Contrasting effects of cover crops on 'hot spot' arbuscular mycorrhizal fungal communities in organic tomato

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Abstract

Arbuscular mycorrhizal fungal (AMF) communities are fundamental in organic cropping systems where they provide essential agroecosystem services, improving soil fertility and sustaining crop production. They are affected by agronomic practices, but still scanty information is available about the role of specific crops, crop rotations and the use of winter cover crops on the AMF community compositions at the field sites. A field experiment was conducted to elucidate the role of diversified cover crops and AMF inoculation on AMF diversity in organic tomato. Tomato, pre-inoculated at nursery with two AMF isolates, was grown following four cover crop treatments: Indian mustard, hairy vetch, a mixture of seven species and a fallow. Tomato root colonization at flowering was more affected by AMF pre-transplant inoculation than by the cover crop treatments. An enormous species richness was found by morphological spore identification: 58 AMF species belonging to 14 genera, with 46 and 53 species retrieved at the end of cover crop cycle and at tomato harvest, respectively. At both sampling times AMF spore abundance was highest in hairy vetch, but after tomato harvest AMF species richness and diversity were lower in hairy vetch than in the cover crop mixture and in the mustard treatments. A higher AMF diversity was found at tomato harvest, compared with the end of the cover crop cycle, independent of the cover crop and pre-transplant AMF inoculation. Our findings suggest that seasonal and environmental factors play a major role on AMF abundance and diversity than short-term agronomic practices, including AMF inoculation. The huge AMF diversity is explained by the field history and the Mediterranean environment, where species characteristic of temperate and subtropical climates co-occur.

Key words: arbuscular mycorrhizal fungal spores; agricultural systems; Glomeromycota; species diversity; AMF diversity hot-spot, biosphere reserves

Introduction

Understanding of diversity and community composition of arbuscular mycorrhizal fungi (AMF) is a necessary prerequisite towards their effective utilization in improving biological soil fertility and crop

production (Jansa et al. 2002, Liu et al. 2014), and might be particularly relevant for organic systems (Oehl et al. 2004). Presently, the role of AMF in influencing soil fertility, crop productivity, yield quality and protection against environmental stresses is widely acknowledged (van der Heijden et al. 1998; Smith and Read 2008; Giovannetti et al. 2012; Berta et al. 2013). AMF contribution to crop growth and productivity can be influenced by both interspecific and intraspecific differences (Munkvold et al. 2004; Vogelsang et al. 2006). Consequently, through sampling or niche complementarity effects, increased AMF diversity may provide agroecological services directly affecting crop production (van der Heijden et al. 2008).

To increase the number of AMF propagules, the AMF root colonization in the field and crop productivity in sustainable agriculture, different approaches are applied, such as use of diverse rotations incorporating mycotrophic crops, cover crops (Higo et al. 2014), and inoculation with exotic AMF isolates (Jeffries et al. 2003). Mycotrophic crops, such as wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.) have been previously reported to increase indigenous AMF propagules, root colonization and growth of the following crop, in contrast to non mycorrhizal crops, such as oilseed rape (*Brassica napus* L.) (Koide and Peoples 2012; Monreal et al. 2011). Karasawa et al. (2001), while comparing mycorrhizal sunflower (*Helianthus annuus* L.) with non mycorrhizal mustard (*Brassica alba* Boiss.) in 17 different soils, unequivocally demonstrated the significance of the mycorrhizal status of the previous crop in influencing the colonization of the subsequent maize crop.

Cover crops reduce seasonal fallow periods, increase diversity in the rotation, improve soil fertility through stimulation of biogeochemical cycles, and suppress weeds (Clark 2007). Additionally, cover crops are hosts to beneficial soil biota including AMF, thus augmenting soil mycorrhizal propagules (Lehman et al. 2012) and colonization of the following crops (Karasawa and Takebe 2012). However, inconsistent results in root colonization have been observed, when AMF non-host cover crops, especially species of *Brassicaceae*, are used (Hill 2006; White and Weil 2010). Although cover crops are known to affect root colonization of the subsequent crop and soil mycorrhizal propagules in organic agroecosystems (Njeru et al. 2013), little is known about their effect on AMF biodiversity. Such information is vital for conservation and better utilization of AMF

especially in organic agroecosystems, where combination of different levels of agrobiodiversity (i.e., genetic, species and habitat biodiversity) play a crucial role (Costanzo and Bàrberi 2013).

Inoculation with exotic AMF constitutes one of the major agronomic practices targeting improvement of AMF symbiosis in sustainable agriculture, having been applied since the 1970s (Mosse 1973; Giovannetti and Avio 2002). Numerous studies have reported positive effects of AMF inoculation on crop production both in pot and field experiments, and especially when indigenous AMF populations are low or not sufficiently infective (Conversa et al. 2013; Douds et al. 2007; Wagg et al. 2011). Whilst the introduced strains are generally considered 'symbiotically superior' (more infective and efficient than the native strains) some studies have reported neutral or even negative results following inoculation, which could be attributed to introduction of less competitive AMF isolates compared to native ones (Garland et al. 2011; Muok et al. 2009). On the other hand, agricultural practices such as intensive fertilizer and pesticide use, frequent tillage or continuous cropping, including crop genotypes which are less susceptible to AMF, may hinder the establishment and effectiveness of the introduced strains (Douds and Millner 1999).

Notwithstanding the increasing practice of AMF inoculation and the rising global trade of AMF commercial inocula, it is still unclear how the introduction of exotic AMF strains either directly in the field or indirectly via transplanting of pre-inoculated plants affects the native AMF diversity and community composition. This may have great ecological consequences, leading to introduction of invasive strains that are deleterious to native AMF biodiversity (Janoušková et al. 2013; Schwartz et al. 2006). Knowledge of how native AMF communities in organic systems are affected by common agronomic practices including cover crop and crop management is a crucial step towards effective utilization of AMF in sustainable crop production.

Biodiversity of AMF can be investigated by morphological spores analyses from the soils or by molecular analyses after DNA extraction from the roots or soils. Both methods have their advantages and disadvantages (Oehl et al. 2010). In the past, major disadvantages for morphological identifications might have been the lack of identification manuals with coloured illustrations, which now are available (e.g. Błaszkowski 2012). Furthermore, spores of different degradation stages can be

found in field samples making the identification sometimes difficult to impossible even for experienced experts, but same would apply also for molecular analyses, when spores, vesicles and hyphae have become empty without any cell contents and any DNA left for the amplification steps. Remarkably, it was repeteadly shown recently that classical spore identification might be even superior to molecular identification for AMF diversity studies (e.g. compare Oehl el al. 2004; 2005 with Hijri et al. 2006; Wetzel et al. 2014).

In the present study, we hypothesized that agronomic practices including diversified winter cover crops and pre-transplant AMF inoculation affect indigenous AMF spore abundance and diversity and root colonization of a subsequent organically managed tomato crop. For this experiment we selected a special environment, a previous grassland fallow field site that had not been cultivated for the previous six years, where we expected that a diverse and strong AMF population had established. Using classical morphological spore identification, the main objectives of this study were: i) to investigate the diversity of indigenous AMF communities following the growth of three winter cover crops differing in species diversity and a no-cover crop fallow; ii) to assess the dynamics of AMF populations following the growth of tomato preceded by the diversified cover crop regime and pre-inoculated with exotic AMF.

Materials and methods

Experimental site and design

This experiment was conducted at CIRAA (Interdepartmental Centre for Agri-environmental Research) "Enrico Avanzi", University of Pisa located at S. Piero a Grado, Pisa, Italy (latitude 43°40' N, longitude 10°19' E), within the UNESCO Man and Biosphere Reserve denominated "Selva Pisana" (http://www.unesco.org/mabdb/br/brdir/directory/biores.asp?code=ITA+08&mode=all). Since 1974, the field site was periodically used for crop production (i.e. maize in 1974-1978, different horticultural crops in 1980-1987, and durum wheat in 1998), grown for several years with perennial alfalfa (1999-2005) and periodically was also an uncultivated, temporary grassland fallow, especially for the last six years preceding the establishment of the field experiment (2006-2011). The climatic

conditions are typical of Mediterranean areas, with rainfall mostly concentrated in autumn (October to December) and spring (March to April). Soil physical and chemical soil characteristics at the experimental site were: clay 11.5%, silt 17.0%, sand 71.5%, pH (H_2O) 6.5, organic C 2.2 %, total N 1.5 g kg^{-1}, and P (Olsen) 4.0 mg kg^{-1}. This experiment was part of the trials conducted under the EU-RTD FP7 funded project SOLIBAM (Strategies for Organic and Low-input Integrated Breeding and Management, 2010-2014), which aimed at investigating the role of species and genetic diversity in promoting mycorrhizal symbiosis, growth and productivity of organic tomato. The trial was laid out in a split-plot design with three blocks serving as replicates. Main plots included four soil cover crop treatments, namely *Brassica juncea* (L.) Czern. cv. ISCI 20 (Indian mustard), *Vicia villosa* Roth cv. Latigo (hairy vetch), a mix of seven species (hereafter, Mix 7) and a no-cover fallow with natural vegetation (Control). The Mix 7 treatment, supplied as a commercial mixture by Arcoiris s.r.l. (Modena, Italy), included *Fagopyrum esculentum* Moench (buckwheat), *Lupinus albus* L. (white lupin), *Phacelia tanacetifolia* Benth. (lacy phacelia), *Pisum sativum* L. (common pea), *Trifolium alexandrinum* L. (berseem clover), *Trifolium incarnatum* L. (crimson clover) and *V. villosa*. The sub-plot factor was AMF inoculation, with two treatments: tomato plantlets grown in substrate inoculated with i) a mix (1:1 vol/vol) of *Funneliformis mosseae* (T.H. Nicolson & Gerd.) C. Walker & A. Schüssler, isolate IMA1 from UK (Collector B. Mosse) and *Glomus intraradices* N.C. Schenck & G.S. Sm., isolate IMA6 from France (Collector V. Gianinazzi-Pearson), or ii) uninoculated control (Mock).

Cover crop management

Cover crops were sown on 19 October 2011 at the rate of 12 kg ha^{-1} (*B. juncea)*, 100 kg ha^{-1} (*V. villosa*), 65 kg ha^{-1} (Mix 7). Weeds were not controlled in any cover crop treatments. Aboveground biomass was sampled on 26 April 2012, separated into cover crop and weed biomass and oven dried at 80 °C until constant weight. Total above-ground biomass was (t ha^{-1}): *V. villosa*, 5.6, Mix 7, 12.1, *B. juncea*, 7.4 and Control, 8.0. Weeds represented 11.8, 17.4, 32.0 and 100% of total above-ground biomass, respectively. The cover crops and weeds were then mown and their residues incorporated

into the soil as green manure by disc harrowing. Seeding beds were then raised and black plastic mulch film and drip irrigation tapes were laid onto the soil.

AM fungal material and inoculation

The AMF species isolates were obtained from pot cultures maintained in the collection of the Soil Microbiology Laboratory of the Department of Food, Agriculture and Environment University of Pisa, Italy. Pots containing a mixture (1:1 by volume) of soil and Terragreen (calcinated clay, OILDRI Chicago, IL, USA) were inoculated with a crude inoculum (500 mL) containing mycorrhizal roots, spores and extraradical mycelium. Mixed seeds of *T. alexandrinum* cv. Tigri, *Medicago sativa* L. cv. Messe and *Plantago lanceolata* L. were surface sterilized, sown in the pots and maintained for six months. At harvest, the shoots were excised and discarded whilst the substrate and roots cut in ca. 1 cm fragments were mixed to form a homogenous crude inoculum mixture, to be used for tomato inoculation. An aliquot of crude inoculum was steam-sterilised to be used as control (Mock).

Tomato seedling inoculation

Tomato (*Solanum lycopersicum* L. cv. Rio Grande) plantlets were grown on turf substrate (Hochmoor Hortus, TERFLOR Capriolo BS, Italy) mixed with crude inoculum (20% by volume). All the plantlets were also supplied with a filtrate obtained by sieving an aliquot of living mixed inocula through a 40 µm sieve to provide the substrate with an equivalent soil microbiota. The plantlets were maintained in the nursery for 40 days and sprayed twice with fertilizer (9:15:30 NPK + 30:10:10 NPK including B, Cu, Fe, Mn and Zn) at the rate of 112.5 g L^{-1}.

Transplanting and field management

Tomato plantlets were transplanted in the field on 30 May 2012, when they had four to five true leaves. Before transplanting, three tomato seedlings from each treatment were uprooted and examined for mycorrhizal colonization by gridline intersect counts (Giovannetti and Mosse 1980) after clearing with 10% KOH and staining with 0.05% trypan blue in lactic acid. Transplanting was done manually

182 at a spacing of 1.5 × 0.5 m (between- and within-row distance, respectively). The tomato plants were
183 watered through drip irrigation and maintained in the field until maturity under standard conditions of
184 organic farming.
185
186 **Soil sampling**
187 Soil samples were taken at the end of the cover crop cycle before incorporation into the soil (10 April
188 2012) and at tomato harvest (20 September 2012). At each sampling date four soil cores were
189 obtained from each sub-plot at a depth of 20 cm and mixed to form a homogenous composite sample.
190 The soil samples were air dried and stored on sealed bags for spore extraction and analysis. Spore
191 extraction and identification was conducted at Agroscope, Institute for Sustainability Sciences, in
192 Zürich-Reckenholz, Switzerland.
193
194 **Mycorrhizal colonization at flowering and harvest**
195 To determine AMF colonization at flowering (17 July 2012) and harvest, root samples were obtained
196 from four randomly selected plants sub-plot^{-1} and stained with 0.05% trypan blue. The percentage root
197 colonization was determined using the gridline intersect method (Giovannetti and Mosse 1980).
198
199 **AMF spore recovery, enumeration and identification**
200 Spore extraction and identification was conducted at Agroscope using the methodology of Sieverding
201 (1991). Spores were isolated from two 25 g of air-dried sub-samples per field plot soil sample. Spores
202 were extracted by wet sieving with tap water through nested sieves (500, 125 and 32 μm mesh size).
203 After sieving, the material obtained from the 125 and 32 μm sieves was transferred to five 50 mL
204 vials sample^{-1} constituting five 25 mL suspensions. The suspensions were under-layered with 25 mL
205 of a 70% wt/vol sucrose solution, and the water/sucrose solution density gradient was centrifuged at
206 2000 rpm for 2 minutes. In the material from the 500 μm sieve no spores or sporocarps were
207 observed. After centrifugation, the supernatant was passed through a 32 μm sieve and washed with tap
208 water. The trapped material, largely containing spores, spore clusters, and sporocarps, was flushed

into 9 cm diameter Petri dishes. The spores were then quantified in Petri dishes with a gridline of 1 cm^2 under an Olympus SZ12 dissecting microscope at up to 90× magnification. For taxonomic identification, the spores were mounted on glass slides and fixed with polyvinyl-lactic acid : glycerol (1:1 vol/vol) (Koske and Tessier 1983). The spores were examined under a compound microscope at up to 400× and identified to species level using all original species descriptions, updated taxonomic studies on the described taxa, and available identification manuals (Błaszkowski 2012; Oehl et al. 2011a, b; Schenck and Pérez 1990). The identified spores were enumerated to determine the community parameters as defined in Table 1. To increase the robustness of results, we performed spore extraction twice for each soil sample. Specimens (spores mounted on slides) of all AMF species identified have been continuously deposited at the mycological herbarium Z+ZT (ETH Zurich) in Zurich. Also, illustrations of the AMF species maintained in living cultures are presented at the continuously updated homepage of the Swiss collection for arbuscular mycorrhizal fungi (SAF; http://www.agroscope.admin.ch/grandes-cultures-systemes-pastoraux/05911/07581/index.html).

Data analyses

Root colonization data were analyzed by two-way ANOVA in a split-plot design with cover crop as the main factor and mycorrhizal inoculation as the sub-plot factor. Spore abundance and AMF community parameters were either analyzed by one-way ANOVA at the end of cover crop cycle (with cover crop as the only factor) or two-way ANOVA at tomato harvest. Moreover, spore abundance and AMF species composition dynamics at the end of cover crop and tomato cycles were analyzed by one-way ANOVA with repeated measures. In addition, rank-abundance plots (Magurran 2004) and species accumulation curves were used to compare AMF community structure and species richness at the end of cover crop and at tomato harvest cycles. Before analyses, data on root colonization were arcsine transformed, while spore abundance data were $\log(x+1)$ transformed to fulfil the assumptions of ANOVA. The data reported in tables and figures are back transformed values. Wherever necessary, Tukey's HSD post hoc comparison was done to test for pairwise mean differences at $P=0.05$. All ANOVA were performed by using SPSS (version 19 software). Redundancy analysis (RDA) was

performed on Hellinger-transformed AMF spore data (Legendre and Gallagher 2001) in order to assess whether AMF community structure was related to the experimental factors. The effects of cover crops and AMF inoculation on AMF community composition were assessed by Montecarlo permutation test. Only species with abundance >1% were included in multivariate analyses. Species accumulation curves and RDA were calculated using the *vegan* package in R version 3.1.0 (R Development Core Team 2013).

Results

Tomato mycorrhizal colonization

At transplant, the pre-inoculated plantlets had an average of 17.5% root colonization while no colonization was observed in the uninoculated plants (Mock). Root colonization percentage at flowering was significantly affected by pre-transplant fungal inoculation ($F_{1,8}$=59.7, P<0.001), but not by cover crop or AMF × cover crop interaction. At harvest, a marginal, non-significant effect of AMF pre-transplant inoculation was still observed, while the cover crop and AMF × cover crop interaction was not significant (Table 2).

AMF spore abundance

At the end of cover crop cycle the fungal spore abundance differed significantly ($F_{3,18}$ = 47.94, P< 0.001) among cover crop treatments. *Vicea villosa* showed the highest spore abundance (14.2±0.9 g^{-1} soil), while *B. juncea* and Control treatments had the lowest densities (Fig. 1). At tomato harvest, the spore abundance was similarly affected by the cover crop treatment ($F_{3,14}$ = 14.47, P< 0.001) but not by the mycorrhizal treatment ($F_{1,14}$ = 2.04, P = 0.175) or mycorrhizal × cover crop interaction. Again, a higher spore abundance was produced after *V. villosa* (18.6±2.7 g^{-1} soil) compared to other treatments (Fig. 1). One-way ANOVA with repeated measures showed that spore abundance was significantly ($F_{1,32}$ = 12.57, P = 0.001) higher at tomato harvest than at the end of the cover crop cycle. However, this increase was not affected by cover crop treatments, since we did not observe a significant interaction between time and cover treatment ($F_{3,32}$ = 0.94, P = 0.431).

AMF species richness and diversity at the end of cover crop

At the end of the cover crop cycle we detected a total of 46 AMF species, belonging to 14 genera of Glomeromycota, with an average of 34 to 40 species in the different cover crop treatments (Table 3). The most common genera were *Glomus* and *Funneliformis* which accounted for 27.8% and 25.0% of the identified spores, respectively. Five species, *Funneliformis geosporus*, *F. mosseae*, *Glomus badium*, and *Septoglomus constrictum* (all in Glomeraceae family), and *Claroideoglomus luteum* were the most evenly distributed across the whole field, with their spores being consistently detected in all soil samples (IF=100%). Similarly, the four aforementioned species of Glomeraceae were the most abundant with the highest spore densities, as determined by relative abundance (RA) and relative abundance index (RAI), in the order *F. geosporus* > *S. constrictum* > *G. badium* > *F. mosseae* (Table 4). The rarest species were *Diversispora przelewicensis*, *Funneliformis fragilistratus* and *Scutellospora aurigloba* which were only detected each in one soil sample.

Species richness did not differ among cover crop treatments (Table 3). There were three species, *F. fragilistratus*, *Gigaspora rosea* and *S. aurigloba* which were only detected in soil samples from the Mix 7 cover crop (Fig. 2a, Table 4). Two species, *Acaulospora laevis* and *D. przelewicensis* were restricted to soil samples from the *V. villosa* cover crop, while *Acaulospora longula* was only detected in *B. juncea* plots (Fig. 2a, Table 4). Further statistical analyses of the Shannon-Wiener diversity index (*H´*) and Simpson's index of dominance (*D*) and evenness (*E*) showed that cover crops did not discernibly affect the structure of AMF communities. Actually, *H´* ranged from 2.60 to 2.76, *D* from 0.90 to 0.91, while *E* was 0.62 to 0.70 in all cover crop treatments. Likewise, the *H´*, *D* and *E* of AMF inoculated and uninoculated plots did not differ statistically. RDA analysis did not reveal any effect of either cover crop treatment or AMF inoculation on AMF community composition ($F_{4,23}$=0.037, P=0.45).

AMF species richness and diversity at tomato harvest

At tomato harvest, we identified a higher AMF species richness (53) than at the end of the cover crop cycle. As in the previous sampling, the *Glomus* genus had the highest AMF species richness, accounting for 22.6% of the identified spores (Table 5). Other genera with a high number of spores identified included: *Funneliformis* (18.8%), *Paraglomus* (11.1%) and *Septoglomus* (10.4%). The most evenly distributed species were *Archaeospora trappei, C. luteum, F. geosporus, F. mosseae, G. badium, Paraglomus* sp. PI5 resembling *Paraglomus majewskii* and *S. constrictum* which were detected in all soil samples (IF= 100%). Based on RAI, *F. geosporus* and *S. constrictum* were the most frequent and abundant, accounting for the highest number (22.3%) of spores identified (Table 6).

Cover crops had no significant effect on AMF species richness. However, we detected the highest species richness (48) in *B. juncea* where five rare species (*Acaulospora spinosa, Archaeospora myriocarpa, Diversispora celata, F. fragilistratus,* and *Funneliformis monosporus*) only present in this treatment were detected (Fig. 2b, Table 6). Although pre-transplant AMF inoculation did not significantly affect AMF species richness, we observed higher numbers of species in the inoculated plots compared to the uninoculated ones (51 *vs* 44 species; Table 6). AMF community composition was similar across all the cover crops and mycorrhizal treatments since ANOVA did not show any significant differences in *H´, D* and *E.* RDA analysis did not reveal any effects of cover crop and AMF inoculation treatments on AMF community composition ($F_{4,23}$=0.78, P=0.72).

AMF community dynamics

Overall, we recovered an extremely high AMF species richness (58): 41 species occurred at both sampling times, 12 species only at tomato harvest and 5 species at the end of cover crop. Most of the species that emerged or disappeared were rare (RA<1%) except *Racocetra* sp. PI7 resembling *Ra. coralloidea* (RA=2.6%), which was interestingly recovered at tomato harvest across all treatments but not at the end of the cover crop cycle. Moreover, two species (*Paraglomus occultum* and *Paraglomus*

sp. PI5 resembling *P. majewskii*) had the greatest increase in RA at tomato harvest, compared to the end of cover crop cycle (4.7 and 3.5, respectively).

The structure of AMF community was relatively similar across cover crop treatments at the end of the cover crop cycle (Fig. 3). However, at tomato harvest one species (*F. geosporus*) became more dominant in *V. villosa* compared to other treatments, although the general AMF community structure in the other cover crop treatments remained unchanged (Fig. 3). The greatest percent rise (33.3%) of species richness was observed in *B. juncea* where 13 new species were recovered at tomato harvest while only 1 species (*Glomus clarum*) disappeared. Moreover, there was a strong positive correlation ($r=0.876$, $P<0.001$) between AMF species abundance at the end of cover crop cycle and at tomato harvest.

The mean species richness retrieved in the tomato field was affected by a significant time x cover crop interaction ($F_{3,32} = 3.66$, $P = 0.023$): more species were recovered after *B. juncea* and Mix 7 (ca. 29 species on average) compared to *V. villosa* and Control (24 species on average) (Table 5). Moreover, at tomato harvest there was a larger increase in species richness with increase in the number of samples in *B. juncea* and Mix 7 cover crops as showed by species accumulation curves (Fig. 4). Analysis of Shannon-Wiener diversity index values indicated a higher AMF diversity ($F_{1,32} = 32.06$, $P < 0.001$) at tomato harvest ($H' = 2.89 \pm 0.04$) than at the end of cover crop cycle, ($H' = 2.70 \pm 0.03$). This increase in diversity was not affected by the cover crop treatment since we did not detect any cover crop x time interaction ($F_{3,32} = 2.39$, $P = 0.087$). Similarly, an effect of time was observed in Simpson's index of dominance values ($F_{1,32} = 9.00$, $P = 0.005$), although there was no significant cover x time interaction. Species evenness did not significantly differ between the two sampling times.

Discussion

AMF species richness, community composition and diversity at field site

Remarkably, 58 AMF species were detected belonging to 14 AMF genera at our single experimental site integrating two sampling dates. This presents the location as a global 'hot-spot' of AMF species

richness. To our knowledge, so far a similar high AMF species richness has never been reported from a single site, and rarely from a well defined region covering several to multiple field sites (Bever et al. 2001; Tchabi et al. 2008). Bever et al. (2001) reported 44 AMF species after multiple years of isolation from the field and additional intensive propagation of AMF in greenhouse pot cultures. We assume that the specific environment combined with the favourable historic land use (see Material and Methods) was decisive for the high species richness found at our study site. We observed several species either adapted to warmer climates (e.g. *Gigaspora gigantea* and *Racocetra fulgida*) or colder climates (e.g. *Cetraspora armeniaca*, *Glomus aureum* and *G. badium*) with sometimes high abundances, while others were so far only recovered from Mediterranean environments (e.g. *Ambispora granatensis* and *Diversispora clara*). On the other hand, species were found that preferably occur either in cultivated (e.g. *F. mosseae*), reduced tillage or undisturbed (e.g. *G. aureum* and *G. badium*) field sites, which well reflects the variable and often clearly extensive land use during the last 40 years. The slightly acidic soil pH is also favourable to optimal for many AMF species and genera (Oehl et al. 2010). In other AMF diversity studies, recently performed in European agro-ecosystems, significantly less AMF species were detected, especially in high-input (e.g. Oehl et al. 2005; Wetzel et al. 2014), but also in low-input, organic or no-tillage farming systems (e.g. Jansa et al. 2002, 2003; Oehl et al. 2004; Maurer et al. 2014). In intensive agricultural systems, lower AMF diversity (e.g. Oehl et al. 2004) might go along with lower root colonization rates (e.g. Mäder et al. 2000), lower extraradical AMF hyphal density and lower nitrogen and phosphorus uptake efficiencies (e.g. Liu et al. 2014). Despite the high species richness and diversity, our results may still be an underestimation since only healthy looking and intact spores were identified and did not consider fungal species that may not have sporulated at the two sampling times in 2011. Above all small-spored species might be difficult to identify by classical spore morphology (Błaszkowski et al. 2010). We are also aware that by sampling at a depth of 0-20 cm we could have missed an important part of sub-soil AMF spore diversity (Oehl et al. 2005). In the later study, however, AMF species that exclusively occurred in the sub-soils, were especially found in the more intensive agricultural systems, while we found all those 'sub-soil' AMF species , e.g. *R. castanea*, *G. invermaium* (Oehl et

368 al. 2005), also present in our topsoils (Table 4, 6). Thus, in our ' hot spot' field site, we consider the
369 AMF biodiversity of the top soil as the most essential for tomato growth, . Moreover, the diversity of
370 AMF communities in the surrounding soil and of those colonizing roots may differ as previously
371 demonstrated in the field and in trap plants (Cesaro et al. 2008; Avio et al. 2013).
372
373 **AMF inoculation and root colonization**
374 In our study, pre-transplant inoculation increased tomato root colonization in the field despite the
375 presence of a rich indigenous AMF biodiversity. This difference was more enhanced at tomato
376 flowering than at harvest when only marginal differences between AMF treatments were detected.
377 Increased root colonization at flowering is particularly important since plants are more
378 physiologically active at this stage, requiring additional nutrients which can be provided by
379 mycorrhizal symbiosis. These results confirm recent reports suggesting increased early root
380 colonization of field grown tomato following pre-inoculation contributing to plant nutrition, growth
381 and yield (Conversa et al. 2013). Although cover crops did not significantly affect root colonization,
382 tomato crop grown after *V. villosa* showed higher root colonization at flowering compared to *B.*
383 *juncea*. While *V. villosa* is a highly mycorrhizal legume, *B. juncea* is a non-host species which can
384 additionally release mycotoxic compounds (usually isothiocyanates, ITC) after the disruption of
385 *Brassica* tissues, that are likely to be deleterious to indigenous AMF populations (Njeru et al. 2013).
386 However, the negative effect of ITC on AMF diminishes gradually, as described previously (Gimsing
387 and Kirkegaard 2006), a probable reason why AMF colonization in *B. juncea* treatment at tomato
388 harvest was similar to the other cover crop treatments. Besides this, tomato crop is a good mycorrhizal
389 host, which is likely to have improved AMF propagules abundance by harvest time across all the
390 treatments.
391
392 **AMF species richness, community composition and diversity in different treatments**
393 At both sampling times, spore abundance was higher in *V. villosa* than in the other cover crops. As a
394 leguminous AMF host crop, *V. villosa* may have been more supportive to AMF during winter,

promoting more rapid sporulation. After incorporation into the soil as green manure, the cover crop is likely to have enhanced tomato growth (Campiglia et al. 2010), indirectly promoting mycorrhizal symbiosis. Our findings confirm previous reports (Galvez et al. 1995) that, as a winter cover crop, *V. villosa* may enhance AMF spore population. In a closely related study (Mathimaran et al. 2005), a higher spore abundance was observed after *P. tanacetifolia* cover crop compared to rapeseed. *B. juncea* and Control plots had the lowest spore abundance after cover crop, possibly because *B. juncea* is a non-mycorrhizal plant, while the Control treatment had both host and non-host weeds. However, since we did not control weeds across all the cover crop treatments, weeds in the *B. juncea* plots of which majority were AMF hosts are likely to have sustained fungal activity during the winter period. At tomato harvest, a higher AMF spore abundance was detected compared to the end of cover crop, which probably was due to seasonal changes. Obviously, while the cover crop cycle (October 2011 to April 2012) was predominantly cold, the tomato cycle (June to September 2012) was by contrast warm, thereby promoting AMF growth and sporulation especially of gigasporalean species which generally are seasonal and prefer warm seasons and climates (Oehl et al. 2009). Moreover, the growth of tomato -a mycorrhizal host- under relatively warm (about 30 °C) and moist conditions as maintained by drip irrigation could have enhanced AMF sporulation by increasing spore abundance, as detected at tomato harvest.

Interestingly, *V. villosa* consistently enhanced spore abundance at the end of cover crop and tomato harvest, yet the same cover crop did not increase AMF species richness and diversity. By contrast, it enhanced the dominance of a few AMF species, especially *F. geosporus* which probably suppressed the emergence of other AMF species. On the other hand, we detected increased AMF species richness in *B. juncea* and Mix 7 plots at tomato harvest. Especially the increase of species richness in *B. juncea* was surprising. However, it is possible that its growth might have been stressful for the AMF communities stimulating the activity and sporulation of multiple indigenous AMF species associating more strongly with subsequent tomato. Another possibility is that the patchiness of weed distribution within *B. juncea* could have led to sporulation of a more diverse AMF community in *Brassica* plots. Overall, these findings demonstrate that increased AMF colonization and even

spore abundance may not be good indicators of increased AMF diversity. Moreover, it is unclear how AMF colonization, spore abundance and also AMF diversity are linked with functionality. Therefore, to enhance AMF diversity and promote delivery of more agroecosystem services more studies on AMF diversity as affected by different cover crops (both hosts and non hosts) and their management are imperative. This 'hot-spot' field site for AMF diversity might serve as an excellent playground for such future studies.

Pre-transplant AMF inoculation did not affect spore abundance, species richness and diversity of fungal communities. While organic agriculture is known to favour AMF abundance and diversity (Bedini et al. 2013; Oehl et al. 2004), our field, which had been extensively used in the last 40 years, and uncultivated in the last 6 years, dominated by grass-rich natural vegetation, is likely to have a higher AMF species composition and diversity owing to its undisturbed recent history. Previously, higher AMF species richness and diversity have been reported in grasslands as compared to arable land (Oehl et al. 2005, 2010). However, we cannot rule out that the establishment of the exotic strains may have occurred, since we only used spore morphology for fungal identification. Actually, direct field inoculation with a mixture of *F. mosseae* and *G. intraradices* has been suggested to affect the diversity of AMF found in the roots of watermelon (Omirou et al. 2013).

Similarly to spore abundance, AMF diversity and species richness exhibited seasonal variations, since we observed more species and a higher diversity at tomato harvest than at the end of the cover crop cycle. This may be due to agro-climatic conditions (summer) favouring more AMF proliferation and sporulation as well as growth of the tomato crop, which is a host. Seasonal fluctuations favouring increased AMF sporulation during spring-summer compared to autumn-winter were recently described (Sivakumar 2013; Zangaro et al. 2013). There was no significant correlation between spore abundance at the end of cover crop cycle and AMF colonization at tomato flowering, confirming previous reports (D'Souza and Rodrigues 2013; Li et al. 2007). Moreover, root colonization or even delivery of agroecosystem services by fungal communities may not depend on AMF spore abundance (Camargo-Ricalde and Dhillion 2003) and spore production does not necessarily indicate the abundance of AMF communities colonizing roots (Oehl et al. 2005).

From our study, *Glomus* and *Funneliformis* were the most frequently occurring genera both at post cover crop and tomato harvest stages. Previous studies (D'Souza and Rodrigues 2013; Songachan and Kayang 2012; Zangaro et al. 2013) showed more sporulation in *Glomus* and *Acaulospora* species compared to *Gigaspora* and *Scutellospora*, which is often attributed to the small spore size that are more rapidly produced and to the shorter life cycle of the former. In our study we did not observe a relatively high number of spores in the *Acaulospora* compared to the other genera. Among those *C. luteum*, *F. geosporus*, *F. mosseae*, *G. badium*, and *S. constrictum* were those appearing as 'generalists' before and after tomato. Two species (*F. geosporus* and *S. constrictum*) were the most abundant and frequent, consistently maintaining the highest RAI at both sampling times.

Conclusion

Our findings show that a very rich AMF diversity was found in an organic tomato agroecosystem. This was mainly reasoned in the special climatic environment and the diversified, extensive land use history of the study site. Pre-transplant AMF inoculation enhanced AMF colonization without affecting native AMF communities, providing evidence that AMF inoculation does not necessarily have negative ecological consequences. Moreover, cover crops showed interesting results, with *V. villosa* affecting spore abundance and dominance of a particular fungal species (*F. geosporus*), while Mix 7 and *B. juncea* increased AMF species richness. Thus, agronomic practices such as pre-transplant fungal inoculation and the right choice of cover crop ('functional identity', *sensu* Costanzo and Bàrberi 2013) may have a great potential in promoting organic crop productivity via enhanced mycorrhizal symbiosis without negatively affecting the dynamics of native AMF communities. Further work is needed to isolate and functionally characterise the native AMF from such 'hot-spot' diversity site. They could be used as inoculants in production sites depleted with AMF to test their potential for crop growth promotion and for restoration of more diverse AMF communities, especially in organic farming.

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Table 1 Parameters used for assessing AMF community structure at the end of cover crop and tomato cycles.

Ecological parameter assessed	Definitions and formulae
Spore abundance (SA)	Number of spores g^{-1} soil
Species richness (S)	Number of AMF species sporulating in each sample
Isolation frequency (IF)	Percentage of samples that contained a particular AMF species
Relative frequency (RF)	IF of a species expressed as a percentage of the sum of IF of all species
Relative abundance (RA)	The ratio between the number of spores of a particular fungal species to the total number of spores expressed as a percentage
Relative abundance index (RAI)	(RA+RF)/2
Shannon-Wiener index (*H´*)	$-\sum(P_i) \ln (P_i)$[a]
Simpson's index of dominance (*D*)	$1-\sum_{i}[n_i (n_i -1)/N(N-1)]$
Species evenness (*E*)	$E=H´/H_{max}$[b]

[a] $P_i=n_i/N$, where n_i is the number of individuals of species $_i$ while N is the total number of individuals of all species in a sample.

[b] H_{max}= ln S, where S (species richness) is the total number of AMF species identified.

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660 **Table 2** Tomato root colonization (%) at flowering and harvest as

661 influenced by cover crops and pre-transplant inoculation.

	Tomato root colonization (%)	
Cover crop	Flowering	Harvest
V. villosa	41.5 (5.61) a	35.6 (4.06) a
Mix 7	35.0 (2.36) a	34.5 (4.41) a
B. juncea	27.6 (2.98) b	31.6 (3.03) a
Control	37.0 (4.41) a	29.8 (2.46) a
AMF		
IMA1+IMA6	41.6 (2.91) a	37.1 (1.96) a
Mock	29.0 (2.04) b	28.6 (2.50) a
P values of the main factors and interaction		
Cover crop	0.191	0.309
AMF	<0.001	0.079
Cover × AMF	0.184	0.893

662 Values followed by the same letter in a column are not significantly different at

663 P<0.05 (Tukey's HSD test). Values in parentheses are standard error of the means.

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Table 3 Number of AMF species detected at the end of the cover crop cycle from the four cover crop treatments.

Cover crop	*V. villosa*	Mix 7	*B. juncea*	Control	Total species
Acaulosporaceae					
Acaulospora	3	2	3	2	4
Ambisporaceae					
Ambispora	3	4	4	4	4
Archaeosporaceae					
Archaeospora	1	1	1	1	1
Diversporaceae					
Diversispora	2	1	1	1	2
Entrophosporaceae					
Claroideoglomus	3	3	3	3	3
Entrophospora	1	1	1	1	1
Gigasporaceae					
Gigaspora	3	2	3	2	4
Glomeraceae					
Funneliformis	3	4	3	3	4
Glomus	10	11	9	10	12
Septoglomus	1	1	1	1	1
Paraglomeraceae					
Paraglomus	2	2	2	2	2
Racocetraceae					
Cetraspora	1	2	2	1	2
Racocetra	2	2	1	1	2
Scutellosporaceae					
Scutellospora	3	4	2	2	4
Total species richness	38	40	36	34	46
Mean±SE (n=6; P=0.854)	21.8±0.95	23.2±1.38	22.3±1.36	22.0±0.73	

Table 4 Relative spore abundance and relative abundance index (RAI) of AMF species in the four cover treatments at the end of the cover crop cycle.

	AMF inoculated plots				Uninoculated plots				
Cover crop	*V. villosa*	Mix 7	*B. juncea*	Control	*V. villosa*	Mix 7	*B. juncea*	Control	RAI
AMF species									
Acaulospora longula			0.4				0.9		0.26
Acaulospora sieverdingii	0.4	0.4	0.7	0.9	0.3	0.3	0.4	1.3	1.40
Acaulospora laevis	0.7				0.3				0.26
Acaulospora sp. PI2[a]	1.1	0.4			0.3		2.2	1.3	1.05
Ambispora gerdemannii	1.4	0.4	1.4	1.3		1.3	0.9	0.9	1.40
Ambispora granatensis	4.2	0.8	7.9	1.3	11.0	2.3	0.4	1.7	3.59
Ambispora sp. PI3[b]		0.4		0.4		1.6	0.4	1.3	0.91
Ambispora sp. PI4	0.4		1.8	0.4		1.3			1.01
Archaeospora trappei	3.5	2.8	3.6	8.2	3.6	2.9	6.0	2.6	4.08
Cetraspora armeniaca		0.4	1.8	0.4		0.7	0.4	0.4	0.91
Cetraspora pellucida	0.4	0.4	0.4			1.3	1.7		1.01
Claroideoglomus claroideum	1.1	2.8	4.7	3.0	5.8	2.9	2.6	3.9	3.66
Claroideoglomus etunicatum	2.8	1.6	2.9	1.7	1.3	0.7	1.7	1.7	2.76
Claroideoglomus luteum	3.5	10.5	3.6	2.2	4.5	4.6	3.9	6.5	4.67
Diversispora przelewicensis	0.4								0.12
Diversispora versiformis	0.4	0.4	0.4	1.3	1.6	0.3		0.9	1.36
Entrophospora infrequens	1.4	2.0	1.8	0.9	1.3	5.9	4.7	3.5	3.30
Funneliformis coronatus	2.4	0.4	3.3	4.8	3.2	2.0	1.3	2.2	2.81
Funneliformis fragilistratus		0.4							0.12
Funneliformis geosporus	14.3	10.9	15.2	13.4	17.5	19.5	15.5	16.5	9.99
Funneliformis mosseae	7.3	10.5	6.1	8.7	4.5	6.2	7.8	6.1	5.75
Gigaspora decipiens					0.3		0.4	0.9	0.37

Gigaspora gigantea	0.7						0.4		0.35
Gigaspora margarita	2.8	1.6	0.4	0.4	1.0	0.3	2.6	4.8	1.94
Gigaspora rosea						0.7			0.14
Glomus aureum	3.1	5.7	4.0	3.5	1.0	0.7	2.2	3.0	3.35
Glomus badium	12.5	11.3	7.2	6.5	8.1	7.5	7.3	7.8	6.53
Glomus clarum		0.4					0.4	0.4	0.35
Glomus diaphanum	3.5	5.3	1.8	3.9	1.6	1.0	4.3	4.3	3.49
Glomus fasciculatum	0.4					0.3			0.23
Glomus intraradices	5.6	2.4	6.9	8.2	2.6	3.6	3.0	3.0	4.34
Glomus invermaium	3.5	7.3	3.6	1.7	0.7	5.5	7.8	2.2	3.57
Glomus irregulare	0.7	0.8	2.5	1.3	1.3	1.3	2.6		2.34
Glomus macrocarpum	2.4	3.6	3.3	2.2	0.7	5.2	3.9	1.7	3.49
Glomus microcarpum						0.7		0.4	0.26
Glomus sinuosum	0.7		0.4			0.7			0.49
Glomus spinuliferum					0.3			0.4	0.23
Paraglomus occultum	0.7	1.2	0.4	3.5	4.9	2.9	1.3	3.5	3.11
Paraglomus sp. PI5[c]		0.4	0.4	0.9	0.3	0.3	0.4	1.3	0.89
Racocetra fulgida	0.4		1.1	0.4		1.3	1.3		1.12
Racocetra sp. PI6	0.4	0.4							0.23
Scutellospora calospora	4.2	7.3	4.7	8.7	7.4	4.2	5.6	5.2	4.51
Scutellospora dipurpurescens					0.3	0.7			0.26
Scutellospora sp. PI9	0.4	1.2	0.4		1.0	1.3		1.7	1.12
Scutellospora aurigloba						0.3			0.12
Septoglomus constrictum	12.9	5.3	7.2	10.0	13.3	7.8	5.6	8.7	6.74
Total AMF species	34	32	31	28	29	36	32	31	

[a] resembling *Acaulospora dilatata*
[b] resembling *Ambispora reticulata*
[c] resembling *Paraglomus majewskii*

Table 5 Number of AMF species detected at tomato harvest across different cover crop treatments.

	V. villosa	Mix 7	*B. juncea*	Control	Sum of species
Acaulosporaceae					
Acaulospora	5	5	5	4	7
Ambisporaceae					
Ambispora	4	4	4	4	4
Archaeosporaceae					
Archaeospora	1	1	2	1	2
Diversporaceae					
Diversipora	2	2	2		3
Entrophosporaceae					
Claroideoglomus	3	3	3	3	3
Entrophospora	1	1	1	1	1
Gigasporaceae					
Gigaspora	2	3	3	4	4
Glomeraceae					
Funneliformis	3	3	5	3	5
Glomus	9	8	8	7	9
Septoglomus	1	1	1	1	1
Paraglomeraceae					
Paraglomus	2	3	3	2	3
Racocetraceae					
Cetraspora		2	2	1	2
Racocetra	2	3	3	3	3
Scutellosporaceae					
Scutellospora	3	4	6	4	6
Total species richness	38	43	48	38	53
Mean±SE (n=6; P=0.133)	24.8± 0.91	28.7± 0.99	29.7± 1.58	24.2± 1.14	

Table 6 Relative spore abundance and relative abundance index (RAI) of AMF species in mycorrhizal and cover crop treatments at tomato harvest.

	AMF inoculated plots				Uninoculated plots				
Cover crop	*V. villosa*	Mix 7	*B. juncea*	Control	*V. villosa*	Mix 7	*B. juncea*	Control	RAI
AMF species									
Acaulospora longula	0.4	0.7		0.5	0.5	1.0	1.1	0.6	1.23
Acaulospora paulinae		0.4				0.3	0.2		0.37
Acaulospora sieverdingii	0.9	1.3	1.0	1.8	0.2	3.2	1.5	2.2	1.93
Acaulospora laevis		0.2			0.4	0.3		0.3	0.38
Acaulospora sp. PI1[a]	0.2								0.09
Acaulospora sp. PI2	1.3	0.9	1.3	1.5	3.3	1.9	0.9	1.6	2.45
Acaulospora spinosa			0.3						0.09
Ambispora gerdemannii	0.2		0.5	1.0	0.4	0.6	0.7		0.98
Ambispora granatensis	3.8	3.1	1.0	2.3	13.1	2.9	2.2	2.9	3.52
Ambispora sp. PI3[b]	0.9	2.2	2.1	2.0	0.7	1.3	1.5	2.9	1.97
Ambispora sp. PI4	2.7	1.3	3.4	2.8	1.0	2.2	1.9	0.6	2.46
Archaeospora myriocarpa			0.3						0.09
Archaeospora trappei	3.3	8.1	6.4	4.8	2.9	4.8	8.0	3.5	4.48
Cetraspora armeniaca		0.2	0.5			1.0	0.4		0.58
Cetraspora pellucida		0.7	0.5			0.6	0.7	0.3	0.71
Claroideoglomus claroideum	1.3	2.4	2.3	3.3	3.6	2.5	0.9	2.2	2.96
Claroideoglomus etunicatum	0.2	0.4	0.5	0.5	0.7	0.3	0.7	0.6	1.11
Claroideoglomus luteum	2.7	4.4	3.9	5.6	3.8	4.1	2.4	3.5	3.74
Diversipora clara					0.2	0.3			0.19
Diversispora celata			0.3						0.09
Diversispora versiformis	0.2		1.3		0.5	0.6			0.78
Entrophospora infrequens	2.0	2.0	0.5	0.8		2.9	1.1	2.2	1.97
Funneliformis coronatus	1.3		0.8	2.3	0.9	1.0	0.7	0.3	1.69
Funneliformis fragilistratus			0.5				0.2		0.2
Funneliformis geosporus	18.3	9.4	9.0	8.1	21.6	5.1	8.8	7.4	7.79
Funneliformis monosporus			0.3						0.09

Funneliformis mosseae	9.7	3.7	6.7	3.8	6.7	4.1	6.3	5.4	4.84
Gigaspora decipiens			0.3					0.6	0.2
Gigaspora gigantea	0.2			0.3		0.3	0.4	0.6	0.49
Gigaspora margarita	0.2	0.2	0.3	1.0	0.2	0.3	0.7	3.2	1.03
Gigaspora rosea		0.2		0.3					0.19
Glomus aureum	4.9	4.0	1.8	5.6	1.9	4.8	5.0	1.6	3.54
Glomus badium	3.5	2.2	2.6	8.1	6.9	6.4	3.5	2.6	4.13
Glomus diaphanum	2.4	6.6	4.4	5.3	2.3	2.2	4.7	3.5	3.67
Glomus fasciculatum	0.2	0.7							0.21
Glomus intraradices	2.7	2.2	3.4	1.5	2.4	1.6	2.2	4.2	2.94
Glomus invermaium	2.9	4.0	3.6	1.5	0.7	3.2	2.4	2.6	3.04
Glomus irregulare	3.5	2.2	3.4	2.5	2.8	2.5	2.8	1.3	3.13
Glomus macrocarpum	1.6	2.6	2.3	2.0	1.2	5.1	2.8	2.6	2.98
Glomus sinuosum	0.7		1.0		0.5				0.38
Paraglomus laccatum		0.4	0.8			0.3	0.2		0.49
Paraglomus occultum	6.2	6.6	5.9	2.5	1.7	9.8	13.2	13.1	5.27
Paraglomus sp. PI5[c]	4.6	5.5	1.8	3.3	2.4	5.1	5.8	3.2	3.84
Racocetra castanea		0.2		0.3		0.6	0.2		0.38
Racocetra sp. PI7[d]	3.8	1.5	4.6	4.3	0.9	3.8	1.1	1.9	1.99
Racocetra fulgida	0.9	1.5	1.0	1.0	2.1	2.9	1.3	0.3	2.02
Scutellospora sp. PI8		0.2				0.3	0.2		0.28
Scutellospora calospora	2.9	3.5	4.9	5.1	2.4	2.9	3.9	7.7	3.45
Scutellospora dipurpurescens	0.4		1.0		0.7		0.2	1.0	0.67
Scutellospora sp. PI9	0.2	1.1	0.3	2.5	0.2	1.0	0.7	0.6	1.24
Scutellospora aurigloba						0.3	0.7		0.37
Scutellospora arenicola				0.3			0.2		0.19
Septoglomus constrictum	8.8	13.2	13.4	11.9	10.4	5.7	8.2	12.8	7.09
Total AMF species	36	37	41	34	34	41	41	34	

[a] resembling *Ac. scrobiculata*, [b] resembling *Am. reticulata*, [c] resembling *Pa. majewskii*, [d] resembling *Ra. coralloidea*

Figure legends

Figure 1. AMF spore abundance g^{-1} of soil at the end of cover crop cycle and at tomato harvest. Means and standard error (bars) within each sampling time sharing the same letter are not statistically different at $P<0.05$ (Tukey's HSD test).

Figure 2. Venn diagrams showing the distribution of (a) 46 AMF species recovered at the end of cover crop cycle, and (b) 53 AMF species recovered at the end of tomato crop cycle.

Figure 3. Rank abundance plots for AMF species recovered (a) at the end of cover crop cycle and (b) at tomato harvest. The y axis indicates the total number of spores assigned to each species, pooled across the samples.

Figure 4. AMF species accumulation curves at (a) the end of cover crop cycle and (b) tomato harvest. The cumulative number of species is plotted for 6 plots for *B.juncea*, Mix 7, Control and *V.villosa*. Bars represent standard deviation.

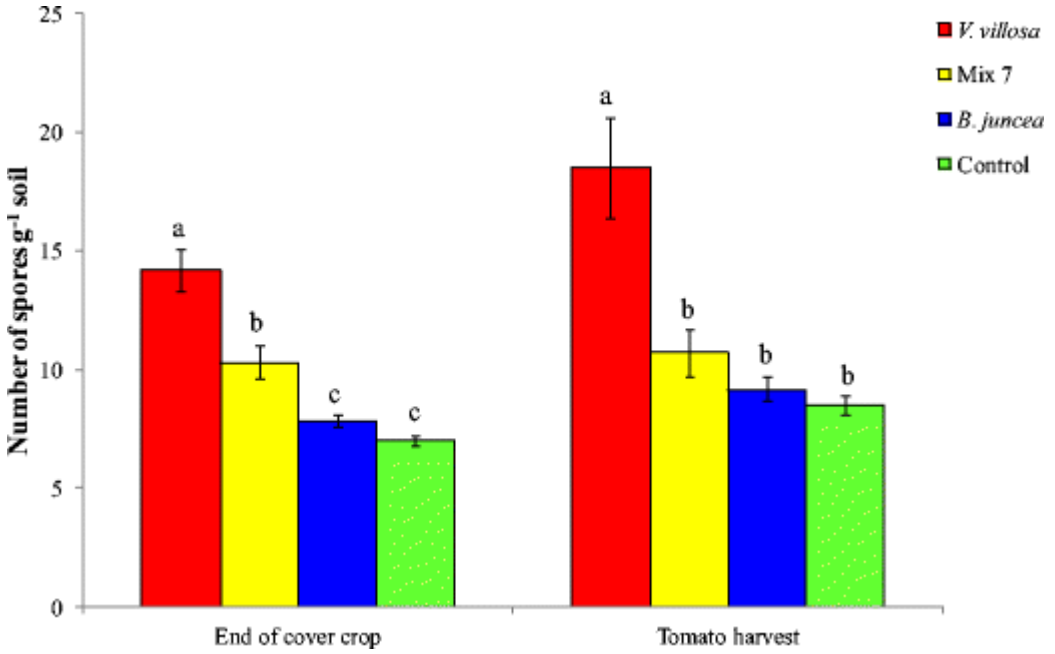
25
20
15
10
5
0
Number of spores g-1 soil
V. villosa
Mix 7
B. juncea
Control
a
b
c
c
a
b
b
b
End of cover crop
Tomato harvest

Fig. 1

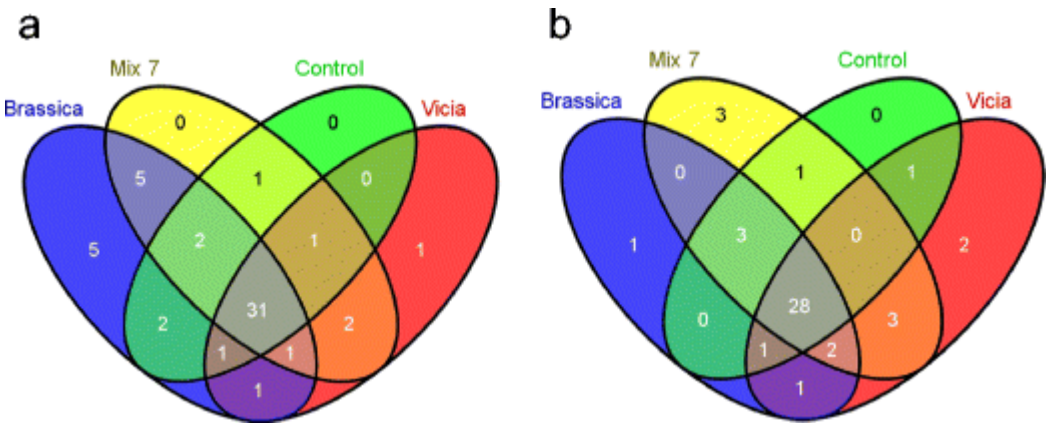
a
Mix 7
Control
Brassica
Vicia
0
0
5
1
0
5
2
1
1
31
2
2
1
1
1
b
Mix 7
Control
Brassica
Vicia
3
0
0
1
1
1
3
0
2
0
28
3
1
2
1

Fig. 2

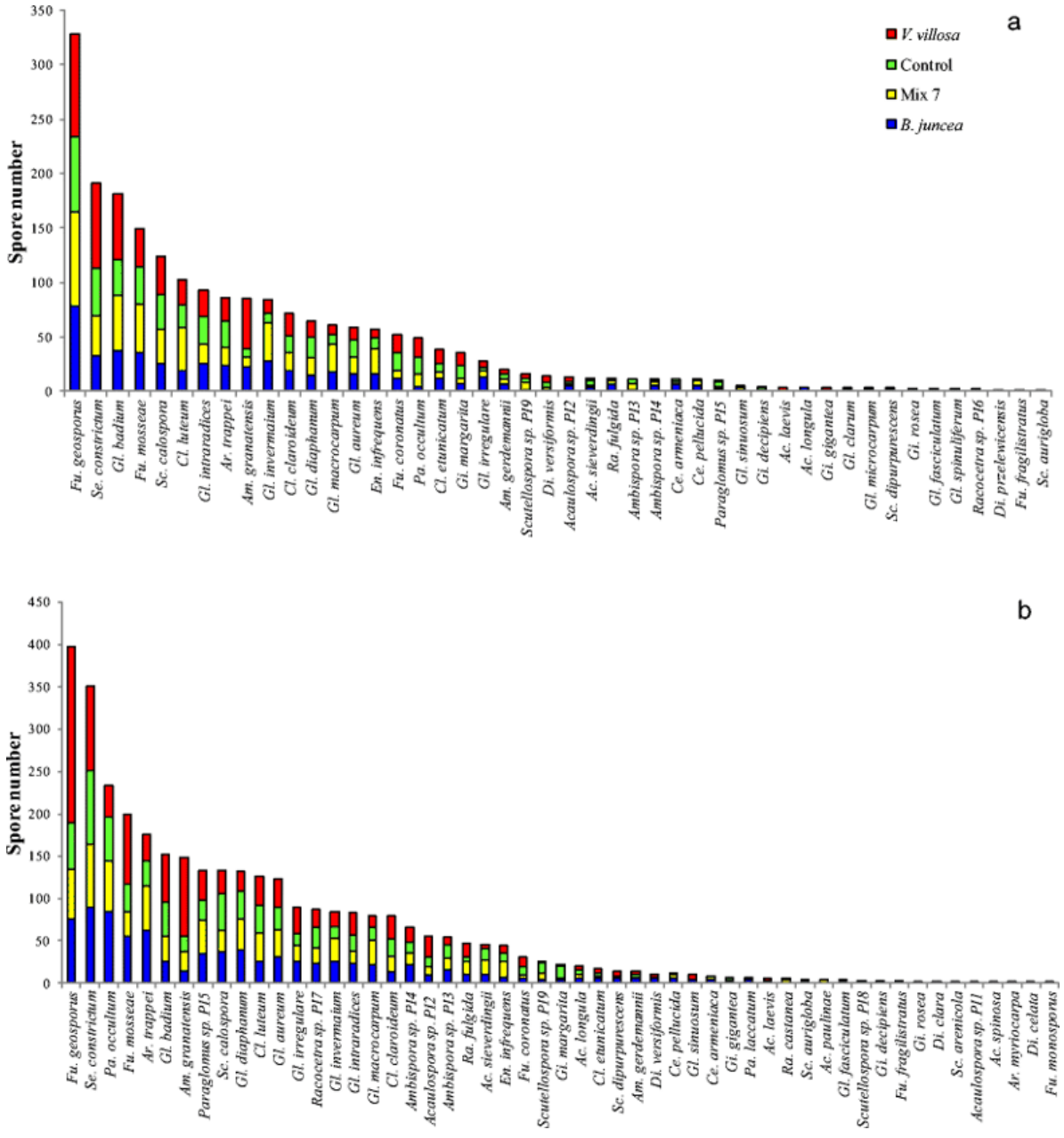
a
V. villosa
Control
Mix 7
B. juncea
Spore number
0
50
100
150
200
250
300
350
Fu. geosporus
Se. constrictum
Gl. badium
Fu. mosseae
Sc. calospora
Cl. luteum
Gl. intraradices
Ar. trappei
Am. granatensis
Gl. invermaium
Cl. claroideum
Gl. diaphanum
Gl. macrocarpum
Gl. aureum
En. infrequens
Fu. coronatus
Pa. occultum
Cl. etunicatum
Gi. margarita
Gl. irregulare
Am. gerdemannii
Scutellospora sp. P19
Di. versiformis
Acaulospora sp. P12
Ac. sieverdingii
Ra. fulgida
Ambispora sp. P13
Ambispora sp. P14
Ce. armeniaca
Ce. pellucida
Paraglomus sp. P15
Gl. sinuosum
Gl. deciplens
Ac. laevis
Ac. longula
Gi. gigantea
Gl. clarum
Gl. microcarpum
Sc. dipurpurescens
Gi. rosea
Gl. fasciculatum
Gl. spinuliferum
Racocetra sp. P16
Di. pizelewicensis
Fu. fragilistratus
Sc. aurigloba
b
Spore number
0
50
100
150
200
250
300
350
400
450
Fu. geosporus
Se. constrictum
Pa. occultum
Fu. mosseae
Ar. trappei
Gl. badium
Am. granatensis
Paraglomus sp. P15
Sc. calospora
Gl. diaphanum
Cl. luteum
Gl. aureum
Gl. irregulare
Racocetra sp. P17
Gl. invermaium
Gl. intraradices
Gl. macrocarpum
Cl. claroideum
Ambispora sp. P14
Acaulospora sp. P12
Ambispora sp. P13
Ra. fulgida
Ac. sieverdingii
En. infrequens
Fu. coronatus
Scutellospora sp. P19
Gi. margarita
Ac. longula
Cl. etunicatum
Sc. dipurpurescens
Am. gerdemannii
Di. versiformis
Ce. pellucida
Gl. sinuosum
Ce. armeniaca
Gi. gigantea
Pa. laccatum
Ac. laevis
Ra. castanea
Sc. aurigloba
Ac. paulinae
Gl. fasciculatum
Scutellospora sp. P18
Gi. deciplens
Fu. fragilistratus
Gi. rosea
Di. clara
Sc. arenicola
Acaulospora sp. P11
Ac. spinosa
Ar. myriocarpa
Di. celata
Fu. monosporus

Fig. 3

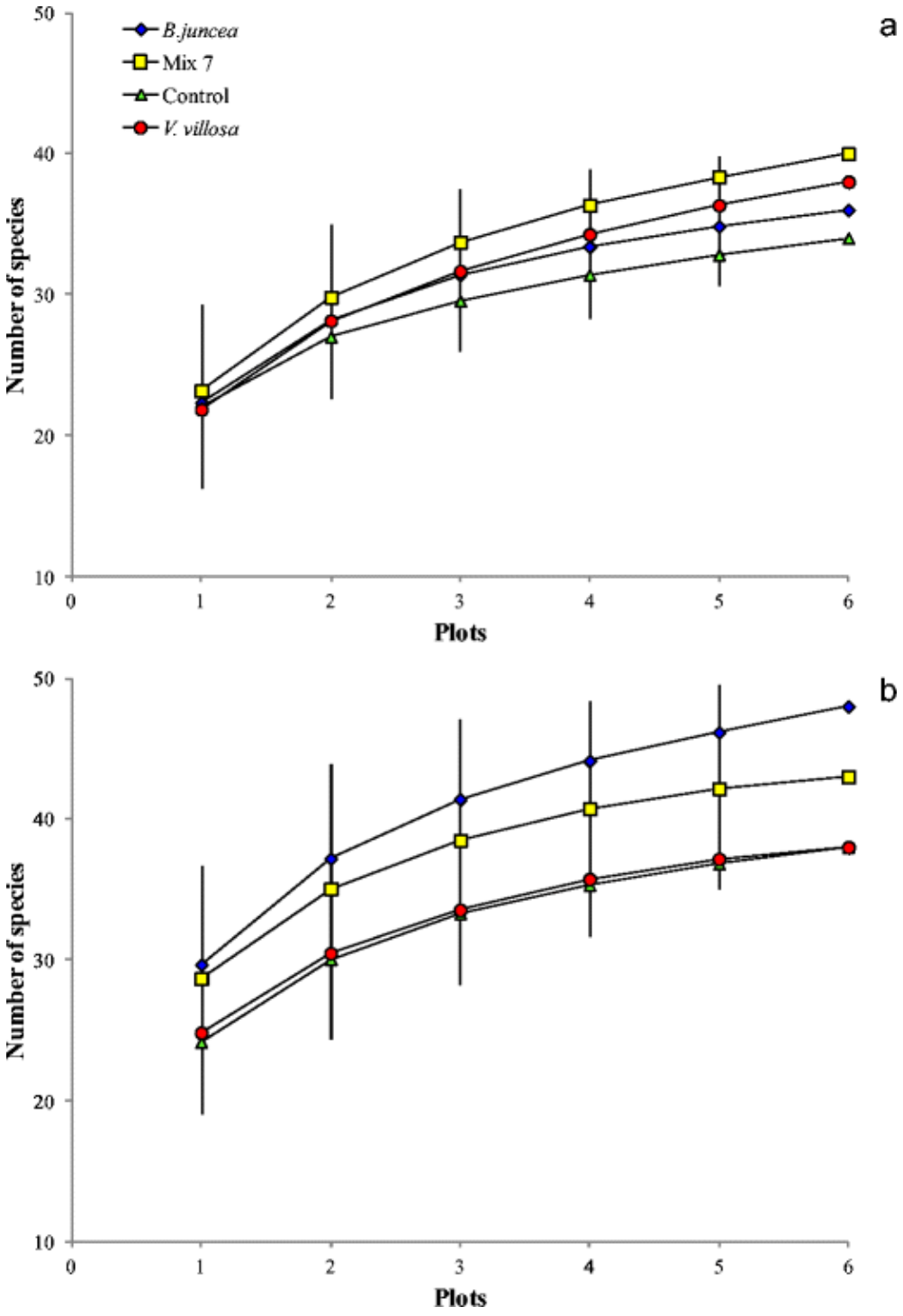
a
B.juncea
Mix 7
Control
V. villosa
Number of species
Plots
b
Number of species
Plots

Fig. 4