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Protective green cover enhances soil respiration and native mycorrhizal potential compared with soil tillage in a high-density olive orchard in a long term study

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Abstract

Arbuscular mycorrhizal fungi (AMF), living in symbiosis with most food crops, improve plant growth and nutrition and provide fundamental ecosystem services. Here, the possibility of increasing root density and native AMF activity through appropriate soil management practices was investigated, comparing the long-term (10 years) effects of a permanent green cover (GC) with shallow tillage (ST) in a high-density olive orchard in a Mediterranean environment. Olive root density, AMF colonization, and soil mycorrhizal inoculum potential (MIP) were determined after trench excavations at different soil depths. Soil respiration was determined by infra-red gas analysis. The activity of native AMF, as assessed by MIP bioassay, was higher in GC plots than in ST ones. Olive roots were well colonized by AMF in both management systems. Soil respiration rates of GC plots were often higher than those of ST, whereas soil moisture and temperature in the topsoil were similar in both treatments. Soil depth significantly affected root density, which peaked at 0.2 m soil depth in both soil treatments. The maintenance of a permanent plant cover appears to be a better option than shallow tillage as a soil management practice to preserve biological soil fertility in olive orchards.

Keywords

Arbuscular mycorrhizal fungi; Mycorrhizal inoculum potential; *Olea europaea* L.; Root density; Soil respiration.

1. Introduction

Soil microorganisms play a key role in soil fertility and plant nutrition, representing fundamental components for the completion of biogeochemical cycles, soil structure improvement and biological control of plant pathogens (Pimentel et al., 1997). Arbuscular mycorrhizal fungi (AMF, phylum Glomeromycota) are beneficial microorganisms living symbiotically in the root system of most plant species (about 80%) providing soil mineral nutrients, in return for plant carbohydrates (Smith and Read, 2008). AMF are able to uptake and translocate soil nutrients to their host plants through a wide extra-radical hyphal system, which extends from colonized roots into the surrounding environment (Giovannetti et al., 2001; Giovannetti et al., 2015) and contribute to deliver important services, acting as biofertilizers, bioenhancers and bioprotectors (Gianinazzi et al., 2010; Rouphael et al., 2015). In addition, spores and hyphae of AMF host diverse communities of mycorrhizosphere bacteria, showing plant growth promoting activities, from production of antibiotics, siderophores and indole acetic acid to P-solubilisation and N-fixation, leading to improved plant nutrition and health (Barea et al., 2002; Philippott et al., 2013; Agnolucci et al., 2015; Battini et al., 2016a). Recent studies have reported that AMF may also modulate the synthesis of health-promoting plant secondary metabolites, contributing to the production of safe and high-quality food (Giovannetti et al., 2013; Battini et al., 2016b).

So far, AMF benefits have been exploited by releasing selected strains into sustainable food production systems (Gianinazzi et al., 2010), while the possibility of increasing the mycorrhizal potential and diversity of native strains through appropriate agronomic practices has been only recently investigated (Njeru et al., 2014, 2015; Turrini et al., 2016). Recent studies reported that organically managed apple orchards, whereby straw mulches and compost were employed, improved AMF symbioses and diversity when compared with conventional ones (Meyer et al., 2015; Van Geel et al., 2015). A number of studies reported that mycorrhizal colonization and activity of AMF were weak in crop management systems subjected to repeated monocultures, high intensity in land use, soil compaction, and/or soil tillage. Deep ploughing disrupts the hyphae of the extraradical mycelial network, reducing the activity and functioning of AMF taxa unable to develop highly interconnected mycelia (Kabir 2005; Avio et al., 2013), often decreasing soil mycorrhizal potential and crop production (Douds et al., 1995; Kabir and Koide, 2002; Jansa et al., 2002, 2003; Oehl et al., 2003; Castillo et al., 2006; Brito et al., 2012).

The use of plant covers, the current recommended practice for inter-row floor management in orchards, has been reported to sustain and enhance native beneficial AMF symbionts, positively affecting mycorrhizal soil potential and crop growth (Kabir and Koide, 2002; Lehman et al., 2012; Njeru et al., 2014). Permanent plant covers contribute to protect the soil from erosion and surface crusting, increase water infiltration and macroporosity in the topsoil, maintain organic matter and nutrients and control soil-borne diseases (Abawi and Widmer, 2000; Dabney et al., 2001; Gómez et al., 2004; Gucci et al., 2012). Plant covers also affect yield, root growth and distribution of fruit trees, depending on plant species and soil

characteristics (Hogue and Neilsen, 1987; Glenn and Welker, 1991; Parker and Meyer, 1996; Yao et al., 2009; Atucha et al., 2011).

In Mediterranean agricultural areas, where over 95% of olive orchards are located, the traditional method of managing the olive orchard floor by periodic tillage causes soil losses, runoff, structure degradation, acceleration of organic matter mineralization, and soil fertility depletion (Hernández et al., 2005; Rodriguez-Lizana et al., 2008; Gómez et al., 2009; Moreno et al., 2009). The alternative method of controlling weeds in the tree row or over the whole orchard floor by herbicides is effective and relatively inexpensive (Hogue and Neilsen, 1987) but, because of the currently increasing concerns about the environmental impact caused by the widespread use of chemicals in fruit growing nowadays it is imperative to reduce herbicides applied in orchards.

Several works showed the important role played by AMF in olive plant performance. Some authors reported increases in the development and nutrition of either nursery-grown olive rooted cuttings or micropropagated plantlets (Citernesi et al., 1998; Estaún et al., 2003; Calvente et al., 2004; Porras-Soriano et al., 2009; Briccoli Bati et al., 2015). Other studies focused on the role played by AMF activity in the protection of olive plantlets from adverse conditions, such as salinity, drought and transplanting stress (Porras-Soriano et al., 2009; Meddad-Hamza et al., 2010; Tugendhaft et al., 2016). On the other hand, there is hardly any information on AMF occurrence and activity in the roots and in the soil of field-grown olive trees managed by different orchard floor practices.

The aim of this work was to compare the long-term (10 years) effects of two different soil management practices (permanent plant cover *versus* shallow tillage) on root activity and soil biological characteristics in a high-density olive orchard under Mediterranean climate conditions. In particular, we determined: i) soil respiration by infra-red gas analysis; ii) the biomass of olive roots less than 5 mm diameter at different soil depths after trench excavations; iii) the activity of native AMF in the soil by the mycorrhizal inoculum potential (MIP) bioassay; iv) colonization of olive roots by native AMF; v) the species composition of the native plants present in the orchard floor. Our hypothesis was that the soil management regime would affect the distribution and respiration of olive roots, as well as the activity of soil mycorrhizal symbionts.

2. Materials and methods

2.1 Plant material and soil type

All measurements and samplings were carried out between 2010 and 2014 in a high-density olive (*Olea europaea* L. cv. Frantoio) orchard planted at a 3.9 x 5 m distance in April 2003 at the Venturina experimental farm of University of Pisa, Italy (43°01'N; 10°36' E). The climate at the study site was sub-humid Mediterranean and climatic variables over the study period were measured using a weather station iMETOS IMT 300 (Pessl Instruments GmbH, Weiz, Austria) installed

on site (Caruso et al., 2013). The average annual precipitation and air temperature during the 2007-2014 period was 825 mm and 15.5 °C, respectively. The soil was a deep (1.5 m) sandy-loam (Typic Haploxeralf, coarse-loamy, mixed, thermic) consisting of 600 g kg^{-1} sand, 150 g kg^{-1} clay and 250 g kg^{-1} silt. The pH was 7.9, average organic matter 1.84% and cation exchange capacity 13.7 meq 100 g^{-1}, all measured at 0.4 m depth (Gucci et al., 2012). The orchard was divided into 12 plots, each consisting of 12 trees (Caruso et al., 2013; Gucci et al., 2012). Prior to planting 147 t ha^{-1} of cow manure were applied into the soil profile. During the 2005-2013 period an average of 50 g of N, P_2O_5 and K_2O per tree were distributed annually by fertigation. Trees were fully-irrigated during the first three years after planting, then they were subjected to deficit irrigation until the 2014 growing season, using subsurface drip lines running parallel to the tree row (south side) at 0.8 m distance from the trunk and a depth of 0.4 m (Caruso et al., 2013). The soil was periodically tilled until October 2004 when two management treatments were established (shallow tillage, ST; permanent green cover, GC), as reported in Gucci et al. (2012). Both treatments were maintained continuously until trench excavations in 2014. In brief, the soil was either tilled at 0.1 m depth, using a power take off-driven harrow with vertical blades, or the plant cover was mown using a mulcher, three or four times a year. Both treatments received the same amount of water and fertilizers throughout the 10-year period.

2.2 Identification of native plant species

In spring 2014 an area of about 20 m^2 in each of the three GC plots was fenced and left undisturbed for identification of the natural flora. Plant samples were taken on three dates from April through November 2014 and species were classified according to Conti et al. (2005).

2.3 Soil respiration

Soil respiration rates (R_s) were measured twice a day (dawn and mid-day) at approximately bi-monthly intervals over almost two consecutive years (2010-2012), using a closed circuit Soil Respiration System (PP Systems, Hitchin Herts, UK) and PVC open collars (0.1 m diameter, 0.12 m high). Collars had been inserted into the soil at four sampling points, varying in orientation and distance from the trunk, below the canopy of three trees per treatment at least six months prior to measurements (Fig. 1). The EGM-4 gas exchange infrared analyser was equipped with a SRC-1 soil respiration chamber and a soil temperature STP-1 probe. Prior to each measurement the respiration chamber was flushed in open air, then fitted carefully and tightly onto a PVC collar. The soil respiration rate was calculated by fitting the rate of increase of the CO_2 concentration inside the chamber over time using a quadratic model. Soil temperature was measured at a depth of 0.08 m with the STP-1 probe, soil moisture at a depth of 0.06 m using a Theta Probe ML2x (Delta-T Devices, UK)

adjacent to each collar every time soil respiration was measured. The Theta Probe had been preliminary calibrated for that soil type following the procedure described in the users' manual.

2.4 Above- and below-ground biomass determination of the orchard floor

The above-ground biomass of the natural plant cover of the orchard floor (GC treatment) was harvested from March 2012 until March 2013 by periodically cutting (every two months) the canopies of native species at ground level from three 1 m^2 square per plot (total of 9 m^2). The three samples per plot were taken along a transect drawn between the first and the fourth tree of two adjacent rows of olive trees. The sampling areas were 0.8 m, 2.5 m (inter-row), and 4.2 m South of the central row of trees in each plot. The dry weight of each sample was measured after oven-drying the freshly-cut biomass at 60°C until constant weight. The above-ground dry weights obtained over the 12-month period were summed to calculate the annual productivity of the orchard floor.

The biomass of above- and below-ground parts of the orchard floor was determined at four sampling points from the three GC plots in May 2013. A 0.3 x 0.3 m square per plot was excavated by hand down to a 0.15 m depth in the inter-row in a position adjacent to the quadrat where above-ground biomass productivity had been assessed (see previous paragraph); an additional quadrat was similarly sampled from one of the GC plots. Samples of the orchard floor were readily transported to the laboratory for biomass determination. After eliminating the above-ground parts, samples were immersed in a Na_2CO_3 solution (2 g l-1) for 12 hours to remove soil particles and debris. Then the organic material (including litter) was recovered using 1 mm mesh sieves and weighed separately after oven-drying at 60 °C until constant weight. The below-ground biomass was then divided into roots of three diameter cohorts (< 1 mm; 1-2 mm; 2-5 mm), and the dry weight of each sample determined after oven-drying at 60°C.

2.5 Trench excavation and determination of olive root biomass

In May 2014 two L-shaped trenches (1 m deep and 0.8 m wide) were excavated on both sides (North and South) of the central row of trees of each plot, as illustrated in Fig. 1. The long side of all trenches was at a 2.1 m distance from the tree row, the L-aisle of the trench reached a minimum distance of 1 m from the tree row (Fig. 1). Trenches were similar in size and position in all six plots (three in the ST plots and three in the GC ones).

Soil cores for olive root density determination were taken at 0.2, 0.4, and 0.6 m depth using a custom-built soil cylinder auger (25 cm^3) at 16 sampling positions in each plot between 14 and 27 May 2014 (Fig. 1). All samples were stored at -20 °C until analysis. Samples were then thawed, immersed in a Na_2CO_3 solution (2 g l^{-1}) to facilitate deflocculation, shaken for 2 hours, and then sieved under running water. Preliminary experiments had shown that 2 hours in the Na_2CO_3 solution were sufficient to separate soil particles from olive roots and, therefore, the standard protocol by

153 Ceccon et al. (2011) was modified accordingly. Olive roots were carefully recovered by tweezers, divided into diameter
154 cohorts (< 1 mm; 1-2 mm; 2-5 mm) using a 1 mm mesh sieve, and the dry weight of each sample determined after oven-
155 drying at 60 °C, until constant weight. Root density was calculated as root dry weight per soil volume.
156
157 *2.6 Mycorrhizal inoculum potential (MIP) bioassay*
158 A total of 16 soil cores (approx. 200 g each) and 16 root sub-samples (approx. 20 g each) were collected at two depths,
159 0.3 and 0.6 m, along the length of each trench 0.8 m apart, at the same date when cores for olive root determination were
160 sampled (see previous section). The sub-samples were then pooled together to obtain six samples per soil depth and soil
161 management treatment. The roots were gently cleaned from the soil and stored at 4°C in polyethylene bags, to be
162 successively analyzed for mycorrhizal colonization.
163 Mycorrhizal inoculum potential (MIP) of the experimental olive orchard soil was carried out using *Cichorium*
164 *intybus* L. cv. Zuccherina di Trieste as host plant. *C. intybus* seeds were sown in 50 ml sterile plastic tubes filled with 40
165 ml of each soil sample. Four replicate tubes per soil sample were prepared (96 tubes in total). The tubes were placed in
166 transparent bags and maintained in a growth chamber at 25 °C and 16/8 h light/dark daily cycle until harvest. One week
167 after germination, *C. intybus* plantlets were thinned to two individuals per tube. Each tube was watered as needed. Plants
168 were harvested 21 days after germination and shoots were excised and discarded. After removing the soil from tubes,
169 roots were gently cleaned with tap water. Roots were then cleared with 10% KOH in a 80°C water bath for 30 min,
170 neutralized in 2% aqueous HCl for 10 min, and stained with Trypan blue in lactic acid (0.05 %). The percentage of AMF
171 root colonization was evaluated using a dissecting microscope (Wild, Leica, Milano, Italy) at x25 or x40 magnification
172 by the gridline intersect method (Giovannetti and Mosse, 1980).
173
174 *2.7 Mycorrhizal colonization*
175 The percentage of AMF root colonization was determined on 20 g of thoroughly washed olive root samples, after clearing
176 and staining, as described above. Samples of colonised roots were selected under the dissecting microscope, mounted on
177 slides and observed at magnification of x125 and x500 under a Polyvar light microscope (Reichert-Jung, Vienna, Austria)
178 for assessing the occurrence of arbuscules and intracellular structures.
179
180 *2.8 Experimental design and statistical analysis*
181 Each treatment consisted in 36 trees, divided into three plots of 12 trees each. Each plot included three rows of trees. To
182 avoid border effects all measurements and samples were taken on the inner trees of the central row of each plot. Treatment
183 means were separated by least significant difference (LSD test) after analysis of variance (ANOVA) for three replicate

184 trees using Costat package (CoHort Software, Monterey, USA). A split plot experimental design (main plot soil
185 management; subplot soil depth) was used to analyze effects on root biomass and density. Since a preliminary analysis
186 showed that there were no differences in root density according to the side (North- South) of the tree or distance from the
187 tree of the sampling position the data were pooled together within the same soil depth and root diameter cohort.
188 Mycorrhizal colonization data were arcsine transformed before analysis of variance.
189
190 **3. Results**
191 3.1 Green cover composition
192 A total of 33 species belonging to 15 families occurred in the green covered plots (Table 1), 55% of which were
193 Therophytes and 39% Hemicryptophytes. Annual species were the most abundant, as expected considering the periodic
194 disturbance by mowing, used as a routine management practice of the sampling areas prior to fencing. Herbaceous
195 biennial and perennial species were also present. Over 40% of the species found were typical of the Mediterranean flora,
196 but 26% of the species had also a European distribution (European Mediterranean); those strictly linked to the
197 Mediterranean environment (Steno-Mediterranean) totalled 10% (Table 1). Overall, all plant taxa are arbuscular
198 mycorrhizal species, except *Sinapis arvensis* (Brassicaceae) and *Beta vulgaris* (Amaranthaceae), which are non-host
199 plants.
200
201 3.2 Root density of olive trees
202 The root density of olive trees was similar within each size cohort (root diameter less than 5 mm) regardless of the soil
203 management treatment. The interaction between soil management and soil depth was never significant, so the two
204 treatments could be separately presented (Table 2). Total root density (dry weight) was 4.79 and 4.38 kg m^{-3} of soil for
205 olive trees grown under GC and ST treatments, respectively. On the other hand, soil depth significantly affected root
206 density: the highest value (5.43 kg m^{-3}) was measured at 0.2 m depth, whereas no differences (4.2 kg m^{-3}) were found
207 between the 0.4 and 0.6 m depth layers. Fine root density (< 1 mm in diameter) was almost twice (1.94) the total of the
208 other diameter cohorts at 0.2 m depth, whereas at 0.4 and 0.6 m depth the ratio between fine roots and other roots was
209 1.78 and 1.43, respectively.
210
211 3.3 Biomass of permanent green cover
212 The above-ground net primary production of the permanent green cover, expressed on a dry weight basis, was 0.42±0.051
213 kg m^{-2} $year^{-1}$ (average of 9 replicates ± standard deviation) corresponding to 4.2±0.51 t ha^{-1} of biomass produced annually

by the orchard floor. The root dry biomass of the green cover, measured nine years after the beginning of the permanent plant cover treatment, was 0.25±0.18 kg m^{-2} (average of four replicates ± standard deviation).

3.4 Soil respiration

The seasonal course of R_s was mainly dependent on changes in soil temperature (Fig. 2). Maximum R_s of 5.42 and 3.36 μmol CO_2 m^{-2} s^{-1} were measured in June for the GC plots and ST ones, respectively, whereas minimum values of 1.23 and 0.91 μmol CO_2 m^{-2} s^{-1} were measured at the last sampling date in November, respectively (Fig. 2a). Soil moisture was below 10% volume in July and August for both treatments (Fig. 2b), soil temperature ranged from 3 to 25 °C from January through August (Fig. 2c). Soil respiration rates of the permanent plant cover treatment were consistently higher (although significantly only at four out of nine dates of measurements) than those measured in ST plots, despite the fact that soil moisture and temperature were similar for both treatments at all but one dates of measurement (Fig. 2).

3.5 Mycorrhizal inoculum potential

Since AMF activity, as assessed by MIP, showed a significant interaction between soil management and soil depth, management effects were separately examined for each depth. Soil tillage negatively affected MIP values, at both soil depths, decreasing mycorrhizal colonization produced by native AMF by 62 and 24% at 0.3 and 0.6 m depth, respectively. The percentage of mycorrhizal root length of biotest plants grown in ST soil was significantly lower (10.7±1.4%) than that of plants grown in GC plots (28.2±3.9%) at 0.3 m depth, ($F_{1,16}$=18.3; P<0.001) (Fig. 3a).

3.6 Mycorrhizal colonization

Olive roots were well colonized by AMF in both treatments and no significant differences were found between the two orchard floor management treatments. However, olive roots originating from GC plots showed higher mycorrhizal colonization levels at 0.3 m depth (29.6 ± 2.6%), than those from ST ones (22.5±2.0%) (Fig. 3b). Accordingly, at 0.6 m depth the percentage of mycorrhizal root length ranged from 30.8 ± 1.7 to 31.3 ± 3.9%. It is interesting to note that olive roots originating from tilled orchards were suberized, highly pigmented and showed knobby, inflated appressoria with septate hyphae, empty germination pegs and many intra-radical coils and vesicles (Fig. 4), while those from GC trees appeared well developed, giving rise to many arbuscules and intraradical hyphae.

4. Discussion

Orchard floor management is important for the economic results and the environmental impact of fruit growing, as it affects tree growth, yield, production costs, soil properties and water resources (Atkinson, 1983; Hogue and Neilsen,

1987; Parker and Meyer, 1996; Gucci et al., 2012). It has also been shown that soil management can modify root growth and distribution down the soil profile (Hogue and Neilsen, 1987; Atucha et al., 2011). For example, apple trees grown under mowed sod grass yielded less, had deeper roots and fewer fine roots (less than 1 mm in diameter) than trees grown in herbicide-treated plots in a long-term study in New York State (Yao et al., 2009). In our work root density peaked at 0.2 m depth and decreased in deeper layers of both soil treatments, consistently with previous reports in orchards where roots were abundant in the top 0.3 m of soil (Hogue and Neilsen, 1987; Parker and Meyer, 1996). In peach root density was higher in vegetation free plots than in plots where weeds were allowed to grow to form a permanent green cover (Parker and Meyer, 1996). In our study the spatial distribution of roots was quite uniform and did not change with either soil management or distance/orientation from the tree trunk. This is not surprising considering that trees had been planted at close distances in a deep, fertile soil and, by the time trenches were excavated, they were fully-grown and their root systems presumably explored thoroughly the soil volume. In addition, the reported effect of a permanent sod forcing tree roots downwards (Hogue and Neilsen, 1987) might have not occurred because, by sub-irrigating, we supplied water directly to the layer where absorbing roots were abundant. The sandy-loam soil texture and deep soil may also explain the high root density of olive trees in our experiment that ranged from 5.4 to 4.2 kg m^{-3} from the 0.2 to the 0.6 m soil depth and averaged 4.6 kg m^{-3} over the whole 0.2-0.6 m profile. These values were higher than the 3.1 kg m^{-3} mean value reported for apple trees growing in a high-density orchard in Northern Italy. The density of roots less than 1 mm in diameter (the most abundant cohort of olive roots) was also greater than values reported for apple trees (Ceccon et al., 2011). In any case since root biomass in fruit trees is highly variable depending on species, soil, climate, and cultural practices, comparisons between studies are sometimes difficult. It should be pointed out that our results were obtained under conditions of a relatively humid climate. The character of latent mesophily was confirmed by the floristic analysis that showed the presence of circumboreal species (10%), Euro-asiatic species (10%), and Paleotemperate (22%) that, although having a North-African distribution, are common in the Euroasiatic supercontinent. The abundance of *Crepis vesicaria* (Subatlantic-Submediterranean) is interesting because, although typical of Mediterranean associations, confirms the existence of a local mesoclimate that was not strictly Mediterranean. The annual productivity of the ground cover vegetation was within the range reported for orchards and vineyards located in peninsular Italy and higher than values reported for orchards in Northern Italy (Scandellari et al., 2016).

Soil respiration rates of GC treated plots were often higher than those of ST ones and in both treatments appeared driven by seasonal changes of environmental parameters. Several studies showed the relationship between soil temperature and R_s, as temperature affects root respiration as well as heterotrophic respiration by microorganisms living in the soil and decomposing material (Huang et al., 2005; Ceccon et al., 2011; Xiao et al., 2014). However, soil temperature and tree root density were similar in GC and ST plots and so differences in R_s between soil treatments were

likely due to respiration either of herbaceous species roots or soil microbiota. In an experiment conducted on perennial grass Carpenter-Boggs et al. (2003) reported that respiration of grass-covered soils was higher than that of tilled soils, because of the higher contents of labile C compounds and microbial biomass. Additional variability in seasonal trends can be attributed to changes in soil moisture. Bryla et al. (2011) reported that R_s increased with soil moisture from values at wilting point until approximately 50-60% of water filled pore space were reached, after which R_s decreased. Soil temperature and moisture often interact in their effects on root respiration. In Concord grapevine root respiration was little affected by soil moisture at soil temperature of 10 °C, while respiration declined with decreasing soil moisture at temperatures between 20 and 30 °C (Huang et al., 2005). In field-grown olive trees Bertolla (2008) found that soil temperature influenced R_s, but the effect was mediated by soil moisture. When soil humidity exceeded 20% (in volume) temperature had a direct effect on R_s, but at humidities less than 10% there was a clear decrease in R_s at T > 22-27 °C. Although soil R_s was higher in GC plots than in ST ones, carbon emissions into the atmosphere were actually less when the soil was permanently covered because of the substantial biomass accumulated in the sod (Bertolla, 2008).

Our work showed that tillage negatively affected AMF activity at both soil depths, decreasing mycorrhizal colonization produced by native AMF in biotest plants, which was significantly lower than in GC managed soil at 0.3 m soil depth. Such a finding is very important, as the MIP bioassay represents a measure of total AMF soil propagules, including extra-radical mycorrhizal hyphae which are functionally active in soil nutrient uptake and transfer to plants, whose functioning may be disrupted by tillage (Giovannetti et al., 2015). The higher levels of MIP values found in the soil under permanent plant cover, compared with tillage, may be attributed to the occurrence of many mycotrophic weed species (94%), contributing to the enhancement and maintenance of native AMF originating from germinated spores and colonized roots. The weeds growing in our GC plots had been previously identified in Tuscan olive groves (Tomei, 2013) and indicated a wide diversity of plant species for our intensively-cultivated ecosystem. Actually, previous studies reported that mycotrophic cover crops may serve as sources of AMF propagules in successive crops (Kabir and Koide, 2002; Karasawa and Takebe, 2012; Lehman et al., 2012), and that non mycotrophic species, such as the non-host *Brassica* spp., did not affect mycorrhizal colonization (Pellerin et al., 2007), while decreasing mycorrhizal soil potential (Njeru et al., 2014). Our results, obtained in a deficit irrigated orchard under sub-humid Mediterranean conditions, also supplement those obtained in traditional, rain-fed groves by Moreno et al. (2009), who reported greater bacterial diversity, as well as increased activities of hydrolytic enzymes involved in the cycling of nutrients (C, N, P, and S) in green managed systems compared with tilled ones. Our MIP data are lower than those obtained by Schwab and Reeves (1981), who reported colonization values of 65, 60 and 36% across vertical transects of 0.01-0.1, 0.2-0.3 and 0.4-0.5 m, respectively, and very low colonization levels (2%) below 0.6 m depth. Recently, Gai et al. (2015) found higher MIP values in the top soil (0-30 cm) than in the subsoil (0.3-0.6 m) of arable fields from two different sites in Northern China. Such data are consistent

with those reported in another recent work whereby AMF biomass, expressed as the concentration of the AMF biomarker C16:1cis11 per soil volume, declined with increasing soil depth, being highest in the 0-0.1 m layer and lowest between 0.7 and 1 m (Higo et al., 2013).

The percentage of colonized root length of olive trees ranged from 22.5 to 31.3%. These values are lower than those reported for olive rooted cuttings of cultivar Arbequina, that ranged from 75 to 80% after artificial inoculation with selected AMF strains (Estaún et al., 2003). Similarly high levels of mycorrhizal colonization (92-97%) were reported in rooted cuttings of the cultivars Cornicabra (Porras-Soriano et al., 2009), Ascolana Tenera, Nocellara del Belice e Carolea (Briccoli Bati et al., 2015). The different values in the percentage of colonized root length found in our olive trees may be ascribed either to the different cultivar investigated or to the AMF inoculum type, which, in our case, was represented by the native AMF occurring in the orchard soil. Actually, large differences in mycorrhizal colonization had been previously reported between olive root cuttings of the two cultivars Arbequina and Leccino, ranging from 52-77% to 3-41%, respectively (Calvente et al., 2004), depending on the identity of the inoculated fungal species. The colonization percentage in our work was similar to that obtained (38%) for the same cultivar Frantoio by Citernesi et al. (1998). Overall, mycorrhizal colonization was not significantly affected by orchard floor management. The relatively stable percentage of olive mycorrhizal colonization for both treatments and depths may be attributed to the very low tillage depth, 0.1 m, which proved to be an effective way to mechanically destroy weeds, but unable to affect the established mycorrhizal symbiosis. Indeed, the roots of perennial species such as *Olea europaea* can maintain intra-radical mycorrhizal propagules capable of spreading to newly-formed roots growing after disturbance. Since no previous work investigated the impact of different soil management practices on AMF colonization of mature olive trees in the field, our results complement those already reported for other crops, such as wheat (Ryan et al., 1994; Mäder et al., 2000), vetch-rye, grass-clover (Mäder et al., 2000), onion (Galván et al., 2009), maize, and soybean (Douds et al., 1993). In particular, tillage was reported to decrease soil AMF spore abundance (Jansa et al., 2002; Oehl et al., 2004; Avio et al., 2013).

Only few studies investigated AMF occurrence in plant roots across the soil profile. Our data on mycorrhizal colonization agree with those obtained by Kabir et al. (1998) in maize roots, where the percentage of colonized root length decreased from 71 to 41 to 20% at soil depths of 0.05-0.10, 0.15-0.20 and 0.20-0.25 m, respectively. Other works reported sharp decreases with increasing soil depth below 0.40 m in rye, barley and peas (Jakobsen and Nielsen, 1983), and in *Bromus hordeaceus* and *Lotus wrangelianus* at two soil depths, topsoil (0-15 cm) and subsoil (15–45 cm) (Rillig and Field 2003). Such differences could be ascribed to the fact that herbaceous species develop superficial root systems, where a large number of fine roots occur in the topsoil, whereas fruit trees (including olive) tend to develop thick roots also in the deeper soil layers.

In conclusion, we showed a beneficial effect of plant covers on soil biological properties, such as mycorrhizal inoculum potential and soil respiration. Our results extend the range of environmental advantages of green covered soils over tilled ones previously observed in olive orchards, such as increases in water infiltration rate, macroporosity, total exchangeable C and total organic C in the topsoil (Gucci et al., 2012), macroaggregate stability and resilience to soil erosion (Gomez et al., 2004; Gomez et al., 2009), as well as bacterial biodiversity (Moreno et al., 2009). The maintenance of a green cover appears a better option than shallow tillage as a soil management practice to alleviate environmental impact and to preserve biological soil fertility in intensively-cultivated olive orchards. Protective green covers should be recommended in marginal soils, in both traditional and intensive olive orchards to improve soil fertility and physical properties.

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References

Abawi, G., Widmer, T., 2000. Impact of soil health management practices on soilborne pathogens, nematodes and root diseases of vegetable crops. Appl. Soil Ecol. 15, 37-47.

Agnolucci, M., Battini, F., Cristani, C., Giovannetti, M., 2015. Diverse bacterial communities are recruited on spores of different arbuscular mycorrhizal fungal isolates. Biol. Fertil. Soils 51, 379-389.

Atkinson, D., 1983. The growth, activity and distribution of the fruit tree root system. Plant Soil 71, 23-35.

Atucha, A., Merwin, I.A., Brown, M.G., 2011. Long-term effects of four groundcover management systems in an apple orchard. HortScience 46, 1176-1183.

Avio, L., Castaldini, M., Fabiani, A., Bedini, S., Sbrana, C., Turrini, A., Giovannetti, M., 2013. Impact of nitrogen fertilization and soil tillage on arbuscular mycorrhizal fungal communities in a Mediterranean agroecosystem. Soil Biol. Biochem. 67, 285-294.

Barea, J.M., Azcon, R., Azcon-Aguilar, C., 2002. Mycorrhizosphere interactions to improve plant fitness and soil quality. A Van Leeuw 81, 343-351.

366 Battini, F., Cristani. C., Giovannetti, M., Agnolucci, M., 2016a. Multifunctionality and diversity of culturable bacterial
367 communities strictly associated with spores of the plant beneficial symbiont *Rhizophagus intraradices*. Microbiol.
368 Res. 183, 68-79.
369 Battini, F., Turrini, A., Quartacci, M., Malorgio, F., Sgherri, C., Picciarelli, P., Pardossi, A., Giovannetti, M., Agnolucci,
370 M., 2016b. Dual inoculation with AMF and associated bacteria improves nutraceutical value of sweet basil grown
371 under commercial conditions. Agrochimica 60, 81-99.
372 Bertolla, C., 2008. Andamento della respirazione del suolo in oliveti con condizioni di diversa disponibilità idrica e
373 gestione del suolo Patterns of soil respiration in olive groves with different soil water availablity and soil management.
374 PhD dissertation, University of Pisa, 219 p.
375 Briccoli Bati, C., Santilli, E., Lombardo, L., 2015. Effect of arbuscular mycorrhizal fungi on growth and on micronutrient
376 and macronutrient uptake and allocation in olive plantlets growing under high total Mn levels. Mycorrhiza 25, 97-
377 108.
378 Brito, I., Goss, M.J., de Carvalho, M., Chatagnier, O., van Tuinen, D., 2012. Impact of tillage system on arbuscular
379 mycorrhiza fungal communities in the soil under Mediterranean conditions. Soil Till. Res. 121, 63-67.
380 Bryla, D.R., Bouma, T.J., Hartmond, U., Eissenstat, D.M., 2011. Influence of temperature and soil drying on respiration
381 of individual roots in citrus, integrating greenhouse observations into a predictive model for the field. Plant Cell
382 Environ. 24, 781-79.
383 Calvente, R., Cano, C., Ferrol, N., Azcón-Aguilar, C., Barea, J.M., 2004. Analysing natural diversity of arbuscular
384 mycorrhizal fungi in olive tree *Olea europaea* L. plantations and assessment of the effectiveness of native fungal
385 isolates as inoculants for commercial cultivars of olive plantlets. Appl. Soil Ecol. 26, 11-19.
386 Carpenter-Boggs, L., Stahl, P.D., Lindstrom, M.J., Schumacher, T.E., 2003. Soil microbial properties under permanent
387 grass, conventional tillage, and no-till management in South Dakota. Soil Till. Res. 71, 15-23.
388 Caruso, G., Rapoport, H.F., Gucci, R., 2013. Long-term effects on yield compenents of young olive trees during the onset
389 of fruit production under different irrigation regimes. Irrig. Sci. 31, 37-47.
390 Castillo, C.G., Rubio, R., Rouanet, J.L., Borie, F., 2006. Early effects of tillage and crop rotation on arbuscular
391 mycorrhizal fungal propagules in an ultisol. Biol. Fertil. Soils 43, 83-92.
392 Ceccon, C., Panzacchi, P., Scandellari, F., Prandi, L., Ventura, M., Russo, B., Millard, P., Tagliavini, M., 2011. Spatial
393 and temporal effects of soil temperature and moisture and the relation to fine root density on root and soil respiration
394 in a mature apple orchard. Plant Soil 342, 195-206.
395 Citernesi, A.S., Vitagliano, C., Giovannetti, M., 1998. Plant growth and root systems morphology of *Olea europaea* L.
396 rooted cuttings as influenced by arbuscular mycorrhizas. J. Hortic. Sci. Biotechnol. 73, 647-654.

Conti, F., Abbate, G., Alessandrini, A., Blasi, C., 2005. An annotated checklist of the Italian vascular flora. Palombi Editore, Roma.

Dabney, S.M., Delgado, J.A., Reeves, D.W., 2001. Using winter cover crops to improve soil and water quality. Commun. Soil Sci. Plant. Annal. 32, 1221-125.

Douds, D.D., Galvez. L., Janke, R.R., Wagoner, P., 1995. Effect of tillage and farming system upon populations and distribution of vesicular-arbuscular mycorrhizal fungi. Agric. Ecosyst. Environ. 52, 111-118.

Douds, D.D., Janke, R.R., Peters, S.E., 1993. VAM fungus spore populations and colonization of roots of maize and soybean under conventional and low-input sustainable agriculture. Agric. Ecosyst. Environ. 43, 325-335.

Estaún, V., Camprubí, A., Calvet, C., Pinochet, J., 2003. Nursery and field response of olive trees inoculated with two arbuscular mycorrhizal fungi, *Glomus intraradices* and *Glomus mosseae*. J. Amer. Soc. Hort. Sci. 128, 767-775.

Gai, J., Gao, W., Liu, L., Chen, Q., Feng, G., Zhang, J., Christie, P., Li, X.J., 2015. Infectivity and community composition of arbuscular mycorrhizal fungi from different soil depths in intensively managed agricultural ecosystems. J. Soils Sediments 15, 1200-1211.

Galván, G.A., Parádi, I., Burger, K., Baar, J., Kuyper, T.W., Scholten, O.E., Kik, C., 2009 Molecular diversity of arbuscular mycorrhizal fungi in onion roots from organic and conventional farming systems in the Netherlands. Mycorrhiza 19, 317–328.

Gianinazzi, S., Gollotte, A., Binet, M.N., van Tuinen, D., Redecker, D., Wipf, D., 2010. Agroecology, the key role of arbuscular mycorrhizas in ecosystem services. Mycorrhiza 20, 519-530.

Giovannetti, M., Avio, L., Sbrana, C., 2013. Improvement of nutraceutical value of food by plant symbionts. In, Ramawat G, Merillon JM eds Handbook of Natural Products. Springer-Verlag, Berlin Heidelberg, pp. 2641-2662.

Giovannetti, M., Avio, L., Sbrana, C., 2015. Functional significance of anastomosis in arbuscular mycorrhizal networks. In Horton TR ed. Mycorrhizal networks. Springer Dordrecht Heidelberg, New York, London, pp. 41-67.

Giovannetti, M., Fortuna, P., Citernesi, A.S., Morini, S., Nuti, M.P., 2001. The occurrence of anastomosis formation and nuclear exchange in intact arbuscular mycorrhizal networks. New Phytol. 151, 717-724.

Giovannetti, M., Mosse, B., 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytol. 84, 489-500.

Glenn, D.M., Welker, W.V., 1991. Soil management affects shoot and root growth, nutrient availability, and water uptake of young peach trees. J. Am. Soc. Hortic. Sci., 116, 238-241.

Gómez, J.A., Romero, P., Giraldez, J.V., Fereres, E., 2004. Experimental assessment of runoff and soil erosion in an olive grove on a Vertic soil in Southern Spain as affected by soil management. Soil. Use Manage. 20, 426-431.

427 Gómez, J.A., Sobrinho, T.A., Giraldez, J.V., Fereres, E., 2009. Soil management effects on runoff, erosion and soil
428 properties in an olive grove of Southern Spain. Soil Till. Res. 102, 5-13.
429 Gucci, R., Caruso, G., Bertolla, C., Urbani, S., Taticchi, A., Esposto, S., Servili, M., Sifola, M.I., Pellegrini, S., Pagliai,
430 M., Vignozzi, N., 2012. Changes in soil properties and tree performance induced by soil management in a high-density
431 olive orchard. Eur. J. Agron. 41, 18-27.
432 Hernández, A.J., Lacasta, C., Pastor, J., 2005. Effects of different management practices on soil conservation and soil
433 water in a rainfed olive orchard. Agric. Water Manage. 77, 232-248.
434 Higo, M., Isobe, K., Yamaguchi, M., Drijber, R.A., Jeske, E.S., Ishii, R., 2013. Diversity and vertical distribution of
435 indigenous arbuscular mycorrhizal fungi under two soybean rotational systems. Biol. Fertil. Soils 49, 1085-1096.
436 Hogue, E.J., Neilsen, G.H., 1987. Orchard floor vegetation management. Hortic. Rev. 9, 377-430.
437 Huang, X., Lakso, A.N., Eissenstat, D.M., 2005. Interactive effects of soil temperature and moisture on Concord grape
438 root respiration. J. Exp. Bot. 56, 2651-2660.
439 Jakobsen, I., Nielsen, N.E., 1983. Vesicular-arbuscular mycorrhiza in field-grown crops. I. Mycorrhizal infection in
440 cereals and peas at various times and soil depths. New Phytol. 93, 401-413.
441 Jansa, J., Mozafar, A., Anken, T., Ruh, R., Sanders, I.R., Frossard, E., 2002. Diversity and structure of AMF communities
442 as affected by tillage in a temperate soil. Mycorrhiza 12, 225-234.
443 Jansa, J., Mozafar, A., Kuhn, G., Anken, T., Ruh, R., Sanders, I.R., Frossard, E., 2003. Soil tillage affects the community
444 structures of mycorrhizal fungi in maize roots. Ecol. Appl. 13, 1164-1176.
445 Kabir, Z. 2005. Tillage or no-tillage, impact on mycorrhizae. Can. J. Plant. Sci. 85, 23-29.
446 Kabir, Z., Koide, R.T., 2002. Effect of autumn and winter mycorrhizal cover crops on soil properties, nutrient uptake
447 and yield of sweet corn in Pennsylvania, USA. Plant Soil 238, 205-215.
448 Kabir, Z., O'Halloran, I.P., Widden, P., Hamel, C., 1998. Vertical distribution of arbuscular mycorrhizal fungi under
449 corn *Zea mays* L. in no-till and conventional tillage systems. Mycorrhiza, 8, 53-55.
450 Karasawa, T., Takebe, M., 2012. Temporal or spatial arrangements of cover crops to promote arbuscular mycorrhizal
451 colonization and P uptake of upland crops grown after nonmycorrhizal crops. Plant Soil 353, 355-366.
452 Lehman, R.M., Taheri, W.I., Osborne, S.L., Buyer, J.S., Jr. DDD., 2012. Fall cover cropping can increase arbuscular
453 mycorrhizae in soils supporting intensive agricultural production. Appl. Soil Ecol. 61, 300-304.
454 Meddad-Hamza, A., Beddiar, A., Gollotte, A., Lemoine, M.C., Kuszala, C., Gianinazzi, S., 2010. Arbuscular mycorrhizal
455 fungi improve the growth of olive trees and their resistance to transplantation stress. Afr. J. Biotechnol. 9, 1159-1167.

456 Mäder, P., Edenhofer, S., Boller, T., Wiemken, A., Niggli, U., 2000. Arbuscular mycorrhizae in a long-term field trial
457 comparing low-input organic, biological and high-input conventional farming systems in a crop rotation. Biol. Fertil.
458 Soils 31, 150-156.
459 Meyer, A.H., Wooldridge, J., Dames, J.F., 2015. Effect of conventional and organic orchard floor management practices
460 on arbuscular mycorrhizal fungi in a 'Cripp's Pink'/M7 apple orchard soil. Agr. Ecosys. Environ. 213, 114-120.
461 Moreno, B., Garcia-Rodriguez, S., Cañizares, R., Castro, J., Benìtez, E., 2009. Rainfed olive farming in south-eastern
462 Spain, long-term effect of soil management on biological indicators of soil quality. Agric. Ecosyst. Environ. 131, 333-
463 339.
464 Njeru, E.M., Avio, L., Sbrana, C., Turrini, A., Bocci, G., Bàrberi, P., Giovannetti, M., 2014. First evidence for a major
465 cover crop effect on arbuscular mycorrhizal fungi and organic maize growth. Agron. Sustain. Dev. 34, 841-848.
466 Njeru, E.M., Avio, L., Bocci, G., Sbrana, C., Turrini, A., Bàrberi, P., Giovannetti, M., Oehl, F., 2015. Contrasting effects
467 of cover crops on 'hot spot' arbuscular mycorrhizal fungal communities in organic tomato. Biol. Fertil. Soils 51, 151-
468 166.
469 Oehl, F., Sieverding, E., Ineichen, K., Mäder, P., Boller, T., Wiemken, A., 2003. Impact of land use intensity on the
470 species diversity of arbuscular mycorrhizal fungi in agroecosystems of central Europe. Appl. Environ. Microbiol. 69,
471 2816-2824.
472 Oehl, F., Sieverding, E., Mader, P., Dubois, D., Ineichen, K., Boller, T., Wiemken, A., 2004. Impact of long-term
473 conventional and organic farming on the diversity of arbuscular mycorrhizal fungi. Oecologia 138, 574-583.
474 Parker, M.L., Meyer J.R., 1996. Peach tree vegetative and root growth respond to orchard floor management. HortScience
475 31, 330-333.
476 Pellerin, S., Mollier, A., Morel, C., Plenchette, C., 2007. Effect of incorporation of *Brassica napus* L. residues in soils on
477 mycorrhizal fungus colonisation of roots and phosphorus uptake by maize *Zea mays* L. Eur. J Agron. 26, 113-120.
478 Philippot, L., Raaijmakers, J.M., Lemanceau, P., van der Putten, W.H., 2013. Going back to the roots, the microbial
479 ecology of the rhizosphere. Nature Rev Microbiol 11, 789-799.
480 Pimentel, D., Wilson, C., Mc Cullum, C., Huang, R., Dwen, P., Flack, J., Tran, Q., Saltman, T., Cliff, B., 1997. Economic
481 and environmental benefits of biodiversity. Bioscience 47, 747-757.
482 Porras-Soriano, A., Soriano-Martín, M.L., Porras-Piedra, A., Azcón, R., 2009. Arbuscular mycorrhizal fungi increased
483 growth, nutrient uptake and tolerance to salinity in olive trees under nursery conditions. J. Plant Physiol. 166, 1350-
484 1359.
485 Rillig, M.C., Field, C.B., 2003. Arbuscular mycorrhizae respond to plants exposed to elevated atmospheric CO_2 as a
486 function of soil depth. Plant Soil 254, 383-391.

Ryan, M.H., Chilvers, G.A., Dumaresq, D.C., 1994. Colonisation of wheat by VA-mycorrhizal fungi was found to be higher on a farm managed in an organic manner than on a conventional neighbour. Plant Soil 160, 33-40.

Rodrıguez-Lizana, A., Espejo-Perez, A.J., Gonzalez-Fernandez, P., Ordonez-Fernandez, R., 2008. Pruning residues as an alternative to traditional tillage to reduce erosion and pollutant dispersion in olive groves. Water Air Soil Pollut. 193, 165-173.

Rouphael, Y., Franken, P., Schneider, C., Schwarz, D., Giovannetti, M., Agnolucci, M., De Pascale, S., Bonini, P., Colla, G., 2015. Arbuscular mycorrhizal fungi act as biostimulants in horticultural crops. Sci. Hort. 196, 91-108.

Scandellari, F., Caruso, G., Liguori, G., Meggio, F., Palese, A.M., Zanotelli, D., Celano, G., Gucci, R., Inglese, P., Pitacco, A., Tagliavini, M., 2016. A survey of carbon sequestration potential of orchards and vineyards in Italy. Eur. J. Hortic. Sci. 81, 106-114.

Schwab, S., Reeves, F.B., 1981. The role of endomycorrhizae in revegetation practices in the semi-arid west. III. Vertical distribution of vesicular-arbuscular VA mycorrhiza inoculum potential. Am. J. Bot. 68, 1293-1297.

Smith, S.E., Read, D., 2008. Mycorrhizal Symbiosis, 3rd edn. Academic Press, London.

Tomei, P.E., 2013. Sulla flora degli oliveti del Monte Pisano. In, Fantoni E. and Narducci R. eds. Gli olivi e l'olio del Monte Pisano, ambiente, storia e attualità, ETS edizioni, Pisa, pp. 39-54.

Tugendhaft, Y., Eppel, A., Kerem, Z., Barazani, O., Ben-Gal, A., Kadereit, J.W., Dag, A., 2016. Drought tolerance of three olive cultivars alternatively selected for rain fed or intensive cultivation. Sci. Hort. 199, 158-162.

Turrini, A., Sbrana, C., Avio, L., Njeru, E.M., Bocci, G., Bàrberi, P., Giovannetti, M., 2016. Changes in the composition of native root arbuscular mycorrhizal fungal communities during a short-term cover crop-maize succession. Bio Fertil. Soils 52, 643-653.

Van Geel, M., Ceustermans, A., Van Hemerlrijck, W., Lievens, B., Honnay, O., 2015. Decrease in diversity and changes in community composition of arbuscular mycorrhizal fungi in roots of apple trees with increasing orchard management intensity across a regional scale. Mol. Ecol. 24, 941-952.

Xiao, W., Xiaogai, G., Zeng, L., Huang, Z., Lei, J., Zhou, B., Li, M., 2014. Rates of litter decomposition and soil respiration in relation to soil temperature and water in different aged *Pinus massoniana* Forests in the three Gorges Reservoir Area, China. Plos One 9 7 e101890.

Yao, S., Merwin, I.A., Brown, M.G., 2009. Apple root growth turnover, and distribution under different orchard groundcover management systems. HortScience 44, 168-175.

Figure captions

Fig. 1 Schematic representation of the two L-shaped trenches excavated South (S-Trench) and North (N-Trench) of the tree row in tilled plots (ST) and green covered (GC) ones of the experimental olive orchard. The solid line represents the sub-irrigation dripline located at a distance of 0.8 m from the tree row and 0.4 m depth. Closed circles represent the sampling points where soil cores for olive root biomass and mycorrhizal studies were sampled. Closed triangles indicate the sampling points where soil respiration rates were measured.

Fig. 2 Soil respiration rates (a), soil moisture (b) and soil temperature (c) measured at four sampling points below the canopy of trees in an olive orchard subjected to two different soil managements: permanent green cover, GC; shallow tillage, ST. Values are means $\pm$ standard deviations of three replicate trees per treatment ($n = 3$) of two daily (dawn and mid-day) measurements. Different letters indicate least significant differences (LSD) between treatments after analysis of variance within each date of measurement ($p \leq 0.05$). Soil temperature and moisture were measured at 0.08 and 0.06 m depth, respectively.

Fig. 3 Soil mycorrhizal inoculum potential (MIP) (a) and mycorrhizal colonization of olive trees (b) under two different soil managements (permanent green cover, GC; shallow tillage, ST) at either 0.3 or 0.6 m depth. Values are means $\pm$ standard errors of six replicates per treatment and soil depth. The asterisks indicates least significant differences (LSD) between treatments after analysis of variance within each soil depth ($P \leq 0.01$).

Fig. 4 Light micrographs showing mycorrhizal colonization patterns in the root cortex of olive (*Olea europaea* L.) by native AMF under permanent green cover (a-c) and shallow tillage (d-h) treatments. (a) Intra-radical hyphae and arbuscules, bar = 120 μm; (b) detail showing an entry point with appressorium, coiled hyphae and arbuscules, bar = 33 μm; (c) detail of arbuscules, bar = 33 μm; (d) suberized root cells showing some intra-radical and extra-radical hyphae (arrow), bar = 120 μm; (e) knobby, inflated appressorium, bar = 33 μm; (f) aborted entry point showing septate infection hyphae, bar = 33 μm; (g) detail of a coil, bar = 33 μm; (h) intercellular vescicles, bar = 80 μm.

549 **Table 1** List of plant species identified in the permanent green cover (GC) plots in 2014. Species are ordered by family
550 (in alphabetical order). The biological form and subform and chorology are also reported. Legend: G, Geophytes; H,
551 Hemicryptophytes; T, Terophytes.

Family	Species	Biological form and subform	Chorology
Amaranthaceae	*Beta vulgaris* L.	T scapose	Euro-Mediterranean
Apiaceae	*Daucus carota* L.	H biennial	Paleotemperate
Araceae	*Arisarum vulgare* Mill.	G rhizomatose	Steno-Mediterranean
Asteraceae	*Cichorium intybus* L.	H scapose	Cosmopolitan
	Coleosthepus myconis (L.) Cass.	T scapose	Steno-Mediterranean
	Crepis vesicaria L.	T scapose	Submediterranean-Subatlantic
	Helminthoheca echioides (L.) Holub.	T scapose	Euro-Mediterranean
	Hypochaeris radicata L.	H rosulate	European-Caucasic
	Picris hieracioides L.	H biennial	Eurasiatic
	Sonchus oleraceus L.	H biennial	Cosmopolitan
	Urospermum dalechampii (L.) F.W. Schimdt	H scapose	Euro-Western Mediterranean
Brassicaceae	*Sinapis arvensis* L.	T scapose	Steno-Mediterranean
Euphorbiaceae	*Euphorbia helioscopia* L.	T scapose	Cosmopolitan
Fabaceae	*Lotus corniculatus* L.	H scapose	Paleotemperate
	Trifolium campestre Schreber	T scapose	Paleotemperate
	Trifolium repens L.	H reptant	Paleotemperate
	Trifolium resupinatum L.	T reptant	Paleotemperate
Geraniaceae	*Geranium dissectum* L.	T scapose	Cosmopolitan
	Geranium rotundifolium L.	T scapose	Paleotemperate
Iridaceae	*Romulea columnae* Sebast et Mauri	G bulbose	Steno-Mediterranean
Malvaceae	*Malva sylvestris* L.	H scapose	Eurasiatic
Plantaginaceae	*Plantago lanceolata* L.	H rosulate	Cosmopolitan
	Veronica persica Poir.	T scapose	Subcosmopolitan
Poaceae	*Avena barbata* Potter	T scapose	Euro-Mediterranean – Turan.
	Bellis perennis L.	H rosulate	Circumboreal
	Bromus madritensis L.	T scapose	Euro-Mediterranean
	Bromus sterilis L.	T scapose	South Mediterranean
	Dactylis glomerata L.	H caespitose	Paleotemperate
	Holcus lanatus L.	H caespitose	Circumboreal
	Hordeum murinum L.	T scapose	Circumboreal
Primulaceae	*Anagallis arvensis* L.	T reptant	Euro-Mediterranean
Ranunculaceae	*Ranunculus parviflorus* L.	T scapose	Euro-Mediterranean
Rubiaceae	*Sherardia arvensis* L.	T scapose	Euro-Mediterranean

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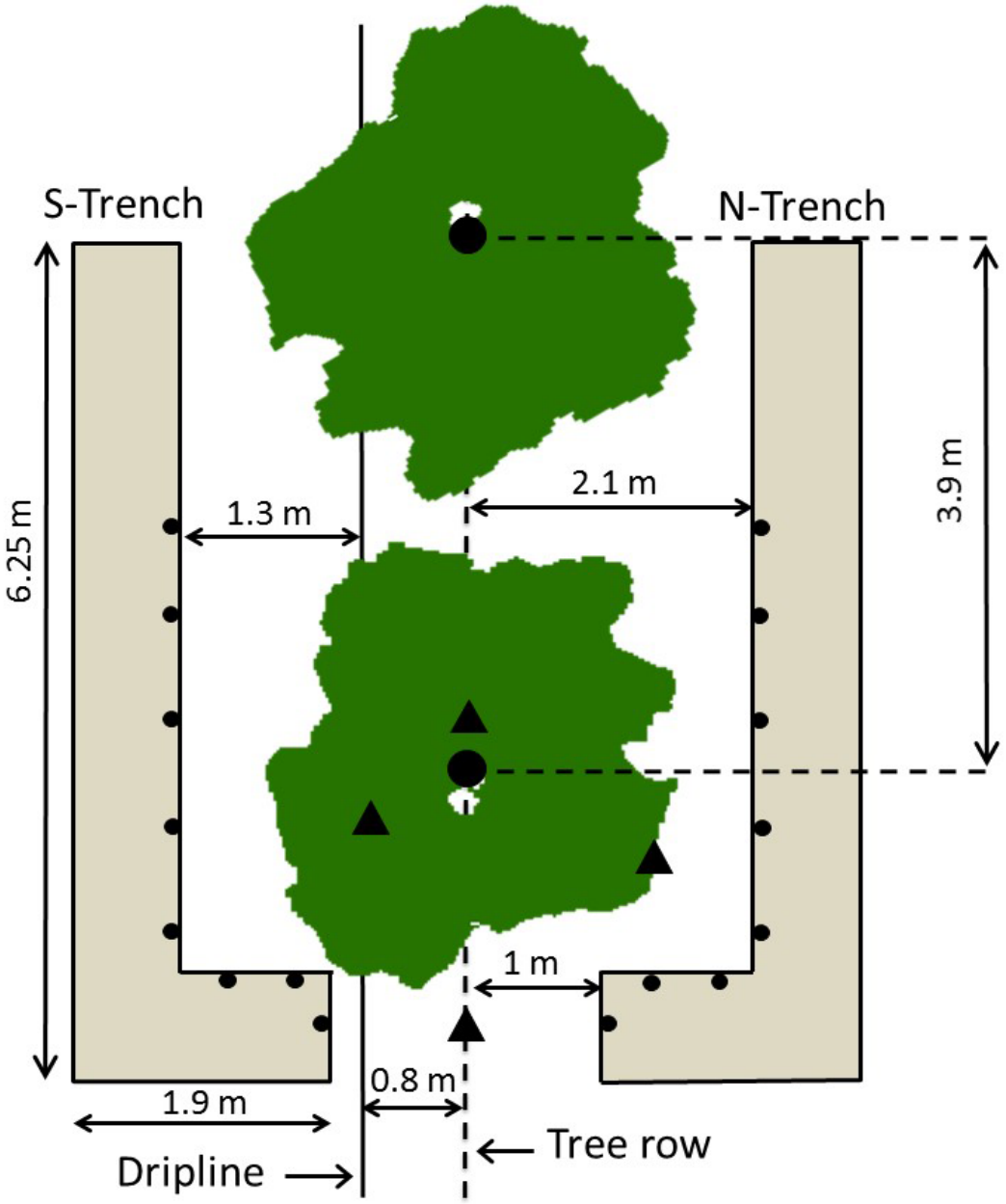
Table 2. Root density (root dry weight per soil volume) of different diameter cohorts sampled at 0.2, 0.4, and 0.6 m soil depth from olive trees subjected to either permanent green cover or shallow tillage for 10 years. Values are means of 16 sampling points per depth and plot (three plots per treatment) along two trenches excavated parallel to the tree row. Values followed by the same letter do not differ significantly ($p < 0.05$).

Variable	Root density (kg m^{-3})		
	< 1 mm	1-2 mm	2-5 mm
Soil management (SM)			
Green cover	3.15	0.86	0.78
Shallow tillage	2.64	1.06	0.68
Soil depth (SD)			
0.2	3.58 a	1.08	0.77
0.4	2.67 b	0.84	0.66
0.6	2.45 b	0.96	0.75
Significance (p value)			
SM	0.321	0.514	0.787
SD	0.001	0.488	0.948
SM x SD	0.184	0.986	0.941

571 FIG.1



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583 FIG.2

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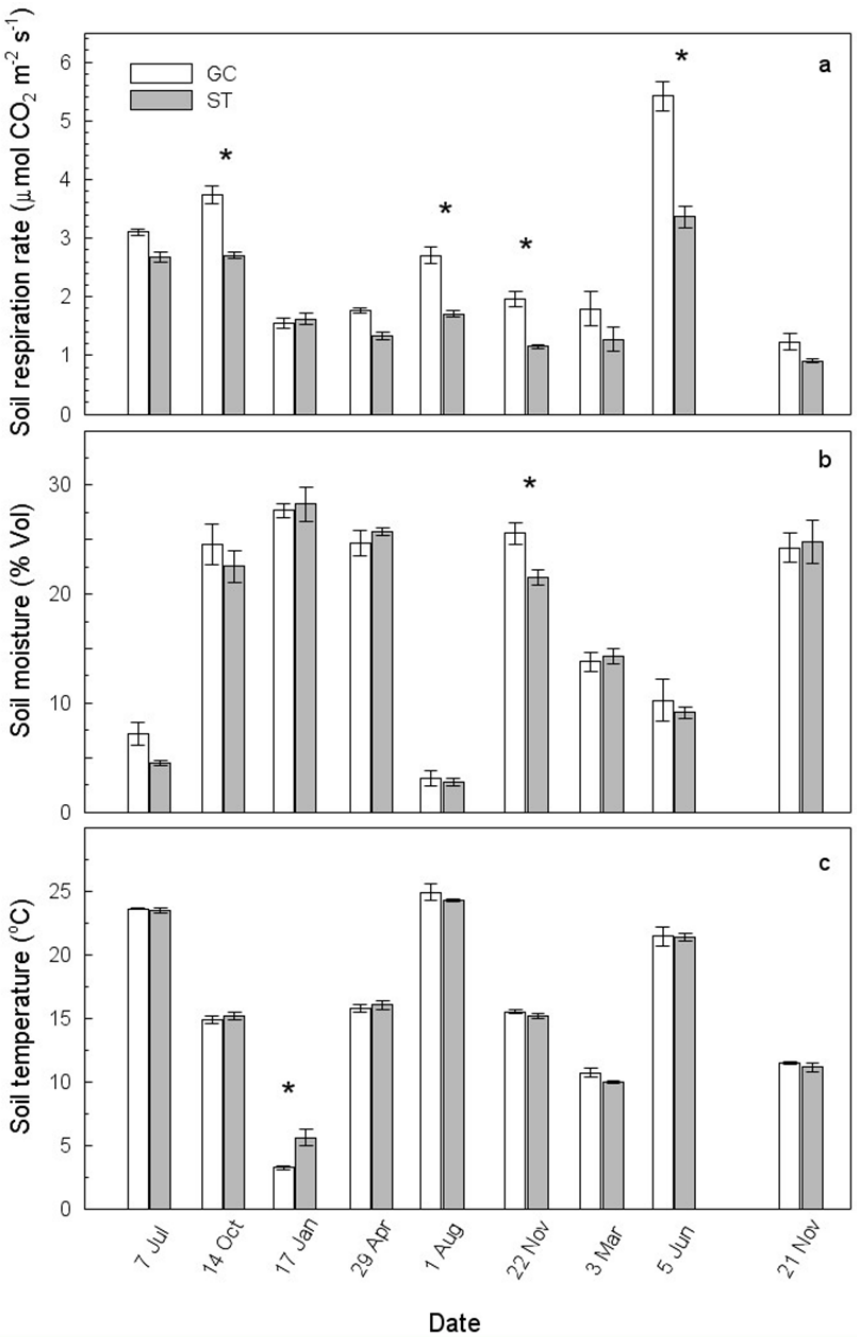

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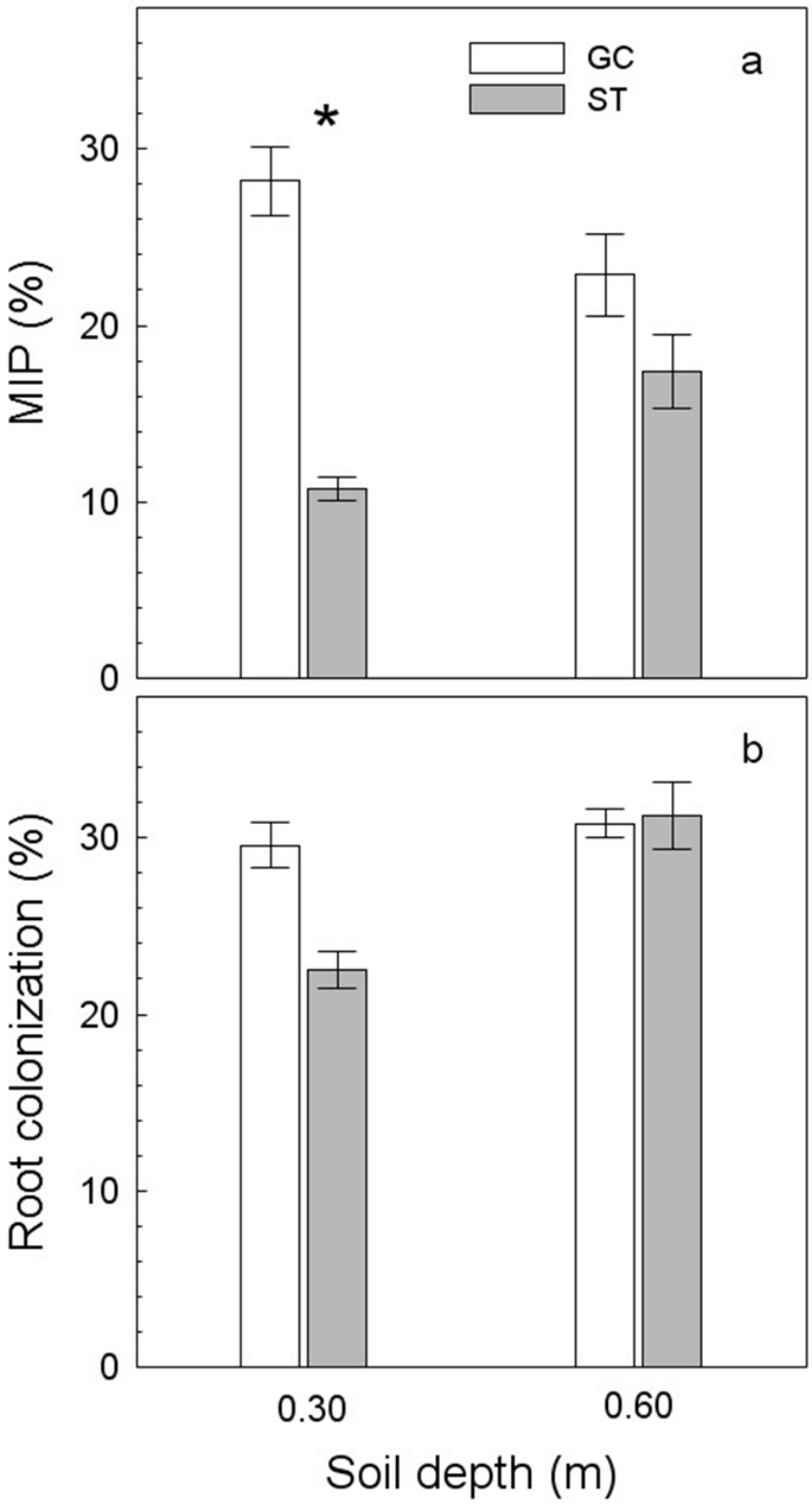
FIG.3


GC
ST
a
*
MIP (%)
30
20
10
0
b
Root colonization (%)
30
20
10
0
0.30
0.60
Soil depth (m)

599 FIG.4

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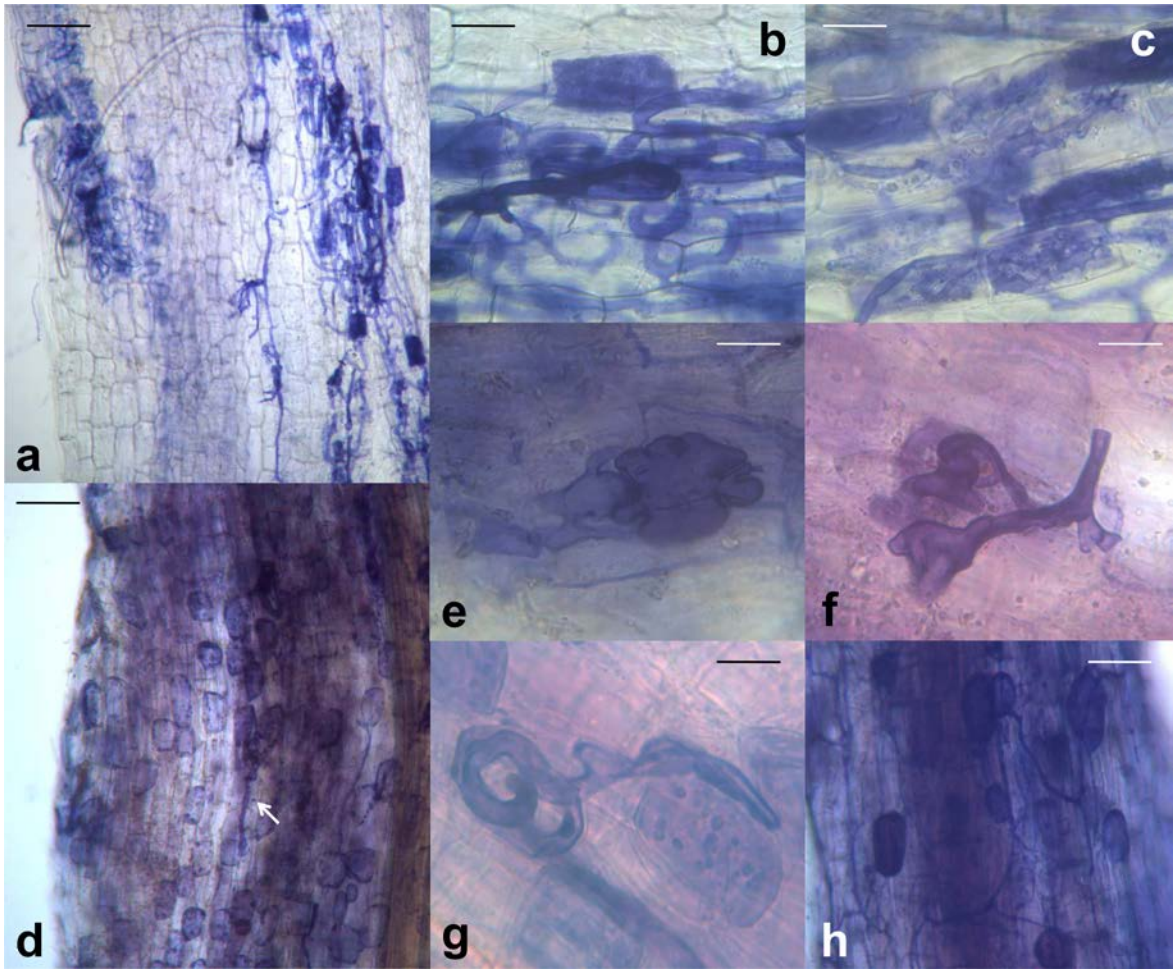

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