

Impact of conservation tillage and organic farming on the diversity of arbuscular mycorrhizal fungi



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ABSTRACT

Communities of arbuscular mycorrhizal fungi (AMF) are strongly affected by land use intensity and soil type. The impact of tillage practices on AMF communities is still poorly understood, especially in organic farming systems. Our objective was to investigate the impact of soil cultivation on AMF communities in organically managed clay soils of a long-term field experiment located in the Sissle valley (Frick, Switzerland) where two different tillage (reduced and conventional mouldboard plough tillage) and two different types of fertilization (farmyard manure & slurry, or slurry only) have been applied since 2002. In addition, a permanent grassland and two conventionally managed croplands situated in the neighborhood of the experiment were analyzed as controls. Four different soil depths were studied including top-soils (0–10 and 10–20 cm) of different cultivation regimes and undisturbed sub-soils (20–30 and 30–40 cm). The fungi were directly isolated from field soil samples, and additionally spores were periodically collected from long-term trap culture (microcosm) systems. In total, >50,000 AMF spores were identified on the species level, and 53 AMF species were found, with 38 species in the permanent grassland, 33 each in the two reduced till organic farming systems, 28–33 in the regularly ploughed organic farming systems, and 28–33 in the non-organic conventional farming systems. AMF spore density and species richness increased in the top-soils under reduced tillage as compared to the ploughed plots. In 10–20 cm also the Shannon–Weaver AMF diversity index was higher under reduced tillage than in the ploughed plots. Our study demonstrates that AMF communities in clay soils were affected by land use type, farming system, tillage as well as fertilization strategy and varying with soil depth. Several AMF indicator species especially for different land use types and tillage strategies were identified from the large data set.

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1. Introduction

Due to the growing demand for productive but sustainable agriculture, different farming systems have been developed. These include various ecologically sound management practices such as reduction or abandonment of soil tillage (so-called conservation or

no-tillage systems, respectively), reduction or abandonment of synthetic crop protection products and/or of easily available mineral fertilizers (so-called integrated production and organic farming systems, respectively), among several other options (Wezel et al., 2014).

To cultivate soils, mouldboard ploughing is traditional. However, in the last decades there was an effort to develop alternative strategies, such as different types of reduced tillage or no-tillage practices, both also denoted as conservation tillage practices. With such systems several advantages come along, above all in ecological as well as in economic aspects like higher biological

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activity, improved soil fertility, reduced soil erosion, less need of energy and labor (Peigne et al., 2007; Soane et al., 2012; Kuntz et al., 2013). On the other hand, the pressure of weeds and soil-borne pathogens are often enhanced in reduced tillage systems, and this might threaten the quantity or the quality of the harvests. This is especially a challenge in organic farming, because no synthetic pesticides, e.g. no synthetic herbicides, insecticides and fungicides, can be applied (Triplett and Dick, 2008; Carr et al., 2011).

Arbuscular mycorrhizal fungi (AMF) are obligate symbionts and associated with the majority of plant species. The fungi have several beneficial effects on their host plants, such as support of nutrient uptake, enhanced resistance against drought or root pathogens (Smith and Read, 2008; van der Heijden et al., 2015). In addition AMF can improve soil structure, soil aggregation and water infiltration and thus, can contribute to the prevention of soil erosion (e.g. Rillig and Mummey, 2006). Furthermore, it was shown that AMF diversity plays an important role for higher plant diversity and for the productivity of plant communities (van der Heijden et al., 1998).

AMF are influenced by farming practices such as soil tillage and fertilization strategy (Jansa et al., 2003; Oehl et al., 2003, 2010; Kabir, 2005). Extensive land use and low-input systems have usually positive effects on AMF, and therefore plants may benefit more from AMF in such agricultural systems (Mader et al., 2000; Njeru et al., 2015). Several studies revealed that community structure and diversity of AMF in soils differ between tilled and reduced or no-tillage soils (e.g. Jansa et al., 2002; Yang et al., 2012; Kohl et al., 2014; Maurer et al., 2014; Wetzel et al., 2014). There was one study focusing on the intra-specific diversity of one AMF species, *Glomus intraradices* (Borstler et al., 2010), however, to our knowledge there has not been any study on AMF communities influenced by tillage intensity in organic farming systems.

AMF communities can be investigated by classical microscopic identification of spores extracted from the soil matrix or from inside the roots (e.g. Douds and Millner, 1999) and by modern molecular analyses in soils or in root systems (e.g. Verbruggen et al., 2012). Both methods have advantages and disadvantages (e.g. Oehl et al., 2004; Njeru et al., 2015), and, whenever possible, both methods should be combined, which has rarely been done in the past due to lack of time, knowledge and experience, respectively (Wetzel et al., 2014).

The objective of the present study was to investigate the influence of soil tillage and type of fertilization on AMF communities in an organically managed long term field experiment running since 2002 (Bernier et al., 2008). While approximately the same amount of nutrients was applied to all plots, there were two fertilization types per tillage strategy: one fertilization regime was mainly with farmyard manure complemented by slurry, while in the other regime only slurry has been applied. In order to compare the AMF communities established in the treatments of the experiment with those communities occurring in the same area in less and more intensively used agricultural soils, one extensively managed permanent grassland subjected to organic farming, and two intensively managed cultivated sites subjected to conventional farming in so-called Integrated Production systems (IP) were also included in our study.

In view of our laboratory research history and the large number of samples (seven treatments/sites and four soil depths per treatment/site, with four field soil replicates per treatment and soil depth) we focused on morphological spore identification and spore quantification. Based on earlier findings obtained in conventionally managed arable fields (e.g. Jansa et al., 2002; Wetzel et al., 2014), we hypothesized that also under organic farming intensive soil tillage, and intensive conventional farming will negatively affect the AMF communities and AMF diversity due to vulnerability of the

AMF mycelia networks by specific soil cultivation techniques. There is little knowledge about AMF spore populations in different soil depths, even from sites of different soil use and cultivation, or from organic farming systems (e.g. Oehl et al., 2005). We hypothesized that AMF diversity decreases with soil depth, and that this decrease varies among different farming and tillage systems. With more than 100,000 AMF spores isolated and more than 50,000 spores identified, the present study has been one of the most extensive AMF diversity studies based on spore morphology presented so far.

2. Materials and methods

2.1. Study sites

For this study, seven sites were selected, all situated in the Sissle valley between the neighbored municipalities Frick and Oeschgen (Canton Aargau, Switzerland) in close vicinity to each other (47°30'–31'N; 8°01'21'–25"E). According to IUSS Working Group WRB (2014, International Union of Soil Sciences), the soils (with about 45% clay content) are all Vertic Cambisols having developed on alluvial and colluvial Jurassic sediments. Mean annual temperature is about 9.0 °C and mean rainfall is about 1000 mm per year. Four sites, located at 47°30'42"N; 8°01'25"E, constituted four treatments of a long-term field experiment of the Research Institute of Organic Agriculture (FiBL), in which reduced tillage and conventional tillage systems under organic farming (RO and CO systems, respectively) have been compared since 2002 (e.g. Bernier et al., 2008; Borstler et al., 2010; Krauss et al., 2010; Sans et al., 2011; Gadermaier et al., 2012; Kuntz et al., 2013; Armengot et al., 2015 for further details). One other site was a permanent, organically managed grassland (GL) at the southern end of the field experiment (47°30'38"N; 8°01'25"E), while two additional cultivated sites were conventional farming systems managed according to the guidelines of Swiss proof of ecological performance and Swiss integrated production (IP). The latter two sites (IP1 and IP2) were located in the North of the field experiment (at 47°30'55"N; 8°01'25"E and 47°30'59"N; 8°01'20"E, respectively). All sites had about 400 m distance to the Sissle river.

The field experiment was designed as a split strip plot (Gadermaier et al., 2012), four times replicated with tillage and fertilization as factors. One fertilization regime was mainly with farmyard manure (M) complemented by slurry, while in the other regime only slurry (S) has been applied (Bernier et al., 2008, Table 1). In two of the treatments, reduced tillage (RO; Reduced tillage under Organic farming) was practiced (5–7 cm depth soil peeling by Skim plough or by overlapping wide chisel sweeps, or 15 cm depth soil loosening by narrow tines of a chisel, depending on the crop in the rotation) to incorporate the harvest residuals and to control weeds, while in two treatments of conventional tillage (CO; Conventional tillage under Organic farming) a mouldboard plough was used (tillage depth 15 cm). For details about the tillage practices in the past, see Gadermaier et al. (2012). The previous crops had been 2004 sunflower, 2005 spelt, 2006–07 grass-clover, 2008 maize, and at sampling time in 2009 winter wheat was grown (Table 1). Seedbed preparation was the same in the reduced and conventionally tilled treatments performed with a rototiller (5 cm depth). In IP1, the ploughing depth was 18–20 cm and a rotary harrow was used before seeding. For the IP2 field, a seeding combination with rotary harrow was used. Depending on the crop, either plough (16–18 cm) or rototiller (5 cm) was chosen to cultivate the soil, which represents another kind of reduced tillage practice by reducing the number of ploughings per crop rotation. Under wet soil conditions, rototiller was replaced with a rotary harrow. The principal agricultural practices, like land use type, farming system, fertilization type and level, crop rotation, standing crop at sampling

Table 1
Principal agricultural practices, standing crop at sampling date, and crop rotation for field sites.

Nr.	Land use type	Farming system	Type and level of fertilization	Site code	Land use intensity (scale)	Standing vegetation at sampling date (crop rotation)
1	Grassland organic	Extensive grassland (GL)	No	GL	0	Arrhenatheretum with randomly dispersed apple trees
2	Arable land	Reduced tillage (RO)	Organic; Manure compost (M), & slurry; 1.4 livestock units ha ⁻¹ y ⁻¹	RO-M	1	Long-term tillage field experiment (Bernier et al., 2008) with winter wheat
3	Arable land	Reduced tillage (RO)	Organic; Slurry (S); 1.4 livestock units ha ⁻¹ y ⁻¹	RO-S	1	(6 year: maize–winter wheat)
4	Arable land	Conventional tillage (CO)	Organic; Manure compost (M), & slurry; 1.4 livestock units ha ⁻¹ y ⁻¹	CO-M	2	(oat–clover intercrop)–sunflower–spelt–2.5 year of grass–clover)
5	Arable land	Conventional tillage (CO)	Organic; Slurry (S); 1.4 livestock units ha ⁻¹ y ⁻¹	CO-S	2	
6	Arable land	Conventional tillage (IP1)	Mineral: 90–120 kg N ha ⁻¹ y ⁻¹	IP1	4	Winter wheat (3 year: winter wheat–winter barley–maize)
7	Arable land	Semi-reduced tillage (IP2)	Mineral: 60 kg N ha ⁻¹ y ⁻¹	IP2	3	Winter wheat– (4 year: winter wheat–rye–pea–maize)
	Conventional farming		Organic: 24 m ³ poultry manure ha ⁻¹ y ⁻¹			

date and the geographic position of all seven study sites are given in Table 1.

2.2. Soil sampling, preparation analyses, and chemical soil analyses

Soil sampling at sites was performed in 2009 in four replicates per site and at four different soil depths (0–10 cm, 10–20 cm, 20–30 cm and 30–40 cm) totaling 112 samples (7 field sites × 4 replicates × 4 soil depths). Soil sampling was performed as described in Oehl et al. (2005). The replicate field plots in the experiment were 12 × 12 m², whereas the net plot sizes, corresponding to the harvest plots 8 × 8 m². One field sample was taken as a composite sample of six soil cores per replicate plot and depth (Oehl et al., 2005). One set of sub-samples was carefully ground by hand and air-dried for subsequent analyses of selected chemical soil parameters (pH, organic carbon and available P), and for the isolation of the AMF spores present. Another set of subsamples was kept at 4 °C for two days, until the samples were used as inocula for the propagation of the AMF communities in so-called AMF trap cultures in the greenhouse. The chemical soil parameters were measured in the laboratory of F.M. Balzer, Wetter-Am  nau, Germany (www.labor-balzer.de), according to standard methods (Oehl et al., 2005). Plant available phosphorus (P) was extracted with double lactate ('P-DL'), a method widely used in Central Europe (Neyroud and Lischer, 2003).

2.3. AMF trap cultures/microcosms

Trap cultures ('microcosms', as called in Oehl et al., 2009) were established for the propagation of the AMF communities for all plots of the field experiment, for the grassland and one of the two IP-sites (IP1). Only the upper layer (0–10 cm) of the top-soils and the lower layer (30–40 cm) of the sub-soils were used, to reduce labor and the number of pots in the greenhouse. The cultures were initiated as described in Oehl et al. (2011b) using four AMF host plants per pot: *Plantago lanceolata*, *Lolium perenne*, *Trifolium pratense* s.l. and *Hieracium pilosella*. In our cultures, 3500 mL pots were filled with 1500 g of an autoclaved substrate Terragreen (American aluminum oxide, Oil Dry US special, type III R; Lobbe Umwelt-technik Iserlohn, Germany) – Loess mixture 3:1; pH-H₂O 6.8; organic carbon 3.0 g kg⁻¹; available P (P-DL) 15.6 mg kg⁻¹; available K (Na-acetate) 350 mg kg⁻¹. As AM fungal inoculum, field soil samples (corresponding to 150 g dry weight) were placed at four equally distributed locations within the pot, at four edges of a 8 × 8 cm² square on the top of the substrate and covered with another 1000 g of autoclaved substrate. Above the inocula on the

substrate surface, about 5–7 seeds of each of the four trap plant species were sown. The growing seedlings were reduced to three per host plant and pot about 4 days after emergence. The cultures were maintained in the greenhouse of Agroscope in Z  rich-Reckenholz at natural light conditions for 20 months from April 2009 until December 2010 under controlled temperature conditions with approx. 20–30/15–22 °C (day/night) during summer and about 15–20/10–12 °C during winter, respectively. The plant consortium was cut 3 cm above the ground four times per season, usually 3–5 days before substrate sampling. An automated watering system (Tropf-Blumat, Weninger GmbH, A-6410 Telfs, Austria) maintaining the water holding capacity at about 80% throughout the experiment, prevented water stress and reduced the risk of cross contamination between the AMF communities established in the different pots by water splashes. Fertilization was not necessary, profiting from the nitrogen fixing rhizobia within the *T. pratense* roots and the well balanced nutrient composition of the substrate. Plant protection was performed with specific bio-control agents against harmful insects and mites when necessary, or with a sulfur-based fungicide against mildew in clover. The formation of spores in the trap cultures was checked at four periods, 4, 8, 16, and 20 months after trap culture establishment, as described in Oehl et al. (2011b).

2.4. Morphological AMF spore analyses

AMF spores were extracted from the soil samples by wet sieving and sucrose density gradient centrifugation (Sieverding, 1991). Spores, spore clusters and sporocarps were picked without pre-selection and mounted together on microscope slides using polyvinyl–lactic acid–glycerol (PLVG) or PLVG mixed 1:1 (v/v) with Melzer's reagent. The slides were examined systematically under a Leitz Laborlux S compound microscope at up to 400-fold magnification to identify all morphologically distinct AMF spore types present. Morphological AMF species identification was based on all existing species descriptions and two identification manuals (Schenck and P  rez, 1990; B  lszkowski, 2012). Classification was based on the Glomeromycota system of Oehl et al. (2011c) recently published by the International Mycological Association (IMA), with a few updates (e.g. Sieverding et al., 2014; B  lszkowski et al., 2015), periodically updated also at the homepage of the Swiss collection for arbuscular mycorrhizal fungi (SAF; <http://www.agroscope.ch/saf>). Relative spore abundance of the AMF species identified in the trap cultures per sampling date was estimated on the microscope slides for at least 100 randomly selected spores per replicate plot. A species was judged as 'rare' when it comprised <5%,

‘frequent’ when it comprised 5–15%, ‘abundant’ when it comprised 15–25%, and ‘dominant’ when it comprised >25% of all spores identified per sampling period.

2.5. Statistical analyses

For all seven sites studied, differences in soil pH, organic carbon, available phosphorus, AMF spore density, AMF species richness and AMF diversity (Shannon–Weaver), all parameters determined from the field soil samples, were statistically tested using one-, two- or three-way ANOVA, with cultivation and fertilization management as one composite or two separate factors and soil depth as the second or third main factor. ANOVA analyses were generally followed by Tukey’s HSD to test for significant differences among treatments or soil depths; Fisher’s Least Significant Difference (LSD) was also calculated. For testing the abundance of selected AMF species at all sites, the non-parametric Kruskal Wallis test was chosen with Bonferroni adjusted p-values. Additionally, for the four treatments of the field experiment, a separate split plot ANOVA was applied (with cultivation, fertilization and soil depth as factors) on AMF spore density, species richness, species diversity, and spore density of selected species. To ordinate AMF community profiles, i.e. species compositions, and environmental parameters, a canonical correspondence analysis (CCA; [Ter Braak, 1986](#)) was performed, applying the model formula AMF communities ~ environmental parameters. All these statistical analyses and the graphical visualizations were computed by using the R software (Ver. 3.1.0, [R Core Team, 2014](#)) packages multcomp ([Hothorn et al., 2008](#)), agricolae ([de Mendiburu, 2014](#)) and vegan ([Oksanen et al., 2013](#)).

3. Results

3.1. Chemical soil parameters in the field experiment and the three study sites around

Soil pH (H₂O) was similar in all treatments of the field experiment and in the adjacent permanent grassland (7.5–7.7 in the top-soils and 7.8–8.2 in the sub-soils; [Table 2](#)). It was about one unit lower in the top- and sub-soils of the conventionally managed IP-sites (6.5–6.8 and 6.7–7.2, respectively), which could at least partly be explained by the long-term use of acidifying mineral fertilizers at the IP-sites. As typical for young, weathering Central

European Cambisol soils, the soil pH generally increased with soil depth. The organic carbon (C_{org}) contents were highest in the top-soil of the grassland (31.8 g kg^{−1}), followed by the top-soil of reduced tillage system fertilized with farmyard manure and slurry (RO-M; 29.5 g kg^{−1}). In the top-soils, C_{org} values were lowest in the IP-sites (23.6–23.7 g kg^{−1}). As expected, C_{org} values were generally lower in the sub- than in the top-soil, however, in the sub-soils, C_{org} was slightly higher in IP-sites (18.5–22.1 g kg^{−1}) than in the field experiment (10.9–16.6 g kg^{−1}) and the grassland (12.5–18.5 g kg^{−1}; [Table 2](#)), which might be explained by the alluvial and colluvial origin of the soils. In the top-soil, available P was higher in the treatments of the organically managed field experiment (126.8–161.0 mg kg^{−1}) due to intensive pig farming before conversion to organic farming. The P values were intermediary in the IP-sites (50.8–56.6 mg kg^{−1}), and lowest in the permanent grassland (32.5 mg kg^{−1}). At all sites, the available P contents decreased towards the sub-soils, and in 30–40 cm they were highest in the IP-sites (42.3–46.8 mg kg^{−1}), intermediate in RO-S, CO-M and CO-S of the field experiment (32.0–41.1 mg kg^{−1}), and lowest in the grassland (17.3 mg kg^{−1}), followed by RO-M (23.4 mg kg^{−1}).

3.2. AMF spore densities in the field experiment and the three study sites around

In the top-soils (0–10 cm) of the RO-plots, AMF spore densities were higher (42.7–48.9 spores g^{−1}) than in the CO-plots (29.5–29.8) of the field experiment ([Fig. 1A](#); [Table 3](#)), while they were similar as those in the adjacent grassland (46.9; [Table 3](#)). In the conventionally managed IP-sites close to the experiment, spore densities were in the range of those of the CO-plots (29.7–32.2 spores g^{−1}). In the grassland and RO-plots, the densities decreased already within 10–20 cm (first uncultivated soil layer in RO-plots; 34.8–37.8 spores g^{−1}), and steadily with increasing soil depths, while in the ploughed CO-plots and at the IP-sites the densities generally did not or only slightly change from 0–10 cm to 10–20 cm (25.2–33.6 spores g^{−1}) which correspond to the ploughing depths ([Table 3](#); [Fig. 2](#)). In the lowest layer (30–40 cm), the densities were highest in the grassland (27.6 spores g^{−1}), followed by those of the RO-plots (19.3–20.1). Lowest spore densities were found in the CO-plots and at the IP-sites (16.5–17.2; [Table 3](#)).

Table 2
Soil pH, organic carbon and available phosphorus in different soil depths at study sites.

Soil depth (cm)	Grassland	Reduced tillage (organic)		Conventional tillage (organic)		Conventional farming (IP)		LSD
	GL	RO-M	RO-S	CO-M	CO-S	IP1	IP2	
Soil pH (H ₂ O)								
0–10	7.5 a B	7.6 a A	7.6 a B	7.6 a A	7.7 a A	6.6 b B	6.5 b B	0.30
10–20	8.1 a A	7.8 ab A	7.8 ab B	7.7 b A	7.7 b A	6.8 c AB	6.8 c A	0.27
20–30	8.2 a A	8.1 ab A	8.2 a A	7.8 b A	7.8 b A	7.2 c A	6.7 d A	0.23
30–40	8.1 a A	8.0 a A	8.2 a A	8.0 a A	7.9 a A	7.1 b A	6.8 b A	0.21
LSD	0.10	0.35	0.21	0.42	0.22	0.26	0.15	
Organic carbon (g kg ^{−1})								
0–10	31.8 a A	29.5 a A	24.5 a A	26.1 a A	25.5 a A	23.7 a A	23.6 a A	7.3
10–20	14.8 b B	23.2 a A	23.7 a A	24.4 a A	24.7 a A	22.4 a AB	23.2 a A	4.6
20–30	12.5 b B	12.5 b B	12.9 b B	14.8 ab B	16.6 ab B	19.6 a B	18.5 ab A	4.2
30–40	18.1 a B	11.3 b B	11.0 b B	10.9 b B	13.1 b B	22.1 a AB	20.0 a A	2.7
LSD	7.6	5.3	5.5	4.3	4.1	2.5	5.7	
Available P (mg kg ^{−1})								
0–10	32.5 b A	161.0 a A	149.3 a A	128.0 a A	126.8 a A	50.8 b A	56.6 b A	23.7
10–20	12.4 c B	120.9 a B	122.6 a B	131.4 a A	137.6 a A	54.1 b A	53.0 b A	19.4
20–30	17.7 b B	33.3 ab C	44.7 a C	55.9 a B	58.1 a B	38.2 ab B	52.4 a A	16.0
30–40	17.3 c B	23.4 bc C	41.1 ab C	32.0 abc B	35.6 ab C	42.3 a B	46.4 a A	11.4
LSD	9.1	21.1	19.2	31.3	21.3	8.3	11.6	

Non-significant differences between the sites (lower case) and between the soil depths (capital letters) are shown by identical letters and were determined with Tukey’s HSD at the 5% level after two one-way ANOVA analyses. Fisher’s LSD (Least Significant Difference) is also given.

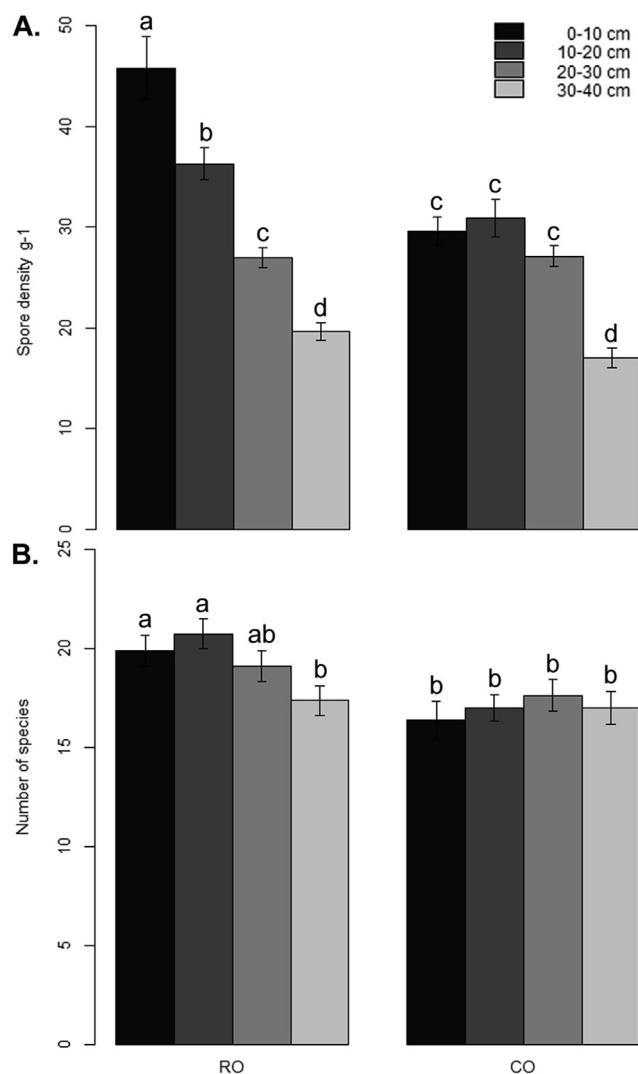


Fig. 1. AMF spore densities (g^{-1} soil) and species richness at different soil depths for reduced tilled (RO; summarized from RO-M and RO-S) and conventionally tilled (CO; summarized from CO-M and CO-S) plots under organic farming in the Frick field experiment. Average and standard deviations are shown. Non-significant differences between different soil depths are shown by identical letters, evidenced by Tukey's HSD test at the 5% level after a two-way ANOVA.

3.3. AMF species richness in the field soil samples of the study area (Sissle valley in Frick)

In total, 48 AMF species were detected at the seven sites and at the four different soil depths. A total of over >31,000 AMF spores

were identified (Table 4, S1). The majority of these species belonged to the Glomeraceae (25 species of *Glomus*, *Dominikia*, *Rhizoglomus*, *Sclerocystis* and *Septoglomus*). Five species each belonged to either Paraglomeraceae (*Paraglomus*) or Archaeosporaceae (*Archaeospora* and *Palaeospora*). Four species were Entrophosporaceae (*Claroideoglomus* and *Entrophospora*). Finally, three species each belonged to Ambisporaceae (*Ambispora*) or Acaulosporaceae (*Acaulospora*), two species to Diversisporaceae (*Diversispora*), and one species to Pacisporaceae (*Pacispora*) and Scutellosporaceae (*Scutellospora*).

3.4. AMF species richness in the field soil samples of the seven study sites

Over all soil depths, total AMF species richness was highest in the permanent grassland (GL) adjacent to the field experiment (38 AMF species, Table 4). In the RO-plots and in IP2, 32 species each were detected, while 31 species were found in CO-M, and lowest richness in CO-S and IP1 (28 and 27 species, respectively). Average AMF species richness, when summarized over all four soil layers analyzed, were also highest in GL (34 species). Average species richness was intermediary in the RO-plots and in IP2 (25–27), and lowest in the CO-plots and in IP1 (22–24 species).

3.5. AMF species richness in the top-soils and sub-soils of the seven study sites

For AMF species richness, a similar order was found in the top-soils (0–10 cm; Table 5): highest species richness in GL (24.3); higher richness in RO-plots (19.5–20.3) than in CO-plots (15.8–17.0) and IP1 (16.0–17.8) and IP2 (17.8–18.8). Remarkably, this order was repeated and even more pronounced in 10–20 cm, where GL (26.0) and the un-ploughed layer of the RO-plots (20.8–21.0) had substantially higher values than the ploughed 10–20 cm layers of the CO-plots (16.5–17.5) and the IP-sites (15.5–18.8). Within the soil profiles, AMF species richness rarely changed significantly (Table 5). However, when data for both RO and CO each were summarized, highest richness was revealed in RO-top-soils (0–10 and 10–20 cm), when compared to RO-subsoils (20–30 and 30–40 cm) and CO-top- and sub-soils (0–40 cm; Fig. 1B). In the lowest layer (30–40 cm), there was no significant difference between RO-plots (16.8–18.0) and CO-plots (17.0 species each; Table 5 and Fig. 1B). Also the IP-sites had similar numbers (16.0 and 19.8, respectively).

3.6. AMF diversity (Shannon–Weaver-index) in the field samples of the seven study sites

When summarized over all four soil depths, the Shannon–Weaver index was similar at all sites (Table 5). There were also no clear tendencies for differences in AMF diversity within the soil

Table 3
AMF spore densities (g^{-1} soil) in different soil depths at study sites.

Soil depth (cm)	Grassland	Reduced tillage (organic)		Conventional tillage (organic)		Conventional farming (IP)		LSD
	GL	RO-M	RO-S	CO-M	CO-S	IP1	IP2	
0–10	46.9 a A	42.7 a A	48.9 a A	29.8 b A	29.5 b AB	29.7 b A	32.2 b A	6.1
10–20	36.5 a B	37.8 a A	34.8 ab B	28.2 bc A	33.6 ab A	25.2 c B	31.5 abc A	4.3
20–30	28.2 a C	27.0 a B	27.0 a C	26.3 a A	27.9 a B	16.9 b C	24.9 a B	3.1
30–40	27.6 a C	19.3 b C	20.1 b C	17.2 b B	16.7 b C	16.5 b C	16.5 b C	3.0
LSD	5.3	5.4	5.3	3.7	3.9	3.2	4.5	

Non-significant differences in AMF spore densities between the sites (lower case) and between the soil depths (capital letters) are shown by identical letters and were determined with Tukey's HSD at the 5% level after two one-way ANOVA analyses. Fisher's LSD (Least Significant Difference) is also given.

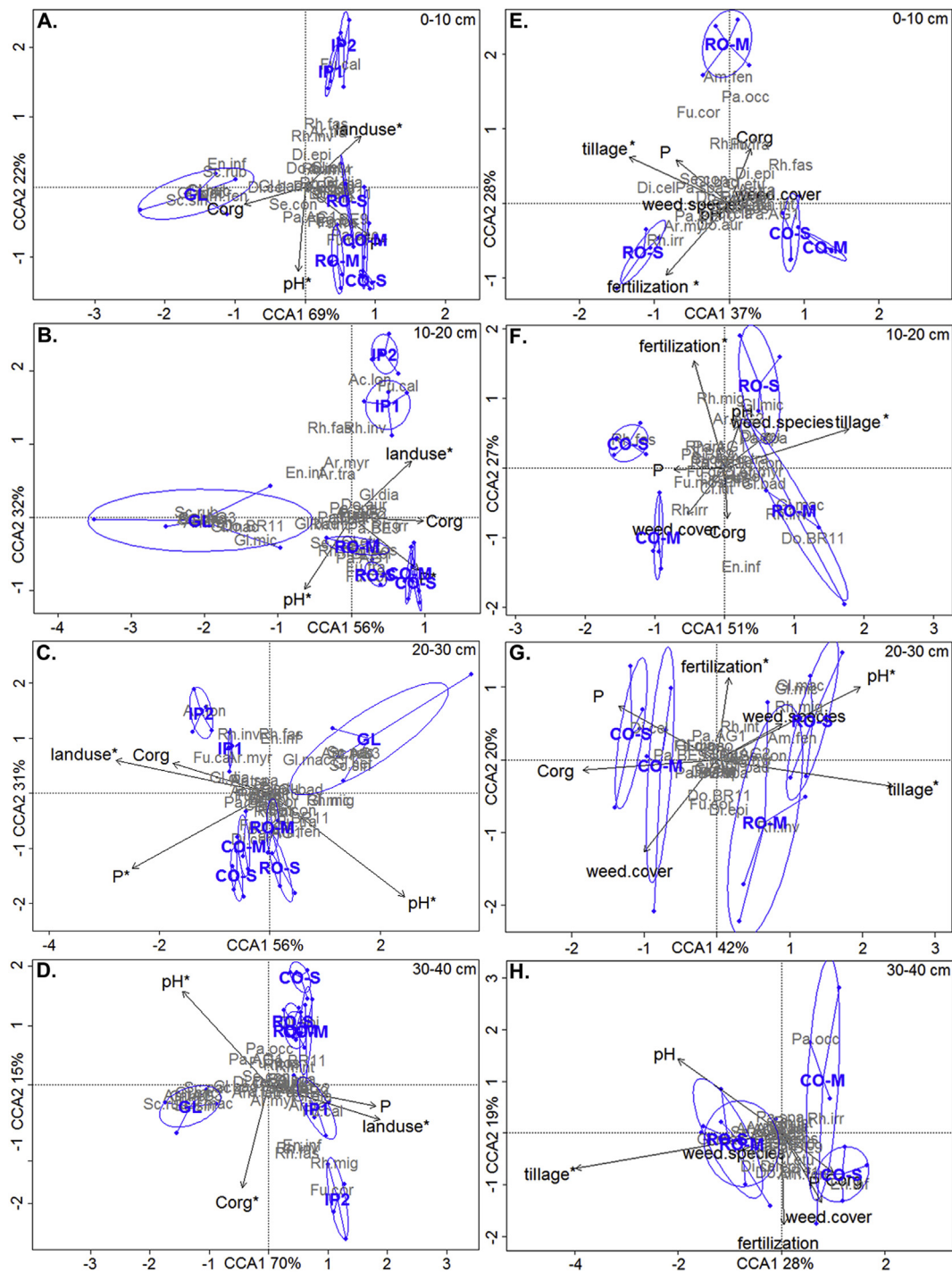


Fig. 2. CCA of AMF community species composition in field samples from the top-soils (0–10 and 10–20 cm) and the subsoils (20–30 and 30–40 cm) respectively. A–D (left): CCA performed on data obtained from all seven sites; E–H (right): CCA performed on data obtained from the field experiment (RO- and CO-plots). Triplots representing the ordination of sites, AMF species and chosen environmental parameters are shown. As environmental parameters, land use intensity ('land use', see Table 1), pH, Corg, and available P ('P') were used (A–D), and tillage, fertilization, pH, Corg, available P, weed species (as species numbers) and weed cover (as %) for E–H. The 2-D plots in the dimensions of the first two CCA axes account for 73–93% of constrained variance of the data (x-axis 45–66%, y-axis 17–42%). * denotes significant effect of the corresponding environmental parameter on the AMF species compositions. Species abbreviations denote: Ac.lon = *Ac. longula*, Am.fen = *Am. fennica*, Ar.myr = *Ar. myriocarpa*, Ar.tri = *Ar. trapezi*, Cl.cla = *Cl. clarioideum*, Cl.etu = *Cl. etunicatum*, Cl.lut = *Cl. luteum*, Di.cel = *Di. celata*, Di.epi = *Di. epigaea*, Do.aur = *Do. aurea*, En.inf = *En. infrequens*, Fu.cal = *Fu. caledonius*, Fu.cor = *Fu. coronatus*, Fu.fra = *Fu. fragilistratus*, Fu.geo = *Fu. geosporus*, Fu.mos = *Fu. mosseae*, Gl.bad = *Gl. badius*, Gl.dia = *Gl. diaphanum*, Gl.het = *Gl. heterosporum*, Gl.mac = *Gl. macrocarpum*, Gl.mic = *Gl. microcarpum*, Rh.fas = *Rhizoglyphus fasciculatus*, Rh.int = *Rhizoglyphus intraradices*, Rh.inv = *Rh. invernium*, Rh.irr = *Rh. irregulare*, Rh.mig = *Rh. microaggregatum*, Sc.pac = *Sclerocystis pachycaulis*, Sc.rub = *Sclerocystis rubiformis*, Sc.sin = *Sc. sinuosa*, Se.con = *Se. constrictum*, Pa.spa = *Palaeospora spainii*, Pa.occ = *Pa. occultum*, Ar.AG2, Do.BR11, Pa.AG1, Pa.BE9, Pa.BE10 are abbreviations for AM fungi that we did not clearly attribute to a known or so far unknown species (see Table 4).

Table 4

Relative spore abundance (%) of AMF species found in different soil depths at the study sites.

	Grassland GL				Reduced tillage (Organic) RO-M				Reduced tillage (Organic) RO-S				Conventional Tillage (Organic) CO-M				Conventional Tillage (Organic) CO-S				Conventional farming (IP) IP1				Conventional farming (IP) IP2			
Soil depth*	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Group A																												
Frequently and abundantly found																												
<i>Funnelformis geosporus</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Fu. mosseae</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Dominikia aurea</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Glomus badium</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Gl. diaphanum</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Septoglomus constrictum</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Archaeospora trappei</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Paraglomus</i> sp. BE9 ^a	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Paraglomus</i> sp. BE10	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Paraglomus</i> sp. AG1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Group B																												
Frequently found but in rather low numbers																												
<i>Clarioideoglomus claroidium</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Cl. etunicatum</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Cl. luteum</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Entrophospora infrequens</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Di. versiformis</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Fu. coronatus</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Fu. fragilistratus</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Rhizoglomus microaggregatum</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Rh. fasciculatum</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Rh. intraradices</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Rh. invermaium</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Rh. irregulare</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Dominikia</i> sp. BR11 ^b	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Ar. myriocarpa</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Archaeospora</i> sp. AG2 ^c	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Palaeospora spainii</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Paraglomus occultum</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Group C																												
Exclusively found in grassland																												
<i>Glomus heterosporum</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Sclerocystis pachycaulis</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Sc.rubiformis</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Ambispora</i> sp. AG3	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Group D																												
Exclusively found in uncultivated or reduced-tillage soil layers																												
<i>Gl. macrocarpum</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Gl. microcarpum</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Sc. sinuosa</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Diversispora celata</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Ambispora fennica</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Group E																												
Exclusively found in conventional farming systems (IP-sites)																												
<i>Fu. caledonius</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Acaulospora longula</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Group F																												
Rarely found species																												
<i>Fu. multiforum</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Archaeospora</i> sp. AG4	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Acaulospora</i> sp. GE2 ^d	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Rh. aggregatum</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Septoglomus</i> sp. AG5	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Ac. cavernata</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Ac. laevis</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Pacispora dominikii</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Scutellospora calospora</i>	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
<i>Paraglomus</i> sp. AG6	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
Total AMF species richness per site ¹	38				32				32				31				28				27				32			

Table 5
AMF species richness and Shannon–Weaver diversity index in different soil depths at study sites.

Soil depth (cm)	Grassland	Reduced tillage (organic)		Conventional tillage (organic)		Conventional farming (IP)		LSD
	GL	RO-M	RO-S	CO-M	CO-S	IP1	IP2	
AMF species richness								
0–40	34.0 a	26.8 b	24.8 bc	23.8 cd	21.8 d	23.3 cd	24.8 bc	2.5
0–10	24.3 a A	20.3 ab A	19.5 bc AB	17.0 bc A	15.8 c A	16.0 bc A	17.8 bc A	2.9
10–20	26.0 a A	21.0 b A	20.8 bc A	17.5 cde A	16.5 de A	15.5 e A	18.8 bcd A	2.1
20–30	25.0 a A	19.3 b A	19.0 b AB	17.5 b A	17.8 b A	16.5 b A	20.3 b A	2.6
30–40	25.3 a A	18.0 bc A	16.8 bc B	17.0 bc A	17.0 bc A	16.0 c A	19.8 b A	2.4
LSD	3.1	2.7	2.2	2.7	2.4	2.5	2.4	
Shannon–Weaver-diversity index								
0–40	2.45 a	2.49 a	2.38 a	2.38 a	2.41 a A	2.13 b	2.44 a	0.24
0–10	2.16 ab A	2.39 a A	2.15 ab B	2.33 ab A	2.24 ab A	2.02 b A	2.16 ab A	0.22
10–20	2.46 a A	2.30 ab A	2.42 ab A	2.21 abc A	2.18 bc A	1.94 c A	2.31 ab A	0.17
20–30	2.26 ab A	2.28 a A	2.41 a A	2.27 ab A	2.21 ab A	1.92 b A	2.39 a A	0.22
30–40	2.18 a A	2.39 a A	2.27 a B	2.22 a A	2.28 a A	2.25 a A	2.38 a A	0.28
LSD	0.25*	0.25	0.20	0.28	0.19	0.27*	0.19*	

Non-significant differences between the sites (lower case) and soil depths (capitals) are shown by identical letters and were determined with Tukey's HSD at the 5% level after two one-way ANOVA analyses. * significant only at the 10% level. Fisher's LSD (Least Significant Difference) is also given.

profile for the different plots and sites, except for 10–20 cm, where the grassland (2.46) and the RO-plots (2.30–2.42) generally had higher index values than the corresponding ploughed layers of the CO-plots (2.18–2.21) and the IP-sites (1.94–2.31). Also remarkably, the most intensively managed site IP1 had lowest values within 0–30 cm soil depth (1.92–2.02). In the field experiment, the exclusive use of slurry might have affected the AMF diversity especially in the top-soil (0–10 cm), since in RO-S and CO-S values (2.15–2.24) were lower than in RO-M and CO-M (2.33–2.39). When comparing for all four soil depths separately, especially CO-S had always lower index values (2.18–2.28) than RO-M (2.28–2.39; Table 5).

3.7. AMF communities in the field soil samples of the seven study sites

The AMF communities differed between all sites according to the Canonical Correspondence Analyses (CCA). When summarized over all four soil depths (data not shown), or analyzed separately in the top-soil layers (0–10 and 0–20 cm) up to the sub-soil layers (20–30 and 30–40 cm), the grassland and the IP-sites separated greatly from each other and from the four organic farming treatments of the field experiment (Fig. 2A–D).

The CCA analyses of the field soil samples from the field experiment suggested a greater impact of tillage than of the fertilization strategy, but both factors affected the AMF communities (Fig. 2E–H). Thus, also the fertilization type had a major influence on the AMF communities of the experiment.

3.8. Distribution pattern of the AMF species detected

According to the occurrences of all species detected in the study area, six groups of species were recognized (Group A–F; Table 4; Fig. 2). Ten species attributed to Group A were generally detected at all sites with abundant spores (e.g. *Dominikia aurea*, *Funnelformis geosporus*, *Fu. mosseae*, *Glomus badium*, *Gl. diaphanum*, and *Septoglomus constrictum*). Seventeen other species also occurred regularly at all sites, but these species were not abundantly found, and thus, they were attributed to Group B in Table 4. Three other species were exclusively found in the permanent grassland (Group C, e.g. *Sclerocystis rubiformis* and *Gl. heterosporum*), while five species (attributed to Group D) were not only found in the grassland, but additionally also in uncultivated soil layers of croplands (e.g. *Sc. sinuosa* and *Gl. macrocarpum*), or also in the top-soils under

reduced tillage (*Am. fennica*), but never in regularly ploughed soil layers of the CO-plots or the IP-sites. These species of the Groups C and D generally clustered close to GL in the CCA analyses performed on all sites (Fig. 2A–D), while species of Group D generally clustered with the RO-plots, when only RO- and CO-plots were considered in the analyses (Fig. 2E–H). *Funnelformis caledonius* and *Acaulospora longula*, attributed to Group E, were only recovered from the IP-site with decreased soil pH (Table 4, Fig. 3), and accordingly they clustered with these sites in the CCA (Fig. 2A–D). Finally, ten other species were not attributed to a species group since those species were only rarely detected (Group F; Table 4).

Several AMF species, although regularly occurring at all sites (attributed to Groups A in Table 4), had a specific distribution pattern (Fig. 3), such as *Glomus badium* (significant lower abundance in croplands, and especially in the field experiment under conventional tillage). Also *Septoglomus constrictum* was detected in GL as well as in croplands, but its spore numbers were significantly reduced in ploughed sites. Accordingly, these two species clustered in the CCA analyses generally closer to GL or the RO-plots than to the CO-plots and the IP-sites. *Funnelformis mosseae* had low abundance in the IP-sites of lower pH, but interestingly it clustered towards the CO-plots when compared to the RO-plots (Fig. 2E–H). For other species, e.g. *Gl. diaphanum* it was the opposite, as spore numbers of this species was very low in GL and highest in the two IP-sites. Other abundant species were not so strongly correlated with specific sites or specific farming systems (e.g. *Dominikia aurea* was very common in all fields with slightly lower abundance in the Frick field trial plots). Sporulation of *Fu. geosporus* was not dependent on environmental and agricultural circumstances, as this species showed the same abundance at all sites.

3.9. Strip split plot ANOVA for effects on AMF communities and selected species in the field experiment

The strip split plot ANOVA analyses on AMF spore density and species richness of the field experiment confirmed results of the overall analysis (Tables 3 and 5) and revealed that soil cultivation and soil depth had a stronger effect on spore populations than fertilization strategy (Table S2). AMF diversity was significantly affected only by soil depth. However, for specific AMF species, the results were variable (Table S3): e.g. *Se. constrictum* and *Gl. badium* spore populations were significantly affected by all three factors, *Ar. trappei* populations only by soil cultivation and soil depth, while *Cl. clarioideum* was significantly affected only by fertilization.

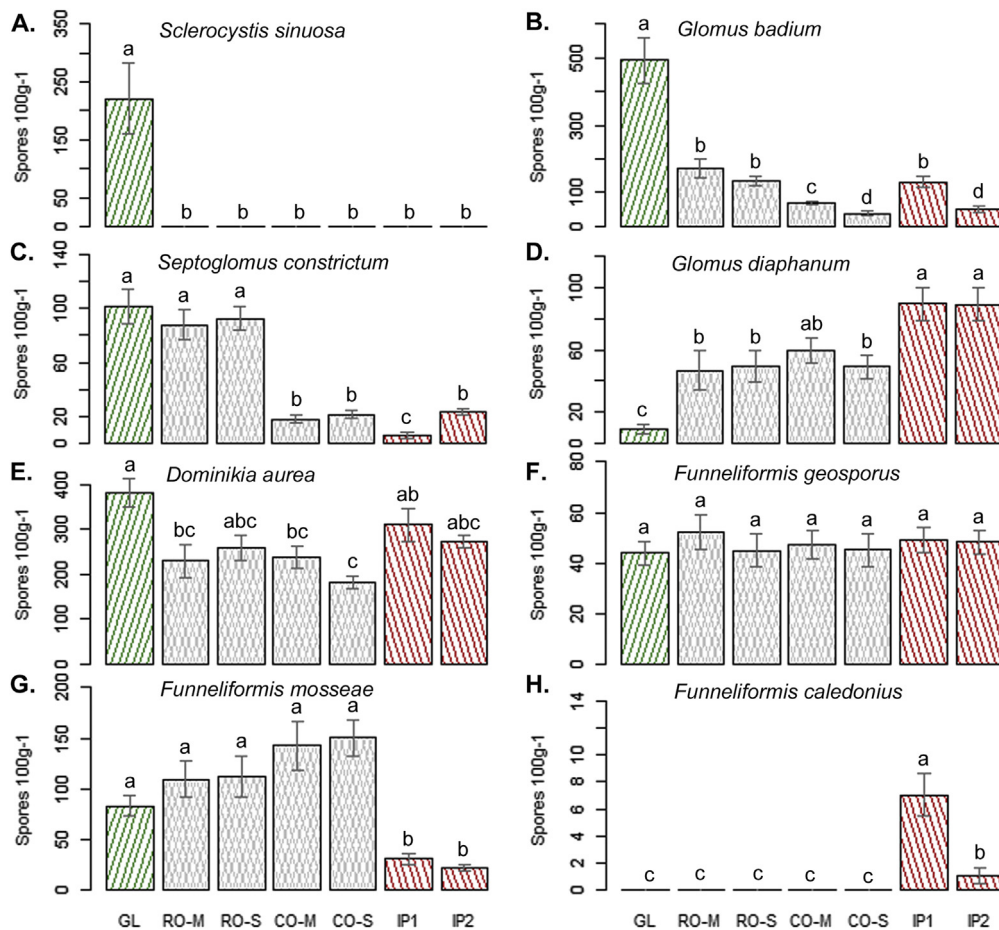


Fig. 3. Spore density (spores 100 g⁻¹) of selected AMF species at sites with different agricultural practices. For testing the abundance of selected AMF species at all sites, the non-parametric Kruskal Wallis test was chosen with Bonferroni adjusted p-values.

3.10. AMF communities in trap cultures and in the overall study

In the trap cultures, >19,000 AMF spores were identified, and 33 AMF species were detected (Table 6). The majority of the species belonged again to the Glomeraceae (22 species of *Glomus*, *Dominikia*, *Funnelformis*, *Rhizoglosum* and *Septoglosum*). Three species were Entrophosporaceae (*Claroideoglosum*). Finally, two species each belonged to Diversisporaceae (*Diversispora*), Paraglomeraceae (*Paraglosum*) and Archaeosporaceae (*Archaeospora*), and one species each belonged to Acaulosporaceae (*Acaulospora*) and Ambisporaceae (*Ambispora*). Five species detected in the trap cultures had not been found in the original field soil samples. These were *Ac. spinosa*, *Fu. monosporus*, *Fu. sp. AG7*, *Glomus sp. AG8*, and *Se. xanthium* (Table 6). Thus, when summarizing, in total 53 AMF species were found at the study sites through the thorough analyses of the field soil samples and the periodical spore isolation from the trap cultures in this study. On the other hand, 20 species, detected in the field soil samples were not recovered in the trap cultures, among them several species exclusively or predominantly found in the grassland (e.g. *Sc. rubiformis*, *Sc. sinuosa*, *Sc. pachycaulis*, *Gl. badium*).

In the top-soils (0–10 cm), AMF species richness was slightly higher in the trap cultures of the RO-plots (11.3–13.3), GL (10.3) and IP1 (10.0) than in the cultures of the CO-plots (6.5–7.8; Table 7). In the sub-soils, this result was similar, but less pronounced: species richness was higher in the trap cultures of GL (9.8) and the RO-plots (8.0–8.8) and IP1 (8.8) than of the CO-plots (7.0–7.8). When summarizing top- and sub-soils a similar order was obtained: AMF

species richness was higher in RO-plots (13.5–16.3), GL (13.0) and IP1 (12.0) than in the cultures of the CO-plots (11.0–11.3 species). Total species richness was higher in GL and in RO-plots (20–21) than CO-plots and IP1 (16–18 species; Table 7). Finally, when summarizing the total species richness data from the field soils and the trap cultures, highest richness was obtained for GL (38), followed by RO-M, RO-S, CO-M (33 each), while lowest values were obtained for CO-S and IP1 (28 each; Table 7).

According to the CCA analyses, the AMF communities differed in the trap cultures 20 months after inoculation with soils from the seven sites. In the top-soil and in the sub-soil layer investigated (0–10 and 30–40 cm, respectively), the grassland and the IP-site (IP1) separated greatly from each other and from the four organic farming treatments of the field experiment (Fig. 4A–B), as it had been found for the field soil analyses. For the spore populations from the field experiment again remarkable results were revealed: Both, tillage strategy and fertilization type had significant impacts on the AMF communities in the trap cultures, and this was not only found for the top-soil but also for the sub-soil cultures.

Several species of Group B (frequently found but in rather low numbers in the field soil samples) abundantly reproduced spores in the trap cultures. These were for example *Cl. claroideum*, *Cl. etunicatum*, *Cl. luteum*, *Rh. intraradices* and *Rh. irregulare* (Table 6). Other species (especially the abundantly sporulating species from Group A) had a similar pattern in the field and in the trap cultures (e.g. *Septoglosum constrictum* was most abundant in GL and in the RO-plots, while *Ar. trappei* was frequently found at almost all sites). Interestingly, *Fu. geosporus* clustered more with

Relative spore abundance (in %) of AMF species detected in trap cultures after 4, 8, 16, and 20 months, initially inoculated with top-soils (soil depth 1) and sub-soils (depth 4) of six study sites.

Relative spore abundances are symbolized as follows: ▫ < 5%, ▣ 5-15%, ▢ 15-25 %; ▤ 25-50%; ▥ > 50 % of total spore numbers.

^a Resembling *Paraglomus*, ^b resembling *Dominikia minuta*, ^c resembling *Archaeospora undulata*, ^d resembling *Acaulospora dilatata*.

Table 7
AMF species richness detected during 20 months in trap cultures inoculated with top- (0–10 cm) or sub-soils (30–40 cm); AMF communities deriving from six of the seven study sites.

	Grassland	Reduced tillage (organic)		Conventional tillage (organic)		Conventional farming (IP)	
	GL	RO-M	RO-S	CO-M	CO-S	IP1	IP2 ^b
Average AMF species richness of top-soil ^a	10.3 (0.5)	13.3 (1.5)	11.3 (1.7)	6.5 (1.7)	7.8 (1.0)	10.0 (1.8)	n.d.
Average AMF species richness of sub-soil ^a	9.8 (1.5)	8.0 (3.5)	8.8 (1.5)	7.8 (1.7)	7.0 (2.4)	8.8 (1.5)	n.d.
Average AMF species richness of top- & subsoil (cumulated) ^a	13.0 (0.8)	16.3 (1.9)	13.5 (1.3)	11.0 (1.8)	11.3 (3.3)	12.0 (0.8)	n.d.
Total AMF species richness of topsoil	15	18	18	11	15	15	n.d.
Total AMF species richness of subsoil	15	15	14	15	12	14	n.d.
Total AMF species richness of top- & subsoil (cumulated)	20	21	20	17	18	16	n.d.
Total AMF species richness in field soils & trap cultures	38	33	33	33	28	28	(32) ^b

^a Data are reported as averages and standard deviations (in brackets) for four replicate plots per field site.

^b No trap cultures were established.

the RO- (especially RO-S) than with the CO-plots in the CCA analyses performed on the AMF communities of the trap cultures (Fig. 4), while it was rather equally abundant at the seven sites in the field soil samples (Fig. 3). Additionally, for *Fu. mosseae*, a fast sporulation was observed in the trap cultures after 4 months, while its abundance steadily decreased with increasing time of culturing in the trap cultures (after 8, 16 and 20 months; Table 6).

4. Discussion

Through AMF species identification by morphological spore analyses from the field soil samples, and periodically from the trap cultures during 20 months, 42 AMF species were found in the tillage field experiment in Frick. Including the grassland and the two IP-sites in the surroundings of the experiment, in total 53 AMF

species were detected. These numbers represent a high AMF species richness in a well-defined small area (here < 1 km²) and are astonishing as at all sites clayey Cambisol soils have developed on the same geology, alluvial and colluvial Jurassic sediments with about 45% clay content and with a relatively small pH (H₂O) range of 6.5–7.7 in the top-soils. Hitherto, such high species richness has rarely been recorded before in temperate or similar cold climates e.g. Bever et al. (2001), Jansa et al. (2002), Wetzels et al. (2014) and Maurer et al. (2014), who found 37, 17, 25 and 39 species, respectively. Even in warmer, mediterranean to (sub-)tropic zones, species richness is often not necessarily higher, e.g. current observations range from 23 to 42 to 58 species (Abdelhalim et al., 2014; de Oliveira Freitas et al., 2014; Guadarrama et al., 2014; Njeru et al., 2015). The high species richness in the present study may be related to the soil properties, as well as to the various ecologically sound agricultural practices included in the

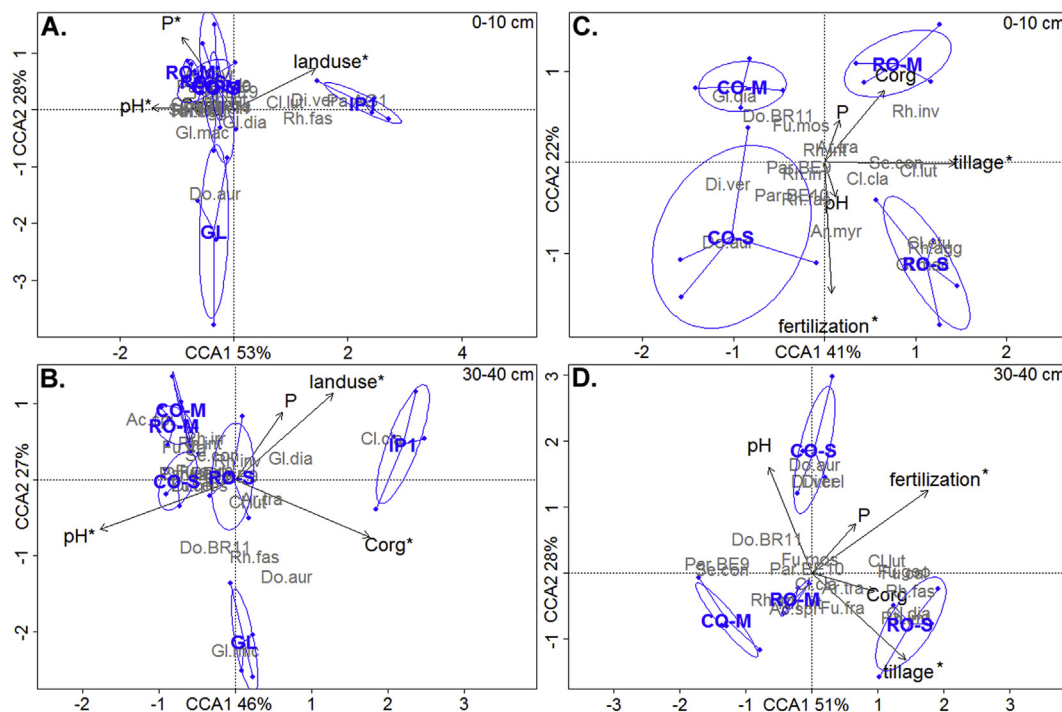


Fig. 4. CCA of AMF community species compositions determined in the trap cultures from the top-soils (0–10 and 10–20 cm) and the subsoils (20–30 and 30–40 cm) respectively. A–B (left): CCA performed on data obtained from all six sites. C–D (right): CCA performed on data obtained from the field experiment (RO- and CO-plots). Tri-plot of sites, AMF species and environmental parameters. Diagrams account for 58–86% of constrained variance of the data (x-axis 32–55%, y-axis 26–32%). As environmental parameters, land use intensity ('land use', see Table 1), pH, Corg, and available P ('P') were used (A–D), and tillage, fertilization, pH, Corg, available P, weed species (as species numbers) and weed cover (as %) for E–H. * denotes significant effect of the corresponding environmental parameter on the AMF species compositions. For species abbreviations see legend of Fig. 2.

experiment, but especially to the extensive sampling design, the combination of field soil and trap culture studies with >50,000 spores identified and the remarkable progresses on AMF spore isolation and identification within the last 2–3 decades (e.g. [Bia  skowski, 2012](#)). It will be interesting to elaborate if, and when in the future, at our study sites a similar high or even higher AMF species richness can be obtained through modern, rapidly developing high-throughput sequencing techniques and subsequent molecular phylogenetic analysis, and if both, traditional and new methods will give complementary or even similar information on the AMF community structure. Following [Wetzel et al. \(2014\)](#), morphological identification might currently be superior to molecular identification in terms of detecting total AMF species richness at a site.

As expected, highest AMF spore densities and species richness was found in the permanent grassland, and species composition was similar as found for other clayey grasslands in the Swiss/French Jura mountain range, even when those grassland soils had developed on limestones (Calcaric Leptosols; [Oehl et al., 2003, 2010](#)). In the croplands of the field experiment and the IP-sites, also typical AMF communities had developed, when compared to two cultivated clayey (Leptosol) soils investigated in the Swiss/French Jura mountain range by [Oehl et al. \(2010\)](#). Our detail analyses in the field experiment revealed that reduced tillage had positive effects on the top-soil AMF communities, since spore densities in RO-plots increased substantially and even fell in the range of spore densities observed in the grassland. Also AMF species richness increased in RO-plots when compared to CO-plots, at least in the soil layers most affected by the tillage practices, but they clearly did not reach the values of the grassland. Several recent studies comparing conventional tillage with reduced or no-till systems in other soils and regions under conventional farming, also reported a negative impact of intensive soil tillage on AMF abundance (e.g. [Jansa et al., 2002](#); [Borie et al., 2006](#); [Brito et al., 2012](#); [Wetzel et al., 2014](#)). The reduced spore abundance in conventional tillage systems has been explained by disruption of hyphal networks and dilution of AMF propagules through tillage (e.g. [Kabir, 2005](#)). Analysis of earthworm and weed communities in the plots of the field experiment indicated also an increase of both earthworm and weed abundance in RO-plots when compared to CO-plots ([Sans et al., 2011](#); [Kuntz et al., 2013](#); [Armengot et al., 2015](#)), and both these factors might have affected also the AMF communities in the tillage systems. However, despite of the almost complete absence of weeds in the conventionally managed IP-sites, also there a similar high AMF species richness and diversity was maintained.

While AMF spore density was clearly affected by tillage, changes in AMF species richness were less pronounced, and AMF diversity changed only slightly between the different top-soils of our study. This observation fits with the results found by [Jansa et al. \(2002\)](#) obtained in sandy-loamy Luvisol soils, although in their study in more acidic and more coarse textured soil, a quite different AMF community had established with a significant higher abundance of *Gigaspora*, *Cetraspora*, *Scutellospora* and *Acaulospora* ([Jansa et al., 2003](#); [Oehl et al., 2010](#)) than in the present study where >95% of the spores were from Glomerales and Paraglomerales in the field soil samples. [Jansa et al. \(2003\)](#) did not detect changes in Shannon–Weaver diversity under different tillage regimes, but differences in the AMF community structure. [Brito et al. \(2012\)](#), however, detected higher AMF diversity in no-till than in tillage systems, applying solely molecular methods. Our multivariate analyses revealed clear differences in the AMF communities established under reduced and conventional tillage. [Jansa et al. \(2003\)](#) proposed several reasons for a shift in AMF communities under conventional tillage: disruption of hyphae by tillage and better adaption of certain species to this treatment, changes in nutrient

availability, in the soil microbial composition and in weed community. The same conclusion might apply to our situation, even though obtained from a quite different soil type and with clearly different AMF communities. The change in AMF community composition in our study compared to [Jansa et al. \(2003\)](#) might be explained by the high clay content ([Lekberg et al., 2007, 2008](#)).

One strength of our study was that we did not only assess the impact of different farming practices on cultivated top-soils, but we also investigated the undisturbed sub-soils beyond the ploughing depth up to 40 cm at all sites. While AMF spore density and species richness decreased at all sites towards the sub-soils, the grassland had the highest numbers in all soil layers, confirming the general observations of [Oehl et al. \(2005\)](#). However, in all the arable sites of our study, including the four treatments of the field experiment, AMF spore density and AMF species richness were very similar in the lowest layer investigated (30–40 cm) suggesting that the differences between the sites diminish with increasing soil depth. Nevertheless, the multivariate analyses revealed a clear difference in the AMF community compositions, and interestingly, these differences continued between RO and CO plots of the field experiment from the differently cultivated top-soils also into the undisturbed sub-soils.

Average AMF spore density, species richness and Shannon–Weaver diversity in the deepest soil layer investigated (30–40 cm) can still be classified as high at all arable fields (16–20 spores g^{−1} soil, 16–20 species and H = 2.21–2.41, respectively), especially when compared with data obtained from other cropland sub- or top-soils investigated in the region (e.g. see [Oehl et al., 2005, 2010](#)) or other regions of more intensive agriculture (e.g. [Oehl et al., 2003](#); [Wetzel et al., 2014](#)). These results might not only depend on the sometimes clearly increased land use intensity in the later studies, but again also on the increased soil clay contents and the abundance of weed species in the organically managed field experiment ([Oehl et al., 2004, 2011a](#), see above).

In the present study, the influence of two types of organic fertilization on AMF communities was compared: manure complemented with slurry (−M), and slurry solely (−S). The −M plots have received higher organic matter input than −S plots due to the straw, needed for bedding animals and as structure element for manure composting ([Berner et al., 2008](#); [Gadermaier et al., 2012](#)). Also other qualities of the two fertilization types might have affected the AMF communities in the field plots. The differences between the AMF communities for AMF spore density, species richness and diversity indices with respect to the fertilization types seemed to be rather small in the field soil samples, even when RO-M often had the highest AMF species richness and diversity indices, and on the other hand CO-S often had the lowest values. The CCA analyses, however, revealed a significant impact of the fertilization type on the AMF spore populations. [Beauregard et al. \(2013\)](#) could not detect differences in AMF abundance in soil due to the application of different organic fertilizers. Effects of fertilization on AMF abundance are mostly caused by different amounts of nutrient input or by the different types of mineral and organic fertilizers applied ([Oehl et al., 2004](#); [Borriello et al., 2012](#); [Avio et al., 2013](#)).

For some time, trap cultures have been seen as an essential tool in morphology based AMF diversity studies, as they might give complementary information (e.g. [Bever et al., 2001](#); [Oehl et al., 2003](#)) to find additional AMF species that had not sporulated in the field before. However, trap cultures as such have been viewed as being unsuitable to characterize AMF communities from field soils as they do not reflect the in-situ reality. However, our AMF results confirm the findings of [Oehl et al. \(2009\)](#) that AMF communities can largely be re-established under long-term growth conditions and using soil pH and climate as close as possible to natural conditions. Our results from the cultures are quite congruent with

those obtained from the field soil analyses, and approximately two-third of the AMF species detected in the overall study reproduced spores in the trap cultures. For several species a similar pattern was found in the CCA analyses obtained from the trap cultures and from the field soil samples (e.g. *Fu. caledonius* and *Am. fennica*), while others were found more or less abundantly but regularly in the field soils and in the trap cultures from the corresponding detection sites (e.g. *Ar. myriocarpa*, *Cl. claroideum* and *Do. aurea*). Importantly, typical grassland AMF species did not sporulate in the trap cultures which is in accordance with the observations of Oehl et al. (2005, 2009). Interestingly, in our trap cultures, fertilization type within the sub-soils of the field experiment obviously played a more important role in determining the AMF communities than revealed from the field soil samples. This might have been biased by the fact that within the trap cultures the AMF communities were not disturbed, or only slightly with the small corers (diameter 1.5 cm) at sampling dates, without any further cultivation difference during 20 months.

In the present study, we classified the AMF species according to their occurrence in the seven study sites following the AMF 'generalist versus specialist' concept of Oehl et al. (2003, 2010). The AMF 'specialists' might also be called 'soil', 'tillage' or 'land use indicators' deduced from Jansa et al. (2009, 2014) and Oehl et al. (2010, 2011a). Some species (so-called 'AMF generalist species') occurred either abundantly or not abundantly at all field sites (species of Groups A and B, respectively). These species were usually also found in previous studies performed in Central Europe (e.g. B  lszkowski, 1993; Oehl et al., 2003, 2010; Wetz  l et al., 2014). Other species, e.g. *Sc. rubiformis* and *Gl. heterosporum*, were exclusively found in the grassland ('grassland specialists'), which were also identified as grassland specialists in previous studies (e.g. Oehl et al., 2003, 2005; B  rstler et al., 2006). Besides in grasslands, species such as *Gl. macrocarpum*, *Sc. sinuosa* and *Am. fennica* were additionally found in the top-soils under reduced tillage and/or undisturbed sub-soil layers (species of Groups C and D, respectively). Also *Septoglomus constrictum* was also more abundant under reduced tillage, which is in accordance with Maurer et al. (2014) and Wetz  l et al. (2014). Finally, other species were exclusively found in conventionally tilled soils (species of Group E), however these two species, *Fu. caledonius* and *Ac. longula*, are often more abundant in acidic than in calcareous soils (e.g. Oehl et al., 2010; Maurer et al., 2014), which also is in accordance with our findings. In the latter two studies, *Fu. caledonius* was found as indicator species for conventional tillage in acidic soils. In our study, *Fu. mosseae* was more abundant in the CO-plots than the RO-plots of the field experiment as it was found for Luvisols and Cambisols (pH about 6.0) in Wetz  l et al. (2014) and Maurer et al. (2014), respectively, but in our study it was also less abundant in the conventionally tilled, but acidic IP-sites, which in respect of soil pH is in strong accordance with previous studies, e.g. with Jansa et al. (2002), Schalamuk et al. (2006) and Oehl et al. (2010). Finally, *Fu. geosporus* occurred abundantly at all sites, which is in accordance with our previous findings from clay soils (Oehl et al., 2003, 2010). However, in the coarse textured substrate of our trap cultures we observed a preferential sporulation of this species in the RO-plots, when compared to the CO-plots, which is in accordance with the findings of Wetz  l et al. (2014) obtained from a silty-loamy Luvisol of Eastern Germany. However, their findings were obtained in a more continental climate than prevailing in Northern Switzerland, which might also have affected the results.

5. Conclusions

Often, AMF communities are not only influenced by one single, but by multiple environmental factors and agricultural

management practices. Here we demonstrated that with increasing land use intensity, i) spore densities are reduced, ii) AMF community compositions are changed, and iii) that several AMF species can be considered as specialists for specific soil or management practices. Soil tillage had a strong impact on AMF, as it was shown here for clay soils. No- and reduced tillage systems are worldwide gaining interest on conventional farms. In organic farming reduced tillage remains challenging, and due to the lack of chemical herbicides ploughing is usually practiced for weed control. Increasing efforts are being made to optimize conservation tillage strategies in organic farming. The recently concluded Era Net CORE Organic project revealed, that in particular in Mediterranean climate, reduced and no-tillage is often applied. In contrast, in temperate climates such as in Switzerland reduced or no-tillage under organic farming is rare. A compiled meta-analysis showed, that reducing tillage depth improved carbon stocks, but weeds were not increased and yields not compromised (Cooper et al., 2014). Reducing tillage intensity and tillage depth is also favorable for AMF, which is advantageous since AMF can contribute to several ecosystems services and are especially important in less intensive agricultural systems like organic farming. It will be interesting to estimate the functional diversity of the AMF species that were found in the agricultural soils with different management practices. An approach to estimate the ecosystem functions of AMF communities was done by K  hl et al. (2014) who showed that AMF communities from different tillage systems can change plant productivity, and AMF communities of non-tilled soils enhanced plant P uptake. In the future, the impact of AMF communities as well as of single AMF species on ecosystem services, especially on plant growth and health, has to be explored, regarding not only soil tillage practices, but also specific farming and crop rotation systems.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.soilbio.2015.02.005>

References

- Abdelhalim, T.S., Finck, M.R., Babiker, A.G., Oehl, F., 2014. Species composition and diversity of arbuscular mycorrhizal fungi in White Nile state, Central Sudan. *Archives of Agronomy and Soil Science* 60 (3), 377–391.
- Armengot, L., Berner, A., Blanco-Moreno, J., M  der, P., Sans, F., 2015. Long-term feasibility of reduced tillage in organic farming. *Agronomy for Sustainable Development* 35, 339–346.
- Avio, L., Castaldini, M., Fabiani, A., Bedini, S., Sbrana, C., Turrini, A., Giovannetti, M., 2013. Impact of nitrogen fertilization and soil tillage on arbuscular mycorrhizal fungal communities in a Mediterranean agroecosystem. *Soil Biology & Biochemistry* 67, 285–294.
- Beauregard, M.S., Gauthier, M.P., Hamel, C., Zhang, T., Welacky, T., Tan, C.S., St-Arnaud, M., 2013. Various forms of organic and inorganic P fertilizers did not negatively affect soil- and root-inhabiting AM fungi in a maize-soybean rotation system. *Mycorrhiza* 23, 143–154.
- Berner, A., Hildermann, I., Flie  bach, A., Pfiffner, L., Niggli, U., M  der, P., 2008. Crop yield and soil fertility response to reduced tillage under organic management. *Soil & Tillage Research* 101, 89–96.
- Bever, J.D., Schultz, P.A., Pringle, A., Morton, J.B., 2001. Arbuscular mycorrhizal fungi: more diverse than meets the eye, and the ecological tale of why. *Bioscience* 51, 923–931.

- Biaszkowski, J., 1993. Comparative studies on the occurrence of arbuscular fungi and mycorrhizae (Glomales) in cultivated and uncultivated soils of Poland. *Acta Mycologica* 28, 93–140.
- Biaszkowski, J., 2012. Glomeromycota. W. Szafer Institute of Botany, Polish Academy of Sciences.
- Biaszkowski, J., Chwat, G., Górska, A., Ryska, P., Kovács, G.M., 2015. Two new genera, *Dominikia* and *Kamienska*, and *D. disticha* sp. nov. in Glomeromycota. *Nova Hedwigia* 100. <http://dx.doi.org/10.1127/0029-5035/2014/00xx>. Online first.
- Borie, F., Rubio, R., Rouanet, J.L., Morales, A., Borie, G., Rojas, C., 2006. Effects of tillage systems on soil characteristics, glomalin and mycorrhizal propagules in a Chilean Ultisol. *Soil & Tillage Research* 88, 253–261.
- Borriello, R., Lumini, E., Giralda, M., Bonfante, P., Bianciotto, V., 2012. Effects of different management practices on arbuscular mycorrhizal fungal diversity in maize fields by a molecular approach. *Biology and Fertility of Soils* 48, 911–922.
- Börstler, B., Renker, C., Kahmen, A., Buscot, F., 2006. Species composition of arbuscular mycorrhizal fungi in two mountain meadows with differing management types and levels of plant biodiversity. *Biology and Fertility of Soils* 42, 286–298.
- Börstler, B., Thiery, O., Sykora, Z., Berner, A., Redecker, D., 2010. Diversity of mitochondrial large subunit rDNA haplotypes of *Glomus* intraradices in two agricultural field experiments and two semi-natural grasslands. *Molecular Ecology* 19, 1497–1511.
- Brito, I., Goss, M.J., de Carvalho, M., 2012. Effect of tillage and crop on arbuscular mycorrhiza colonization of winter wheat and triticale under Mediterranean conditions. *Soil Use and Management* 28, 202–208.
- Carr, P.M., Mäder, P., Creamer, N.D., Beeby, J.S., 2011. Editorial: overview and comparison of conservation tillage practices and organic farming in Europe and North America. *Renewable Agriculture and Food Systems* 27, 2–6.
- Cooper, J.M., Baranski, M., Nobel de Lange, M., Barberi, P., Fließbach, A., Peigne, J., Berner, A., Brock, C., Casagrande, M., Crowley, O., Davide, C., De Vlieghe, A., Döring, T.F., Entz, M., Grosse, M., Haase, T., Halde, C., Hammer, V., Huiting, H., Leithold, G., Messmer, M., Schloter, M., Sukkel, M., van der Heijden, M., Willekens, K., Wittwer, R., Mäder, P., 2014. Effects of reduced tillage in organic farming on yield, weeds and soil carbon: meta-analysis results from the TILMAN-ORG project. In: Rahmann, G., Aksoy, U. (Eds.), *Building Organic Bridges*. Johann Heinrich von Thünen-Institut, Braunschweig, Germany, pp. 1163–1166. Thünen Report, No. 20.
- de Mendiburu, F., 2014. *agricolae: Statistical Procedures for Agricultural Research*. R Package Version 1.2-0. <http://CRAN.R-project.org/package=agricolae>.
- de Oliveira Freitas, R., Buscado, E., Nagy, L., dos Santos Maciel, A.B., Carrenho, R., Luzião, R.C.C., 2014. Arbuscular mycorrhizal fungal communities along a pedo-hydrological gradient in a Central Amazonian terra firme forest. *Mycorrhiza* 24, 21–32.
- Douds, D., Millner, P., 1999. Biodiversity of arbuscular mycorrhizal fungi in agroecosystems. *Agriculture, Ecosystems and Environment* 74, 77–93.
- Gadermaier, F., Berner, A., Fließbach, A., Friedel, J.K., Mäder, P., 2012. Impact of reduced tillage on soil organic carbon and nutrient budgets under organic farming. *Renewable Agriculture and Food Systems* 27, 68–80.
- Guadarrama, P., Castillo, S., Ramos-Zapata, J.A., Hernández-Cuevas, L.V., Camargo-Ricalde, S.L., 2014. Arbuscular mycorrhizal fungal communities in changing environments: the effects of seasonality and anthropogenic disturbance in a seasonal dry forest. *Pedobiologia* 57, 87–95.
- Hothorn, T., Bretz, F., Westfall, P., 2008. Simultaneous inference in general parametric models. *Biometrical Journal* 50, 346–363.
- IUSS Working Group WRB, 2014. World Reference Base for Soil Resources 2014. International Soil Classification System for Naming Soils and Creating Legends for Soil Maps. World Soil Resources Report No. 106. FAO, Rome, 181 pp.
- Jansa, J., Erb, A., Oberholzer, H.R., Smilauer, P., Egli, S., 2014. Soil and geography are more important determinants of indigenous arbuscular mycorrhizal communities than management practices in Swiss agricultural soils. *Molecular Ecology* 23, 2118–2135.
- Jansa, J., Mozafar, A., Anken, T., Ruh, R., Sanders, I.R., Frossard, E., 2002. Diversity and structure of AMF communities as affected by tillage in a temperate soil. *Mycorrhiza* 12, 225–234.
- Jansa, J., Mozafar, A., Kuhn, G., Anken, T., Ruh, R., Sanders, I.R., Frossard, E., 2003. Soil tillage affects the community structure of mycorrhizal fungi in maize roots. *Ecological Applications* 13, 1164–1176.
- Jansa, J., Oberholzer, H.R., Egli, S., 2009. Environmental determinants of the arbuscular mycorrhizal fungal infectivity of Swiss agricultural soils. *European Journal of Soil Biology* 45, 400–408.
- Kabir, Z., 2005. Tillage or no-tillage: impact on mycorrhizae. *Canadian Journal of Plant Science* 85, 23–29.
- Köhl, L., Oehl, F., van der Heijden, M.G.A., 2014. Agricultural practices indirectly influence plant productivity and ecosystem services through effects on soil biota. *Ecological Applications* 24, 1842–1853.
- Krauss, M., Berner, A., Burger, D., Wiemken, A., Niggli, U., Mäder, P., 2010. Reduced tillage in temperate organic farming: implications for crop management and forage production. *Soil Use and Management* 26, 12–20.
- Kuntz, M., Berner, A., Gatterer, A., Scholberg, J.M., Mader, P., Pfiffner, L., 2013. Influence of reduced tillage on earthworm and microbial communities under organic arable farming. *Pedobiologia* 56, 251–260.
- Lekberg, Y., Koide, R.T., Rohr, J.R., Aldrich-Wolfe, L., Morton, J.B., 2007. Role of niche restrictions and dispersal in the composition of arbuscular mycorrhizal fungal communities. *Journal of Ecology* 95, 95–105.
- Lekberg, Y., Koide, R.T., Twomlow, S.J., 2008. Effect of agricultural management practices on arbuscular mycorrhizal fungal abundance in low-input cropping systems of southern Africa: a case study from Zimbabwe. *Biology and Fertility of Soils* 44, 917–923.
- Mäder, P., Edenhofer, S., Boller, T., Wiemken, A., Niggli, U., 2000. Arbuscular mycorrhizae in a long-term field trial comparing low-input (organic, biological) and high-input (conventional) farming systems in a crop rotation. *Biology and Fertility of Soils* 31, 150–156.
- Maurer, C., Rüdy, M., Chervet, A., Stürny, W., Flisch, R., Oehl, F., 2014. Diversity of arbuscular mycorrhizal fungi in field crops using no-till and conventional tillage practices. *Agrarforschung Schweiz* 5, 398–405.
- Neyroud, J.A., Lischer, P., 2003. Do different methods used to estimate soil phosphorus availability across Europe give comparable results? *Journal of Plant Nutrition and Soil Science* 166, 422–431.
- Njeru, E., Avio, L., Bocci, G., Sbrana, C., Turrini, A., Barberi, P., Giovannetti, M., Oehl, F., 2015. Contrasting effects of cover crops on 'hot spot' arbuscular mycorrhizal fungal communities in organic tomato. *Biology and Fertility of Soils* 51, 151–166.
- Oehl, F., Jansa, J., Ineichen, K., Mader, P., van der Heijden, M., 2011a. Arbuscular mycorrhizal fungi as bio-indicators in Swiss agricultural soils. *Agrarforschung Schweiz* 2, 304–311.
- Oehl, F., Laczko, E., Bogenrieder, A., Stahr, K., Bosch, R., van der Heijden, M., Sieverding, E., 2010. Soil type and land use intensity determine the composition of arbuscular mycorrhizal fungal communities. *Soil Biology & Biochemistry* 42, 724–738.
- Oehl, F., Schneider, D., Sieverding, E., Burga, C.A., 2011b. Succession of arbuscular mycorrhizal communities in the foreland of the retreating Morteratsch glacier in the Central Alps. *Pedobiologia* 54, 321–331.
- Oehl, F., Sieverding, E., Ineichen, K., Mader, P., Boller, T., Wiemken, A., 2003. Impact of land use intensity on the species diversity of arbuscular mycorrhizal fungi in agroecosystems of Central Europe. *Applied and Environmental Microbiology* 69, 2816–2824.
- Oehl, F., Sieverding, E., Ineichen, K., Maeder, P., Wiemken, A., Boller, T., 2009. Distinct sporulation dynamics of arbuscular mycorrhizal fungal communities from different agroecosystems in long-term microcosms. *Agriculture, Ecosystems & Environment* 134, 257–268.
- Oehl, F., Sieverding, E., Ineichen, K., Boller, T., Wiemken, A., 2005. Community structure of arbuscular mycorrhizal fungi at different soil depths in extensively and intensively managed agroecosystems. *New Phytologist* 165, 273–283.
- Oehl, F., Sieverding, E., Mäder, P., Dubois, D., Ineichen, K., Boller, T., Wiemken, A., 2004. Impact of long-term conventional and organic farming on the diversity of arbuscular mycorrhizal fungi. *Oecologia* 138, 574–583.
- Oehl, F., Sieverding, E., Palenzuela, J., Ineichen, K., Silva, G., 2011c. Advances in Glomeromycota taxonomy and classification. *IMA Fungus* 2, 191–199.
- Oksanen, J., Blanchet, F.G., Kindt, R., Legendre, P., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Wagner, H., 2013. *vegan: Community Ecology Package*. R Package Version 2.0-10. <http://CRAN.R-project.org/package=vegan>.
- Peigne, J., Ball, B.C., Roger-Estrade, J., David, C., 2007. Is conservation tillage suitable for organic farming? A review. *Soil Use and Management* 23, 129–144.
- R Core Team, 2014. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria.
- Rillig, M.C., Mummey, D.L., 2006. Mycorrhizas and soil structure. *New Phytologist* 171, 41–53.
- Sans, F.X., Berner, A., Armengot, L., Maeder, P., 2011. Tillage effects on weed communities in an organic winter wheat-sunflower-spelt cropping sequence. *Weed Research* 51, 413–421.
- Schalamuk, S., Velazquez, S., Chidichimo, H., Cabello, M., 2006. Fungal spore diversity of arbuscular mycorrhizal fungi associated with spring wheat: effects of tillage. *Mycologia* 98, 16–22.
- Schenck, N.C., Pérez, Y., 1990. *Manual for the Identification of VA Mycorrhizal Fungi*, third ed. Synergistic Publications, Gainesville, FL, USA.
- Sieverding, E., 1991. Vesicular-arbuscular mycorrhiza management in tropical agroecosystems. In: Smith, S.E., Read, D. (Eds.), *Mycorrhizal Symbiosis*. Academic Press, Oxford.
- Sieverding, E., Silva, G.A., Berndt, R., Oehl, F., 2014. *Rhizoglossus*, a new genus in the Glomeraceae. *Mycotaxon* 129 (2), 373–386.
- Smith, S.E., Read, D., 2008. *Mycorrhizal Symbiosis*. Academic Press, Oxford.
- Soane, B.D., Ball, B.C., Arvidsson, J., Basch, G., Moreno, F., Roger-Estrade, J., 2012. No-till in northern, western and south-western Europe: a review of problems and opportunities for crop production and the environment. *Soil and Tillage Research* 118, 66–87.
- Ter Braak, C., 1986. Canonical correspondence analysis: a new eigenvector technique for multivariate direct gradient analysis. *Ecology* 67, 1167–1179.
- Triplett, G.B., Dick, W.A., 2008. No-tillage crop production: a revolution in agriculture! *Agronomy Journal* 100, S153–S165.
- van der Heijden, M.G.A., Klironomos, J.N., Ursic, M., Moutoglou, P., Streitwolf-Engel, R., Boller, T., Wiemken, A., Sanders, I.R., 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* 396, 69–72.
- van der Heijden, M.G.A., Martin, F., Selosse, M.A., Sanders, I.R., 2015. Mycorrhizal ecology and Evolution: the past, the present and the future. *New Phytologist* 205, 1406–1423.

- Verbruggen, E., van der Heijden, M.G.A., Weedon, J.T., Kowalchuk, G.A., Rling, W.F.M., 2012. Community assembly, species richness and nestedness of arbuscular mycorrhizal fungi in agricultural soils. *Molecular Ecology* 21, 2341–2353.
- Wetzel, K., Silva, G., Matczinski, U., Oehl, F., Fester, T., 2014. Superior differentiation of arbuscular mycorrhizal fungal communities from till and no-till plots by morphological spore identification when compared to T-RFLP. *Soil Biology & Biochemistry* 72, 88–96.
- Wezel, A., Casagrande, M., Celette, F., Vian, J.F., Ferrer, A., Peigne, J., 2014. Agro-ecological practices for sustainable agriculture. A review. *Agronomy for Sustainable Development* 34, 1–20.
- Yang, A.N., Hu, J.L., Lin, X.G., Zhu, A.N., Wang, J.H., Dai, J., Wong, M.H., 2012. Arbuscular mycorrhizal fungal community structure and diversity in response to 3-year conservation tillage management in a sandy loam soil in North China. *Journal of Soils and Sediments* 12, 835–843.