF species. Recently developed molecular methods for the detection of AMF species directly in the plant roots (Helgason et al. 1998; Redecker 2000; Schüssler et al. 2001) are therefore promising and in the future they will be combined with the morphological tools available. However, the molecular tools are costly and time-consuming, and AMF populations cannot as yet be quantified by these techniques. It is always uncertain whether there are other AMF that remain undetected because their percentage of intraradical structures are low, or if there are other reasons for these techniques still partly to fail. In this study, we assessed the AMF community structure based on the classic spore morphology characteristics in order to highlight both qualitative and

Table 1 Fertilization and plant protection strategies of the farming systems investigated in the long-term bio-dynamic, bio-organic, and conventional (DOC) field trial

	Mineral-conventional	Conventional	Bio-organic	Bio-dynamic	Control
	(MIN)	(CON)	(ORG)	(DYN)	(NON)
Type of manure/fertilizer	Mineral fertilizers only[a]	Anaerobically rotted farmyard manure and slurry[b], plus mineral fertilizers	Slightly aerobically rotted farmyard manure and slurry[b]	Aerobically composted farmyard manure and slurry[b] amended with bio-dynamic preparations	Non-fertilised
Nutrient input (kg ha^{-1}year^{-1})[c]	124 N 41.2 P 254 K	154 N 39.0 P 258 K	88 N 25.1 P 139 K	91 N 22.0 P 159 K	0 N 0 P 0 K
Plant protection	Synthetic pesticides respecting thresholds; according to the guidelines of Swiss Integrated Production since 1985		No synthetic pesticides; following the Swiss guidelines of bio-organic farming	No synthetic pesticides; applying mineral and plant preparations according to bio-dynamic farming practices	

[a]Onefold Swiss Standard recommendation since 1991; for details of the previous crop rotation periods see Oehl et al. (2002)

[b]Rate 1.4 livestock units ha^{-1} year^{-1} since 1991; for details of previous periods see Oehl et al. (2002)

[c]Mean nutrient input since 1985

quantitative differences in organic and conventional farming systems of the long-term field trial mentioned above.

Materials and methods

The long-term bio-dynamic, bio-organic, and conventional trial: a comparison of farming systems

The bio-dynamic, bio-organic, and conventional (DOC) field experiment to compare organic and conventional farming systems was set up in 1978, with four field plot replicates per treatment, on a loess soil at Therwil near Basel (Switzerland). As described in detail in a recent publication (Mäder et al. 2002), two organic farming systems [bio-dynamic (DYN); bio-organic (ORG)] and two conventional systems [using mineral fertilizers plus farmyard manure (CON); using mineral fertilizers exclusively (MIN)] were established in a replicated field plot experiment. The 7-year crop rotation (potatoes, winter wheat 2×, beetroots, some intercrops and a grass-clover meadow lasting for 2.5 years), crop varieties and tillage were identical in all systems. In addition to the four distinct fertilization treatments, a non-fertilized control (NON) was included in the experiment. Table 1 provides an overview of the five treatments. Soil physical (Mäder et al. 2002), chemical (Oberson et al. 1993; Mäder et al. 2002; Oehl et al. 2002), microbiological (Fliessbach and Mäder 2000; Fliessbach et al. 2000; Oehl et al. 2001) and weed and crop yield parameters (Mäder et al. 2002) have been investigated extensively. At the end of three crop rotations (in 1998 after 21 years), nine weed species belonging to several plant families (Poaceae, Rubiaceae, Papaveraceae, Hydrophyllaceae, Polygonaceae, Asteraceae and Scrophulariaceae) were observed in the organically managed wheat plots, whereas only four species, three species of Poaceae plus scarcely *Papaver rhoeas*, occurred in the conventionally managed plots (D. Dubois and B. Streit, unpublished data).

Soil sampling, chemical soil analysis and AMF inocula preparation

Soil samples were taken from each of four replicate plots per treatment (plot sizes of 5×20 m) in March 2000, i.e. at the beginning of the vegetation period. All the plots sampled carried an 18-months-old grass-clover stand, as determined by the crop rotation scheme. From each of the four plots per treatment, six soil cores were taken up to a depth of 10 cm using a soil corer of 8 cm diameter. As an inoculum for the trap cultures (see below), undisturbed soil crumbs were taken from these soil cores (20 g per trap plant, representing as uniformly as possible all six soil cores at 5–7 cm soil depth). The rest of each soil core was carefully ground by hand, mixed and air-dried. Each composite sample, representing one plot, was a mixture of six such soil cores. It was kept at 4°C until AMF spores were isolated by wet-sieving and analysed as described below.

Trap cultures

For each of the four replicate plots of each treatment, two trap cultures were set up as previously described in detail (Oehl et al. 2003a). Briefly, an autoclaved substrate was used consisting of a mixture of Terragreen (American aluminium oxide, oil dry US special, type III R, <0.125 mm; Lobbe Umwelttechnik, Iserlohn, Germany) and loess from a local site (3:1, w/w). The AMF inocula (see above; 20 g per trap plant, nine plants per trap culture) were positioned in a 27×17×20-cm (length×width×height) box beneath the subsequently planted AMF-free trap plants consisting of *Lolium perenne*, *Trifolium pratense* and *Plantago lanceolata*, three of each species per trap culture (Oehl et al. 2003a). There were two reasons for choosing these trap plant species: firstly, all three species are well-known AMF host plants frequently used for trap cultures and secondly, at sampling time they were the dominant plant species in the grass-clover stand representing three important functional groups (a grass, a legume, a forb). Eight non-mycorrhizal controls were also included, as previously described (Oehl et al. 2003a). Finally, an automated watering system controlled by soil moisture (Tropf-Blumat; Weninger, Telfs, Austria) was installed. The trap cultures were set up in March 2000 and kept in the greenhouse under ambient natural light and temperature conditions for 20 months. During this time, the trap plants were cut back seven times 3 cm above the ground.

Sampling in the trap cultures

During two vegetation periods in the greenhouse (2000 and 2001), every 2 months (i.e. after 2, 4, 6, 8 months and again after 14, 16, 18 and 20 months) two soil-cores were taken at the same locations of each box for AMF spore identification. The core volume was 15 cm^3 and the sampling depth 10 cm. The sampling holes in the boxes were immediately re-filled each time with the sterile substrate used in the experiment. As a measure of the infection potential of the field samples, the initial mycorrhizal root colonization was determined in the trap cultures after 2 months using trypan blue as a stain for the AMF structures (Brundrett et al. 1994).

AMF spore isolation and identification

AMF spores were extracted from the field samples and from the trap cultures by wet sieving and sucrose density gradient centrifugation (Oehl et al. 2003a). Spores were counted using a dissecting microscope at up to 90-fold magnification. The AMF spore abundance (number of spores per gram of soil) refers to the spore count before the selection process used for identification of AMF species, which was performed as follows: healthy-looking spores (about 50–70% of the total number) were picked under the dissecting microscope and mounted in polyvinyl-lactic acid-glycerine (PVLG) (Koske and Tessier 1983) or PVLG mixed 1:1 (v/v) with Melzer's reagent (Brundrett et al. 1994). These spores were then examined using a compound microscope at up to 400-fold magnification. About 70% of the spores fixed on slides could be identified at the species level, while the rest consisted of dead and decaying spores, lacking clear morphological features. The identification was based on current species descriptions and identification manuals (Schenck and Perez 1990; Morton http://invam.caf.wvu.edu/Myc_Info/Taxonomy/species.htm). In this study, a "species" may be a clearly identified morphospecies based on spore morphology, a not yet described morphospecies, or an already described morphospecies that is not yet known to the authors. A "species group" comprises more than one morphospecies due to the fact that the features were not clear enough to clearly attribute the majority of the spores to one of the morphospecies in the group.

The Shannon-Weaver (H') index was calculated as an additional measure of AMF species diversity, combining two components of diversity, species richness and evenness. Since diversity measures may change with changes in the number of individuals in a sample, a correction of H' was performed as described (Fager 1972). For the same reason, the number of species, i.e. the species richness E, was corrected using the rarefaction method (Legendre and Legendre 1988). (Note: to present the species richness and the H', the AMF species grouped into one species group had been counted as one single species.)

In the trap cultures, dominant AMF spores were estimated in a chronosequence by identifying at least 100 mounted spores that were randomly selected from samples taken at intervals over a period of 20 months. An AMF species was considered as "dominant" with respect to sporulation at a given sampling date

when it comprised at least 20% of all spores identified in at least one of the four replicate plots per treatment.

Statistics

Significance of differences between treatments with respect to spore abundance, species numbers and AMF diversity (Shannon-Weaver index) was tested using Fisher's least significant difference at $P < 0.05$ after one-way ANOVA. In the trap cultures inoculated with soil from the different treatments, the same statistical tests were applied for initial mycorrhizal root colonization. A hierarchical cluster analysis using Ward's minimum variance method (Legendre and Legendre 1988) was applied to determine the similarity (more precisely: dissimilarity) with respect to AMF species composition between treatments, based on the χ^2 distance (Legendre and Legendre 1988).

Results

AMF species found in the DOC field trial

In the soil samples taken from the plots representing the different farming systems applied in the long-term DOC field trial, 19 AMF species (*Glomales* species) could be distinguished (Table 2). Seventeen spore types could be unequivocally assigned to known species of the Glomales, namely ten species of the genus *Glomus* Tulasne & Tulasne, five of the genus *Acaulospora* Gerdemann & Trappe and two of the genus *Scutellospora* Walker & Sanders. Two spore types could not be readily distinguished at the species level and comprised one species group. One comprised *Glomus fasciculatum* and *Glomus clarum,* and the other *Glomus albidum* and *Paraglomus occultum* (*P. occultum*) Morton & Redecker. Spores belonging to species from the genera *Entrophospora* Ames & Schneider, *Archaeospora* Morton & Redecker and *Gigaspora* Gerdemann & Trappe (emend. Walker & Sanders) were not found in the present study.

Abundance and diversity of AMF spores in soils from the different DOC plots

As shown in Table 2, all of the 19 AMF species found in the present study were present in the samples from ORG, and 18 and 17 AMF species, respectively, in NON and DYN. Only 15 species were observed in the samples of the conventionally managed plots (CON and MIN). Regarding relative spore abundance, most of the *Glomus* species showed no significant differences between the conventional and organic treatments. However, spores of *G. caledonium* and *G. diaphanum* accounted for a significantly higher proportion of the spores identified in the conventional treatments. Spores of *G. mosseae* tended to be more abundant in the organic treatments (Table 2). Spores of the genus *Scutellospora* (*S. pellucida* and *S. calospora)* accounted for a relatively large proportion of the spores identified in MIN whereas in CON the lowest numbers of these spores were found. Spores of *Acaulospora* spp. were generally more abundant in the organic treatments ORG and DYN as well as in NON. Interestingly, spores of *A. laevis, A. longula* and *Acaulospora* sp. BR1 were never found in the conventional treatments. Only the abundance of *A. thomii* spores seemed not to be influenced by the farming systems.

The number of AMF spores per gram soil (Table 3A) and the number of AMF species found per plot (Table 3B, C) was significantly higher in the organic and non-

Table 2 Relative spore abundance (%)(mean of four field plot replicates) from each of the arbuscular mycorrhizal fungi (AMF) species distinguished in the soil samples from the different treatments in the long-term DOC field trial. Data *within a row* followed by the *same letter* are not statistically different ($P>0.05$). *LSD* Fisher's least significant difference at the 5% level after one-way ANOVA; for other abbreviations, see Table 1

Glomales species	MIN	CON	ORG	DYN	NON	LSD
Glomus diaphanum	13.9 a	22.4 b	6.2 a	7.8 a	7.24 a	9.2
Glomus caledonium	4.6 b	4.9 b	1.7 a	0.5 a	0.8 a	2.6
Glomus dominikii	0.9 a	0.3 a	0.7 a	4.2 b	1.6 ab	3.2
Glomus etunicatum	3.3 a	4.4 a	10.3 a	12.1 a	5.8 a	7.5
Glomus fasciculatum group[a] (two species)	2.1 a	4.1 a	2.6 a	5.7 a	2.0 a	3.6
Glomus mosseae	3.0 ab	3.7 abc	5.9 c	5.4 bc	1.1 a	2.8
Glomus sp. BR9	4.7 a	7.8 a	8.8 a	7.0 a	3.2 a	6.8
Glomus geosporum	5.6 a	6.8 a	4.9 a	6.6 a	3.9 a	6.1
Glomus albidum group[b] (two species)	14.3 a	8.0 a	6.2 a	6.4 a	10.0 a	12.2
Glomus constrictum	3.3 a	8.6 a	7.4 a	8.4 a	2.7 a	9.9
Glomus invermaium	5.7 a	15.8 a	12.9 a	6.4 a	12.2 a	8.7
Glomus aureum	–a	–a	0.4 b	–a	–a	0.3
Scutellospora calospora	16.9 b	3.1 a	6.3 a	8.7 ab	8.9 ab	10.4
Scutellospora pellucida	11.6 b	5.2 a	3.9 a	3.4 a	10.0 ab	6.3
Acaulospora paulinae	8.9 a	4.4 a	13.2 b	10.7 b	20.8 c	5.8
Acaulospora thomii	2.3 a	1.7 a	2.8 a	2.6 a	3.1 a	2.7
Acaulospora laevis	–a	–a	5.2 c	2.2 ab	3.2 bc	2.5
Acaulospora longula	–a	–a	0.9 ab	2.1 bc	2.7 c	1.6
Acaulospora sp. BR1[c]	–a	–a	0.4 a	–a	0.6 a	1.0
Total number of spores identified	226	325	776	420	610	–

[a]Comprises *G. fasciculatum* and *G.clarum*
[b]Comprises *G. albidum* and *Paraglomus occultum*
[c]Resembling *Acaulospora scrobiculata*

fertilized treatments (ORG, DYN and NON) as compared to the conventional ones (CON and MIN). When the total number of AMF species contained in the four replicate plots for each treatment were considered (Table 4D), the species numbers decreased in the order: ORG>NON> DYN>CON=MIN. The AMF species diversity as expressed by the *H ′* decreased in the order: DYN=ORG≥ NON≥CON=MIN (Table 3E, F).

A hierarchical cluster analysis showed the highest similarity in AMF species composition between the two organic treatments (ORG and DYN) on the one hand and between the two conventional treatments (CON and MIN) on the other hand (Fig. 1). The NON, where the plant protection strategy was as in the DYN treatment, resembled more the organic than the conventional treatments.

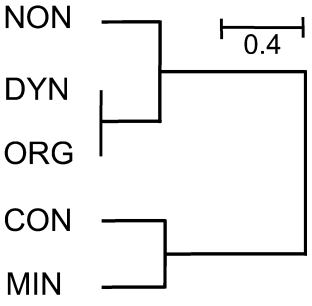

Fig. 1 Hierarchical cluster analysis of arbuscular mycorrhizal fungi species composition in the investigated treatments of the long-term bio-dynamic, bio-organic, and conventional (DOC) field trial based on the χ^2 distance, using Ward's minimum variance method (Legendre and Legendre 1998). The corresponding units (dimensionless) were calculated on the basis of spore numbers per gram soil of each species identified per treatment. *Scale bar* =0.4 units. *NON* Non-fertilized control, *DYN* bio-dynamic, *ORG* bio-organic, *CON* mineral fertilizers plus farmyard manure, *MIN* mineral fertilizers exclusively

AMF trap cultures: inoculum potential of soils from the different DOC plots

The inoculum potential of soil samples from the different treatments in the DOC field trial was determined in AMF trap cultures by measuring the proportion of root length of the trap plants (*Lolium*, *Plantago* , *Trifolium*) colonized by AMF after 2 months of culture. It was found to be highest in the samples from DYN and decreased in the same order as the spore abundances observed: DYN>NON≥ORG≥ CON≥MIN (Fig. 2).

AMF trap cultures: species composition

The AMF species composition was monitored in the trap cultures at bimonthly intervals. As shown in Table 4, there

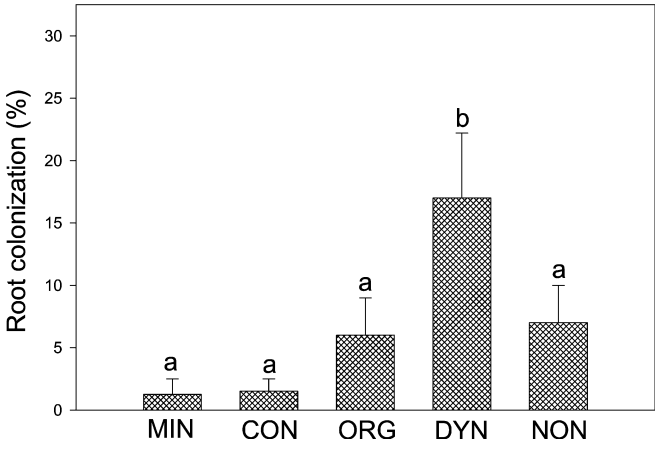

Fig. 2 Mycorrhizal root colonization after 2 months of trap culturing. The trap cultures had been inoculated with soil derived from the plots of the different treatments of the long-term DOC field trial. Average and SD of four field plot replicates per site are shown. Columns marked by the *same letter* are not significantly different (P >0.05)

Table 3 AMF spore abundance and species richness found in the field samples of the different treatments of the long-term DOC field trial. The AMF diversity is expressed by the Shannon-Weaver diversity index (H') calculated from the no. of spores identified for each species. Data *within a row* followed by the *same letter* are not statistically different (P>0.05). For abbreviations, see Table 1

	MIN	CON	ORG	DYN	NON	LSD[b]
No. of AMF spores per gram soil (average of four field plot replicates)	7.9 a	10.0 a	12.5 b	14.0 b	13.5 b	2.34
No. of AMF species[a] found (average of four field plot replicates)	12.5 a	13.0 a	16.5 b	15.5 b	15.8 b	1.68
No. of AMF species[a] (average of four field plot replicates) corrected for unequal numbers of spores identified at different plots (see Table 3) using the rarefaction method (Legendre and Legendre 1998)	12.3 a	12.9 a	16.3 b	15.4 b	15.5 b	1.66
Total No. of AMF species[a] found at the field sites (sum of four field plot replicates)	15	15	19	17	18	-
H'	2.16 a	2.26 ab	2.51 c	2.51 c	2.36 bc	0.16
Corrected H': corrected for unequal numbers of spores identified at different plots (see Table 2) according to Fager (1972)	0.23 a	0.43 ab	0.79 c	0.81 c	0.56 bc	0.27

[a]Species groups counted as only one species (see Material and methods and Table 2)

[b]LSD at the 5% level after one-way ANOVA

Table 4 Time (months) after trap culture initiation at which spores of different AMF species were detected for the first time. The inocula for the trap cultures were derived from the different treatments of the long-term DOC field trial. For abbreviations, see Tables 1 and 2

Glomales species	Months after setting up trap cultures				
	MIN	CON	ORG	DYN	NON
AMF species that started sporulation during the first vegetation period in the greenhouse (0–8 months of trap culturing)					
G. mosseae	2	2	2	2	2
G. caledonium	2	2	4	4	2
G. geosporum	2	4	2	2	2
G. etunicatum	4	4	2	2	2
G. diaphanum	16	6	6	14	2
G. lamellosum	4	4	6	4	6
G. albidum	14	4	4	4	6
Archaeospora trappei	4	8	6	2	8
G. aggregatum	8	8	4	8	8
G. fasciculatum	8	8	8	8	6
Entrophospora infrequens			8	8	6
Glomus sp. BR11[a]	8	16	8	8	8
S. calospora		20	8	8	6
G. versiforme	20	20	6	6	
Glomus sp. BR12	8	16	16		
G. aureum	16	8	14	6	8
G. sp. BR9			6	6	16
G. constrictum	20	18	6	8	20
A. paulinae	14	14	6	8	8
A. thomii	8	14	8	14	14
A. longula	14	14	8	8	8
G. microcarpum	16	20	8	20	
AMF species that started sporulation during the second vegetation period in the greenhouse (11–20 months of trap culturing)					
A. laevis	14	14	14	14	14
G. invermaium	16	16	16	14	16
Acaulospora sp. BR13			16	14	
G. monosporum				14	
G. clarum			20		16
S. pellucida	20	20	20		
G. intraradices	20	14	16		16
Glomus sp. BR14[b]				18	
Glomus sp. BR4			20		20
Glomus sp. BR15			14		

[a]Resembling *Glomus arborense*

[b]Resembling *Glomus nanolumen*

was a gradual appearance of spores belonging to the different AMF species. Spores of *G. mosseae*, *G. caledonium*, *G. geosporum* and *G. etunicatum* appeared within the first 4 months after setting up the trap cultures, independently of the soil used for inoculation. A number of species formed spores only later: In fact, ten of them started to sporulate only during the second vegetation period, >12 months after setting up the trap cultures. After 20 months in the greenhouse, all the trap cultures combined yielded 32 AMF species (Table 4). Remarkably, this is 52% more than in all the field samples combined. From the *G. fasciculatum* group (Table 2), three different species could be identified: *G. fasciculatum*, *G. clarum* and *G. lamellosum* (spores of the latter were not recognized in the field samples). Spores of *Archaeospora trappei* and *Glomus* sp. BR11 had not been detected in the field samples where they might have been erroneously attributed to the *G. albidum* group. Both species are small-spored (40–80 μm) with fragile spore walls and hence difficult to identify directly from the field. Spores of some species that formed singly or in loose clusters in the soil or roots could not be related to any species found in the field samples: e.g. *Entrophospora infrequens*, *G. aggregatum* and *G. intraradices*. Although no sporocarp-forming AMF were observed in the field samples, such species appeared in the trap cultures at the end of the study (after 18–

20 months; Table 4), namely *G. microcarpum*, *Glomus* sp. BR4, *Glomus* sp. BR12 and *Glomus* sp. BR15. Only three of the species found in the field samples were never observed in the trap cultures during the whole period of the study (*G. dominikii*, *P. occultum* and *Acaulospora* sp. BR1). In one of the non-mycorrhizal control pots, *G. intraradices* was found at the end of the study, obviously as a contaminant.

During the first 4 months of trap culturing, the differences in the number of AMF species between the treatments were negligible (Table 5). However, at the end of the first vegetation period (after 6–8 months trap culturing), almost twice as many AMF species were found in the trap cultures based on soils from the organic farming systems (and from the non-fertilized control) than from conventional farming systems. Additional AMF species appearing in this period of time were: *S. calospora*, *Acaulospora paulinae*, *A. longula*, *E. infrequens*, *Glomus constrictum* and *G. versiforme* (Table 4). The number of species found in trap cultures based on soils from conventional farming systems increased considerably in the second vegetation period, but it remained smaller compared to the organic farming systems up till the end of the experiment (Table 5).

AMF trap cultures: dominant sporulating AMF species

The species that sporulated during the first 4 months (Tables 4, 5) remained dominant throughout the first vegetation period up to 8 months (Table 6). Although the trap cultures were established with the same host plants in the same Terragreen-loess substrate, different AMF community structures developed in trap cultures inoculated with soils from the different farming systems. *Glomus caledonium* was dominant in the trap cultures based on soils from the conventional treatments whilst *G. geosporum* and *G. mosseae* spores were more abundant in the trap cultures based on soils from the organic treatments (Table 6). Others, e.g. *G. etunicatum* and *G. lamellosum* sporulated indifferently with respect to the treatment.

Table 5 No. of AMF species that formed spores in the trap cultures based on soils from the different treatments of the long-term DOC field trial. AMF spores were isolated and identified from the trap cultures repeatedly over a period of 20 months. For abbreviations, see Table 1

Duration of trap culture	MIN	CON	ORG	DYN	NON
2 Months	3	2	3	4	5
4 Months	6	6	6	7	5
6 Months	6	7	13	10	10
8 Months	11	11	20	18	16
14 Months	14	16	23	24	18
16 Months	18	19	27	24	22
18 Months	18	20	27	26	22
20 Months	22	24	30	26	24

Spore domination changed dramatically in the second vegetation season of trap culturing in the greenhouse, 11–16 months after setting up the trap cultures. While *G. caledonium* remained the most dominant sporulating species in the cultures of the conventional treatments, the three most dominant sporulating species in the cultures based on soils from the organic treatments were from the genus *Acaulospora* (*A. longula*, *A. paulinae* and *A. thomii*). From the Acaulosporaceae, only *A. thomii* also sporulated abundantly in the trap cultures based on soils from the conventional treatments (Table 6).

At the end of the second greenhouse season (months 16–20) differences between the treatments became less evident. A shift to a prevalence of *G. fasciculatum* and *G. invermaium* spores occurred in all five treatments. The main difference between the treatments during that period was the dominance of *G. constrictum* spores in the cultures based on soils from the organic treatments, while *Glomus* sp. BR12 spores prevailed—as already in springtime—in the trap cultures based on soils from the conventional MIN treatment (Table 6).

Discussion

Taking into account all the AMF species identified directly in the field samples and in the trap cultures, a total of 35 AMF species were detected at the single field site of the long-term field trial under study. This is considerably higher than the 8–20 species that are usually reported from arable lands (Land and Schönbeck 1991; Douds and Millner 1999; Fitter 2001; Jansa et al. 2002) and is closer to the numbers reported for grasslands (Douds and Millner 1999; Bever et al. 2001; Fitter 2001; Oehl et al. 2003a). The 7-year crop rotation applied to all plots, including 3 years of grass-clover and the cultivation of sunflower/vetch mixtures as intercrops in order to bridge longer vegetation-free periods in autumn, is highly desirable from an ecological point of view and may have contributed to this high AMF species richness.

The fact that such a high number of AMF species has been detected is certainly also due to the design of our trap cultures in the greenhouse. The cultures were kept for almost 2 years, and a consortium of three different trap plant species rather than only a single one was used, factors that are known to potentially influence AMF propagation and sporulation (Bever et al. 1996, 2001). The extension of trap culturing for a second vegetation period, from 8 to 20 months, resulted in an increase in AMF species recovery of >50%. In an arid ecosystem, Stutz and Morton (1996) found only 25% of the AMF species after the first of a total of three trap culture cycles (cycles of four months each).

It became clear that certain AMF species could not be readily identified in the field samples; the morphological features of the spores were not distinct enough and, thus, these spores could only be grouped into a species group (e.g. *G. fasciculatum* group; Table 2). Moreover, the sporocarpic AMF species *G. microcarpum*, *Glomus* sp.

Table 6 Dominant[a] sporulating AMF species found in the trap cultures during two vegetation periods (0–8 and 11–20 months). The inocula for the trap cultures were derived from the different treatments of the long-term DOC field trial. *G.cal G. caledonium*, *G. mos G. mosseae*, *G.etu G. etunicatum*, *G.geo G. geosporum*, *G.lam G. lamellosum*, *G.s12 Glomus* sp. BR12, *G.fas G. fasciculatum*, *G. inv G. invermaium*, *G.con G. constrictum*, *G.alb G. albidum*, *G.aur G. aureum*, *Ar.tra Archaeospora trappei*, *A.pau Acaulospora paulinae*, *A.lon Acaulospora longula*, *A.tho Acaulospora thomii*; for other abbreviations, see Tables 1and 2

Age of the trap cultures (months)	MIN	CON	ORG	DYN	NON	Age of the trap cultures (months)	MIN	CON	ORG	DYN	NON
First vegetation period (0–8 months)						Second vegetation period (11–20 months)					
0–2	*Gcal*[c]	*G.cal*	*G.etu*	*G.geo*	*G.geo*	11–14	*G.cal*	*G.cal*	*A.pau*	*A.lon*	*A.lon*
		G.mos	*G.mos*	*G.mos*	*G.mos*		*G.lam*	*G.mos*	*A.lon*	*A.pau*	*A.pau*
					G.etu		*G.s12*	*A.tho*	*A.tho*	*A.tho*	*A.tho*
							Ar tra		*G.con*	*G.mos*	*Ar.tra*
							A.tho		*G.etu*		
2–4	*G.cal*	*G.cal*	*G.etu*	*G.geo*	*G.etu*	14–16	*G.fas*	*G.inv*	*A.pau*	*A.lon*	*A.lon*
	G.etu	*G.mos*	*G.geo*	*G.etu*	*G.mos*		*G.cal*	*G.mos*	*A.lon*	*A.pau*	*Ar tra*
		G.etu	*G.cal*	*G.mos*	*G.cal*		*G.s12*	*G.lam*	*A.tho*	*G.geo*	*G.inv*
							Ar.tra	*A.tho*	*Ar tra*	*G.con*	*G.fas*
							A.tho		*G.inv*		
									G.lam		
4–6	*G.cal*	*G.cal*	*G.etu*	*G.mos*	*G.etu*	16–18	*G.fas*	*G.inv*	*G.con*	*G.fas*	*G.fas*
	G.etu	*G.mos*	*G.mos*	*G.geo*	*G.lam*		*G.inv*	*G.fas*	*G.inv*	*G.inv*	*G.etu*
			G.geo	*G.etu*			*G.s*12	*G.alb*	*G.fas*	*G.con*	*G.aur*
				G.lam			*G.cal*		*A.lon*		
									A.pau		
6–8	*G.cal*	*G.cal*	*G.etu*	*G.geo*	*G.lam*	18–20	*G.fas*	*G.inv*	*G.con*	*G.con*	*G.fas*
	G.etu	*G.mos*	*G.lam*	*G.mos*	*G.etu*		*G.s*12	*G.fas*	*G.inv*	*G.inv*	*G.aur*
	G.lam	*G.etu*	*G.alb*	*G.fas*	*G.cal*		*G.inv*	*G.con*	*G.fas*	*G.mic*	*G.inv*
		G.lam	*Ar tra*	*G.lam*	*G.mos*						
		G.fas		*G.alb*							

[a]AMF species comprising >20% of all spores identified in at least one of the four field plot replicates per treatment; names are given in the order of diminishing relative frequency

BR4, *Glomus* sp. BR12 and *Glomus* sp. BR15 were not detected in the field samples, but appeared upon trap culturing.

The results of 20 months of trap culturing under natural light and ambient temperature conditions allow some conclusions to be drawn concerning the time required for different AMF species to initiate sporulation and/or the seasonality and succession patterns of AMF sporulation. Species most often described from arable lands (*Glomus caledonium*, *G. mosseae*, *G. etunicatum*, *G. geosporum*, *G. albidum* (Land et al. 1993; Kurle and Pfleger 1996; Jansa et al. 2002) were the first species to sporulate. A second ecological group of *Glomus* began to sporulate in low numbers at the end of the first vegetation period after 8 months culturing in the greenhouse, namely *G. constrictum*, *G. fasciculatum* and *Glomus* sp. BR9. However, the main sporulation of these species (*G. constrictum* and *G. fasciculatum*) as well as of others like *G. invermaium* and *G. aureum* (Oehl et al. 2003b) occurred only during the second half of the second vegetation period (14–20 months of culturing; Table 6). Together with *Glomus* sp. BR12 this species group succeeded in sporulation species like *G. caledonium* and *G. etunicatum* in the two conventional treatments (CON and MIN). The *Acaulospora* species mainly sporulated at the beginning of the second vegetation period after having passed the winter in the greenhouse. This suggests that these species might need either a winter before they sporulate or just a longer time period to reach sporulation than most of the *Glomus* species present. *Scutellospora* species sporulated late in the season, i.e. only between October and December in both years of trap culturing. This is in accordance with Lee and Koske (1994) who found—in a sand dune ecosystem—an increased late season abundance of spores of *Gigaspora gigantea*, another member of this family (Gigasporaceae). Studies on AMF community dynamics are still extremely rare (Hart et al. 2001).

The species richness in the field trial was slightly, but significantly lower in the conventional than in the organic treatments despite the fact that the same crop rotation had been applied. This result was confirmed in the trap cultures. It is generally assumed that AMF are more affected by the quantity and quality of fertilizers applied than by plant protection strategies and, thus, our finding suggests that the AMF diversity might have decreased in the conventionally managed plots (MIN and CON) because of the conspicuously higher input of mineral

fertilizer that is instantly available (mineral fertilizers versus farmyard manure). Previously, the organic treatments (ORG and DYN) have been found to induce an enhanced mycorrhizal root colonization in the field and in pot experiments (Mäder et al. 2000); here it is shown that they also exhibit a higher AMF spore abundance in the field (Table 3) and an enhanced infection potential as measured in the trap cultures (Fig. 2).

The most striking effect of the different farming systems concerned the spore abundance of AMF species not belonging to the genus *Glomus*. Many of these species appeared to prefer or even to be restricted to the plots managed by organic farming. This was the case for most species of *Acaulospora* identified from the field as well as for *Acaulospora* sp. BR13 and *Entrophospora infrequens* that were found only in the trap cultures of ORG and DYN and NON. A previous study indicated a trend towards an increase in AMF belonging to the genera *Acaulospora*, *Scutellospora* and *Entrophospora* under long-term reduced tillage managements (Jansa et al. 2002). Our study complements this finding, indicating that not only reduced tillage but also organic farming may increase the diversity of AMF species and genera in arable lands. Our findings indicate that some AMF species, especially *Acaulospora* spp., find an ecological niche in the soils of organic farming systems, and that this may be connected with the characteristically low level of readily available P in these soils (Oehl et al. 2002). We hypothesize that these AMF species are functionally important for natural ecosystems and low-input sustainable farming; if this is true, their loss under conventional farming would be alarming.

The finding of higher AMF diversity (Table 3) and plant infection potential (Fig. 2) in the organic as compared to the conventional treatments fits well with previous observations of an increased overall microbial diversity and biomass (Fliessbach and Mäder 2000), a higher faunal (earthworms and carabides) (Pfiffner and Niggli 1996; Pfiffner and Mäder 1997) and floral (weeds) diversity (Mäder et al. 2002), higher soil respiration rates and enzyme activities (Oberson et al. 1996; Fliessbach and Mäder 2000), an increased amount and turnover of microbially bound P (Oehl et al. 2001) that accompanies a higher potential for organic P mineralization (Oehl 1999) and finally a higher soil aggregate stability (Mäder et al. 2002).

In European organic farming, fertilization is mainly or exclusively based on animal manure produced on-farm, and it is common practice to apply nutrients in the form of manure at a rate of 1.2–2.0 lifestock units ha^{-1} $year^{-1}$. This practice usually leads to a negative nutrient input/output balance and, in the long-term, could cause nutrient deficiencies, especially with respect to P (Oehl et al. 2002) and K (Mäder et al. 2002). This is often accepted because of an enhanced weathering and concomitant nutrient release from minerals due to an increase in soil biological activity induced by organic farming. It has been reported also that AMF consortia isolated from organic farms are more effective in plant growth promotion under conditions of low nutrient availability than consortia from conventional farms (Scullion et al. 1998). Moreover, fertilizing low-nutrient soils has been found to select AMF strains that are inferior mutualists (Johnson 1993). It will be a challenging task, therefore, to now test the AMF species isolated from the different farming treatments of the long-term field trial with respect to their efficacy in crop growth promotion under conditions of low nutrient availability. If it turns out that indeed less benign AMF species are selected under intensive agriculture this would have to be taken into account in the widespread current attempts to convert high-input, intensively managed agroecosystems into ecologically less harmful and more sustainable agroecosystems that still provide satisfactory crop yields.

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