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

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Abstract:	<p>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.</p>
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1 Mycorrhizal activity and diversity in a long-term organic

2 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

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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.

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77 2011; Daniell et al. 2001; Douds and Johnson 2007), activity - evaluated as colonization ability
78 (Mozafar et al. 2000; Ryan et al. 2000) - and GRSP production (Wright et al. 1999; Wright and
79 Anderson 2000; Bedini et al. 2007, 2009; Wilson et al. 2009). On the other hand several studies
80 showed that AMF diversity (Oehl et al. 2003, 2004; Verbruggen et al. 2010), root colonization
81 (Mäder et al. 2000; Smukler et al. 2008) and spore abundance (Galvez et al. 2001; Oehl et al. 2003,
82 2004) were higher in organically managed soils, suggesting that AMF may play an essential
83 functional role in the maintenance of soil biological fertility and structure, compensating for the
84 reduced use of chemical fertilizers and pesticides (Galvez et al. 2001; Lekberg and Koide 2005;
85 Gosling et al. 2006; Ryan and Tibbet 2008).

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

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

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103 **Materials and methods**

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105 *Description of the long term experiment*

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

- 111 a) “Old Organic” (OldO) with an area of 5.2 ha, divided into 4 fields under organic
112 management (former EC Regulation 2092/91 and now EC reg. 834/07) since 1991;
- 113 b) “Young Organic” (YngO) with an area of 5.2 ha, divided into 4 fields under EC regulations
114 2078/92 (integrated farming) from 1991 to 2000 and converted into organic management since
115 2001;
- 116 c) “Conventional” (Conv) with an area of 2.6 ha divided into 2 conventional fields, where
117 farming techniques were those normally used in the territory of the study area for conventional
118 management.

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

123 Fields (260 x 50 m) were tilled by ploughing at 25-30 cm of depth. The crops were rainfed. The
124 mineral and organic fertilizers used in OldO and YngO systems were: guano (N:P:K ratio,
125 06:15:03), chicken dung (N:P:K ratio, 4:4:0), organic N fertilizer in pellets of diverse origin. The
126 mineral and synthetic fertilizers used in Conv were: liquid fertilizer (N:P:K ratio, 30:0:0),
127 ammonium polyphosphate (N:P:K ratio, 10:34:0), urea (N:P:K ratio, 46:0:0), triple superphosphate
128 (N:P:K ratio, 0:46:0), ammonium nitrate (N:P:K ratio, 27:0:0) and diammonium phosphate (N:P:K

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129 ratio, 18:46:0). Since 2001, the fertilization rate (N-P₂O₅, kg ha⁻¹y⁻¹) was: OldO 12-13 for winter
130 cereals and 18-20 for spring crops; YngO 17-20 for winter cereals and 14-2 for spring crops; Conv
131 120-70 for winter cereals and 95-65 for spring crops. In organic fields weed control was performed
132 by mechanical cultivation and plant diseases were controlled by indirect means (crop rotation and
133 ecological infrastructures represented by natural and planted vegetation in mixed hedgerows and
134 grass strips), while in the conventional field weeds control was performed using the chemical
135 herbicides Zodiac Dicuran (AI: Diflufenican 0.06 and Clortoluron 1.36 Kg ha⁻¹) and Primigran TZ
136 (AI: Terbutylazine 0.9 and Metolachlor 1.8 Kg ha⁻¹).

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

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

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142 Climate and soil characteristics

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

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

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155 method (Nelson and Sommers 1996), total N by Kjeldahl digestion (Bremner 1996), available P
156 (Olsen P) by extracting soil with 0.5 M NaHCO₃ at pH 8.5 (Olsen and Sommers 1982),
157 exchangeable K using ammonium nitrate method (Mehlich, 1984), following official methods (DM
158 13/09/1999 SO - GU n°248 21/10/1999 Met III.1) in an external laboratory (accredited by SINAL,
159 www.accredia.it).

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161 Field sampling for mycorrhizal assessment

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

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170 Mycorrhizal root colonization

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

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176 Mycorrhizal Inoculum Potential bioassay

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

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

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189 Analysis of GRSP

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

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202 Fungal spore extraction and identification

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

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4 207 dishes, examined under a dissecting microscope (Wild, Leica) at magnifications up to x50 with
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6 208 illumination by incident light from a fibre-optic quartz-halogen light source. When present,
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8 209 sporocarps were dissected with forceps and the released spores were counted. Spores were manually
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10 210 isolated using capillary pipettes according to their morphology and colour, mounted on microscope
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12 211 slides in polyvinyl alcohol lactoglycerol (PVLG), and examined under the Polyvar light
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14 212 microscope. For taxonomical identification, which was morphologically based, spores were
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16 213 mounted both in PVLG and in PVLG + Melzer's reagent (1:1, v:v) as media. Qualitative spore
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18 214 traits (spore shape, colour and size, spore wall ornamentation, wall structure and shape, colour and
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20 215 size of the subtending hypha) were examined on at least 20 spores for each morphotype, except for
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22 216 *Glomus badium*, *Glomus* spp. 3 and 5 and *Acaulospora* sp., which were found in low number. Spore
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24 217 morphotypes were compared with original diagnoses of AMF species and with the reference culture
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26 218 descriptions at <http://invam.caf.wvu.edu/fungi/taxonomy/speciesID.htm> and
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28 219 <http://www.agro.ar.szczecin.pl/~jblaszkowski/index.html>. Since important changes of AMF
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30 220 nomenclature have been recently proposed by different authors (Oehl et al. 2011; Krüger et al.
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32 221 2012), with some taxa differently named, we utilised the new binomials only for consistent species
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34 222 names and maintained the previous ones for the others.

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36 223 Total spore densities were determined as spore number 100 g⁻¹ soil. Abundance of each species
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38 224 was then converted into biovolume, calculated as $V = 1/6\pi D^3$ (D = spore diameter) for species with
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40 225 spherical spores, or as $V = 1/6\pi D_1 D_2^2$ (D₁ = larger diameter; D₂ = smaller diameter) for species
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42 226 with elongated spores. Total spore biovolume per sample was obtained by summing spore
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44 227 biovolumes of all species recorded in the sample. Relative abundance was calculated as the spore
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46 228 biovolume of an individual AMF taxon divided by the total spore biovolume within a sample.

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48 229 Species richness was measured as the number of AMF species recorded in each sample, and
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50 230 Shannon diversity index (H) and equitability (E = H divided by the logarithm of number of taxa)
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52 231 and Simpson index of diversity (D) were calculated on spore number data using PAST 1.99
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54 232 software (Hammer et al. 2001).

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6 234 Statistical analyses
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10 236 Data of all experiments were analysed by one-way analysis of variance (ANOVA) using
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12 237 management treatment as factor. The data were logarithm or arcsine transformed when needed to
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14 238 fulfil the assumptions of the ANOVA. Analyses were performed with the SPSS 18.0 software
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16 239 (SPSS Inc., Chicago, IL, USA). Means and standard errors (S.E.) given in tables are for
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18 240 untransformed data.
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22 241 Principal component analysis (PCA) was performed to show the abundance of AMF species in
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24 242 the different microagroecosystems, using spore numbers after logarithmic transformation, with the
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26 243 PAST software. Species occurring only in one sample and with a spore number lower than 1% of
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28 244 total spores, were excluded from the analyses.
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33 246 **Results**

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37 248 Soil characteristics

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42 250 Soil chemical analyses showed that in the experimental area the pH was moderately alkaline with a
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44 251 low level of organic matter, total N and available phosphate content and a normal content of
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46 252 exchangeable potassium. No significant differences were observed among the three
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48 253 microagroecosystems except for organic C content, which was significantly higher in YngO than in
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50 254 Conv soil (Table 1).
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55 256 Mycorrhizal root colonization

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4 258 Percentages of maize colonized root length at the end of the cultivation cycle were significantly
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6 259 different among the management systems ($F_{2,7} = 5.94$, $P = 0.031$) and were 60.2 ± 2.8 , 50.3 ± 5.2
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8 260 and 43.4 ± 3.9 in OldO, YngO and Conv fields, respectively. Tukey HSD Post hoc test showed a
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10 261 clear separation between OldO and Conv values ($P < 0.05$).
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15 263 Mycorrhizal Inoculum Potential bioassay
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19 265 Mycorrhizal Inoculum Potential was significantly different among the three microagroecosystems.
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21 266 Fungal entry points in *C. intybus* roots were significantly higher in OldO and YngO soils ($0.59 \pm$
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23 267 0.05 and $0.60 \pm 0.10 \text{ mm}^{-1}$, respectively), than in Conv soil ($0.23 \pm 0.04 \text{ mm}^{-1}$), 20 days after
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25 268 emergence ($F_{2,9} = 4.964$; $P < 0.05$). A similar pattern was observed for mycorrhizal root
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27 269 colonization, which showed significant differences among management systems, 30 days after
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29 270 emergence ($F_{2,9} = 21.436$; $P < 0.001$) (Fig. 1).
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35 272 Analysis of GRSP
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39 274 The concentration of T-GRSP was significantly higher in organic microagroecosystems ($F_{2,8} =$
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41 275 7.398 , $P = 0.015$). OldO soil showed the highest T-GRSP content, whereas the lowest value was
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43 276 found in Conv soil (Table 2). On the contrary, no differences were detected in the EE-GRSP content
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45 277 among the three management systems ($F_{2,8} = 0.033$, $P = 0.967$) (Table 2). Regression analyses
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47 278 showed a significant positive effect of organic management on T-GRSP content. The time since
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49 279 conversion from conventional to organic farming explained 58% of the variance in T-GRSP
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51 280 concentration ($R=0.797$; $P=0.003$). Positive correlations were also observed between T-GRSP and
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53 281 root colonization values of 30 days' old *C. intybus* plantlets in the MIP test ($R=0.691$; $P = 0.018$)
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55 282 and between T-GRSP values and the percentage of colonized root length in the host crop, maize
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57 283 ($R=0.485$; $P=0.155$).
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285 Fungal spore number and morphotypes

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287 Spore numbers (spore number 100 g⁻¹ soil) in YngO (542.5 ± 61.2) and Conv fields (395.5 ± 93.1)

288 were significantly higher (F_{2,9} = 9.809, P = 0.005) than in OldO (136.0 ± 23.2). Total spore volume,

289 ranging from 220 ± 27 to 296 ± 10 nL, did not differ significantly among management treatments

290 (F_{2,9} = 3.396, P = 0.08).

291 Overall, in the three microagroecosystems fifteen AMF spore morphotypes were identified,

292 belonging to *Acaulospora*, *Claroideoglossum*, *Funneliformis*, *Glomus*, *Pacispora*, *Scutellospora*.

293 Five *Glomus* morphotypes were not assigned to any described species, as well as the morphotypes

294 belonging to *Acaulospora*, *Pacispora* and *Scutellospora*. The number of AMF species was

295 significantly higher in YngO than in OldO and Conv (F_{2,9} = 9.300, P = 0.006). Diversity indices

296 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,

297 P = 0.007, Simpson's, Shannon's index and equitability, respectively), OldO showing the highest

298 value (Table 3).

299 *Glomus viscosum* was the most abundant species in terms of spore number (Table 4), while

300 *Funneliformis geosporus* was the most abundant morphotype in terms of biovolume (data not

301 shown). Overall, 7 different species were common to the three microagroecosystems:

302 *Claroideoglossum claroideum*, *F. geosporus*, *Funneliformis mosseae*, *Glomus* sp. 1, *Pacispora* sp.,

303 *Scutellospora* sp. and *G. viscosum*. *Glomus sinuosum* and *Glomus rubiforme* were detected only in

304 Conv, while *Acaulospora* sp. occurred only in the YngO (Table 4).

305 We performed PCA analysis to show differences in AMF species abundance. The first two

306 principal component axes described most of the total variation (77.4%) in the data (Fig. 2). The

307 PCA biplot showed that the distribution of samples on axis 1 (61.3% of the total variation) is

308 mainly determined by *G. viscosum*, whereas on axis 2 (16.1% of variation), samples are mainly

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4 309 segregated by abundance of fungal spores belonging to *C. claroideum*. The third axis explains
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6 310 mainly *Pacispora* distribution (10.4% of variation).
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10 312 **Discussion**

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15 314 In this work we assessed the differences in AMF activity and diversity among conventionally and
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17 315 organically managed Mediterranean arable soils. Our work shows that: i) AMF population activity,
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19 316 assessed as MIP and root colonization, and GRSP content were higher in organic
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22 317 microagroecosystems and increased with time since transition to organic farming, ii) AMF diversity
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24 318 was affected by the different managements.
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27 319 The conversion from conventional to organic management affected AMF activity, as assessed by
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29 320 soil mycorrhizal potential, indicating a progressive improvement in the activity of the resident
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31 321 AMF, which, even in YngO - 6 years after conversion - was higher than in conventionally
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33 322 cultivated soils. Our data contrasts with those obtained by Purin et al. (2006), who did not find
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35 323 differences in MIP values between conventional and organic apple orchards, 4 years after
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38 324 conversion. However, our findings fit well with a recent study of Gosling et al. (2010) who found
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40 325 higher colonization potential, 2-3 years after conversion to organically managed soils, in 11
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42 326 different farms across England. In MOLTE, a combination of factors could have determined the
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44 327 higher MIP values of organically managed soils. First, the type of weed control utilized - that
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47 328 resulted in highest weed diversity in organic microagroecosystems (Migliorini and Vazzana 2007) -
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49 329 may have led to a higher occurrence of AMF propagules originating from mycorrhizal weeds,
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52 330 whose control was shown to cause changes in diversity, abundance and functioning of AMF
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54 331 (Feldmann and Boyle 1999; Kabir and Koide 2000). Accordingly, Purin et al. (2006) suggested that
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56 332 highest MIP values detected in a native grassland surrounding conventional and organic orchards
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59 333 could be explained by the higher amount of mycorrhizal plant species in grassland than under the
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61 334 canopy of the orchards. Gosling et al. (2010) ascribed the increased colonization potential in
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335 organically managed soils to the two years ley required in UK during the conversion period.

336 Second, probably AMF colonization levels of the main crop could have played an important role in
337 soil colonization potential. Our data show that, at harvest, AMF root colonization of maize was
338 higher in organic than in conventional fields and it correlated well with soil MIP values ($R=0.68$,
339 $P=0.029$).

340 Higher levels of AMF root colonization detected in maize under organic management are in
341 agreement with several previous studies on maize and other crops (Ryan et al. 1994; Mäder et al.
342 2000; Galván et al. 2009; Verbruggen et al. 2010). High AMF colonization in organic fields has
343 been often attributed to low levels of available soil P (Mäder et al. 2000; Ryan et al. 2000) which, at
344 high concentrations, negatively affect mycorrhizal establishment (Thingstrup et al. 1998; Kahiluoto
345 et al. 2000, 2001). However, since in our assessment available soil P concentration was not different
346 among the microagroecosystems, other factors, such as crop rotation (Gavito and Miller 1998),
347 cover crops and AMF population diversity (Scullion et al. 1998) could have contributed to enhance
348 mycorrhizal colonization in organically managed fields.

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

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

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361 strong correlation of GRSP with colonized root volume and with soil aggregation (Bedini et al
362 2009). Bradford assay could be sensitive to polyphenols and humic compounds (Whiffen et al.
363 2007; Gillespie et al. 2011) and GRSP quantification biased by organic fertilisation (Rosier et al.
364 2006). However, since no significant differences were found in soil organic matter between Oldo
365 and Conv (Table 1) as well as in humic and fulvic acids soil content (Canali, personal
366 communication), the different GRSP concentrations might be ascribed to the symbiotic activity of
367 AMF, as assessed by MIP and root colonization.

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

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

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4 387 Interestingly, the two AMF *G. sinuosum* and *G. rubiforme* were restricted to Conv. Such species
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6 388 have been reported to occur in grasslands and to disappear in disturbed soils (Oehl et al. 2010;
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8 389 Sieverding 1989), although sometimes retrieved in conventionally managed soils as spores (Na
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10 390 Bhadalung et al. 2005; Bedini et al. 2007; Rasmann et al. 2009) or sequences (Alguacil et al. 2008).
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13 391 In Conv we also found several spores of the genus *Scutellospora*, which is considered highly
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15 392 vulnerable to disturbances and agricultural practices (Giovannetti and Gianinazzi-Pearson 1994;
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17 393 Johnson 1993). The occurrence of such sensitive species in conventionally managed arable soils
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19 394 may be explained by the dispersal of AMF propagules from natural undisturbed sites close to
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21 395 cultivated soils, which, dispersed by mammals and wind, may rapidly colonize crop plants growing
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23 396 nearby (Allen et al. 1989; Püschel et al. 2008; Fracchia et al. 2011). Indeed, MOLTE site includes
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25 397 ecological infrastructures and is surrounded by woods and riparian areas, which could have
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27 398 functioned as sources of inoculum. Another species of *Scutellospora*, *S. calospora*, was found in
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29 399 organically and conventionally managed fields in Tuscany, close to a natural site, where such a
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31 400 species also occurred (Mazzoncini et al 2010; Turrini et al 2008).

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36 401 Overall, all our data suggest that AMF occurring in conventional agricultural soils respond to the
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38 402 transition to organic farming by a progressive enhancement of their activity, that seems independent
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40 403 from the species richness of sporulating AMF communities. The improved GRSP accumulation
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42 404 indicates that, in organic systems, AMF may provide ecosystem services in sustainable
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44 405 agroecosystems, in terms of maintaining soil structure, reducing erosion and contributing to below-
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46 406 ground carbon pools. Further long-term studies should be performed in order to assess the
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48 407 population dynamics of AMF and the specific functional role played by these beneficial soil
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50 408 organisms in organic agriculture.

51 52 53 54 409 55 56 410 **Acknowledgements**

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Table 1

Main soil properties (0 - 20 cm depth) of the three microagroecosystems of the Montepaldi Long Term Experiment (mean \pm 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 ₂ O)	8.23 \pm 0.03a	8.08 \pm 0.09a	7.98 \pm 0.08a	0.075
Organic C (g kg ⁻¹)	10.76 \pm 0.25ab	11.16 \pm 0.38b	9.84 \pm 0.11a	0.021
Total N (g kg ⁻¹) ⁽¹⁾	1.22 \pm 0.01a	1.14 \pm 0.02a	1.12 \pm 0.01a	0.055
Available P ₂ O ₅ ⁽²⁾ (mg kg ⁻¹)	14.75 \pm 2.10a	18.00 \pm 3.19a	17.50 \pm 0.65a	0.563
C/N	8.55 \pm 0.24a	9.45 \pm 0.45a	8.82 \pm 0.13a	0.243
Exchangeable K ₂ O (mg kg ⁻¹)	129.50 \pm 13.35a	125.75 \pm 6.99a	115.50 \pm 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.

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Table 2

Glomalin-related soil protein (mg g^{-1}) in the three microagroecosystems of the Montepaldi Long Term Experiment (mean \pm 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 \pm 0.02a	0.95 \pm 0.02 ab	0.89 \pm 0.03 b
EE-GRSP	0.40 \pm 0.02a	0.42 \pm 0.03a	0.41 \pm 0.03a

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

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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.

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Table 4

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

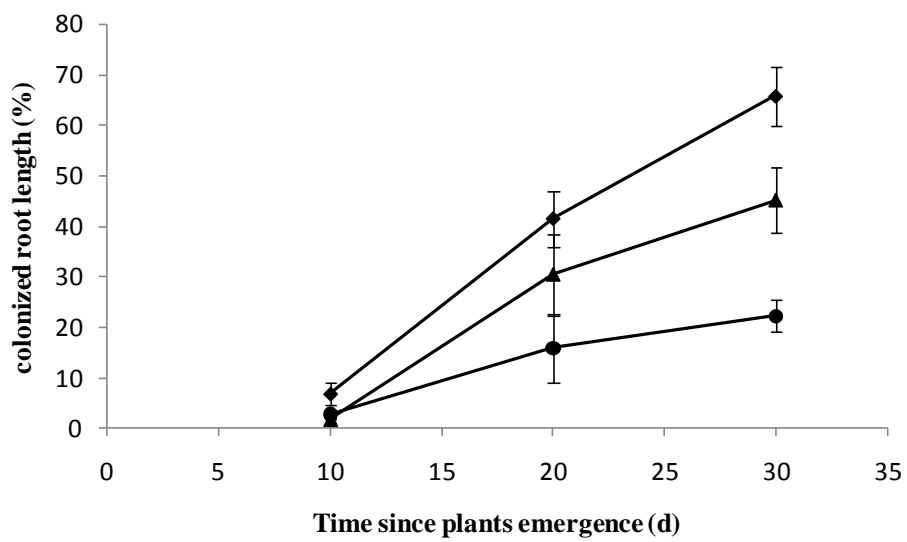
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638 **Figure captions**

639
640 **Fig. 1** Percentage of mycorrhizal root length in *Cichorium intybus* after 10, 20 and 30 days' growth
641 in the soil from the three microagroecosystems of Montepaldi Long Term Experiment (MOLTE).
642 (◆) OldO, fields converted into organic management since 1991; (▲) YngO, fields converted into
643 organic management since 2001; (●) Conv, fields under conventional farming management. Bars
644 represent standard errors of the means (S.E.).

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

Figure 1



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