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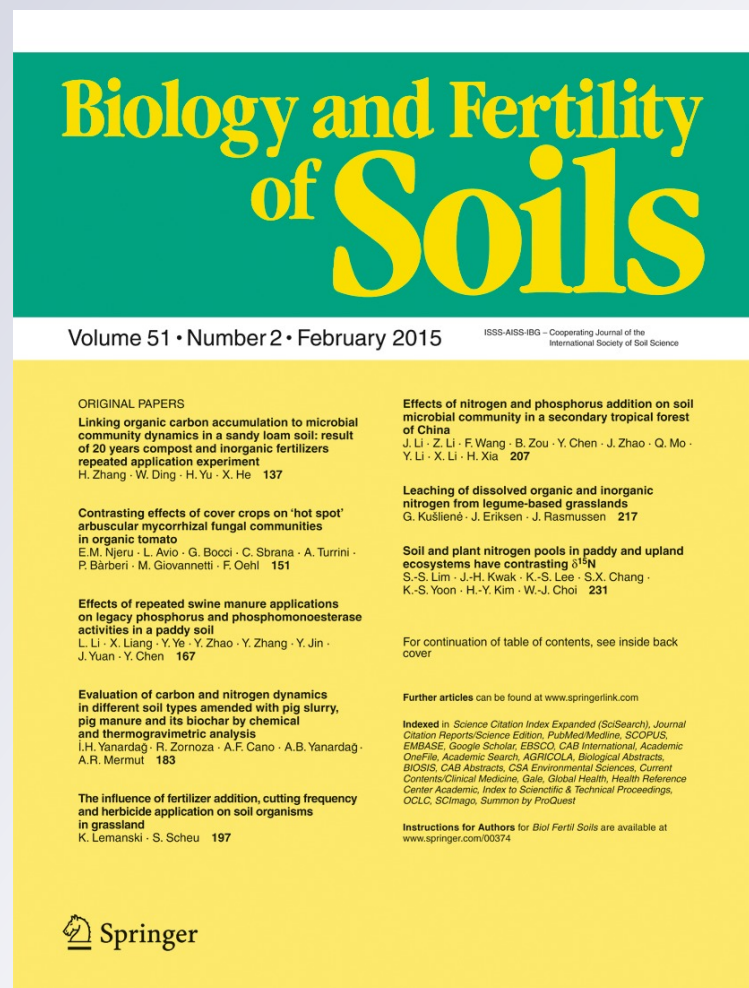
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Contrasting effects of cover crops on ‘hot spot’ arbuscular mycorrhizal fungal communities in organic tomato

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Abstract Arbuscular mycorrhizal fungal (AMF) communities are fundamental in organic cropping systems where they provide essential agro-ecosystem 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 sub-tropical climates co-occur.

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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 agro-ecological 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 agro-ecosystems (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 agro-ecosystems, where a combination of different levels of agrobiodiversity (i.e. genetic, species and habitat biodiversity) plays a crucial role (Costanzo and Barberi 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 the 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 spore 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. Błaszowski 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 repeatedly shown recently that classical spore identification might be even superior to molecular identification for AMF diversity studies (e.g. compare Oehl et 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 6 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 as follows: (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 and (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 Interdepartmental Centre for Agri-environmental Research ‘Enrico Avanzi’ (CIRAA), 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/bioes.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 6 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 characteristics at the experimental site were as follows: clay 11.5 %, silt 17.0 %, sand 71.5 %, pH (H₂O) 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 Strategies for Organic and Low-input Integrated Breeding and Management (SOLIBAM 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 v/v) 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*) and 65 kg ha⁻¹

(mix 7). Weeds were not controlled in any cover crop treatment. Above-ground 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 Terra-Green (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 6 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-sterilized 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 nitrogen, phosphorus and potassium (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 at a spacing of 1.5×0.5 m (between- and within-row distance,

respectively). The tomato plants were watered through drip irrigation and maintained in the field until maturity under standard conditions of organic farming.

Soil sampling

Soil samples were taken at the end of the cover crop cycle before incorporation into the soil (10 April 2012) and at tomato harvest (20 September 2012). At each sampling date, four soil cores were obtained from each sub-plot at a depth of 20 cm and mixed to form a homogenous composite sample. The soil samples were air dried and stored on sealed bags for spore extraction and analysis. Spore extraction and identification was conducted at Agroscope, Institute for Sustainability Sciences, in Zürich-Reckenholz, Switzerland.

Mycorrhizal colonization at flowering and harvest

To determine AMF colonization at flowering (17 July 2012) and harvest, root samples were obtained from four randomly selected plants per sub-plot and stained with 0.05 % trypan blue. The percentage root colonization was determined using the gridline intersect method (Giovannetti and Mosse 1980).

AMF spore recovery, enumeration and identification

Spore extraction and identification were conducted at Agroscope using the methodology of Sieverding (1991). Spores were isolated from two 25 g of air-dried sub-samples per field plot soil sample. Spores were extracted by wet sieving with tap water through nested sieves (500, 125 and 32- μm mesh size). After sieving, the material obtained from the 125 and 32- μm sieves was transferred to five 50-mL vials per sample constituting five 25-mL suspensions. The suspensions were under-layered with 25 mL of a 70 % *w/v* sucrose solution, and the water/sucrose solution density gradient was centrifuged at 2,000 rpm for 2 min. In the material from the 500- μm sieve, no spores or sporocarps were observed. After centrifugation, the supernatant was passed through a 32- μm sieve and washed with tap 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 0.5 cm^2 under a Leica M165 dissecting microscope at up to $\times 90$ magnification. For taxonomic identification, the spores were mounted on glass slides and fixed with polyvinyl-lactic acid/glycerol (1:1 *v/v*) (Koske and Tessier 1983). The spores were examined under a Leica DM750 compound microscope at up to $\times 400$ and identified to species level using all original species descriptions, updated taxonomic studies on the described taxa and available identification manuals (Błaszczkowski 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 the 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 analyzed by 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,

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 per gram 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)	$1 - \sum[n_i(n_i - 1)/N(N - 1)]$
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

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 ANOVAs 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 Monte Carlo 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 \times cover crop interaction. At harvest, a marginal, non-significant effect of AMF pre-transplant inoculation was still observed, while the cover crop and AMF \times cover crop interaction were not significant (Table 2).

Table 2 Tomato root colonization (%) at flowering and harvest as influenced by cover crops and pre-transplant inoculation

Cover crop	Tomato root colonization (%)	
	Flowering	Harvest
<i>V. villosa</i>	41.5 (5.61) a	35.6 (4.06) a
Mix 7	35.0 (2.36) a	34.5 (4.41) a
<i>B. juncea</i>	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
<i>P</i> values of the main factors and interaction		
Cover crop	0.191	0.309
AMF	<0.001	0.079
Cover \times AMF	0.184	0.893

Values followed by the same letter in a column are not significantly different at $P<0.05$ (Tukey's HSD test). Values in parentheses are standard error of the means.

AMF spore abundance

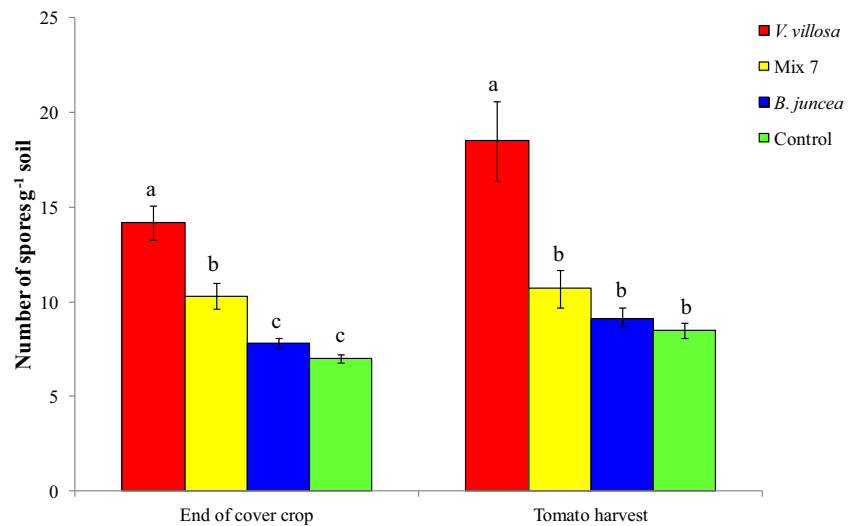
At the end of cover crop cycle, the fungal spore abundance differed significantly ($F_{3,18}=47.94$, $P<0.001$) among cover crop treatments. *V. villosa* showed the highest spore abundance ($14.2\pm 0.9\text{ g}^{-1}$ soil), while *B. juncea* and control treatments had the lowest densities (Fig. 1). 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 \times cover crop interaction. Again, a higher spore abundance was produced after *V. villosa* ($18.6\pm 2.7\text{ g}^{-1}$ 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*, *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 (isolation frequency (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 and D from 0.90 to 0.91, while E was 0.62 to 0.70 in all cover crop treatments.

Fig. 1 AMF spore abundance per gram 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)



Likewise, the *H'*, *D* and *E* of AMF-inoculated and AMF-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

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

Cover crop	<i>V. villosa</i>	Mix 7	<i>B. juncea</i>	Control	Total species
Acaulosporaceae					
<i>Acaulospora</i>	3	2	3	2	4
Ambisporaceae					
<i>Ambispora</i>	3	4	4	4	4
Archaeosporaceae					
<i>Archaeospora</i>	1	1	1	1	1
Diversosporaceae					
<i>Diversispora</i>	2	1	1	1	2
Entrophosporaceae					
<i>Claroideoglomus</i>	3	3	3	3	3
<i>Entrophospora</i>	1	1	1	1	1
Gigasporaceae					
<i>Gigaspora</i>	3	2	3	2	4
Glomeraceae					
<i>Funneliformis</i>	3	4	3	3	4
<i>Glomus</i>	10	11	9	10	12
<i>Septoglomus</i>	1	1	1	1	1
Paraglomeraceae					
<i>Paraglomus</i>	2	2	2	2	2
Racocetraceae					
<i>Cetraspora</i>	1	2	2	1	2
<i>Racocetra</i>	2	2	1	1	2
Scutellosporaceae					
<i>Scutellospora</i>	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

Cover crop	AMF-inoculated plots				Uninoculated plots				RAI
	<i>V. villosa</i>	Mix 7	<i>B. juncea</i>	Control	<i>V. villosa</i>	Mix 7	<i>B. juncea</i>	Control	
AMF species									
<i>Acaulospora longula</i>			0.4				0.9		0.26
<i>Acaulospora sieverdingii</i>	0.4	0.4	0.7	0.9	0.3	0.3	0.4	1.3	1.40
<i>Acaulospora laevis</i>	0.7				0.3				0.26
<i>Acaulospora</i> sp. PI2 ^a	1.1	0.4			0.3		2.2	1.3	1.05
<i>Ambispora gerdemannii</i>	1.4	0.4	1.4	1.3		1.3	0.9	0.9	1.40
<i>Ambispora granatensis</i>	4.2	0.8	7.9	1.3	11.0	2.3	0.4	1.7	3.59
<i>Ambispora</i> sp. PI3 ^b		0.4		0.4		1.6	0.4	1.3	0.91
<i>Ambispora</i> sp. PI4	0.4		1.8	0.4		1.3			1.01
<i>Archaeospora trappei</i>	3.5	2.8	3.6	8.2	3.6	2.9	6.0	2.6	4.08
<i>Cetraspora armeniaca</i>		0.4	1.8	0.4		0.7	0.4	0.4	0.91
<i>Cetraspora pellucida</i>	0.4	0.4	0.4			1.3	1.7		1.01
<i>Claroideoglossum claroideum</i>	1.1	2.8	4.7	3.0	5.8	2.9	2.6	3.9	3.66
<i>Claroideoglossum etunicatum</i>	2.8	1.6	2.9	1.7	1.3	0.7	1.7	1.7	2.76
<i>Claroideoglossum luteum</i>	3.5	10.5	3.6	2.2	4.5	4.6	3.9	6.5	4.67
<i>Diversispora przelewicensis</i>	0.4								0.12
<i>Diversispora versiformis</i>	0.4	0.4	0.4	1.3	1.6	0.3		0.9	1.36
<i>Entrophospora infrequens</i>	1.4	2.0	1.8	0.9	1.3	5.9	4.7	3.5	3.30
<i>Funneliformis coronatus</i>	2.4	0.4	3.3	4.8	3.2	2.0	1.3	2.2	2.81
<i>Funneliformis fragilistratus</i>		0.4							0.12
<i>Funneliformis geosporus</i>	14.3	10.9	15.2	13.4	17.5	19.5	15.5	16.5	9.99
<i>Funneliformis mosseae</i>	7.3	10.5	6.1	8.7	4.5	6.2	7.8	6.1	5.75
<i>Gigaspora decipiens</i>					0.3		0.4	0.9	0.37
<i>Gigaspora gigantea</i>	0.7						0.4		0.35
<i>Gigaspora margarita</i>	2.8	1.6	0.4	0.4	1.0	0.3	2.6	4.8	1.94
<i>Gigaspora rosea</i>						0.7			0.14
<i>Glomus aureum</i>	3.1	5.7	4.0	3.5	1.0	0.7	2.2	3.0	3.35
<i>Glomus badium</i>	12.5	11.3	7.2	6.5	8.1	7.5	7.3	7.8	6.53
<i>Glomus clarum</i>		0.4					0.4	0.4	0.35
<i>Glomus diaphanum</i>	3.5	5.3	1.8	3.9	1.6	1.0	4.3	4.3	3.49
<i>Glomus fasciculatum</i>	0.4					0.3			0.23
<i>Glomus intraradices</i>	5.6	2.4	6.9	8.2	2.6	3.6	3.0	3.0	4.34
<i>Glomus invermaium</i>	3.5	7.3	3.6	1.7	0.7	5.5	7.8	2.2	3.57
<i>Glomus irregulare</i>	0.7	0.8	2.5	1.3	1.3	1.3	2.6		2.34
<i>Glomus macrocarpum</i>	2.4	3.6	3.3	2.2	0.7	5.2	3.9	1.7	3.49
<i>Glomus microcarpum</i>						0.7		0.4	0.26
<i>Glomus sinuosum</i>	0.7		0.4			0.7			0.49
<i>Glomus spinuliferum</i>					0.3			0.4	0.23
<i>Paraglomus occultum</i>	0.7	1.2	0.4	3.5	4.9	2.9	1.3	3.5	3.11
<i>Paraglomus</i> sp. PI5 ^c		0.4	0.4	0.9	0.3	0.3	0.4	1.3	0.89
<i>Racocetra fulgida</i>	0.4		1.1	0.4		1.3	1.3		1.12
<i>Racocetra</i> sp. PI6	0.4	0.4							0.23
<i>Scutellospora calospora</i>	4.2	7.3	4.7	8.7	7.4	4.2	5.6	5.2	4.51
<i>Scutellospora dipurpurescens</i>					0.3	0.7			0.26
<i>Scutellospora</i> sp. PI9	0.4	1.2	0.4		1.0	1.3		1.7	1.12
<i>Scutellospora aurigloba</i>						0.3			0.12
<i>Septoglossum constrictum</i>	12.9	5.3	7.2	10.0	13.3	7.8	5.6	8.7	6.74

Table 4 (continued)

Cover crop	AMF-inoculated plots				Uninoculated plots				RAI
	<i>V. villosa</i>	Mix 7	<i>B. juncea</i>	Control	<i>V. villosa</i>	Mix 7	<i>B. juncea</i>	Control	
Total AMF species	34	32	31	28	29	36	32	31	

^a Resembling *Acaulospora dilatata*

^b Resembling *Ambispora reticulata*

^c Resembling *Paraglomus majewskii*

species richness, accounting for 22.6 % of the identified spores (Table 5). Other genera with a high number of spores identified included the following: *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 (Table 6), accounting for the highest number (22.3 %) of spores identified.

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 *Racocetra 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 richness 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 × 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. 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

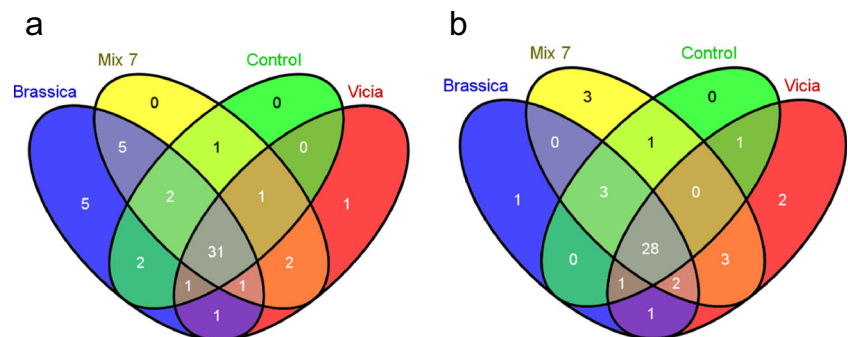


Table 5 Number of AMF species detected at tomato harvest across different cover crop treatments

	<i>V. villosa</i>	Mix 7	<i>B. juncea</i>	Control	Sum of species
Acaulosporaceae					
<i>Acaulospora</i>	5	5	5	4	7
Ambisporaceae					
<i>Ambispora</i>	4	4	4	4	4
Archaeosporaceae					
<i>Archaeospora</i>	1	1	2	1	2
Diversisporaceae					
<i>Diversispora</i>	2	2	2		3
Entrophosporaceae					
<i>Claroideoglossum</i>	3	3	3	3	3
<i>Entrophospora</i>	1	1	1	1	1
Gigasporaceae					
<i>Gigaspora</i>	2	3	3	4	4
Glomeraceae					
<i>Funneliformis</i>	3	3	5	3	5
<i>Glomus</i>	9	8	8	7	9
<i>Septoglomus</i>	1	1	1	1	1
Paraglomeraceae					
<i>Paraglomus</i>	2	3	3	2	3
Racocetraceae					
<i>Cetraspora</i>		2	2	1	2
<i>Racocetra</i>	2	3	3	3	3
Scutellosporaceae					
<i>Scutellospora</i>	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	

(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 \times 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 \times 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" section) 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 adapted to 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 in either 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

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

Cover crop	AMF-inoculated plots				Uninoculated plots				RAI
	<i>V. villosa</i>	Mix 7	<i>B. juncea</i>	Control	<i>V. villosa</i>	Mix 7	<i>B. juncea</i>	Control	
AMF species									
<i>Acaulospora longula</i>	0.4	0.7		0.5	0.5	1.0	1.1	0.6	1.23
<i>Acaulospora paulinae</i>		0.4				0.3	0.2		0.37
<i>Acaulospora sieverdingii</i>	0.9	1.3	1.0	1.8	0.2	3.2	1.5	2.2	1.93
<i>Acaulospora laevis</i>		0.2			0.4	0.3		0.3	0.38
<i>Acaulospora</i> sp. PI1 ^a	0.2								0.09
<i>Acaulospora</i> sp. PI2	1.3	0.9	1.3	1.5	3.3	1.9	0.9	1.6	2.45
<i>Acaulospora spinosa</i>			0.3						0.09
<i>Ambispora gerdemannii</i>	0.2		0.5	1.0	0.4	0.6	0.7		0.98
<i>Ambispora granatensis</i>	3.8	3.1	1.0	2.3	13.1	2.9	2.2	2.9	3.52
<i>Ambispora</i> sp. PI3 ^b	0.9	2.2	2.1	2.0	0.7	1.3	1.5	2.9	1.97
<i>Ambispora</i> sp. PI4	2.7	1.3	3.4	2.8	1.0	2.2	1.9	0.6	2.46
<i>Archaeospora myriocarpa</i>			0.3						0.09
<i>Archaeospora trappei</i>	3.3	8.1	6.4	4.8	2.9	4.8	8.0	3.5	4.48
<i>Cetraspora armeniaca</i>		0.2	0.5			1.0	0.4		0.58
<i>Cetraspora pellucida</i>		0.7	0.5			0.6	0.7	0.3	0.71
<i>Claroideoglossum claroideum</i>	1.3	2.4	2.3	3.3	3.6	2.5	0.9	2.2	2.96
<i>Claroideoglossum etunicatum</i>	0.2	0.4	0.5	0.5	0.7	0.3	0.7	0.6	1.11
<i>Claroideoglossum luteum</i>	2.7	4.4	3.9	5.6	3.8	4.1	2.4	3.5	3.74
<i>Diversispora clara</i>					0.2	0.3			0.19
<i>Diversispora celata</i>			0.3						0.09
<i>Diversispora versiformis</i>	0.2		1.3		0.5	0.6			0.78
<i>Entrophospora infrequens</i>	2.0	2.0	0.5	0.8		2.9	1.1	2.2	1.97
<i>Funneliformis coronatus</i>	1.3		0.8	2.3	0.9	1.0	0.7	0.3	1.69
<i>Funneliformis fragilistratus</i>			0.5				0.2		0.2
<i>Funneliformis geosporus</i>	18.3	9.4	9.0	8.1	21.6	5.1	8.8	7.4	7.79
<i>Funneliformis monosporus</i>			0.3						0.09
<i>Funneliformis mosseae</i>	9.7	3.7	6.7	3.8	6.7	4.1	6.3	5.4	4.84
<i>Gigaspora decipiens</i>			0.3					0.6	0.2
<i>Gigaspora gigantea</i>	0.2			0.3		0.3	0.4	0.6	0.49
<i>Gigaspora margarita</i>	0.2	0.2	0.3	1.0	0.2	0.3	0.7	3.2	1.03
<i>Gigaspora rosea</i>		0.2		0.3					0.19
<i>Glomus aureum</i>	4.9	4.0	1.8	5.6	1.9	4.8	5.0	1.6	3.54
<i>Glomus badium</i>	3.5	2.2	2.6	8.1	6.9	6.4	3.5	2.6	4.13
<i>Glomus diaphanum</i>	2.4	6.6	4.4	5.3	2.3	2.2	4.7	3.5	3.67
<i>Glomus fasciculatum</i>	0.2	0.7							0.21
<i>Glomus intraradices</i>	2.7	2.2	3.4	1.5	2.4	1.6	2.2	4.2	2.94
<i>Glomus invermaium</i>	2.9	4.0	3.6	1.5	0.7	3.2	2.4	2.6	3.04
<i>Glomus irregulare</i>	3.5	2.2	3.4	2.5	2.8	2.5	2.8	1.3	3.13
<i>Glomus macrocarpum</i>	1.6	2.6	2.3	2.0	1.2	5.1	2.8	2.6	2.98
<i>Glomus sinuosum</i>	0.7		1.0		0.5				0.38
<i>Paraglomus laccatum</i>		0.4	0.8			0.3	0.2		0.49
<i>Paraglomus occultum</i>	6.2	6.6	5.9	2.5	1.7	9.8	13.2	13.1	5.27
<i>Paraglomus</i> sp. PI5 ^c	4.6	5.5	1.8	3.3	2.4	5.1	5.8	3.2	3.84
<i>Racocetra castanea</i>		0.2		0.3		0.6	0.2		0.38
<i>Racocetra</i> sp. PI7 ^d	3.8	1.5	4.6	4.3	0.9	3.8	1.1	1.9	1.99
<i>Racocetra fulgida</i>	0.9	1.5	1.0	1.0	2.1	2.9	1.3	0.3	2.02

Table 6 (continued)

Cover crop	AMF-inoculated plots				Uninoculated plots				RAI
	<i>V. villosa</i>	Mix 7	<i>B. juncea</i>	Control	<i>V. villosa</i>	Mix 7	<i>B. juncea</i>	Control	
<i>Scutellospora</i> sp. PI8		0.2				0.3	0.2		0.28
<i>Scutellospora calospora</i>	2.9	3.5	4.9	5.1	2.4	2.9	3.9	7.7	3.45
<i>Scutellospora dipurpureascens</i>	0.4		1.0		0.7		0.2	1.0	0.67
<i>Scutellospora</i> sp. PI9	0.2	1.1	0.3	2.5	0.2	1.0	0.7	0.6	1.24
<i>Scutellospora aurigloba</i>						0.3	0.7		0.37
<i>Scutellospora arenicola</i>				0.3			0.2		0.19
<i>Septogloium constrictum</i>	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 *Acaulospora scrobiculata*

^b Resembling *Ambispora reticulata*

^c Resembling *Paragloium majewskii*

^d Resembling *Racocetra coralloidea*

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 2012. Above all, small-spored species might be difficult to identify by classical spore morphology (Błaszczkowski 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 that all those ‘sub-soil’ AMF species, e.g. *Racocetra castanea*, *Glomus invermaium* (Oehl et al. 2005), are also present in our top soils (Tables 4 and 6). Thus, in our hot spot field site, we consider the AMF biodiversity of the top soil as the most essential for tomato growth. Moreover, the diversity of AMF communities in the surrounding soil and of those colonizing roots may differ as previously demonstrated in the field and in trap plants (Cesaro et al. 2008; Avio et al. 2013).

AMF inoculation and root colonization

In our study, pre-transplant inoculation increased tomato root colonization in the field despite the presence of a rich

indigenous AMF biodiversity. This difference was more enhanced at tomato flowering than at harvest when only marginal differences between AMF treatments were detected. Increased root colonization at flowering is particularly important since plants are more physiologically active at this stage, requiring additional nutrients which can be provided by mycorrhizal symbiosis. These results confirm recent reports suggesting increased early root colonization of field grown tomato following pre-inoculation contributing to plant nutrition, growth and yield (Conversa et al. 2013). Although cover crops did not significantly affect root colonization, tomato crop grown after *V. villosa* showed higher root colonization at flowering compared to *B. juncea*. While *V. villosa* is a highly mycorrhizal legume, *B. juncea* is a non-host species which can additionally release mycotoxic compounds (usually isothiocyanates, ITC) after the disruption of *Brassica* tissues that are likely to be deleterious to indigenous AMF populations (Njeru et al. 2013). However, the negative effect of ITC on AMF diminishes gradually, as described previously (Gimsing and Kirkegaard 2006), a probable reason why AMF colonization in *B. juncea* treatment at tomato harvest was similar to the other cover crop treatments. Besides this, tomato crop is a good mycorrhizal host, which is likely to have improved AMF propagule abundance by harvest time across all the treatments.

AMF species richness, community composition and diversity in different treatments

At both sampling times, spore abundance was higher in *V. villosa* than in the other cover crops. As a 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

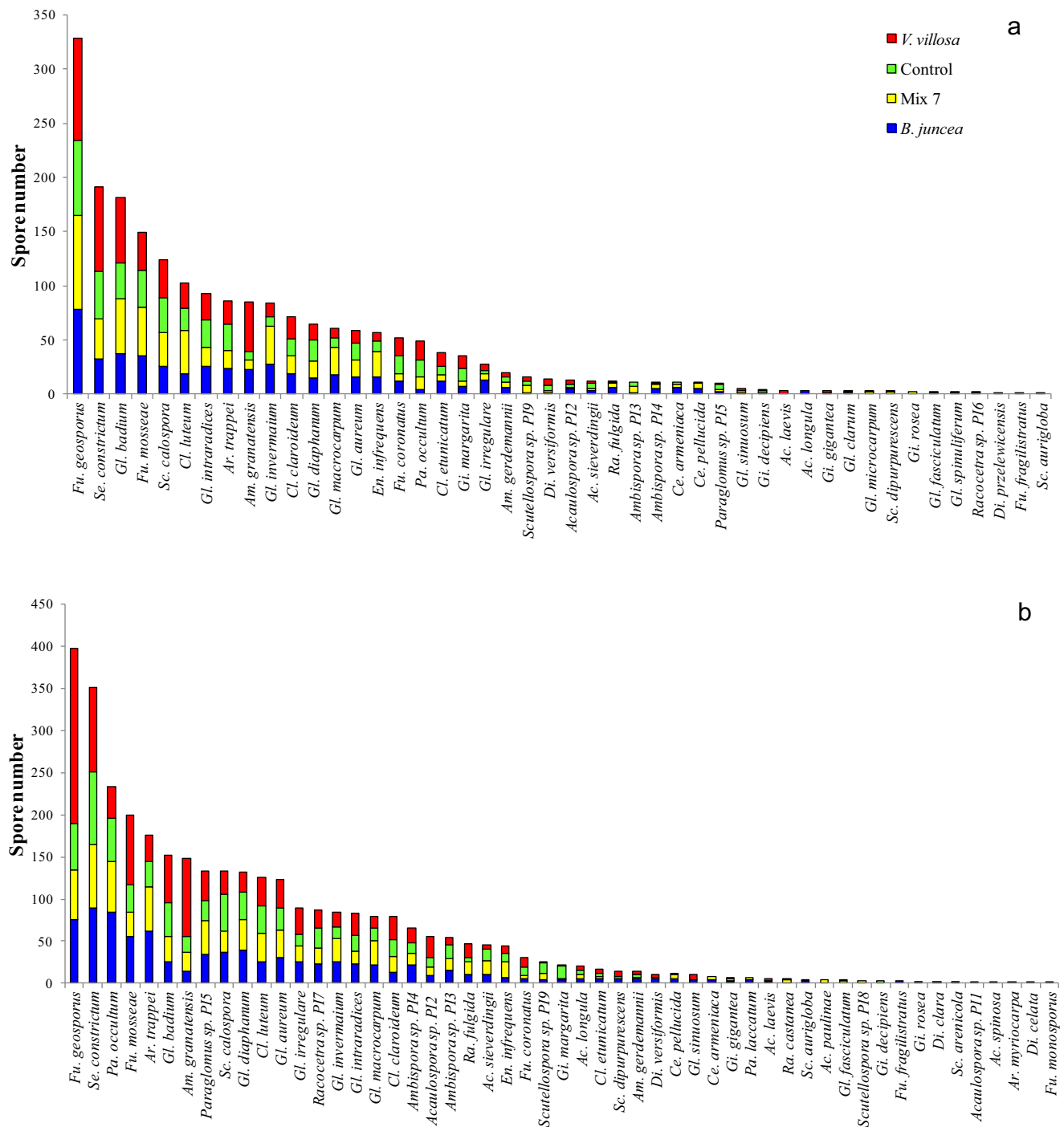
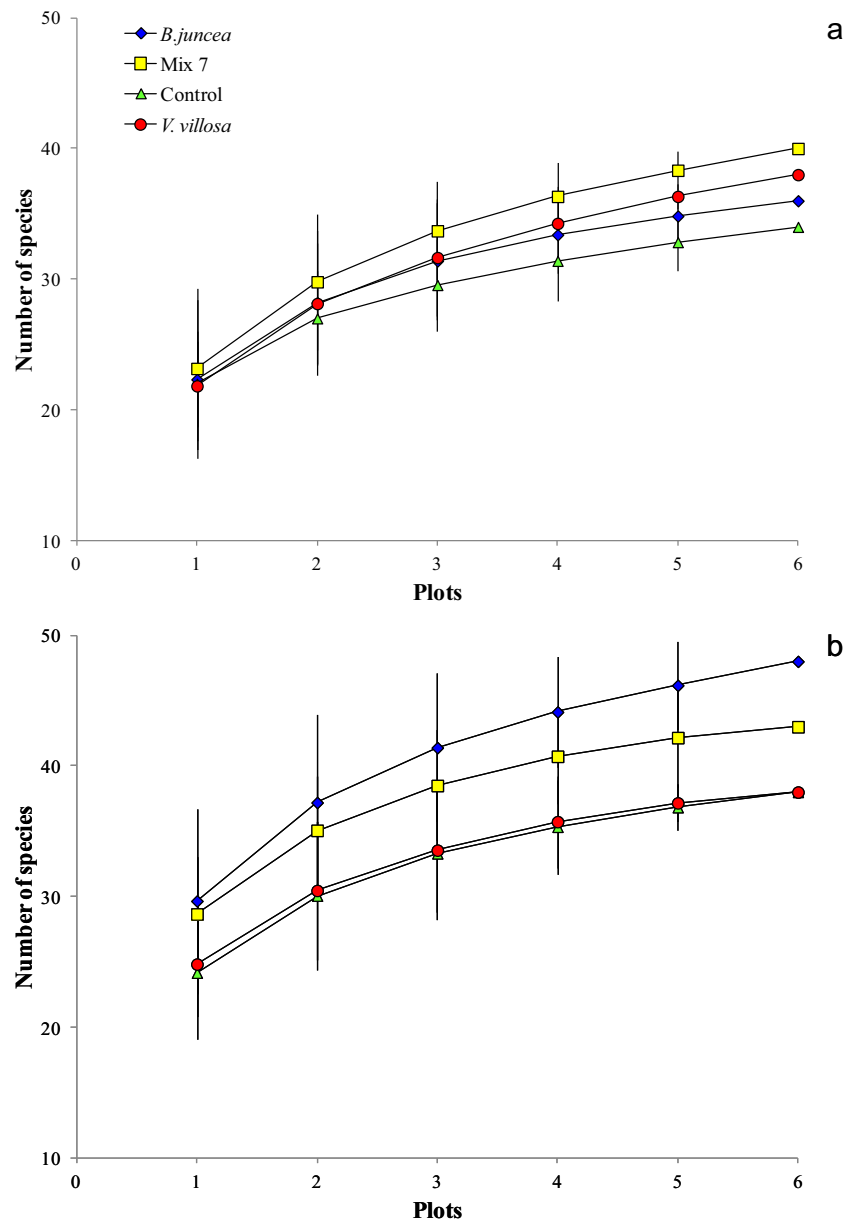


Fig. 3 Rank abundance plots for AMF species recovered **a** at the end of cover crop cycle and **b** at tomato harvest. The y axis indicates the total number of spores assigned to each species, pooled across the samples

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 the 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

Fig. 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 six plots for *B. juncea*, mix 7, control and *V. villosa*. Bars represent standard deviation



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 agro-

ecosystem 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 agroclimatic 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 is 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 agro-ecosystem. 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 Barberi 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 characterize the native AMF from such hot spot diversity sites. 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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