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Mycorrhizal activity and diversity in a long-term organic Mediterranean agroecosystem

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Abstract:	In organic agriculture, soil fertility and productivity rely on biological processes carried out by soil microbes, which represent the key elements of agroecosystem functioning. Arbuscular mycorrhizal fungi (AMF), fundamental microorganisms for soil fertility, plant nutrition and health, may play an important role in organic agriculture, by compensating for the reduced use of fertilizers and pesticides. Though, AMF activity and diversity following conversion from conventional to organic farming is poorly investigated. Here we studied AMF abundance, diversity and activity in short- and long-term organically and conventionally managed Mediterranean arable agroecosystems. Our results show that both AMF population activity, as assessed by the mycorrhizal inoculum potential (MIP) assay, the percentage of colonized root length of the field crop (maize) and glomalin-related soil protein (GRSP) content were higher in organically managed fields and increased with time since transition to organic farming. Here, we showed an increase of GRSP content in arable organic systems and a strong correlation with soil MIP values. The analysis of AMF spores showed differences among communities of the three microagroecosystems in terms of species richness and composition, as suggested by a multivariate analysis. All our data indicate that AMF respond positively to the transition to organic farming, by a progressive enhancement of their activity, that seems independent from the species richness of the AMF communities. Our study contributes to the understanding of the effects of agricultural managements on AMF, which represent a promising tool for the implementation of sustainable agriculture.
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Mycorrhizal activity and diversity in a long-term organic Mediterranean agroecosystem

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Abstract

In organic agriculture, soil fertility and productivity rely on biological processes carried out by soil microbes, which represent the key elements of agroecosystem functioning. Arbuscular mycorrhizal fungi (AMF), fundamental microorganisms for soil fertility, plant nutrition and health, may play an important role in organic agriculture, by compensating for the reduced use of fertilizers and pesticides. Though, AMF activity and diversity following conversion from conventional to organic farming is poorly investigated. Here we studied AMF abundance, diversity and activity in short- and long-term organically and conventionally managed Mediterranean arable agroecosystems. Our results show that both AMF population activity, as assessed by the mycorrhizal inoculum potential (MIP) assay, the percentage of colonized root length of the field crop (maize) and glomalin-related soil protein (GRSP) content were higher in organically managed fields and increased with time since transition to organic farming. Here, we showed an increase of GRSP content in arable organic systems and a strong correlation with soil MIP values. The analysis of AMF spores showed differences among communities of the three microagroecosystems in terms of species richness and composition, as suggested by a multivariate analysis. All our data indicate that AMF respond positively to the transition to organic farming, by a progressive enhancement of their activity, that seems independent from the species richness of the AMF communities. Our study contributes to the understanding of the effects of agricultural managements on AMF, which represent a promising tool for the implementation of sustainable agriculture.

Key Words: Organic agriculture; Arbuscular mycorrhizal fungi; Glomalin-related soil protein; Mycorrhizal inoculum potential; Soil fertility

Introduction

Organic agriculture, defined as "a production system that sustains the health of soils, ecosystems and people" by the International Federation of Organic Agriculture Movements (http://www.ifoam.org/growing_organic/definitions/doa/index.html), is a broad group of farming systems characterized by strict limitation of chemical fertilizers, herbicides and pesticides, by soil management through addition of organic materials and by the use of crop rotation (IFOAM, 2006). As a consequence, soil fertility and productivity of organic farming systems largely rely on biological processes carried out by soil microbes, which represent the key elements of agroecosystem functionality and, therefore, a critical factor for the success of organic agriculture (Lampkin 1990; Gosling et al. 2006).

Arbuscular mycorrhizal fungi (AMF) are considered the most important soil organisms for agro-ecosystem sustainability, as they establish root symbioses with most crop plants and, acting as a living interface between plant roots and soil, translocate mineral nutrients - mainly P, N, Zn, Ca, Cu - from soil to plants by the large extraradical mycelial network spreading from mycorrhizal roots into the surrounding environment (Giovannetti and Avio 2002; Smith and Read 2008). AMF also affect soil quality, improving soil structure by promoting the accumulation of the extracellular proteinaceous substance named glomalin-related soil protein (GRSP), which largely contributes to the formation of water stable macroaggregates of soil particles (Wright and Upadhyaya 1998; Rillig 2004; Bedini et al. 2009). Recently, a role of AMF in the synthesis of plant secondary metabolites has been reported, contributing to the production of safe and high-quality food (Ceccarelli et al. 2010; Giovannetti et al. 2012).

Intensive agricultural practices, such as monocropping, deep ploughing, chemical fertilization and pesticide use are detrimental to soil microbial linkages (Matson et al. 1997; Mäder et al. 2002; Tilman et al 2002), negatively affecting AMF populations in terms of biodiversity (Sasvári et al.

2011; Daniell et al. 2001; Douds and Johnson 2007), activity - evaluated as colonization ability (Mozafar et al. 2000; Ryan et al. 2000) - and GRSP production (Wright et al. 1999; Wright and Anderson 2000; Bedini et al. 2007, 2009; Wilson et al. 2009). On the other hand several studies showed that AMF diversity (Oehl et al. 2003, 2004; Verbruggen et al. 2010), root colonization (Mäder et al. 2000; Smukler et al. 2008) and spore abundance (Galvez et al. 2001; Oehl et al. 2003, 2004) were higher in organically managed soils, suggesting that AMF may play an essential functional role in the maintenance of soil biological fertility and structure, compensating for the reduced use of chemical fertilizers and pesticides (Galvez et al. 2001; Lekberg and Koide 2005; Gosling et al. 2006; Ryan and Tibbet 2008).

The conversion from conventional to organic management, with the resulting changes in AMF communities, is a slow and gradual process (Göllner et al. 2005); in particular, in Mediterranean and semi-arid soils, such a process may be even longer, as a result of the low content and high turn-over of organic matter (Raviv 2010). Besides, most of the mycorrhiza-related literature originates from temperate regions and little is known about the effects of long-term organic management on AMF communities of arable soil in the Mediterranean area, where about 5 million hectares are organically managed, one of which in Italy (ISMEA 2008). Investigations on the effects of organic farming on AMF communities in Mediterranean arable soils could contribute to the understanding of the behaviour of these symbiotic fungi and their role as provider of ecological services in sustainable agriculture.

In this work we utilized different parameters to evaluate AMF abundance, diversity and activity in arable soils of Central Italy, 6 and 16 years after conversion from conventional to organic farming. We assessed: i) AMF activity in the soil by the mycorrhizal inoculum potential (MIP) bioassay; ii) AMF colonization of the crop plant (maize); iii) soil GRSP content; iv) correlations between GRSP and different AMF activity parameters; v) AMF spore number, biomass and diversity.

Materials and methods

Description of the long term experiment

The Montepaldi Long Term Experiment (MOLTE) is active since 1991 in the farm of the University of Florence (San Casciano Val di Pesa, 11° 09' 08''E, 43° 40' 16'' N, 90 m a.s.l.), covering a slightly sloping surface of about 15 hectares (Vereijken 1994, 1997, 1999; Migliorini and Vazzana 2007). MOLTE includes three microagroecosystems:

a) "Old Organic" (OldO) with an area of 5.2 ha, divided into 4 fields under organic management (former EC Regulation 2092/91 and now EC reg. 834/07) since 1991;

b) "Young Organic" (YngO) with an area of 5.2 ha, divided into 4 fields under EC regulations 2078/92 (integrated farming) from 1991 to 2000 and converted into organic management since 2001;

c) "Conventional" (Conv) with an area of 2.6 ha divided into 2 conventional fields, where farming techniques were those normally used in the territory of the study area for conventional management.

A four-year crop rotation has been adopted in OldO and YngO since 2001: green manure+spring crop (maize or sunflower)/winter cereal (barley or wheat)+red clover/red clover II/winter cereal (barley or wheat). A biennial rotation has been adopted in Conv: spring crop (maize or sunflower)/winter cereal (barley or wheat).

Fields (260 x 50 m) were tilled by ploughing at 25-30 cm of depth. The crops were rainfed. The mineral and organic fertilizers used in OldO and YngO systems were: guano (N:P:K ratio, 06:15:03), chicken dung (N:P:K ratio, 4:4:0), organic N fertilizer in pellets of diverse origin. The mineral and synthetic fertilizers used in Conv were: liquid fertilizer (N:P:K ratio, 30:0:0), ammonium polyphosphate (N:P:K ratio, 10:34:0), urea (N:P:K ratio, 46:0:0), triple superphosphate (N:P:K ratio, 0:46:0), ammonium nitrate (N:P:K ratio, 27:0:0) and diammonium phosphate (N:P:K

ratio, 18:46:0). Since 2001, the fertilization rate ($N\text{-}P_2O_5$, kg $ha^{-1}y^{-1}$) was: OldO 12-13 for winter cereals and 18-20 for spring crops; YngO 17-20 for winter cereals and 14-2 for spring crops; Conv 120-70 for winter cereals and 95-65 for spring crops. In organic fields weed control was performed by mechanical cultivation and plant diseases were controlled by indirect means (crop rotation and ecological infrastructures represented by natural and planted vegetation in mixed hedgerows and grass strips), while in the conventional field weeds control was performed using the chemical herbicides Zodiac Dicuran (AI: Diflufenican 0.06 and Clortoluron 1.36 Kg ha^{-1}) and Primigran TZ (AI: Terbuthylazine 0.9 and Metolachlor 1.8 Kg ha^{-1}).

The microagroecosystems were surrounded by ecological infrastructures such as natural and artificial hedges, in order to minimise interaction effects and cross-contaminations among the differently managed fields.

Soil sampling was carried out in the fields where maize was grown.

Climate and soil characteristics

Climatic conditions of the experimental area are typical of the Mediterranean sub-Apennines zone. The annual rainfall is about 770 mm with maximum in autumn and spring and minimum in the period June-August. The annual mean temperature was 14°C with maximum above 30°C in summer and minimum about -1°C in winter, in the period 1992-2008.

The soil of MOLTE is composed of parent rock material derived from Pliocene sediments (slope zones) and river Pesa fluvial deposit from Holocene (plane zones), classified as *Fluventic Xerochrepts* (Lulli et al. 1980). Texture varies from “silty clay loam” to “clay loam”. Soil samples for chemical analyses were collected in October 2007, after maize harvest, from the OldO, YngO and Conv fields where maize was grown. Four samples were collected at 0-25 cm depth from each field, air-dried, crushed and passed through a 2-mm sieve. Chemical soil characteristics were analysed as follows: pH in a 1:2.5 (w/v) soil water ratio, total organic C by the Walkley-Black

method (Nelson and Sommers 1996), total N by Kjeldahl digestion (Bremner 1996), available P (Olsen P) by extracting soil with 0.5 M $NaHCO_3$ at pH 8.5 (Olsen and Sommers 1982), exchangeable K using ammonium nitrate method (Mehlich, 1984), following official methods (DM 13/09/1999 SO - GU n°248 21/10/1999 Met III.1) in an external laboratory (accredited by SINAL, www.accredia.it).

Field sampling for mycorrhizal assessment

After maize harvest, in October 2007, 4 plots (plot size, 130 x 25 m) were sampled within each maize field in each microagroecosystem. Each sample was obtained by combining four sub-samples, collected from each plot, 25 m apart, using a soil corer (5 cm diameter, 20 cm deep). Then, soil samples (12), containing also maize roots, were transferred to the laboratory in individual polyethylene bags, carefully ground by hand, and after separating maize roots, were air dried and kept at 4 °C until analysed.

Mycorrhizal root colonization

AMF colonization was assessed on maize roots and percentage of colonized root length was determined after root staining with 0.05% Trypan blue in lactic acid (Phillips and Hayman 1970), using the gridline intersect method (Giovannetti and Mosse 1980).

Mycorrhizal Inoculum Potential bioassay

Mycorrhizal Inoculum Potential (MIP) of the experimental field soils was assessed using *Cichorium intybus* L. as host plant. *C. intybus* seeds were sown in 50 mL sterile plastic tubes filled with 40 mL of each soil sample. Three replicate tubes per soil sample were prepared. Five days after emergence,

C. intybus plants were thinned to 2 individuals per tube. Ten, 20 and 30 days after emergence, plants were removed from tubes and root systems were washed, stained and mounted on microscope slides. Root length and colonized root length were measured on stained roots at magnification of x40 using a grid eyepiece under a dissecting microscope (Wild, Leica, Milano, Italy). Number of entry points were assessed on stained 10 and 20 days old roots at magnification of x125 and verified at magnification of x500 under a Polyvar light microscope (Reichert-Jung, Vienna, Austria).

Analysis of GRSP

Glomalin-related soil protein was extracted following Wright and Upadhyaya (1998). Easily extractable GRSP (EE-GRSP) was extracted from 1 g of 2 mm sieved dry soil for each sample using 8 ml of a 20 mM citrate solution, pH 7.0 at 121°C for 30 min. The extracts were centrifuged at 5000 g for 20 min to pellet soil particles and the supernatant was decanted and stored at 4°C until analysed. Total GRSP (T-GRSP) was extracted after EE-GRSP from the same samples by repeated cycles of extraction with 50 mM citrate, pH 8.0 at 121°C for 60 min. Extraction of T-GRSP continued until the supernatant content of GRSP was under the method detection limits (ca. 2 mg ml^{-1}). Extracts of T-GRSP from each cycle were pooled and centrifuged at 10000 g for 10 min to remove residual soil particles. Glomalin-related soil protein content was determined by Bradford assay (Sigma-Aldrich, Inc.) with bovine serum albumin as the standard.

Fungal spore extraction and identification

Spores and sporocarps of AMF were extracted from duplicate sievings of 25 g of each sample, by wet-sieving and decanting, through a set of nested sieves (Gerdemann and Nicolson 1963). Spores and sporocarps retained on sieves of pore size 400, 250, 100 and 50 µm were flushed into Petri

dishes, examined under a dissecting microscope (Wild, Leica) at magnifications up to x50 with illumination by incident light from a fibre-optic quartz-halogen light source. When present, sporocarps were dissected with forceps and the released spores were counted. Spores were manually isolated using capillary pipettes according to their morphology and colour, mounted on microscope slides in polyvinyl alcohol lactoglycerol (PVLG), and examined under the Polyvar light microscope. For taxonomical identification, which was morphologically based, spores were mounted both in PVLG and in PVLG + Melzer's reagent (1:1, v:v) as media. Qualitative spore traits (spore shape, colour and size, spore wall ornamentation, wall structure and shape, colour and size of the subtending hypha) were examined on at least 20 spores for each morphotype, except for *Glomus badium*, *Glomus* spp. 3 and 5 and *Acaulospora* sp., which were found in low number. Spore morphotypes were compared with original diagnoses of AMF species and with the reference culture descriptions at http://invam.caf.wvu.edu/fungi/taxonomy/speciesID.htm and http://www.agro.ar.szczecin.pl/ ~jblaszkowski/index.html. Since important changes of AMF nomenclature have been recently proposed by different authors (Oehl et al. 2011; Krüger et al. 2012), with some taxa differently named, we utilised the new binomials only for consistent species names and maintained the previous ones for the others.

Total spore densities were determined as spore number 100 g^{-1} soil. Abundance of each species was then converted into biovolume, calculated as $V = 1/6\pi D^3$ (D = spore diameter) for species with spherical spores, or as $V = 1/6\pi D_1 D_2^2$ (D_1 = larger diameter; D_2 = smaller diameter) for species with elongated spores. Total spore biovolume per sample was obtained by summing spore biovolumes of all species recorded in the sample. Relative abundance was calculated as the spore biovolume of an individual AMF taxon divided by the total spore biovolume within a sample.

Species richness was measured as the number of AMF species recorded in each sample, and Shannon diversity index (H) and equitability (E = H divided by the logarithm of number of taxa) and Simpson index of diversity (D) were calculated on spore number data using PAST 1.99 software (Hammer et al. 2001).

Statistical analyses

Data of all experiments were analysed by one-way analysis of variance (ANOVA) using management treatment as factor. The data were logarithm or arcsine transformed when needed to fulfil the assumptions of the ANOVA. Analyses were performed with the SPSS 18.0 software (SPSS Inc., Chicago, IL, USA). Means and standard errors (S.E.) given in tables are for untransformed data.

Principal component analysis (PCA) was performed to show the abundance of AMF species in the different microagroecosystems, using spore numbers after logarithmic transformation, with the PAST software. Species occurring only in one sample and with a spore number lower than 1% of total spores, were excluded from the analyses.

Results

Soil characteristics

Soil chemical analyses showed that in the experimental area the pH was moderately alkaline with a low level of organic matter, total N and available phosphate content and a normal content of exchangeable potassium. No significant differences were observed among the three microagroecosystems except for organic C content, which was significantly higher in YngO than in Conv soil (Table 1).

Mycorrhizal root colonization

Percentages of maize colonized root length at the end of the cultivation cycle were significantly different among the management systems ($F_{2,7} = 5.94$, $P = 0.031$) and were 60.2 ± 2.8, 50.3 ± 5.2 and 43.4 ± 3.9 in OldO, YngO and Conv fields, respectively. Tukey HSD Post hoc test showed a clear separation between OldO and Conv values ($P < 0.05$).

Mycorrhizal Inoculum Potential bioassay

Mycorrhizal Inoculum Potential was significantly different among the three microagroecosystems. Fungal entry points in *C. intybus* roots were significantly higher in OldO and YngO soils (0.59 ± 0.05 and 0.60 ± 0.10 mm^{-1}, respectively), than in Conv soil (0.23 ± 0.04 mm^{-1}), 20 days after emergence ($F_{2,9} = 4.964$; $P < 0.05$). A similar pattern was observed for mycorrhizal root colonization, which showed significant differences among management systems, 30 days after emergence ($F_{2,9} = 21.436$; $P < 0.001$) (Fig. 1).

Analysis of GRSP

The concentration of T-GRSP was significantly higher in organic microagroecosystems ($F_{2,8} = 7.398$, $P = 0.015$). OldO soil showed the highest T-GRSP content, whereas the lowest value was found in Conv soil (Table 2). On the contrary, no differences were detected in the EE-GRSP content among the three management systems ($F_{2,8} = 0.033$, $P = 0.967$) (Table 2). Regression analyses showed a significant positive effect of organic management on T-GRSP content. The time since conversion from conventional to organic farming explained 58% of the variance in T-GRSP concentration (R=0.797; P=0.003). Positive correlations were also observed between T-GRSP and root colonization values of 30 days' old *C. intybus* plantlets in the MIP test (R=0.691; $P = 0.018$) and between T-GRSP values and the percentage of colonized root length in the host crop, maize (R=0.485; P=0.155).

Fungal spore number and morphotypes

Spore numbers (spore number 100 g^{-1} soil) in YngO (542.5 ± 61.2) and Conv fields (395.5 ± 93.1) were significantly higher ($F_{2,9} = 9.809$, $P = 0.005$) than in OldO (136.0 ± 23.2). Total spore volume, ranging from 220 ± 27 to 296 ± 10 nL, did not differ significantly among management treatments ($F_{2,9} = 3.396$, $P = 0.08$).

Overall, in the three microagroecosystems fifteen AMF spore morphotypes were identified, belonging to *Acaulospora*, *Claroideoglomus*, *Funneliformis*, *Glomus, Pacispora*, *Scutellospora.* Five *Glomus* morphotypes were not assigned to any described species, as well as the morphotypes belonging to *Acaulospora*, *Pacispora* and *Scutellospora*. The number of AMF species was significantly higher in YngO than in OldO and Conv ($F_{2,9} = 9.300$, $P = 0.006$). Diversity indices differed among management systems ($F_{2,9} = 5.866$, $P = 0.023$; $F_{2,9} = 5.527$, $P = 0.027$; $F_{2,9} = 8.883$, $P = 0.007$, Simpson's, Shannon's index and equitability, respectively), OldO showing the highest value (Table 3).

Glomus viscosum was the most abundant species in terms of spore number (Table 4), while *Funneliformis geosporus* was the most abundant morphotype in terms of biovolume (data not shown). Overall, 7 different species were common to the three microagroecosystems: *Claroideoglomus claroideum, F. geosporus, Funneliformis mosseae, Glomus* sp. 1, *Pacispora* sp., *Scutellospora* sp. and *G. viscosum*. *Glomus sinuosum* and *Glomus rubiforme* were detected only in Conv, while *Acaulospora* sp. occurred only in the YngO (Table 4).

We performed PCA analysis to show differences in AMF species abundance. The first two principal component axes described most of the total variation (77.4%) in the data (Fig. 2). The PCA biplot showed that the distribution of samples on axis 1 (61.3% of the total variation) is mainly determined by *G. viscosum*, whereas on axis 2 (16.1% of variation), samples are mainly

segregated by abundance of fungal spores belonging to *C. claroideum*. The third axis explains mainly *Pacispora* distribution (10.4% of variation).

Discussion

In this work we assessed the differences in AMF activity and diversity among conventionally and organically managed Mediterranean arable soils. Our work shows that: i) AMF population activity, assessed as MIP and root colonization, and GRSP content were higher in organic microagroecosystems and increased with time since transition to organic farming, ii) AMF diversity was affected by the different managements.

The conversion from conventional to organic management affected AMF activity, as assessed by soil mycorrhizal potential, indicating a progressive improvement in the activity of the resident AMF, which, even in YngO - 6 years after conversion - was higher than in conventionally cultivated soils. Our data contrasts with those obtained by Purin et al. (2006), who did not find differences in MIP values between conventional and organic apple orchards, 4 years after conversion. However, our findings fit well with a recent study of Gosling et al. (2010) who found higher colonization potential, 2-3 years after conversion to organically managed soils, in 11 different farms across England. In MOLTE, a combination of factors could have determined the higher MIP values of organically managed soils. First, the type of weed control utilized - that resulted in highest weed diversity in organic microagroecosystems (Migliorini and Vazzana 2007) - may have led to a higher occurrence of AMF propagules originating from mycorrhizal weeds, whose control was shown to cause changes in diversity, abundance and functioning of AMF (Feldmann and Boyle 1999; Kabir and Koide 2000). Accordingly, Purin et al. (2006) suggested that highest MIP values detected in a native grassland surrounding conventional and organic orchards could be explained by the higher amount of mycorrhizal plant species in grassland than under the canopy of the orchards. Gosling et al. (2010) ascribed the increased colonization potential in

organically managed soils to the two years ley required in UK during the conversion period. Second, probably AMF colonization levels of the main crop could have played an important role in soil colonization potential. Our data show that, at harvest, AMF root colonization of maize was higher in organic than in conventional fields and it correlated well with soil MIP values (R=0.68, *P*=0.029).

Higher levels of AMF root colonization detected in maize under organic management are in agreement with several previous studies on maize and other crops (Ryan et al. 1994; Mäder et al. 2000; Galván et al. 2009; Verbruggen et al. 2010). High AMF colonization in organic fields has been often attributed to low levels of available soil P (Mäder et al. 2000; Ryan et al. 2000) which, at high concentrations, negatively affect mycorrhizal establishment (Thingstrup et al. 1998; Kahiluoto et al. 2000, 2001). However, since in our assessment available soil P concentration was not different among the microagroecosystems, other factors, such as crop rotation (Gavito and Miller 1998), cover crops and AMF population diversity (Scullion et al. 1998) could have contributed to enhance mycorrhizal colonization in organically managed fields.

Along with differences in soil inoculum potential and crop root colonization, we detected different GRSP concentrations among the different microagroecosystems. The concentration of T-GRSP in MOLTE soils was higher in organically than in conventionally managed plots, but differences became significant only in the long-term trial (OldO). Consistently with our results, other studies indicated that, in the short-term, organic farming did not significantly affect GRSP concentrations. Bedini et al. (2008) did not find significant changes in GRSP concentrations in organically managed soils (5 years after conversion) compared with conventional fields, while Purin et al. (2006) obtained contrasting data in GRSP concentration between organic and conventional apple orchards in Brazil (4 years after conversion).

We detected a strong correlation between GRSP and soil MIP, a moderate correlation between GRSP and maize mycorrhizal colonisation and no correlation between GRSP and spore number or biovolume. These findings, obtained in the field, confirm previous pot experiment data showing a

strong correlation of GRSP with colonized root volume and with soil aggregation (Bedini et al 2009). Bradford assay could be sensitive to polyphenols and humic compounds (Whiffen et al. 2007; Gillespie et al. 2011) and GRSP quantification biased by organic fertilisation (Rosier et al. 2006). However, since no significant differences were found in soil organic matter between Oldo and Conv (Table 1) as well as in humic and fulvic acids soil content (Canali, personal communication), the different GRSP concentrations might be ascribed to the symbiotic activity of AMF, as assessed by MIP and root colonization.

Since GRSP is primarily involved in the formation of water-stable soil macro-aggregates (Wright and Upadhyaya 1998; Bedini et al. 2009) as well as in soil C storage (Rillig et al. 2003; Bedini et al. 2007), our data suggest that long-term organic management may improve soil quality, by enhancing GRSP production. This represents one of the ecosystem services supplied by AMF, even in the absence of positive impacts on yields (Ryan and Kirkegaard 2012).

Here, the biodiversity of AMF communities was investigated by counts and identification of spores occurring in field soil after maize harvest, representing a subset of the entire AMF diversity. The numbers of AMF species detected were consistent with those previously found in Mediterranean agroecosystems by spore analyses (Calvente et al. 2004; Bedini et al. 2007, 2008) or molecular methods (Cesaro et al. 2008; Brito et al. 2012). However, the analysis of AMF spores showed differences among communities of the three microagroecosystems in terms of species richness, which in YngO was higher than in the other systems, and in terms of species composition, as suggested by PCA analysis. Our results compare well with data on microarthropods species obtained from the same site (Simoni, personal communication). Other authors reported contrasting effects of organic agriculture on AMF biodiversity. Some studies showed a higher AMF species richness in organically managed soils (Oehl et al. 2004; Hijri et al. 2006; Verbruggen et al. 2010), while others found no or only slight differences after 5 years (Bedini et al. 2008) or 15 years of organic cultivation (Franke-Snyder et al. 2001; Galván et al. 2009), compared with conventional farming.

Interestingly, the two AMF *G. sinuosum* and *G. rubiforme* were restricted to Conv. Such species have been reported to occur in grasslands and to disappear in disturbed soils (Oehl et al. 2010; Sieverding 1989), although sometimes retrieved in conventionally managed soils as spores (Na Bhadalung et al. 2005; Bedini et al. 2007; Rasmann et al. 2009) or sequences (Alguacil et al. 2008). In Conv we also found several spores of the genus *Scutellospora*, which is considered highly vulnerable to disturbances and agricultural practices (Giovannetti and Gianinazzi-Pearson 1994; Johnson 1993). The occurrence of such sensitive species in conventionally managed arable soils may be explained by the dispersal of AMF propagules from natural undisturbed sites close to cultivated soils, which, dispersed by mammals and wind, may rapidly colonize crop plants growing nearby (Allen et al. 1989; Püschel et al. 2008; Fracchia et al. 2011). Indeed, MOLTE site includes ecological infrastructures and is surrounded by woods and riparian areas, which could have functioned as sources of inoculum. Another species of *Scutellospora*, *S. calospora*, was found in organically and conventionally managed fields in Tuscany, close to a natural site, where such a species also occurred (Mazzoncini et al 2010; Turrini et al 2008).

Overall, all our data suggest that AMF occurring in conventional agricultural soils respond to the transition to organic farming by a progressive enhancement of their activity, that seems independent from the species richness of sporulating AMF communities. The improved GRSP accumulation indicates that, in organic systems, AMF may provide ecosystem services in sustainable agroecosystems, in terms of maintaining soil structure, reducing erosion and contributing to below-ground carbon pools. Further long-term studies should be performed in order to assess the population dynamics of AMF and the specific functional role played by these beneficial soil organisms in organic agriculture.

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Table 1

Main soil properties (0 - 20 cm depth) of the three microagroecosystems of the Montepaldi Long Term Experiment (mean ± S.E.). OldO, fields converted into organic management since 1991; YngO, fields converted into organic management since 2001; Conv, fields under conventional farming management.

	OldO	YngO	Conv	P value
pH (H_2O)	8.23 ± 0.03a	8.08 ± 0.09a	7.98 ± 0.08a	0.075
Organic C (g kg^{-1})	10.76 ±0.25ab	11.16 ±0.38b	9.84 ±0.11a	0.021
Total N (g kg^{-1})(1)	1.22 ±0.01a	1.14 ±0.02a	1.12 ±0.01a	0.055
Available P_2O_5(2) (mg kg^{-1})	14.75 ± 2.10a	18.00 ± 3.19a	17.50 ± 0.65a	0.563
C/N	8.55 ± 0.24a	9.45 ± 0.45a	8.82 ± 0.13a	0.243
Exchangeable K_2O (mg kg^{-1})	129.50 ± 13.35a	125.75 ± 6.99a	115.50 ± 1.94a	0.530

(1) Kjeldahl method; (2) Olsen method. Values in row followed by different letters are significantly different ($P \leq 0.05$) as determined by the Tukey's HSD test.

630

Table 2

Glomalin-related soil protein (mg g^{-1}) in the three microagroecosystems of the Montepaldi Long Term Experiment (mean ± S.E.). OldO, fields converted into organic management since 1991; YngO, fields converted into organic management since 2001; Conv, fields under conventional farming management. T-GRSP, Total glomalin-related soil protein; EE-GRSP, Easily extractable glomalin-related soil protein.

	OldO	YngO	Conv
T-GRSP	1.01 ± 0.02a	0.95 ± 0.02 ab	0.89 ± 0.03 b
EE-GRSP	0.40 ± 0.02a	0.42 ± 0.03a	0.41 ± 0.03a

Values in row followed by different letter are significantly different ($P \leq 0.05$) as determined by the Tukey's HSD test.

Table 3

Species richness, diversity, and equitability in AM fungi communities of the three micro agroecosystems of the Montepaldi Long Term Experiment (mean ± S.E.). OldO, fields converted into organic management since 1991; YngO, fields converted into organic management since 2001; Conv, fields under conventional farming management.

	OldO	YngO	Conv
No. observed species	8.0 ± 0.4 a	10.5 ± 0.5 b	7.5 ± 0.6 a
Shannon index	1.705 ± 0.03 b	1.087 ± 0.13 a	1.422 ± 0.18 ab
Simpson index	0.772 ± 0.01 b	0.475 ± 0.07 a	0.658 ± 0.09 ab
Equitability	0.822 ± 0.02 b	0.467 ± 0.07 a	0.705 ± 0.08 ab

Values in row followed by different letter are significantly different ($P \leq 0.05$) as determined by the Tukey's HSD test.

Table 4

Abundance (mean ± S.E.) of arbuscular mycorrhizal fungal species by spore number (spores 100 g^{-1}) from the three micro agroecosystems of the Montepaldi Long Term Experiment. OldO, fields converted into organic management since 1991; YngO, fields converted into organic management since 2001; Conv, fields under conventional farming management.

	OldO	YngO	Conv
Acaulospora sp.		1.0 ± 1.0	
Claroideoglomus claroideum	10.0 ± 4.7	7.0 ± 3.1	49.5 ± 14.8
Funneliformis geosporus	50.5 ± 9.0	46.0 ± 8.3	40.5 ± 7.3
Funneliformis mosseae	18.0 ± 3.2	53.5 ± 4.6	29.5 ± 4.6
Glomus badium	1.5 ± 1.5	2.5 ± 0.5	
Glomus rubiforme			25.0 ± 25.0
Glomus sp1	11.5 ± 3.9	12.5 ± 4.3	2.5 ± 1.5
Glomus sp2		4.5 ± 1.3	6.5 ± 4.7
Glomus sp3		1.0 ± 0.6	0.5 ± 0.5
Glomus sp4	10.0 ± 5.3	2.5 ± 1.0	
Glomus sp5	4.0 ± 1.8	0.5 ± 0.5	
Glomus sinuosum			25.0 ± 25.0
Glomus viscosum	16.5 ± 11.8	390.0 ± 69.1	200.0 ± 76.7
Pacispora sp.	11.0 ± 6.6	5.5 ± 2.5	2.5 ± 1.0
Scutellospora sp.	3.0 ± 1.3	16.0 ± 2.2	14.0 ± 3.4

Figure captions

Fig. 1 Percentage of mycorrhizal root length in *Cichorium intybus* after 10, 20 and 30 days' growth in the soil from the three microagroecosystems of Montepaldi Long Term Experiment (MOLTE). (♦) OldO, fields converted into organic management since 1991; (▲) YngO, fields converted into organic management since 2001; (●) Conv, fields under conventional farming management. Bars represent standard errors of the means (S.E.).

Fig. 2 Principal component analysis (PCA) of AMF spore distribution in the three microagroecosystems of the Montepaldi Long term Experiment (MOLTE). (♦) OldO, fields converted into organic management since 1991; (▲) YngO, fields converted into organic management since 2001; (●) Conv, fields under conventional farming management. The percentage of the total variance explained by each principal component is indicated in brackets.

Figure 1

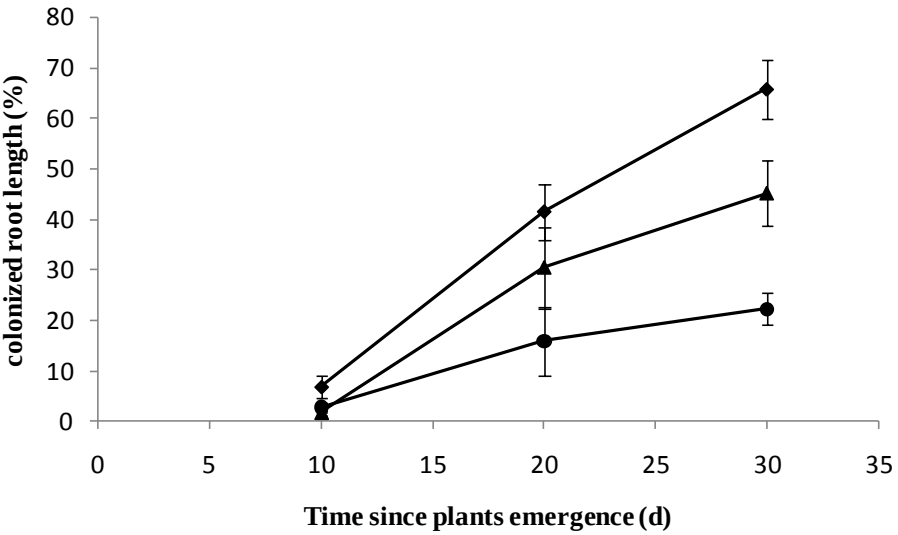
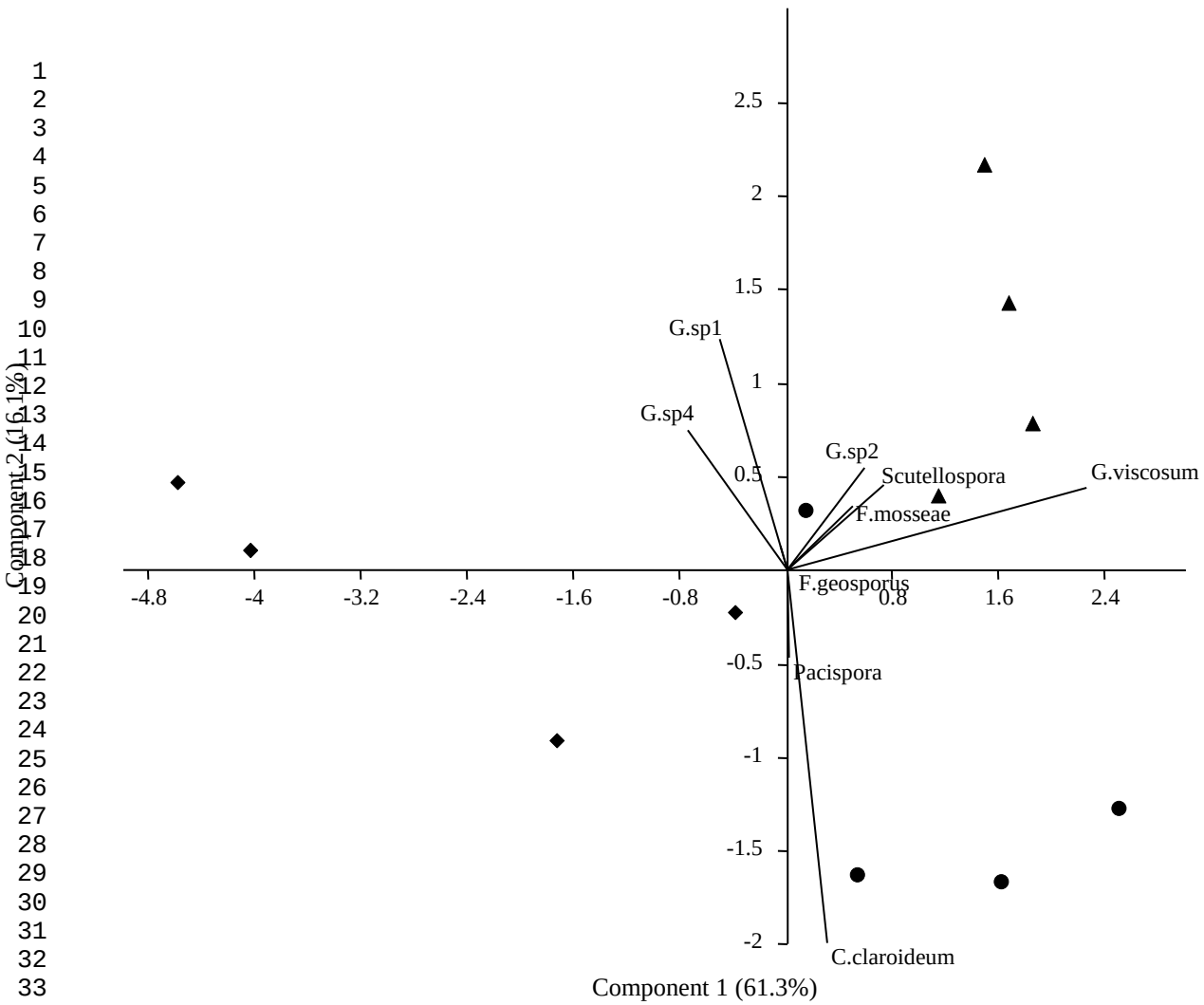

colonized root length (%)
80
70
60
50
40
30
20
10
0
0
5
10
15
20
25
30
35
Time since plants emergence (d)

Figure 2


2.5
2
1.5
1
0.5
-0.5
-1
-1.5
-2
-4.8
-4
-3.2
-2.4
-1.6
-0.8
0.8
1.6
2.4
G.sp1
G.sp4
G.sp2
Scutellospora
G.viscosum
F.mosseae
F.geosporus
Pacispora
C.claroideum
Component 1 (61.3%)
Component 2 (16.1%)