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Organic farming induces changes in soil microbiota that affect agro-ecosystem functions

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ABSTRACT

Cultivation of vegetables under plastic tunnels is a steadily growing farming system in the agricultural sector but raises concerns about its environmental sustainability. The aim of the present work was to assess the impact of organic farming, compared to conventional cultivation, on agro-ecosystem functions and soil microbial communities. Two farms that practiced organic cultivation for 10 and 20 years were compared with one conventional farm, all with cultivations under plastic tunnel. Soil functions were assessed with multi-species bioassays on plant growth and organic matter decomposition, and microbial communities were characterized by high-throughput sequencing of bacterial and eukaryotic rRNA gene markers. Plant growth and organic matter decomposition were higher in organic compared to conventionally management soils. Agronomical practices showed a significant effect on microbial diversity and composition. Soil bacterial diversity was lower in both organic farms than in the conventional farm. Soil eukaryotic diversity was slightly higher in the 10-year organic farm but lower in the 20-year organic farm compared to the conventional farm. At phylum level, Acidobacteria, Firmicutes, and TM7 were higher, while Planctomycetes, Verrucomicrobia, and Actinobacteria were lower in organic-farmed soils compared to conventional farm. Noteworthy, Metazoa raised from 0.1% of eukarya relative abundance in conventional-farmed to 20.9% in 20 year organic-farmed soil. Correlation analysis between microbial OTUs relative abundance and ecosystem functions suggest that eukarya play a major role, compared to bacteria, in controlling plant growth and organic carbon cycling. Integration of microbial diversity data with plant growth and decomposition of organic matter decomposition allowed us to provide a linkage between agricultural management, microbial community composition, and soil functionality.

1. Introduction

Microbes are key soil components that control agro-ecosystem functioning. Soil microbes provide nutrients by mineralizing organic matter (Hodge et al., 2000), control soil organic carbon storage (Moorhead and Sinsabaugh, 2006), contribute to the structural stability of soil aggregates (Abiven et al., 2009), suppress soilborne plant pathogens (Janvier et al., 2007; Senechkin et al., 2014; van Bruggen et al., 2015) and, as a consequence, affect plant health and crop yield. Investigation of the relationships between microbial community composition and ecosystem functioning has received growing attention in recent years, and it is now well recognized that microbial diversity affects ecosystem processes such as primary productivity (van Der Heijden et al., 2008), organic carbon cycling (Bell et al., 2005), resistance to perturbations (Dang et al., 2005), and suppression of soilborne pathogens (van Bruggen et al., 2006). A number of studies, moreover, pointed out that cultivation management practices such as tillage regime (Shi et al., 2013), fertilization (Fierer et al., 2012), and pest management (Wei et al., 2016) profoundly affect soil microbial diversity and its taxonomic composition.

In the last decades a significant decrease in soil quality and crop productivity has been observed worldwide because of salinization (Sumner, 1995), pollution by heavy metals and xenobiotics (Moolenaar et al., 1997), reduction of soil organic carbon (Loveland and Webb, 2003), and loss of natural soil suppressiveness towards plant pathogens (Weller et al., 2002). Soil quality loss has been related to intensive cultivation systems based on repeated tillage, and heavy application of synthetic fertilizers as well as fumigants

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and fungicides (Tilman et al., 2002). In this regards, organic farming that relies on organic amendment for plant nutrition and natural products for protection from plant pathogens and pests has been proposed as an environmentally friendly, low intensive cultivation approach (Reganold and Wachter, 2016). In 2015, about 44 million ha worldwide were managed organically and about 12 million in the European Union (Willer and Lernoud, 2016), and the dedicated cultivation area is still growing.

Most of the available studies investigated the impact of conventional *vs.* organic farming on crop productivity. The meta-analysis by Seufert et al. (2012) shows that organic yields are on average lower than conventional yields, but such a difference is larger for vegetable and cereal productions compared to rain-fed legume and perennial crops. To compete with conventional systems in terms of crop yield, organic farming uses several approaches focusing on the improvement of soil physical structure, enhancement of enzymatic activity and beneficial microbes such as plant-growth promoting rhizobacteria and arbuscular mycorrhizal fungi (Drinkwater et al., 1995; Mäder et al., 2002). There have been several studies comparing microbial community taxonomic composition and diversity of soils under organic and conventional management (van Diepeningen et al., 2006). Such studies reported either a higher microbial diversity in organically managed soils (Hiddink et al., 2005; Hartmann et al., 2015) or similar species richness in both types of cultivation (Sugiyama et al., 2010). The contradictory effect of organic farming on microbial diversity has been related to the methodological differences (*i.e.* culture *vs.* culture-independent methods), and to the different agro-ecosystems studied ranging from tropical cultivations (Caldwell et al., 2015), to annual crops in temperate environment (Hartmann et al., 2015), and forage grasslands in cold climate (Pershina et al., 2015). Most of the available studies focused on field crops (Li et al., 2012) such as potato (Orr et al., 2015), with far less attention paid to vegetable cultivations (Drinkwater et al., 1995; van Bruggen et al., 2016), despite their economic importance. In detail, no studies addressed the long-term impact of conventional and organic farming on soil microbial communities in intensive cultivation under permanent plastic tunnels.

Cultivation under plastic tunnels is a steadily growing agricultural sector all over the world, and at present, it covers more than 2,000,000 ha worldwide and about 200,000 ha in the Mediterranean Basin (Scarascia-Mugnozza et al., 2011). This type of cultivation affects soil quality because it drastically modifies water, carbon, and nutrient cycles. The almost complete rainfall restriction and the consequent requirement of localized irrigation to support crop water demand increases soil salinity, while the widespread use of mineral fertilizer induces soil acidification (Ju et al., 2007). In addition, the systematic elimination of crop residues to limit plant diseases, the optimal temperature and water content that promote mineralization of organic matter are all factors that induce a reduction of soil organic carbon content, with a negative feedback on soil microbial communities (Bonanomi et al., 2014). Organic farming that promotes high microbial diversity and functionality is expected to positively affects also crop yield and organic carbon cycling.

Most of the previous studies comparing soils under organic and conventional management described the diversity of soil microbial communities, missing the key link between microbiome richness and composition with its functional roles (Hättenschwiler et al., 2005; Mendes et al., 2015). In this study, we hypothesized that the long-term organic farming under the permanent plastic tunnel, compared with conventional cultivation, modifies soil microbiome and its functionality with positive cascade effects on plant productivity and carbon cycling. To verify this hypothesis a multidisciplinary approach was used that combined microbial community characterization by high-throughput sequencing of bacterial and eukaryotic rRNA gene markers, with multi-species bioassays assessing key agro-ecosystem functions (*i.e.* plant growth and organic matter decomposition) in organic and conventional managed soils. The main objectives of our study were (i) to reveal the differences in microbiota of soils managed organically and conventionally; (ii) to assess the impact of the different microbial communities on plant growth and organic matter decomposition; (iii) to explore the relationships between microbiota composition and agro-ecosystem functions.

2. Materials & methods

2.1. Study site

The study site is a productive area of about 5000 ha cultivated under greenhouses located in the Salerno area (Southern Italy). Low-technology, unheated polyethylene-covered greenhouses (height ~4 m) are the main crop protection structures used in this area. The study site had a Mediterranean climate with a mean annual temperature of 15.9 °C and mean monthly temperatures ranging from 23.6 °C in August to 9.0 °C in January. The climate has a mean annual rainfall of 988 mm with a relatively dry summer (84 mm).

2.2. Soil sampling

Three large farms (cultivated surface > 25 ha) were chosen among a group of 20 farms that were previously characterized in a study aimed to monitor the effects of plastic tunnel farming systems on soil quality (Bonanomi et al., 2011). The three farms were subject to a similar intensive farming system adopted for at least 20 years. The agricultural system was based on cultivation under the plastic tunnel, intensive tillage with an average of 6 plowing treatments every year including rototilling, spading and harrowing. The three farms, moreover, had a similar soil type (Table S1) because they are located in a small area with a maximal planar distance among sites of ~11 km. The three farms adopt similar crop rotations with tomato (*Solanum lycopersicon*), zucchini (*Cucurbita pepo*), and fourth range cultivations such as lettuce (*Lactuca sativa*), sugar beet (*Beta vulgaris*), and rocket (*Eruca sativa*).

The specific aim of this study was to compare three soil management regimes: i. Conventional cultivation (hereafter indicated as conventional); ii. Organic farming for 10 years (organic - 10 y), and; organic farming for 20 years (organic - 20 y). The main differences between conventional and organic farming were largely due to pest management and plant nutrition practices. In the organic farms, no synthetic pesticides were used and plant nutrition was based on organic fertilizers such as compost and animal manure (i.e. average application rate of ~5 t ha^{-1} $year^{-1}$). In the conventional farm, instead, soil disinfestation was performed once a year (i.e. solarization or application of Metham-Na), as well as the application of a herbicide (Kerb, Dow AgroSciences with propyzamide as active ingredient). Plant nutrition was based on mineral fertilizers by fertigation (240, 70 and 120 kg ha^{-1} $year^{-1}$ of N, P, and K, respectively).

In each farm, three plastic tunnels were considered for a total of nine tunnels. In each tunnel, five soil sub-samples were collected following a W scheme (four sampling plots near to corners and one sampling plot in the center of the tunnel) and pooled together in order to have three composite soil samples for each farm. A total of 9 independent soil samples were collected (three farms with three tunnels each). The sampling strategy was designed to minimize the variability due to soil types and allowed a direct comparison of management regime effect. Soil samples (~50 kg) from each tunnel