Alessandra Turrini[1], Cristiana Sbrana[2], Luciano Avio[2], Ezekiel Mugendi Njeru[3], Gionata Bocci[4], Paolo Bàrberi[4], Manuela Giovannetti[1]

# Changes in the composition of native root arbuscular mycorrhizal fungi communities during a short-term cover crop-maize succession

[1] Dipartimento di Scienze Agrarie, Alimentari e Agro-ambientali, Università di Pisa, Via del Borghetto 80, 56124 Pisa, Italy.

[2] Istituto di Biologia e Biotecnologia Agraria, CNR, UO Pisa, Via del Borghetto 80, 56124 Pisa, Italy.

[3] Department of Microbiology, Kenyatta University, P.O. Box 43844-00100, Nairobi, Kenya.

[4] Istituto di Scienze della Vita, Scuola Superiore Sant'Anna, P.za Martiri della Libertà 33, 56127 Pisa, Italy.

Corresponding author: Alessandra Turrini

phone: +39 050 2216646

e-mail: alessandra.turrini@ unipi.it

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**Authorship and contributorship**

A.T., C.S., L.A. and M.G. conceived and designed the experiments. A.T. and C.S. performed molecular and data analyses. L.A. performed data analyses. A.T., L.A. and M.G. wrote the paper. P.B., G.B. and E.M.N. provided the agronomic management and revised the paper. M.G. contributed reagents/materials/analysis tools.

**Abstract**

Arbuscular mycorrhizal fungi (AMF) establish mutualistic associations with the most important agricultural food and feed crops, sustaining plant growth, nutrient uptake and tolerance of biotic and abiotic stresses. Scanty information is available on the role played by crop identity and diversity as a driving force shaping AMF species communities in the field, in particular in low-input and organic farming, where crop rotation and the use of cover crops are common practices. Here, using a molecular approach, we investigated whether plant communities established in low and high diversity cover crop treatments affect composition of native AMF root communities of subsequent maize in a Mediterranean organic agroecosystem. A total of 16 AMF sequence types were detected, with *Acaulospora cavernata* as the most abundant phylotype, accounting for 37.4% of the sequences, followed by *Funneliformis mosseae*, *Claroideoglomus lamellosum* and *Rhizoglomus intraradices*. Sequences matching to *Funneliformis caledonium*, *Diversispora aurantia*, *Diversispora epigaea* and *Archaeospora schenckii* corresponded to less than 2.0% of the total. The most abundant sequences retrieved in plants from cover crop treatments were represented by *A. cavernata*, while sequences in maize roots were related to *F. mosseae*, *R. intraradices* and *Glomus* sp. Such data show for the first time a change in the composition of native AMF communities colonizing maize roots, which was independent of the identity and diversity of the preceding crop. Our findings suggest that host preference may represent a strong driver of AMF community dynamics in agroecosystems, differentially boosting or depressing AMF species, possibly in relation to their functional significance.

**Keywords**

Arbuscular mycorrhizal fungi; cover crop diversity; AMF diversity; Glomeromycota; small ribosomal subunit (SSU rDNA); native AMF communities.

## Introduction

Soil microorganisms play a key role in natural and agricultural ecosystems by providing fundamental ecological services, such as biogeochemical cycling, soil structure formation and improvement, C sequestration and turnover, nutrient availability, control of soil-borne pests and diseases. As such, they represent essential elements of fertility and productivity maintenance in agricultural soils, an issue which is particularly relevant for low external input and organic farming systems (Pimentel et al. 1997). A thoroughly investigated group of beneficial soil biota is represented by arbuscular mycorrhizal fungi (AMF, Glomeromycota), which establish mutualistic associations with the roots of most terrestrial plants (about 80%), including the most important food and feed crops. AMF are widely recognised as key drivers of plant performance, sustaining plant growth, nutrient uptake and tolerance of biotic and abiotic stresses,

reducing the need of chemical fertilizers and pesticides and consequently the environmental impact of agriculture (Smith and Read, 2008). Moreover, AMF provide key ecosystem services, such as soil aggregation and C sequestration (Gianinazzi et al. 2010) and may stimulate the biosynthesis of beneficial plant secondary metabolites, contributing to the production of safe and high quality food (Sbrana et al. 2014). A large body of investigations showed that AMF uptake and transfer soil mineral nutrients, such as phosphorus (P), nitrogen (N), sulphur (S), potassium (K), calcium (Ca), iron (Fe), copper (Cu) and zinc (Zn) by means of extraradical mycorrhizal hyphae, which spread from colonised roots into the soil (Giovannetti et al. 2001; Avio et al. 2006; Blanke et al. 2011). In addition, AMF enhance the availability of soil resources through the synergistic action of a large community of beneficial bacteria that live associated to their spores and mycelium (Hildebrandt et al. 2006; Agnolucci et al. 2015).

Several studies reported that AMF affect the structure and composition of plant communities, depending on the quantity and quality of fungal taxa occurring in natural and experimental soils, as AMF function and activity vary largely among different taxa (van der Heijden et al. 1998; Munkvold et al. 2004; Angelard et al. 2010). By contrast, poor information is available on the role played by plant diversity as a driving force shaping AMF species composition and population dynamics (Davison et al. 2011; Vályi et al. 2015), given the obligate biotrophic status of AMF and their variable host preference.

Differences in AMF community composition along spatial or land-use intensity gradients in agricultural fields were previously reported. For example, different crop management systems involving high intensity of tillage or chemical fertilization use have been shown to affect AMF species composition, spore abundance and mycorrhizal colonization (Douds et al. 1995; Jansa et al. 2003; Oehl et al. 2004; Castillo et al. 2006; Brito et al. 2012) by stimulating the growth of AMF populations more adapted to high input conditions (Johnson et al. 2003; Na Bhadalung et al. 2005; Toljander et al. 2008).

The identity and diversity of crops may represent a strong driving force shaping AMF communities in the field, in particular in low-input and organic farming, where crop rotation and the use of cover crops are common practices, and where effective agricultural management strategies supporting crop plant-beneficial soil microbiota associations should be implemented. Cover crops, which are well known to provide several important ecological services to sustainable and organic agriculture (see e.g. Kabir and Koide 2002; Weil and Kremen 2007; Lehman et al. 2012), play an important role in maintaining and enhancing the number of AMF propagules, the extent of root colonisation, and the soil mycorrhizal potential, e. g. by providing nourishment during winter periods to AMF, which are obligate mutualists (Kabir and Koide 2002). Recently we provided the first evidence of the important role played by winter cover crop identity in promoting early mycorrhizal colonisation and growth of the subsequent crop and soil mycorrhizal activity in organic agroecosystems (Njeru et al. 2014). Such data showed that the right choice of cover crop species opens the

possibility of raising inoculum potential of AMF native strains, a fundamental approach in low-input and organic farming, which rely more on the efficient use of natural soil resources than on the use of external inputs.

Here, we carried out further studies to understand how different levels of cover crop diversity can affect the dynamics of native AMF communities, differentially boosting or depressing different species. Using a molecular approach, we investigated whether the identity and diversity of preceding cover crops are able to shape native AMF communities colonizing the roots of subsequent maize crops in a Mediterranean organic agroecosystem. Achieving this objective can provide insights into strategies for a more sustainable land use aimed at conserving soil beneficial microbial diversity.

## Materials and Methods

### Study site

The experimental fields were located at the Interdepartmental Centre for Agri-environmental Research "Enrico Avanzi" (CIRAA) of the University of Pisa, located at S. Piero a Grado, Pisa (latitude 43°40' N, longitude 10°20' E) in Italy, 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).

Physical and chemical characteristics of soil are: pH (water) 7.66±0.1, total N (Kjeldahl method, g $kg^{-1}$) 1.38±0.14, conductivity (μS) 48±6.96, Clay (%) 18.13±1.72, Silt (%) 19.47±0.6, Sand (%) 62.43±1.65, Available P (Olsen method, mg $kg^{-1}$) 11.4±1.97, organic matter (%) 2.05±0.06. Climatic conditions at the experimental station are typical for Mediterranean regions, with rainfall mostly concentrated in autumn (October to December) and spring (March to April). The experimental field was part of the trials carried out within 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 cover crop diversity in promoting both the mycorrhizal symbiosis and the growth and productivity of subsequent different maize genotypes (Njeru et al. 2014). The field was under a 5-year stockless arable crop rotation, managed as an organic system: weeds were not controlled and unfertilized maize was sown after cover crops were mown and incorporated into the soil by disc harrowing at a depth of 15 cm, at the end of April 2013.

### Experimental design and sampling

Within the scheme of the original experiment we selected two winter cover crop treatments, characterized by low and high plant species diversity, followed by the maize hybrid Pioneer® PR64Y03. We sampled three plots (3×2.5 m each) within each treatment, at the end of the cover crop cycle (end of April 2013) and at the fourth leaf (juvenile) phenological stage of maize plants (June 2013). At the first sampling we selected the most represented plant species of

the communities established in low (LD) and high (HD) diversity cover crop treatments and collected three plants of *Vicia villosa* Roth cv. Latigo plants (hairy vetch) from each of the three LD cover crop plots and three plants of *Phacelia tanacetifolia* Benth. (lacy phacelia), *Trifolium alexandrinum* L. (berseem clover), *Trifolium incarnatum* L. (crimson clover), *Avena* sp. and *V. villosa* from each of the three HD cover crop plots. The second sampling was performed collecting three maize plants from each of the three LD and three HD cover crop plots. Plants were placed in polythene bags and transported to the laboratory for analyses. Fine roots of the three individual plants per species collected in each plot were pooled for DNA extraction, obtaining 18 analytical samples in the first sampling and 6 samples in the second one, and stored at -80°C.

**DNA extraction, PCR, cloning and sequencing**

Fine root material (100 mg) of each sample was ground in liquid nitrogen and genomic DNA was extracted using DNeasy Plant Mini Kit (Qiagen Milan, Italy). Several dilution of extracted DNA were prepared (1:1, 1:10, 1:100) and 1 µl was used as template. Partial small subunit (SSU) of ribosomal RNA gene fragments were amplified in volumes of 25 µl with 0.125 U of GoTaq Flexi DNA Polymerase (Promega, Milan, Italy), 0.4 µM of each primer (AML1/AML2, Lee et al. 2008), 0.2 mM of each dNTP, 1.5 mM of $MgCl_2$ and 1× the manufacturer's reaction buffer. The thermal cycler (Eppendorf Mastercycler personal, Eppendorf, Milano, Italy) was programmed as follows: a manual "hot start" at 94°C for 3 min, 30 cycles at 94°C for 30 sec, 58°C for 40 sec, 72°C for 55 sec and a final extension step at 72°C for 10 min. Reactions yields were estimated by using a 1% agarose gel containing ethidium bromide (0.5 µg $ml^{-1}$).

The SV Wizard® (Promega) PCR-purified amplicons of DNA from the root samples were ligated into the pGem-T Easy vector (Promega) to transform XL10-Gold Ultracompetent *Escherichia coli* cells (Stratagene, La Jolla, CA, USA). The composition of the AM fungal communities was determined using PCR-RFLP screening of clone libraries (AML1/AML2 primers and HinfI and AluI restriction enzymes). Nine to 25 clones were screened by PCR-RFLP analysis per clone library. Plasmids of representative clones of each RFLP pattern in each library were purified by Wizard® Plus SV Minipreps (Promega) and sequenced using T7 vector primers at BMR Genomics s.r.l. (University of Padova, Italy). Ninety two unique cloned sequences generated in this study have been deposited in EMBL Nucleotide Sequence Database (www.ebi.ac.uk/embl/) under the accession numbers (LN906495- LN906586).

**Phylogenetic analysis**

Sequences were edited in MEGA6 and their similarities were determined using the Basic Local Alignment Search Tool (BLASTn) provided by NCBI. The detection of chimeric sequences was performed using USEARCH 6.0 (http://fungene.cme.msu.edu/FunGenePipeline/chimera_check/form.spr).

153 Sequences were aligned with those corresponding to the closest matches from GenBank as well as with
154 sequences from major clades of Glomeromycota using MUSCLE as implemented in MEGA6. Phylogenetic tree were
155 inferred by Neighbour-joining analysis. The evolutionary distances were computed using the Maximum Composite
156 Likelihood method. The confidence of branching was assessed using 1000 bootstrap resamplings. Sequences were
157 assigned to operational taxonomic units (OTUs) on the basis of RFLPs, Blast and phylogenetic analyses. Such analysis
158 was carried out in MEGA6.
159
160 **Statistical analyses**
161 Richness and diversity of AMF communities were evaluated for each set of plots (LD and HD) and each individual
162 host. We determined the rarefaction curves with the EstimateS 9.1.10 software to estimate whether the number of
163 screened sequenced were sufficient to capture AMF diversity of each host.
164 Estimates of community diversity were determined as Shannon entropy (H) and Simpson dominance. The
165 Pielou evenness (H/log S, S = number of species) was used as a measure of how far the Shannon diversity departs from
166 the maximum possible value. In addition, we used the Hill numbers of order 1 (the exponential of Shannon diversity)
167 and 2 (the reciprocal of Simpson dominance), which are the number of equally abundant species necessary to produce
168 the observed value of the relevant diversity index (Chao et al. 2014). The indices were calculated using PAST 3.0. and
169 1000 bootstraps to determine confidence intervals.
170 Non parametric Mann-Whitney U test was used to determine differences in the diversity indices between AMF
171 communities in maize after LD and HD cover crops. The same test was performed to assess differences in AMF
172 communities in vetch roots, grown in LD and HD plots. Kruskal Wallis test was used to compare diversity indices
173 among AMF communities retrieved in the five cover crops.
174 Community similarities among all host species were then visualized through nonmetric multidimensional
175 scaling (NMS) plots, using OTUs abundance data, with dissimilarities calculated using the adjusted Bray-Curtis
176 method, and statistically evaluated by permutational multivariate analysis of variance (PERMANOVA), to test the
177 effect of the diversity (HD or LD) of preceding cover crops on AMF communities of maize, and to test the effect of host
178 species among cover crops. Non parametric tests (Mann-Whitney U and Kruskal Wallis) were performed in SPSS, all
179 other analyses were performed in PAST 3.0 except for NMS which was performed using Canoco 5.0.
180
181 **Results**
182 **Identification of root colonizing AMF**

The AMF communities occurring in the roots of all the samples analysed (18 samples from cover crops and 6 samples from maize) were successfully amplified using the primer pair AML1/AML2, obtaining a fragment of the expected size (~800 bp). A total of 505 clones from the 24 clone libraries were screened by PCR-RFLP analysis, obtaining 19 different RFLP patterns. For each RFLP group several clones originating from different libraries were sequenced, giving a total of 173 Glomeromycota sequences. All non-redundant sequences from the 24 clone libraries (92 out of 173) and 23 references from GenBank were used for neighbour-joining phylogenetic analyses (Fig. 1). After RFLPs, BLASTn and phylogenetic analyses the sequences were grouped into 12 OTUs supported by a bootstrap value >95%, and 4 OTUs (Fun1, Fun2, Rh2, Div1) which were distinguished by the HinfI/AluI RFLP patterns.

Among the 16 OTUs, we retrieved sequences belonging to six out of the eleven Glomeromycota families (Redecker et al. 2013). The most abundant family was represented by Acaulosporaceae, with *Acaulospora cavernata* as the only phylotype, accounting for 37.4% of the sequences, followed by Glomeraceae (37.2%) and Claroideoglomeraceae (23.6%). Diversisporaceae, Paraglomeraceae and Archaeosporaceae sequences corresponded to less than 2.0 % of total sequences. Within the Glomeraceae family we retrieved 7 OTUs (Table 1), 4 of which were identified as *Funneliformis caledonius* (Fun2, 0.4%), *Funneliformis mosseae* (Fun3, 10.7%), *Rhizoglomus intraradices* (synonym *Rhizophagus intraradices*, formerly known as *Glomus intraradices*) (Rh1, 6.7%) and *Rhizoglomus irregulare* (synonym *Rhizophagus irregularis*) (Rh2, 3%) (Table 1); the remaining 3 OTUs (Glo1, Glo2 and Fun1) represented sequences of uncultured *Glomus* and *Funneliformis* species (Table 1). Four OTUs were ascribed to Claroideoglomeraceae family: one was identified as *Claroideoglomus lamellosum* (Cl3, 9.9%), the others (Cl1, Cl2, Cl4) representing uncultured *Claroideoglomus* species. OTUs assigned to Acaulosporaceae, Diversisporaceae, Paraglomeraceae and Archaeosporaceae (Acau, Div1, Div2, Par, Arch) matched to sequences of either known (*Acaulospora cavernata*, *Diversispora aurantia*, *Diversispora epigaea* and *Archaeospora schenckii*) or unknown species (*Paraglomus* sp.) (Table 1). MaarjAM database (http://maarjam.botany.ut.ee/, accessed on 20/03/2015) was used to confirm the assignment of our OTUs to sequences of Glomeromycota. Rarefaction analyses indicated that the number of analysed sequences was sufficient to capture the AMF diversity in the roots of most plant species, since the curves almost reached the asymptote (Fig. S1).

**AMF diversity in cover crop roots**

The total number of OTUs for each plant species varied from 6 to 10 (Fig. 2). The AM fungal communities occurring in *P. tanacetifolia* and *Avena* sp. showed the highest H, Hill1 and Hill2 indices and the least D values among cover crop plants, while legume plants (*V. villosa*, *T. alexandrinum*, *T. incarnatum*) showed an opposite trend (Table 2). All OTUs retrieved in LD were also found in HD plots. The most abundant OTUs - affiliated to *A. cavernata* (Acau), *C.*

214 *lamellosum* (Cl3), *Funneliformis* sp. (Fun1) and *Claroideoglomus* sp. (Cl1) - occurred in all cover crop hosts, though
215 differently distributed in the diverse plant species. Acau and Cl3 sequences were the most frequently retrieved in all
216 hosts, except in *P. tanacetifolia*, where Fun1 and Cl1 were dominant (Fig. 2). In *Avena* sp. such four OTUs were evenly
217 distributed (Fig. 2), while Acau was dominant in legume plants (from 51.6% to 79.7%). The other OTUs were rarely
218 represented: Rh1, Rh2 and Cl2 were peculiar to both *P. tanacetifolia* and *Avena* sp., Fun3 to *T. alexandrinum* and *T.*
219 *incarnatum*, Arch to *T. alexandrinum* and Div2 to *Avena* sp. (Fig. 2). AMF communities detected in the roots of *V.*
220 *villosa* grown either as single cover crop species in LD or mixed with the other four species in HD, did not show
221 significant differences, as revealed by PERMANOVA (P=0.8). By contrast, in HD treatment AMF communities
222 detected in the roots of the five cover crop hosts were significantly different (P=0.035).
223
224 **AMF diversity in maize roots**
225 The total number of OTUs (6) found in maize subsequent to *V. villosa* grown as single cover crop was lower than in
226 maize following the HD cover crop treatment (11). Other diversity indices, such as H, Hill1 and Hill2 also showed a
227 similar trend (Table 2). Sequences related to *F. mosseae* (Fun3), *R. intraradices* (Rh1) and Glo2 represented the most
228 abundant OTUs, accounting for 81% and 69% of sequences in LD and HD cover crop treatments, respectively. All
229 OTUs, except Glo1, were found in both LD and HD maize roots (Fig. 2). AMF communities occurring in maize roots
230 were similarly independent of cover crop treatment, as shown by PERMANOVA analysis.
231
232 **Maize host plants change AMF community associated with preceding cover crop hosts**
233 Nonmetric multidimensional scaling (NMS) performed on OTUs data of both cover crop and subsequent maize root
234 systems revealed large differences in the composition of AMF communities among the different cover crop hosts and a
235 change in AMF root communities in maize (Fig. 3). In particular, AMF communities occurring in the roots of legume
236 plants (*T. alexandrinum*, *T. incarnatum* and *V. villosa*) clustered close in the left part of the plot along axis 2, while
237 those of *Z. mays* and *P. tanacetifolia* plotted in the right and middle part of the graph, respectively.
238 Among the 16 OTUs detected in this study, 11 were common to cover crops and subsequent maize plants, one
239 OTU (Par) was retrieved only in maize roots and the remaining 4 OTUs (Arch, Cl2, Div2, Fun2) were found only in
240 cover crop plants (Fig. 2). Several phylotypes shared among all host plants showed large abundance differences. *A.*
241 *cavernata* sequences (Acau), the most abundant in cover crops (37.4%), were retrieved in very small numbers in maize
242 roots following HD treatment (1.5%) and were absent after LD treatment. Accordingly, other sequences common to
243 cover crop plants (Fun1, Cl1 and Cl3), highly represented in some hosts, were sporadically found in maize plants (Fig
244 2). For instance, Fun1 was detected in *Avena* sp. and *P. tanacetifolia* at high levels, 19.4% and 42.6% respectively,

while represented only 0% and 1.5% of the sequences occurring in *Z. mays* following LD and HD treatments, respectively. On the other hand, the three most abundant sequence types found in maize roots (Fun3, Rh1 and Glo2), affiliated to *F. mosseae*, *R. intraradices* and *Glomus* sp., were sporadically retrieved in cover crop hosts (3.7%).

## Discussion

In this work we showed for the first time a definite change in the composition of native AMF communities colonizing maize roots, which was independent of the identity and diversity of preceding cover crops, in a Mediterranean organic agroecosystem.

### Identification of root colonizing AMF

In the experimental plots, we detected 16 OTUs in the roots of host plants, a higher number than previously found in plants growing in Mediterranean agroecosystems, using molecular methods (Cesaro et al. 2008; Brito et al. 2012). A higher species richness than in our study was detected in Mediterranean semi-arid environments (Torrecillas et al. 2012). Using a morphological approach a previous study on the occurrence of AMF spores in an organic field located in the same area detected a very high number of AMF species (58), never reported from a single site (Njeru et al. 2015). The lower number of species detected in the present work, compared with such a study, may be a consequence of the specific environment of the "hot spot area", the methodology used (morphological *vs* molecular) or the material analysed (spores *vs* roots). Actually, in root material only sequences of colonizing AMF are retrieved, leading to the detection of lower species richness than in soil (Hempel et al. 2007, Balestrini et al. 2010, Alguacil et al. 2014).

In this work we retrieved four phylotypes ascribed to the generalist species *F. mosseae*, *R. intraradices*, *R. irregulare* and *C. lamellosum*, commonly found both in low and high-input farming systems (Oehl et al. 2003, 2005). Interestingly, we found sequences of *D. aurantia*, an AMF species rare in arable soils, in the roots of 3 out of 5 cover crop species and in the subsequent maize plants (both HD and LD treatments). Spores of this species were found in a Mediterranean sand dune system, near to our experimental site (Błaszkowski et al. 2004). The occurrence of *A. cavernata* at high levels in our organic system is worth of notice, since Acaulosporaceae were reported to be sensitive to fertilizers and pesticides commonly used in conventional agriculture (Błaszkowski 1993; Johnson 1993; Helgason et al. 1998). Phylotypes affiliated to *A. trappei*, *D. epigaea* and *Paraglomus* sp. were also detected, although rarely and associated to single plant species, confirming their occurrence in the area (Njeru et al. 2015).

### Effect of LD and HD cover crop treatments on AMF diversity

In cover crop species and subsequent maize the total number of OTUs was higher in HD than in LD treatments and other diversity indices (H, Hill1 and Hill2) showed a similar trend. No significant effects of the cover crop treatments (LD *vs* HD ) on AMF community composition of *V. villosa* and maize were found, as shown by PERMANOVA analyses. Little is known about the effects of diversified cover crop treatments on AMF diversity. A previous work, based on morphological identification of spores, showed increases of AMF species richness in plots with high plant diversity (Njeru et al. 2015). Other studies reported that a mixture of cover crops had a positive effect on AMF propagules and biomass (Lehman et al. 2012) and on mycorrhizal colonization (Njeru et al. 2014), compared to monocultures. We found that nearby plant species did not influence root AMF community composition in *V. villosa*, that hosted nearly the same AMF community when cultivated alone (LD) and together with other cover crop species (HD), in contrast with other data (Mummey et al. 2005; Hausmann and Hawks 2009).

**Changes in AMF communities colonising the roots of cover crops and subsequent maize**

In our study AMF communities colonising cover crop and subsequent maize roots were completely different, in terms of relative abundance of single species. Other works reported either consistent findings (Mathimaran et al. 2007) or contrasting results, showing that winter cover crops and subsequent crops shared similar AMF communities (Higo et al. 2014, 2015). Possible explanations of changes in AMF species abundance found in our work may entail either host preference or seasonal variations. Indeed, cover crop and maize samplings were carried out in late spring and in early summer, respectively, a very limited temporal change; in addition, we detected variability in AMF communities among cover crop species in the same season. Some studies showed seasonal variations of root AMF communities in natural ecosystems, especially between winter and summer (Helgason et al. 1999; Dumbrell et al. 2011, Montero Sommerfeld et al. 2013). In cover crop species belonging to Fabaceae (*V. villosa* and *Trifolium* spp) we retrieved a great abundance of *A. cavernata* sequences, confirming the occurrence of Acaulosporaceae in early season, as reported by other authors (Hijri et al. 2006; Mello et al. 2015). However, as such sequences were very rare in maize roots, the question as to whether their sporadic occurrence depends on season or host preference remains to be answered.

AMF community composition has long been known to be more dependent on host plant identity (Helgason et al. 2002; Vandenkoornhuyse et al. 2003; Gollotte, et al. 2004; Scheublin et al. 2004; Sýkorová et al. 2007) than on habitat (Becklin et al. 2012), season (Davison et al. 2011), phosphorus levels (Gosling et al. 2013) or land use intensity (Vályi et al. 2015). Indeed, some authors hypothesized that AMF communities are not random assemblages, but are associated with ecological groups of plant species showing specific traits (habitat generalist *vs* forest specialist) (Davison et al. 2011). We found that legume plants (*V. villosa* and *Trifolium* spp.) hosted similar communities, compared with phylogenetically divergent species (*Avena* sp., *P. tanacetifolia*), confirming previous data (Scheublin et

al. 2004). By contrast, other studies showed that closely related plants have distinct mycorrhizal communities (Montesinos-Navarro et al. 2012; Reinhart et al. 2014; Veresoglou and Rillig 2014). Plant host preference may be explained by findings showing that plant transcriptional responses to AMF vary in gene expression levels, depending on plant and fungal genotypes involved in the symbiosis (Hohnjec et al. 2005). Accordingly, plasticity in the expression of a core set of plant genes has been detected in the interaction with different AMF taxa from Glomeraceae, Acaulosporaceae, Gigasporaceae and Diversisporaceae (Massoumou et al. 2007; Feddermann et al. 2008). Moreover, during plant/fungus interactions preceding the establishment of the symbiosis, chemical signals released by plant roots may differentially affect recognition process and colonization by different AMF symbionts (Giovannetti et al. 1993; Akiyama et al. 2005; Scervino et al. 2005).

## Conclusions

In this work, in a Mediterranean organic experimental agroecosystem and using molecular methods, we showed that diversified cover crop treatments did not represent significant ecological drivers shaping native AMF community diversity and composition in the roots of subsequent maize. The differential abundance of native AMF species in the preceding and subsequent crops and the prevalence of AMF generalists, such as *F. mosseae* and *R. intraradices* in maize roots support the view that host preference may represent a strong driver of AMF community dynamics in agroecosystems. Our work provides a prime starting point for ecological studies concerning host preferential changes of AMF communities in roots, where fungal species may be boosted or depressed, possibly in relation to their functional significance. A better understanding of the complex interactions between AMF dynamics and diversity and plant performance represents a great challenge for future studies aimed at exploiting all the potential benefits of native AMF communities to crop productivity and ecosystem health.

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474

475 **Table1.** Sequence types of arbuscular mycorrhizal fungi, identified using AML1-AML2 primers pair, in the roots of

476 plants from cover crop treatments and subsequent maize.

| OTU name | Identity (%) | Taxonomic affiliation |
|---|---|---|
| Acau | AJ306442 (99) | *Acaulospora cavernata* |
| Arch | FR773150 (98) | *Archaeospora schenckii* |
| Cl1 | HG004500 (99) | *Claroideoglomus* sp. |
| Cl2 | HE576833 (99) | *Claroideoglomus* sp. |
| Cl3 | AJ276087 (99) | *Claroideoglomus lamellosum* |
| Cl4 | JN791123 (99) | *Claroideoglomus* sp. |
| Div1 | AM713432 (99) | *Diversispora aurantia* |
| Div2 | X86687 (99) | *Diversispora epigaea* |
| Fun1 | JN644423 (100) | *Funneliformis* sp. |
| Fun2 | Y17635 (99) | *Funneliformis caledonium* |
| Fun3 | FR750227 (99) | *Funneliformis mosseae* |
| Glo1 | FN869768 (99) | *Glomus* sp. |
| Glo2 | FR848608 (99) | *Glomus* sp. |
| Par | HE613466 (99) | *Paraglomus* sp. |
| Rh1 | FR750206 (99) | *Rhizoglomus intraradices* |
| Rh2 | HF968850 (99) | *Rhizoglomus irregulare* |

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**Table 2.** Simpson (D), Shannon-Weaver (H), Equitability (J), the exponential of Shannon diversity (Hill1) and the reciprocal of Simpson dominance (Hill2) indices (95% confidence limits) calculated from AMF community phylotypes associated to different cover crop and maize plants growing in low diversity (LD) and high diversity (HD) cover crop plots.

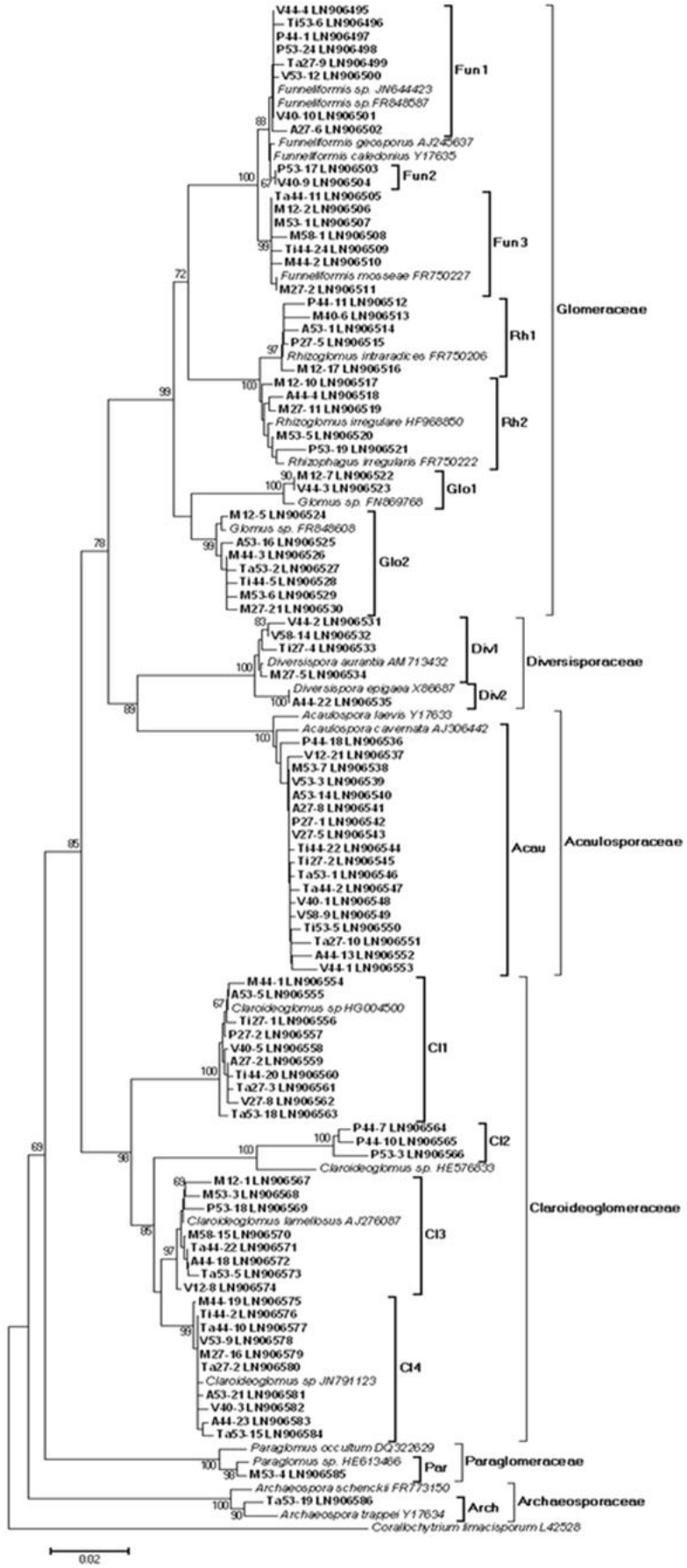
| **Cover crop treatment** | **Plant species** | **Dominance -D** | | **Shannon-H** | | **Equitability-J** | | **Hill1** | | **Hill2** | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Lower | Upper | Lower | Upper | Lower | Upper | Lower | Upper | Lower | Upper |
| LD | *V. villosa* | 0.313 | 0.532 | 0.906 | 1.367 | 0.569 | 0.816 | 2.210 | 3.922 | 1.846 | 3.208 |
| HD | *V. villosa* | 0.381 | 0.641 | 0.778 | 1.305 | 0.433 | 0.687 | 2.118 | 3.653 | 1.556 | 2.618 |
| HD | *T. alexandrinum* | 0.498 | 0.771 | 0.528 | 1.086 | 0.307 | 0.578 | 1.602 | 2.888 | 1.256 | 1.988 |
| HD | *T. incarnatum* | 0.232 | 0.440 | 1.229 | 1.717 | 0.608 | 0.826 | 3.352 | 5.446 | 2.288 | 4.249 |
| HD | *P. tanacetifolia* | 0.179 | 0.254 | 1.514 | 1.898 | 0.741 | 0.881 | 4.548 | 6.585 | 3.886 | 5.550 |
| HD | *Avena* sp. | 0.178 | 0.351 | 1.453 | 1.926 | 0.680 | 0.879 | 4.106 | 6.688 | 2.820 | 5.461 |
| LD | *Z. mays* | 0.221 | 0.329 | 1.322 | 1.614 | 0.741 | 0.901 | 2.527 | 5.077 | 3.023 | 4.536 |
| HD | *Z. mays* | 0.188 | 0.375 | 1.500 | 1.996 | 0.634 | 0.833 | 4.017 | 7.008 | 3.626 | 4.994 |

**Figure captions**

**Fig. 1** Neighbor-Joining phylogenetic tree of glomeromycotan sequences derived from plant roots of cover crops and subsequent maize plants. Bootstrap values are shown when they exceed 70% (1,000 replications). The analysis is based on partial nuclear small subunit ribosomal RNA gene sequences (SSU; ~ 800 bp; AML1/AML2 fragment) and involved 115 nucleotide sequences. Different sequence types are indicated in brackets and names are reported in Table 1. AMF family are also reported. Sequences obtained in the present study are shown in bold and their accession numbers are prefixed with plant species/field plot clone identifiers (V= *V. villosa*; P=*P. tanacetifolia*; Ti= *T. incarnatum*; Ta= *T. alexandrinum*, A=*Avena* sp.; M= *Z. mays*). The tree is rooted with a reference sequence of *Corallochytrium lymacisporum* (L42528).

**Fig. 2** Relative abundance (%) of AMF phylotypes detected in the roots of the different host plants occurring in the low diversity (LD) and high diversity (HD) cover crop treatments.

**Fig. 3** Nonparametric multidimensional scaling (NMS) ordination of AMF colonizing host species occurring in plots managed with low diversity (LD) (green circles) and high diversity (HD) (red circles) cover crop treatments. A, *Avena* sp.; P, *Phacelia tanacetifolia*; Ti, *Trifolium incarnatum*; Ta, *Trifolium alexandrinum*; V, *Vicia villosa* and M, *Zea mays*. The names of the different sequence types are reported in Table 1.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Ti53-6 LN906496
P44-1 LN906497
P53-24 LN906498
Ta27-9 LN906499
V53-12 LN906500
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Funneliformis sp. FR848587
V40-10 LN906501
A27-6 LN906502
Funneliformis geosporus AJ245637
Funneliformis caledonius Y17635
P53-17 LN906503
V40-9 LN906504
Fun1
Fun2
Ta44-11 LN906505
M12-2 LN906506
M53-1 LN906507
M58-1 LN906508
Ti44-24 LN906509
M44-2 LN906510
Funneliformis mosseae FR750227
M27-2 LN906511
Fun3
P44-11 LN906512
M40-6 LN906513
A53-1 LN906514
P27-5 LN906515
Rhizoglomus intraradices FR750206
M12-17 LN906516
Rh1
M12-10 LN906517
A44-4 LN906518
M27-11 LN906519
Rhizoglomus irregulare HF968850
M53-5 LN906520
P53-19 LN906521
Rhizophagus irregularis FR750222
Rh2
Glomeraceae
M12-7 LN906522
V44-3 LN906523
Glomus sp. FN869768
Glo1
M12-5 LN906524
Glomus sp. FR848608
A53-16 LN906525
M44-3 LN906526
Ta53-2 LN906527
Ti44-5 LN906528
M53-6 LN906529
M27-21 LN906530
Glo2
V44-2 LN906531
V58-14 LN906532
Ti27-4 LN906533
Diversispora aurantia AM713432
M27-5 LN906534
Diversispora epigaea X86687
A44-22 LN906535
DiM1
DiM2
Diversisporaceae
Acaulospora laevis Y17633
Acaulospora cavernata AJ306442
P44-18 LN906536
V12-21 LN906537
M53-7 LN906538
V53-3 LN906539
A53-14 LN906540
A27-8 LN906541
P27-1 LN906542
V27-5 LN906543
Ti44-22 LN906544
Ti27-2 LN906545
Ta53-1 LN906546
Ta44-2 LN906547
V40-1 LN906548
V58-9 LN906549
Ti53-5 LN906550
Ta27-10 LN906551
A44-13 LN906552
V44-1 LN906553
Acau
Acaulosporaceae
M44-1 LN906554
A53-5 LN906555
Claroideoglomus sp HG004500
Ti27-1 LN906556
P27-2 LN906557
V40-5 LN906558
A27-2 LN906559
Ti44-20 LN906560
Ta27-3 LN906561
V27-8 LN906562
Ta53-18 LN906563
Cl1
P44-7 LN906564
P44-10 LN906565
P53-3 LN906566
Cl2
Claroideoglomus sp. HE576833
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M53-3 LN906568
P53-18 LN906569
Claroideoglomus lamellosus AJ276087
M58-15 LN906570
Ta44-22 LN906571
A44-18 LN906572
Ta53-5 LN906573
V12-8 LN906574
Cl3
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Ta44-10 LN906577
V53-9 LN906578
M27-16 LN906579
Ta27-2 LN906580
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A53-21 LN906581
V40-3 LN906582
A44-23 LN906583
Ta53-15 LN906584
Cl4
Claroideoglomeraceae
Paraglomus occultum DQ322629
Paraglomus sp. HE613486
M53-4 LN906585
Par
Paraglomeraceae
Archaeospora schenckii FR773150
Ta53-19 LN906586
Archaeospora trappei Y17634
Arch
Archaeosporaceae
Corallochytrium limacisporum L42528
0.02

**Fig. 1**

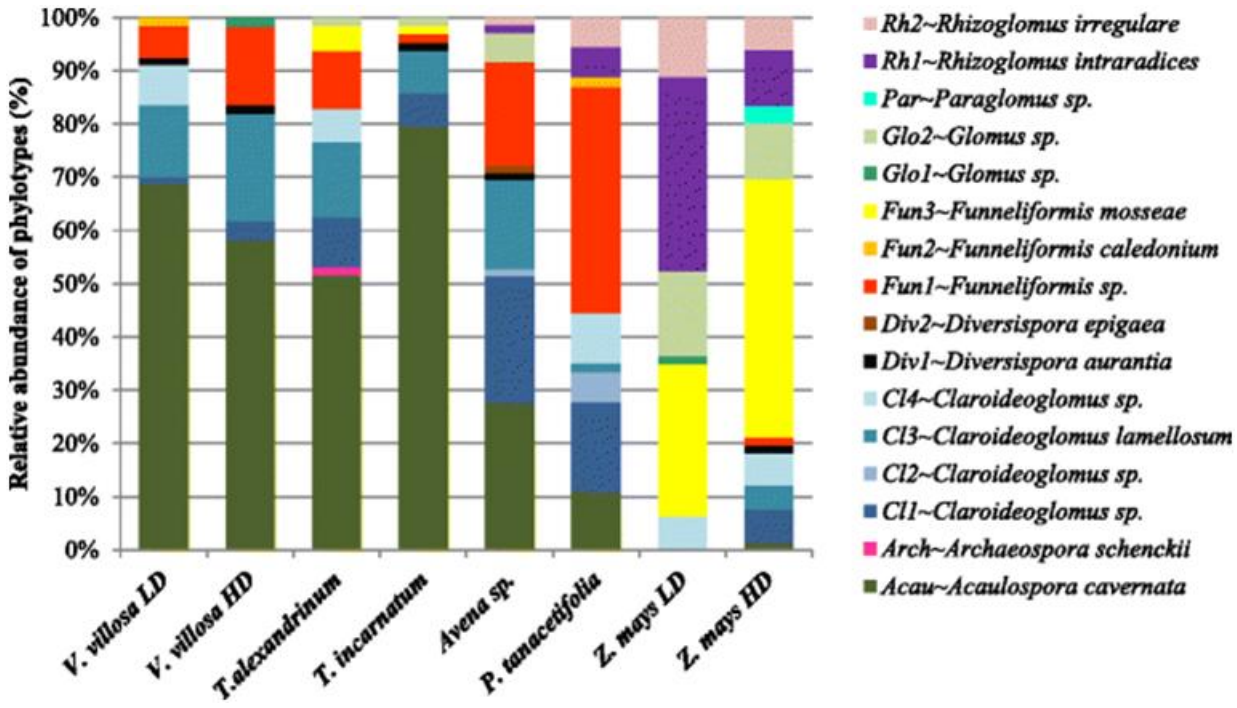
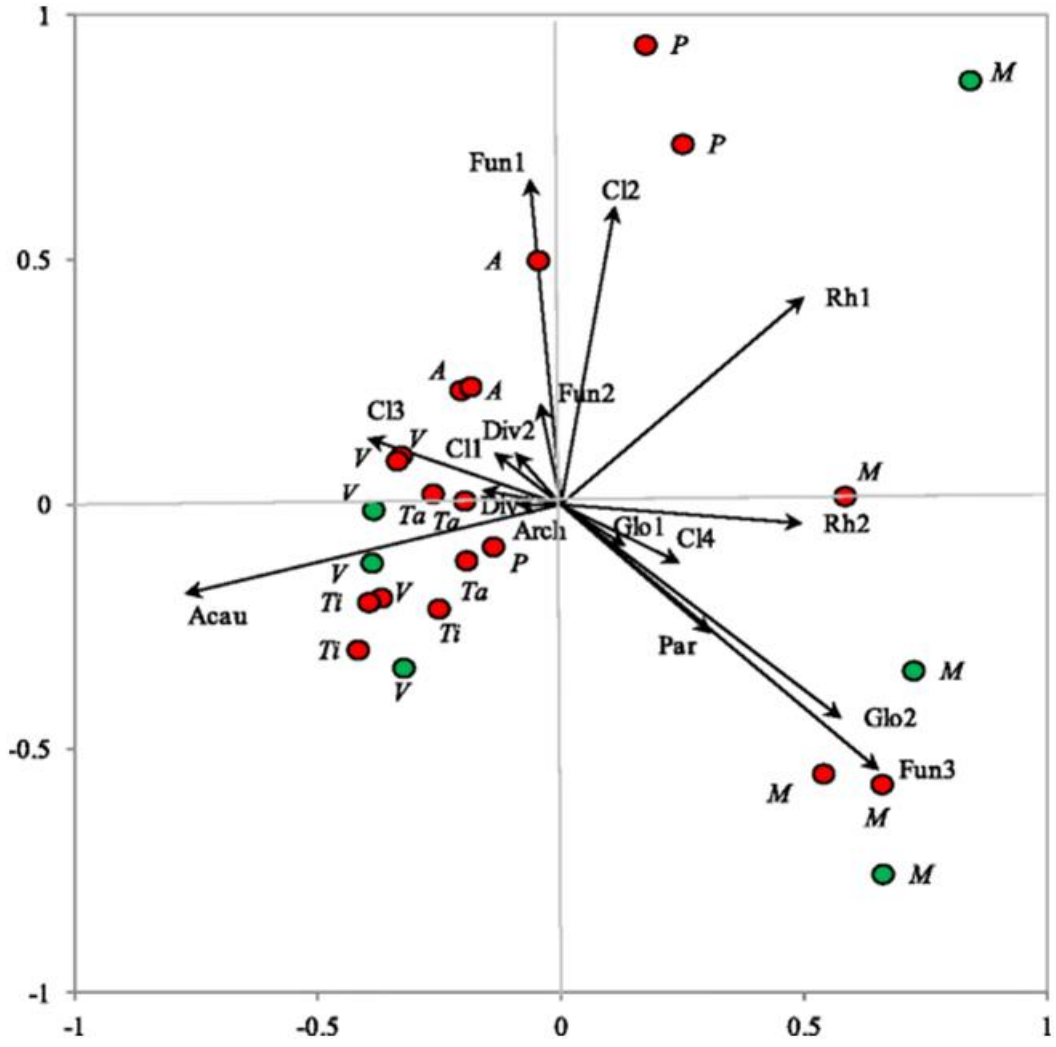

Relative abundance of phylotypes (%)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
V. villosa LD
V. villosa HD
T.alexandrinum
T. incarnatum
Avena sp.
P. tanacetifolia
Z. mays LD
Z. mays HD
Rh2~Rhizoglomus irregulare
Rh1~Rhizoglomus intraradices
Par~Paraglomus sp.
Glo2~Glomus sp.
Glo1~Glomus sp.
Fun3~Funneliformis mosseae
Fun2~Funneliformis caledonium
Fun1~Funneliformis sp.
Div2~Diversispora epigaea
Div1~Diversispora aurantia
Cl4~Claroideoglomus sp.
Cl3~Claroideoglomus lamellosum
Cl2~Claroideoglomus sp.
Cl1~Claroideoglomus sp.
Arch~Archaeospora schenckii
Acau~Acaulospora cavernata

**Fig. 2**


1
0.5
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-1
-0.5
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M
P
Fun1
Cl2
A
Rh1
A
A
Fun2
Cl3
V
Div2
V
Cl1
M
V
Div1
Ta
Ta
Arch
Glo1
Cl4
Rh2
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Acau
Ti
Par
Ti
M
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Fun3
M
M
M

**Fig. 3**