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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 infrared 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.

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, 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; Philippot 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.,

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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; Rodrıguez-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 × 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.