

The metabolic and genetic diversity of soil bacterial communities depends on the soil management system and C/N dynamics: The case of sustainable and conventional olive groves

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ABSTRACT

Under Mediterranean type climates, a sustainable soil management aimed at increasing soil organic carbon and microbiological diversity is of key importance. The aim of this study was to characterize and compare soils from a mature Mediterranean olive (*Olea europaea* L.) orchard subjected to two different soil management systems (sustainable, S_{mng} , and conventional, C_{mng}) for 18 years. Composite soil samples were analyzed qualitatively (pedological determinations) and quantitatively (soil C, N and pH). Bacterial metabolic activity and diversity were evaluated spectrophotometrically by the Biolog® assay, while 16S-based metagenomics analysis was used to analyse the abundance of N-cycling bacteria. From the general analysis of the data obtained by the study of soil profiles and litter, C and N dynamics, and the genetic and functional diversity of soil bacteria, it emerged that the S_{mng} system caused an improvement in soil organic matter in the topsoil layers, compared to the C_{mng} system, with consequent increases in the abundance of soil N-cycling bacteria but without affecting the indices related to total bacterial metabolic activity and diversity. The results obtained could promote the development of new approaches for optimizing soil carbon cycling, managing nutrient transport, and sustaining and improving olive yield and quality.

1. Introduction

In Mediterranean orchards, a sustainable soil management (S_{mng}) aimed at increasing soil organic carbon (SOC) stocks (e.g., by no-tillage, increased C and N inputs, recycling of pruning residuals, cover crops) can affect many soil parameters, such as physical characteristics (Palese et al., 2014), chemical parameters (Sofo et al., 2010; Montanaro et al., 2010), water content (Celano et al., 2011), and CO₂ fluxes (Montanaro et al., 2012). Soil management, if not well planned and conducted, can provoke decreases in soil organic matter (SOM), mainly due to SOM mineralization (Montanaro et al., 2010). In Mediterranean olive orchards, semi-arid climates, via stimulated microbial metabolism and respiration, cause decreases of SOC and other nutrients (primarily soil N; Pascasio et al., 2018). In these agrosystems, a S_{mng} that includes a localized and evapotranspiration-based irrigation can improve plant

physiological status, olives and oil production and quality in the mid-term (Palese et al., 2009; Sofo et al., 2010). In olive groves, the S_{mng} also gives considerable economic advantages to the farmers (Pergola et al., 2013) and provides efficient ecosystemic and sociocultural services (Montanaro et al., 2017). In addition, the S_{mng} applied to olive orchards can reduce the negative repercussions on the environment linked to nutrient leaching/runoff, particularly weighty for nitrates (Palese et al., 2015).

Soil microorganisms' dynamics (e.g., mobility, growth, nutrient absorption and respiration), major responsible of soil fertility and quality (Bünemann et al., 2018), are strongly affected both by the type of soil management and irrigation (Enwall et al., 2007; Jeanbille et al., 2016), and this has been widely demonstrated in fruit crops (Palese et al., 2009; Sofo et al., 2014a,b; Pascasio et al., 2018). The functionality and metabolism of soil microorganisms are related to soil quality

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and fertility, as they influence and at the same time are influenced by the soil C and N contents, being bacteria an essential part of C and, even more, of N cycling processes (de Vries and Shade, 2013; de Vries and Wallenstein, 2017; Mooshammer et al., 2014; Zhang et al., 2014; Li et al., 2018).

In the case of olive orchards, that are a conspicuous part of the Mediterranean agricultural landscape (about 9.5 Mha in 2010, according to Pergola et al., 2013) and are often characterized by low levels of soil organic matter, knowing if the agronomic practices adopted are beneficial or not for soil microbial communities assumes a key importance (Sofó et al., 2014b; Pascasio et al., 2018). One of the easiest and reliable techniques for defining soil microbiological status is the determination of bacterial metabolic diversity by the Biolog® method, having a high discriminating power between bacterial soil communities from different soil environments and from soils subjected to various agronomic treatments (Zhang et al., 2014). Culture-based and genetic techniques have been used successfully in olive orchards to ascertain the presence of N-cycling functional bacteria and better characterize the portion of soil microbiota involved in soil N metabolism (Sofó et al., 2010). This is particularly important for olive orchards, where N is often a limiting factor, even when soils are managed sustainably and have high organic C inputs (Montanaro et al., 2010; Celano et al., 2011). Besides microbiological and genetic analysis, nowadays, next-generation sequencing (NGS), coupled with bioinformatic tools and metagenomic approaches, have made it easier to comprehensively analyze microbial communities on any type of matrix, including soils (Lakshmanan et al., 2014; Jansson and Hofmockel, 2018).

From the opposite side, qualitative soil profile descriptions, and the analysis of C/N ratios and pH values both in topsoil and subsoil can be very useful when interpreting soil C and N data for understanding the general context of the environment soil microorganisms live in (FAO, 2006; Mooshammer et al., 2014). Particularly, the characteristics of the litter layer are a very informative addition to the soil profile description, as they reflect the equilibrium between litter production, litter microbial decomposition and interaction with the mineral soil (Zanella et al., 2018a).

Because of the complexity and site-specificity of soils, defining soil quality is not an easy task (Bünemann et al., 2018). The study of the soil N-cycling bacteria and of the N dynamics could help to understand how soil management can affect soil status (Pascasio et al., 2018; Li et al., 2018). On this basis, the aim of this study was to characterize and compare soils from a Mediterranean olive orchard subjected to two different soil management systems (sustainable, S_{mng} , and locally conventional management, C_{mng}) for 18 years. Considering the higher C and N inputs in the S_{mng} , compared to the C_{mng} , bacterial diversity, particularly of N-cycling bacteria, was analyzed and discussed in relation to soil properties, in order to understand how eventual C/N and pH imbalances can regulate soil C dynamics and N cycling.

2. Materials and methods

2.1. Experimental site, orchard management and soil sampling

The trial was done in a 2-ha olive orchard (*Olea europaea* L., cv. ‘Maiatica’; 70-year-old plants with a distance of 8×8 m; NE orientation) located in Ferrandina (Southern Italy, Basilicata region; N 40°29'; E 16°28'). The area has a semi-arid climate, with an annual rainfall of 565 mm (mean 1995–2015), concentrated mostly in the winter, and a mean annual temperature of 16.1 °C. The soil is a sandy loam, a Haplic Calcisol, according to the World Reference Base for Soil Resources, with a mean bulk density of 1.30 g cm^{-3} and sediment as parental material. The major landform is plain, the slope form is classified as convex-straight and the slope gradient class as gently sloping (2–5%) (FAO, 2006). The depth of the groundwater at the moment of the analysis was > 1.5 m.

Half of the orchard (1 ha) has been managed using organic agricultural practices for 17 years (2000–2017) (sustainable management, S_{mng}) (Table S1). Trees were drip-irrigated from March to October ($2800 \text{ m}^3 \text{ ha}^{-1} \text{ year}^{-1}$) with urban wastewater. The average values of organic C, N, P and K contained in the treated wastewater were 124, 54, 3 and $50 \text{ kg ha}^{-1} \text{ year}^{-1}$, respectively (mean 2000–2016). Six drip emitters (8 L h^{-1}) over a 1-m radius were placed around each plant. A light pruning was carried out every year during winter. The soil was permanently covered by spontaneous self-seeding weeds, that were mowed twice a year. Cover crop residues and prunings were shredded and left along the row as mulch. An integrative amount of $40 \text{ kg ha}^{-1} \text{ year}^{-1}$ of N-NO_3^- was distributed by fertigation once per year, during the fruit set and pit hardening phase (early spring), in order to entirely satisfy olive nutrient needs.

The other half of the orchard (1 ha) was kept as ‘control’ plot. It was rainfed and conducted with a locally conventional management (C_{mng}) for 17 years (2000–2017), according to the practices usually adopted by farmers in the region (Table S1). The C_{mng} was managed by tillage (milling at 10 cm depth) performed 2–3 times per year to control weeds. Intensive pruning was carried out every two years, but pruned residues were removed from the olive orchard. A mineral fertilization was carried out once per year, during the fruit set and pit hardening phase (early spring), using ternary compounds (NPK 20-10-10 fertilizer at doses ranging from 300 to $500 \text{ kg ha}^{-1} \text{ year}^{-1}$).

In March 2017, soil samplings was performed in both the treatments (S_{mng} and C_{mng}) in the non-irrigated inter-row area, in order to avoid interferences due to water regime between the two treatments. Soil sub-samples were picked in 10 points over a 1-m radius area around each olive tree, at different soil depths (0, 5, 10, 20, 30, 60 and 90 cm) for chemical analysis, and from the topsoil layer (0–30 cm) for bacterial communities’ analysis (Biolog® assay and 16S-based metagenomics analysis). The 10 sub-samples were pooled on site to constitute a composite soil sample of about 1 kg. For both the soil management systems (S_{mng} and C_{mng}), three composite samples ($n = 3$), each composed of 10 different sub-samples, were prepared. This sampling techniques allowed to minimize spatial variability, according to Bacon and Hudson (2001) and Tian et al. (2004). After removing visible crop residues, the soil composite samples were immediately stored in sterilized plastic bags at 4 °C for chemical and microbiological analysis, and at –20 °C for DNA extraction, and subsequently analysed within 10 days.

2.2. Soil and litter layer description

Before soil description analysis, the litter layer in the S_{mng} spot was removed and ground vegetation was cut. Excavation was made with an auger at a soil depth of 1.5 m. The chips from the auger were carefully laid out on a black piece of plastic, next to a meter, in order to check depth regularly. This procedure was done three times for each soil treatment (S_{mng} and C_{mng}). Soil changes, such as compacted or heavier layers, shiny surface of chips from the auger, indicative for a higher clay content, were observed. Master horizons were identified and named, according to FAO (2006). FAO guidelines were used for soil profile description of both S_{mng} and C_{mng} . At the end, the holes were closed and the surface tramped for avoiding damages to humans or animals.

For litter layer description, the litter layer was carefully observed and described using the reference guidelines of Zanella et al. (2018b). In brief, a ‘micro-pit’ (30–50 cm wide and 20 cm deep) was dug for describing the boundary between litter layer and mineral soil, and the structure of the A-horizon. A straight, clean cut with the spade at one side of the profile was done, disturbing soil and litter layer as little as possible. A loupe was used to examine zoogenic pellets. For the sake of consistency with the soil description analysis described above, the same FAO protocol (2006) was used to describe the structure of the A horizon and its lower boundary in both S_{mng} and C_{mng} .

2.3. Soil chemical analysis

All the chemical reagents were purchased from Sigma-Aldrich (Saint Louis, MI, USA) unless differently reported. On soil composite samples (soil depths of 0, 5, 10, 20, 30, 60 and 90 cm), total organic carbon (TOC), total N (TN) and pH were determined. All the soil samples were air-dried at approximately 25 °C and then sieved through a 2-mm stainless steel sieve. The size fraction smaller than 2 mm was used for soil chemical analyses. Soil pH was measured by a glass electrode (Basic 20[®]; Crison Instruments SA, Barcelona, Spain) both in distilled water and CaCl₂ using a suspension 1:2.5 soil to liquid phase ratio (Pansu and Gautheyrou, 2006). Total organic carbon (TOC) was determined by Walkley and Black method by oxidation at 170 °C with potassium dichromate (K₂Cr₂O₇) in presence of sulfuric acid (H₂SO₄), and the excess K₂Cr₂O₇ was measured by Mōhr salt titration (Pansu and Gautheyrou, 2006). Total nitrogen (TN) was measured by Kjeldahl method (Pansu and Gautheyrou, 2006).

2.4. Soil biolog[®] analysis

Three replicates of 10 g-subsamples (dry weight equivalent) of each soil composite sample (0–30 cm) were suspended in Ringer solution and sonicated for 2 min to disperse microbial cell, according to (Sofo et al., 2018), till having a final value of 10⁴ CFU (colony-forming units) mL⁻¹.

Sole carbon source utilization patterns of soil microbial communities were assessed using the Biolog[®] 96-well Eco-Microplates™ (AES Laboratoire, France), containing 31 different carbon sources. An aliquot of 100 µl of the soil dilution at 10⁻³ dilution was used for the assay. The microplates were incubated at 25 °C in darkness and the substrate-utilization profiles were analyzed on well-absorbance values at the 96-h observation period using a Microplate E-Max Reader (Bio-Rad; Hercules, CA USA) with 590- (primary) and 750-nm (secondary) wavelength filters.

Data were analyzed to determine the metabolic diversity indices accurately described by Sofo et al. (2018). The indices examined were the following: average well color development (AWCD), a measure of total bacterial metabolic activity, Shannon's diversity index (*H'*), Shannon's evenness index (*E*), i.e. the equitability of activities across all the utilized substrates, McIntosh's diversity index (*U*), and McIntosh's evenness index (*Z*). In addition to total AWCD, the carbon substrates of the Biolog[®] plates were divided into eight main groups of compounds, respectively, and the AWCD value for each group was calculated.

2.5. Extraction of soil total DNA and identification of specific bacterial taxa

DNA was extracted from an amount of 0.5 g of soil using the method described by Pascazio et al. (2018). The quality and concentration of the extracted DNA extracts were determined spectrophotometrically at 260, 280 and 230 nm using a NanoDrop[®]ND-1000 UV-Vis spectrophotometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA).

For each sample of soil DNA, the taxonomic characterization up to the species level of bacterial communities were carried out through a 16S-based metagenomics approach. 16S library preparation and sequencing were performed by IGA Technology Services S.r.l. (Udine, Italy). Bacterial 16S library preparation workflow included two PCR amplifications. An initial PCR amplification was performed on genomic DNA to amplify the variable V3-V4 region of 16S ribosomal RNA gene. Gene-specific primers with overhang adapters for compatibility with Illumina index and sequencing adapters were used. The primer sequences, without the adapters, were: 16S-341F 5'-CCTACGGGNGGCWGCAG-3' and 16S-805R 5'-GACTACHVGGGTATCTAATCC-3' (Klindworth et al., 2013). The PCR products were purified and used as target for a limited cycle amplification to add Illumina flow-cell binding domains and dual index adapters using NexteraXT Index Kit (FC-131-1001/FC-131-1002). The resulting libraries were purified, quantified and normalized. Up to 96 libraries were pooled and

sequenced from both ends on an Illumina MiSeq and more than 100,000 reads of about 300 bp were generated.

2.6. Statistical analysis

The statistical analysis of the soil chemical data was performed using Sigmastat 3.1 SPSS Inc. software (SPSS Inc., Quarry Bay, Hong Kong). The means of all the measured parameters were treated by one-way analysis of variance (ANOVA) with the orchard management type (*S*_{mng} and *C*_{mng}) as factor. Means were separated according to Fisher's LSD test at *p* ≤ 0.05. In this experiment, three analytical replicates for each treatment from the three independent composite soil samples (*n* = 3) were considered.

Regarding the metagenomic analysis, the most abundant and long reads in each OTU (operational taxonomic unit) were selected as representative sequences. These sequences were then used for the taxonomy assignments using the GreenGene database, version 2013.8 (<http://greengenes.secondgenome.com/>) as a reference database. Rarefaction curves end-points and normalization of counts for diversity analysis were set to 50% of the target sequencing coverage. Samples not satisfying the count threshold were not included. The results were compared using a nonparametric two-sample *t*-test and the *p* values were calculated through 999 Monte Carlo permutations. Only the OTUs of the bacteria involved in N-cycling processes (see references in the discussion) and significantly different between *S*_{mng} and *C*_{mng} at *p* ≤ 0.05 were considered and here discussed. The OTUs belonging to the same bacterial taxon were grouped.

3. Results

3.1. Soil, litter and humus description

The soil profile and description reported in Table 1 indicates in both the treatments an A horizon of 0–45 cm, a B horizon of 45–90 cm and a C horizon < 90 cm. The mineral nature is mainly carbonatic. Soil plasticity was defined as 'plastic' in the A horizons, 'slightly plastic' in the B horizons and 'non-plastic' in the C horizons. Soil consistence was defined as 'firm' in the A horizons and 'friable' in the B horizons of both the management systems. The A horizon of the *S*_{mng} was 'sticky', compared to the *C*_{mng}, that was 'slightly sticky'. Differences in the degree of soil stickiness between the two management systems were observed also in the B horizon.

The different soil management in the two agricultural systems caused the lack of organic horizons in the *C*_{mng}, while *S*_{mng} presented defined OL and OH horizons (Table 2). With reference to Agro humus systems and forms (Zanella et al., 2018c), the topsoil of *C*_{mng} was attributed to a 'massive Agro Mull' (absence of OH horizon and presence of massive, poorly zoogenic A horizon) while *S*_{mng} was attributed to a 'biomacro Agro Amphi' (presence of biomacrostructured A and OH horizons). Only the *S*_{mng} topsoil can be classified even with reference to Terrestrial natural humus systems and forms (Zanella et al. 2018b). The presence of zoogenic A and OH horizons allows to assign the profile to an Amphi system; more precisely, the transition (OH/A < 5 mm) and OH thickness (OH ≥ 1 cm) corresponded to the ones of an Eumesoamphi form. The classification of *C*_{mng} is impossible if reference is made to Terrestrial natural humus systems and forms because the A horizon does not show signs of macro- or mesofauna activity. Not having organic horizons, it would end up in a Mull system; not showing biological activity in A horizon, it would be located in a Mor system. This is a typical situation in stressed agricultural soils (Zanella et al., 2018c). Intensive agriculture pushes an original Mull system to another poor of pedofauna system that resembles a natural Mor, a system that develops in very difficult conditions of cold climate and/or strong acidity of the substratum.

Table 1
Soil profile and classification in the sustainable (S_{mng}) and the conventional (C_{mng}) systems, according to FAO guidelines for profile description (2006).

Soil management	Soil horizon	Depth (cm)	Field texture	Color matrix (Munsell)	Nature of mineral concentrations	Carbonate reaction (HC)	Plasticity	Consistence when moist	Stickiness	Observations
Sustainable (S_{mng})	A1	0–30	Sandy Loam	10.0YR-2/2	Carbonates	Moderately calcareous	Plastic	Firm	Sticky	Presence of roots (herbaceous)
	A2	30–45	Sandy Loam	10.0YR-3/2	Carbonates	Moderately calcareous	Plastic	Firm	Sticky	Presence of roots (herbaceous)
	Bk	45–90	Sandy Loam	5.0Y-3/2	Carbonates	Moderately calcareous	Slightly plastic	Friable	Slightly sticky	
	Ck	< 90	Sandy Loam	5.0Y-4/4	Carbonates-silica	Strongly calcareous	Non-plastic	Friable	Non-sticky	
Conventional (C_{mng})	Ap1	0–30	Sandy Loam	10.0YR-2/2	Carbonates	Moderately calcareous	Plastic	Firm	Slightly sticky	
	Ap2	30–45	Sandy Loam	10.0YR-3/2	Carbonates	Moderately calcareous	Plastic	Firm	Slightly sticky	
	Bk	45–90	Sandy Loam	5.0Y-3/2	Carbonates	Moderately calcareous	Slightly plastic	Friable	Non-sticky	
	Ck	< 90	Sandy Loam	5.0Y-4/4	Carbonates-silica	Strongly calcareous	Non-plastic	Friable	Non-sticky	

3.2. Soil chemical parameters

The profiles of TOC and TN levels in the two management systems, and particularly in the topsoil (0–5 cm), were considerably different (Fig. 1). In the first 5 cm of soils, TOC was significantly higher ($p \leq 0.05$) in the S_{mng} , compared to the C_{mng} , while the trend was reversed at 10, 20 and 30 cm, and became not statistically different between the two treatments at 60 and 90 cm. Regarding TN, its levels were significantly higher in the S_{mng} at 0 and 5 cm, while the differences in the remaining soil depths were not statistically significant. The C/N ratios in the S_{mng} were significantly lower at 5, 10, 20, 30 and 60 cm, and not significantly different at 0 and 90 cm, compared to the respective values of the C_{mng} . In the litter of the S_{mng} , the values of TOC, TN and C/N were 443.90 ± 17.93 (SD) $g\ kg^{-1}$, $5.60 \pm 0.58\ g\ kg^{-1}$, and 79.27 ± 8.91 , respectively.

In the S_{mng} , the values of soil pH measured both in water and $CaCl_2$ increased with rising soil depth from 0 to 10 cm, then remained relatively stable, and increased again at 90 cm, whereas soil pH in the C_{mng} increased constantly from 0 to 90 cm in water (excepting for the value at 60 cm), in $CaCl_2$ the trend was similar but with a slight decrease at 90 cm (Fig. 2).

The values of TOC, TN and pH cumulated for the soil depths 0–30, 30–60 and 60–90 cm are reported in Table 3.

3.3. Soil metabolic diversity of soil bacteria

The Biolog® analysis showed that total AWCD and S were not significantly different between the S_{mng} and C_{mng} (Table 4). No significant differences between the two management systems were found for the two indices of bacterial community diversity here used (H' and U) nor for evenness indices (E and Z) (Table 4). As for total AWCD, the AWCD calculated for the principal classes of bacterial carbon substrates were not significantly different between the S_{mng} and C_{mng} (Fig. S1).

3.4. Identification of N-cycling bacteria

The abundance of the bacterial taxa involved in N transformations statistically different ($p \leq 0.05$) between S_{mng} and C_{mng} are reported in Fig. 3. In the C_{mng} system, the total abundance of free-living N-fixing Proteobacteria, Actinobacteria and Cyanobacteria was statistically higher than in the S_{mng} system. Differently, N-fixing symbiont Proteobacteria belonging to the Rhizobiales order were more abundant in the S_{mng} . The abundance of two OTUs of denitrifying bacteria (*Denitrobacter* spp. and *Pseudomonas* spp.) was different between the two soil management systems, being higher in the S_{mng} . The abundance of the three bacterial taxa involved in nitrogen oxidation from ammonia to nitrates (two Bacteria OTUs belonging to *Nitrospira* spp. and *Nitrosovibrio* spp., and the Archea OTU *Nitrososphaera* spp.) was higher in the S_{mng} , compared to C_{mng} .

Overall raw results of the metagenomic analysis, with relative abundance of a) all the total OTUs and, specifically, b) of the OTUs belonging to N-cycling bacteria and statistically different ($p \leq 0.05$) between the two management systems are presented in Supplementary Tables S2 and S3, respectively.

4. Discussion

The description of the different soil layers (horizons) gives a good general idea of what is going on in a soil profile in terms of soil genesis, leaching, bioturbation and water table dynamics. This is particularly important for agro-ecosystems, that originate from natural or semi-natural environments successively disturbed by original vegetation change and agronomic practices (Zanella et al., 2018c). Despite the conversion of a soil from natural to agronomic causes a certain level of disturbance in the activity of many soil invertebrates, such as earthworms and enchytraeids, microorganisms are less affected by the

Table 2
Little layer and humus description in the sustainable (S_{mng}) and the conventional (C_{mng}) systems using the classification of Zanella et al. (2018b).

Soil management	Sustainable (S_{mng})	Conventional (C_{mng})
Is there a litter layer present?	Yes	No
Is there an OL layer present (layer characterised by the accumulation of leaves and needles. Although possibly discolored and slightly fragmented, the leaves or needles are still easily discernible to the naked eye)?	Yes	No
Average thickness (cm)	3	–
Is it an nOL layer (litter age < 1 year)?	Yes	No
Is it an vOL layer (leaves or needles are slightly altered: i.e. slightly discolored, bleached, matted, skeletonized, or slightly fragmented)?	Discontinuous	No
Average thickness (cm)	1	–
Is there an OF layer present (layer characterised by the accumulation of partly decomposed litter, i.e. clearly fragmented and bleached, skeletonized leaves or needles)?	No	No
Is the material mainly fragmented by soil fauna (zoOF - e.g., mite pellets visible) or by fungi (nozOF - layer is matted and permeated by hyphae)?	Intermediate	No
Is there an OH layer present (layer characterised by black, grey-brown or reddish-brown, well decomposed litter. Individual leaves or needles are no longer visible)?	Yes	No
Average thickness (cm)	1	–
Dig a micro-pit ca 20 cm deep and note the transition of the litter layer to the mineral soil. How is this transition?	sharp (< 5mm)	–
How deep is the A horizon? (cm)	45	45
What is the structure of the mineral A horizon?	Grade: moderate Type: granular Size: medium	Grade: moderate Type: granular Size: medium
How is the transition between the mineral A horizon and the underlying soil (E or B horizon)?	Distinctness: gradual Topography: wavy	Distinctness: gradual Topography: wavy
Are there any droppings of earthworms identifiable in the A horizon?	Yes	No
Are there any droppings of other soil fauna (enchytraeids and arthropods) identifiable in the A-horizon?	Yes	No
Humus classification according to Zanella et al. (2018b)	System Form	AMPHI –
Humus classification according to Zanella et al. (2018c)	System Form	AGRO AMPHI Biomacro Agro Amphi
		– AGRO MULL Massive Agro Mull

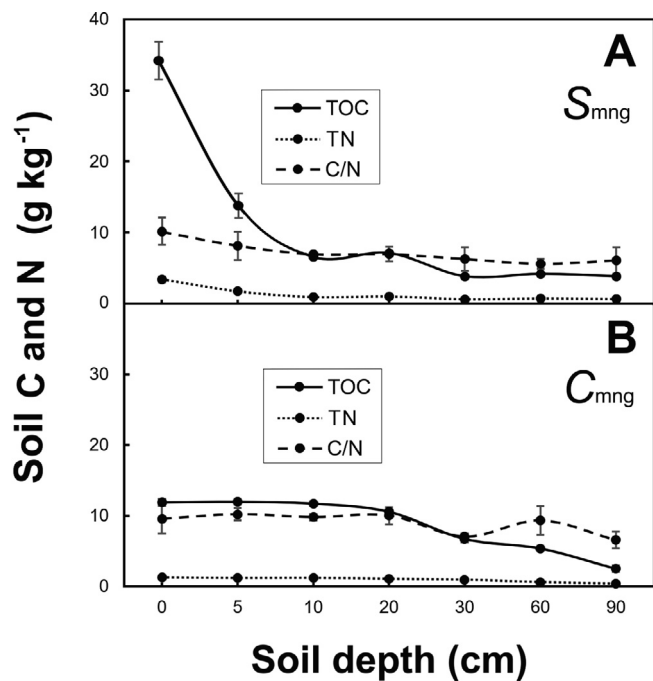


Fig. 1. Soil total organic carbon (TOC; continuous line), total nitrogen (TN; dotted line) and C/N ratio (dashed line) of soils from (A) the sustainable (S_{mng}) and (B) the conventional (C_{mng}) systems measured at different soil depths. Each value represents the mean (\pm SD) from composite soil samples ($n = 3$).

agronomic practices adopted and continue to play a key role in the maintenance of soil fertility (Bünemann et al., 2018). This trend was confirmed by our results, where in both the soil management systems a high bacterial metabolic activity in the topsoil (0–30 cm) (Table 4 and Fig. 3) was observed, while only the A horizon of the S_{mng} system there was the presence of droppings of earthworms and of other soil fauna

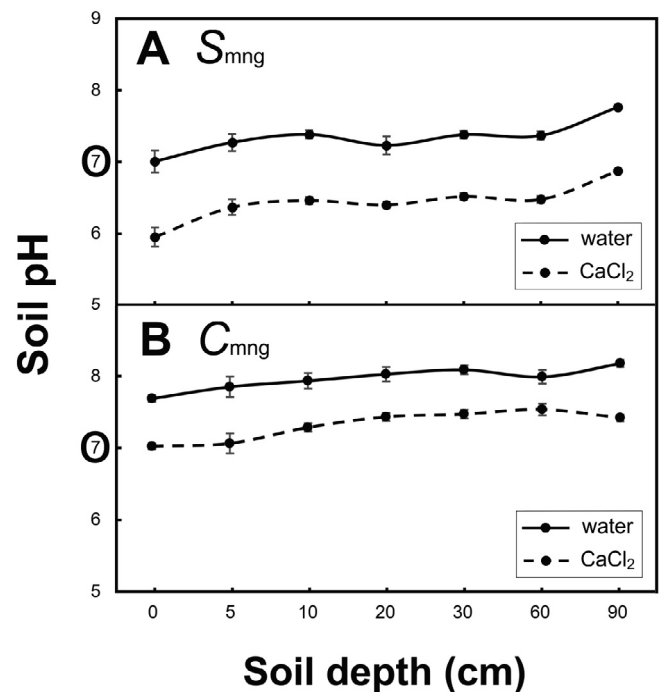


Fig. 2. pH measured in water (continuous line) and in $CaCl_2$ (dashed line) of soils from (A) the sustainable (S_{mng}) and (B) the conventional (C_{mng}) systems measured at different soil depths. Each value represents the mean (\pm SD) from composite soil samples ($n = 3$).

identifiable (Tables 1 and 2).

In the case the agriculture techniques adopted are not sustainable, changes in land use towards agriculture are often accompanied by changes in soil structure, reduced edaphic biodiversity, soil erosion, nutrients runoff, ground and surface-water use, and soil contamination (Celano et al., 2011; Palese et al., 2014; Zanella et al., 2018c). In this

Table 3

Soil total organic carbon (TOC), total nitrogen (TN), C/N ratio. pH measured in water and pH measured in CaCl₂ of soils from the sustainable (*S_{mng}*) and the conventional (*C_{mng}*) systems measured at different soil depths. Each value represents the mean (± SD) from composite soil samples (*n* = 3). Average values were cumulated from the values of Tables 1 and 2, and referred to grouped soil depths (0–30, 30–60 and 60–90 cm). Values followed by different letters are statistically different (*p* ≤ 0.05) within columns.

Soil system	Soil depth (cm)	TOC (g kg ⁻¹)	TN (g kg ⁻¹)	C/N	pH _{water}	pH _{CaCl₂}
Sustainable (<i>S_{mng}</i>)	0–30	13.18 ± 0.22 a	1.56 ± 0.20 a	7.69 ± 0.37 b	7.23 ± 0.09 c	6.34 ± 0.08 d
	30–60	4.02 ± 0.08 d	0.70 ± 0.11 c	5.94 ± 0.17c	7.37 ± 0.06 bc	6.50 ± 0.05 cd
	60–90	4.03 ± 0.12 d	0.71 ± 0.13 c	5.84 ± 0.26c	7.57 ± 0.05b	6.68 ± 0.04 c
Conventional (<i>C_{mng}</i>)	0–30	10.59 ± 0.32 b	1.13 ± 0.09 b	9.33 ± 0.10 a	7.91 ± 0.09 a	7.26 ± 0.07 b
	30–60	6.04 ± 0.43 c	0.77 ± 0.05 c	8.21 ± 0.23 ab	8.02 ± 0.08 a	7.50 ± 0.07 a
	60–90	3.94 ± 0.29 d	0.49 ± 0.08 d	7.96 ± 0.61 b	8.07 ± 0.07 a	7.47 ± 0.06 a

perspective, the need of sustainable agricultural techniques and soil management systems, like the high C-input set of practices used in this experiment (i.e., the *S_{mng}* system described in the Materials and Methods section), becomes urgent for soil preservation and conservation, especially in Mediterranean soils (Sofó et al., 2014a,b).

From the soil qualitative analyses (Table 1), it appears that the presence of roots from spontaneous cover crops was observed only in the A horizon of the *S_{mng}*. This resulted in increases in soil stickiness, that was higher in the A and Bk horizons of the *S_{mng}*, where the suffix 'k' indicates the accumulation of pedogenic carbonates. This effect was likely due to the higher levels of water (held by the roots of grasses), root exudates and organic acids, whose synergic action on soil structure is able to modify soil stickiness (Paese et al., 2015; Zanella et al., 2018b). Another clear aspect emerging from the comparison of the two management systems was the absence of litter in the *C_{mng}* system (Table 2).

The litter layer refers to all the dead organic debris (such as leaves, needles, branches and twigs, either still recognizable or significantly altered) on top of the mineral soil surface, and is an important visual clue for characterizing the rate of recycling of organic matter, that enters the A horizon through the litter. The sustainable practices of the *S_{mng}* system, especially no-tillage, allowed to maintain a well-developed litter layer (OL) with an average thickness of 3 cm (including 1 cm of new litter, nOL, and 2 cm of old litter, vOL) and tracks of the activity of soil fauna (zoOF) and fungal hyphae (nozOF) (specific terms and diagnostic horizons according to Zanella et al., 2018a). Due to its high content of organic matter deriving from crop and pruning residues, litter provided *S_{mng}* topsoil (0–5 cm) with higher TOC and TN contents, compared to the *C_{mng}* (Fig. 1). In both the soil management systems, the trends of TOC and TN generally decreased with increasing soil depth (Fig. 1). The higher content of organic matter, likely transported in the deeper soil layers in a dissolved form by soil solution, caused lower soil pH in the *S_{mng}* system (Fig. 2), whose values ranged from 7.68 to 8.16 (in water) due to the carbonatic nature of the soil (Table 1). The average difference of topsoil (0–5 cm) pH measured in water and CaCl₂ was higher in *S_{mng}* (0.98) than in *C_{mng}* (0.71) (Fig. 2), so demonstrating that the soil exchangeable H⁺ in *S_{mng}* mainly derived from organic matter functional groups (Fig. 1).

The topsoil, the outermost layer of soil (0–30 cm), is known to have the highest concentration of organic matter and microorganisms, with consequent high biological soil activity. For this reason, in this soil

layer, bacterial metabolic diversity indices were estimated by Biolog® (Table 4) and the relative abundances of the bacterial (OTUs) were measured by 16S-based metagenomic analysis (Tables S1 and S2, and Fig. 3).

Biolog® indices are related to the metabolic activity, number, variety and diversity of bacteria, including diversity within and between functional groups (Sofó et al., 2018), being affected by agricultural practices and C-inputs in the agro-ecosystem (Sofó et al., 2014a,b). From the results (Table 4 and Fig. S1), it did not appear a significant metabolic inhibition of bacterial metabolism in the soil managed conventionally (*C_{mng}*), compared to the *S_{mng}* system. The long-term application of sustainable management did not lead to a significantly higher total bacterial metabolic activity (*AWCD* and *S*) and diversity (*H'* and *U*), nor to the predominance of few metabolic groups of bacteria (*E* and *Z*). The same trend was found within the main groups of bacterial carbon substrates (Fig. S1). This suggests that the difference between the *C_{mng}* and *S_{mng}* systems likely resides in the abundance of bacterial taxonomic groups having specific functions (qualitative differences) more than in parameters based on total bacterial metabolic activity (quantitative differences).

Contrary to soil C, whose reactions are overlapped and difficult to attribute to specific soil microorganisms, transformations among N forms are mostly mediated by bacteria (Robertson and Groffman, 2015; Van Groenigen et al., 2015). As an example, nitrification, denitrification and fixation, are carried out by a relatively restricted number of bacterial genera (de Vries and Shade, 2013; Mooshammer et al., 2014), whose genes are good markers for N availability for plants and indices of soil fertility, as they can be unequivocally interpreted and reference values are available (see Pascazio et al., 2018, for a study in olive). Our results depicted a scenario where N-cycling was generally up-regulated in the *S_{mng}*, compared to *C_{mng}* (Table S2 and Fig. 3). This is in accordance with a previous culture-based analysis carried out in the same system by Sofó et al. (2010), who found that the number of ammonifying bacteria, proteolytic bacteria, and *Azotobacter* spp. under a sustainable management system was significantly higher, compared to a conventional one. The increase in soil TN, with the consequent reduced C/N, in the topsoil (0–30 cm) of the *S_{mng}* (Table 3 and Fig. 1) likely had a priming effect on N-cycling bacteria, as also recently highlighted by Pascazio et al. (2018). Interestingly, even if the total abundance of N-fixing bacteria was comparable in the two systems (610 in the *S_{mng}* and 567 in the *C_{mng}*), significant differences were found in the abundance of

Table 4

Bacterial community indices, identified by Biolog® 96-well Eco-Microplates™, of soils from the sustainable (*S_{mng}*) and the conventional (*C_{mng}*) systems. Each value represents the mean (± SD) from composite soil samples (*n* = 3). Values followed by different letters are statistically different (*p* ≤ 0.05) within columns. *AWCD* = average well colour development; *S* = richness; *H'* = Shannon's diversity index; *E* = Shannon's evenness index; *U* = McIntosh's diversity index; *Z* = McIntosh's evenness index.

Soil management	<i>AWCD</i>	<i>S</i>	<i>H'</i>	<i>E</i>	<i>U</i>	<i>Z</i>
Sustainable (<i>S_{mng}</i>)	0.720 ± 0.039 a	15.111 ± 0.928 a	3.179 ± 0.085 a	2.661 ± 0.130 a	4.692 ± 0.300 a	0.963 ± 0.014 a
Conventional (<i>C_{mng}</i>)	0.852 ± 0.076 a	16.778 ± 2.386 a	3.228 ± 0.175 a	2.629 ± 0.086 a	5.557 ± 0.358 a	0.964 ± 0.012 a

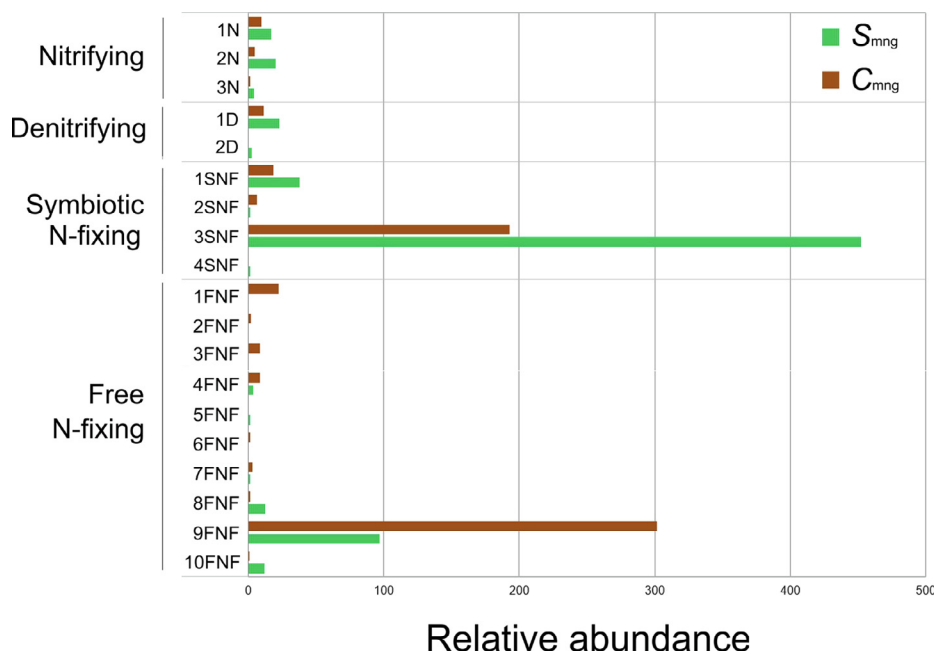


Fig. 3. Relative abundances of the operational taxonomical units (OTUs) of N-cycling bacteria that were statistically different in soils from the sustainable (S_{mng}) and the conventional (C_{mng}) systems. Only the OTUs with a statistical difference ($p \leq 0.05$) between S_{mng} and C_{mng} were considered. k: kingdom; p: phylum; c: class; o: order; f: family; g: genus; s: species. **Legend:** Nitrifying – 1N: k: Bacteria; p: Nitrospirae; c: Nitrospira; o: Nitrospirales; f: Nitrospiraceae; g: Nitrospira; 2N: k: Bacteria; p: Proteobacteria; c: Betaproteobacteria; o: Nitrosomonadales; f: Nitrosomonadaceae; g: Nitrososphaerales; f: Nitrososphaeraceae; g: Nitrososphaera. Denitrifying – 1D: k: Bacteria; p: Proteobacteria; c: Gammaproteobacteria; o: Pseudomonadales; f: Pseudomonadaceae; g: Pseudomonas; s: corrugata; 2D: k: Bacteria; p: Proteobacteria; c: Betaproteobacteria; o: Burkholderiales; f: Alcaligenaceae; g: Denitrobacter. Symbiotic N-fixing – 1SNF: k: Bacteria; p: Proteobacteria; c: Alphaproteobacteria; o: Rhizobiales; f: Phyllobacteriaceae; g: Mesorhizobium; 2SNF: k: Bacteria; p: Proteobacteria; c: Alphaproteobacteria; o: Rhizobiales; f: Bradyrhizobiaceae; g: Bradyrhizobium; s: elkanii; 3SNF: k: Bacteria; p: Proteobacteria; c: Alphaproteobacteria; o: Rhizobiales; f: Bradyrhizobiaceae; g: Bradyrhizobium; s: pachyrhizi. Free N-fixing – (Cyanobacteria) 1FNF: k: Bacteria; p: Cyanobacteria; c: Oscillatorioophycidae; o: Chroococcales; f: Phormidiaceae; g: Microcoleus; s: vaginatus; 2FNF: k: Bacteria; p: Cyanobacteria; c: Synechococcophycidae; o: Synechococcales; f: Acaryochloridaceae; g: Acaryochloris; 3FNF: k: Bacteria; p: Cyanobacteria; c: Nostocophycidae; o: Nostocales; f: Nostocaceae; g: Nostoc; (Other bacteria) 4FNF: k: Bacteria; p: Actinobacteria; c: Actinobacteria; o: Actinomycetales; f: Frankiaceae; g: Frankia; 5FNF: k: Bacteria; p: Proteobacteria; c: Alphaproteobacteria; o: Rhodospirillales; f: Rhodospirillaceae; g: Azospirillum; 6FNF: k: Bacteria; p: Proteobacteria; c: Alphaproteobacteria; o: Rhodospirillales; f: Rhodospirillaceae; g: Phaespirillum; s: fulvum; 7FNF: k: Bacteria; p: Proteobacteria; c: Alphaproteobacteria; o: Rhodospirillales; f: Rhodospirillaceae; 8FNF: k: Bacteria; p: Proteobacteria; c: Alphaproteobacteria; o: Rhodospirillales; f: Acetobacteraceae; g: Acidisphaera; 9FNF: k: Bacteria; p: Proteobacteria; c: Alphaproteobacteria; o: Rhodospirillales; f: Acetobacteraceae; g: Roseomonas; 10FNF: k: Bacteria; p: Proteobacteria; c: Alphaproteobacteria; o: Rhodospirillales; f: Acetobacteraceae.

Rhizobiales; f: Rhizobiaceae; g: Sinorhizobium; s: meliloti; 3SNF: k: Bacteria; p: Proteobacteria; c: Alphaproteobacteria; o: Rhizobiales; f: Bradyrhizobiaceae; g: Bradyrhizobium; s: elkanii; 4SNF: k: Bacteria; p: Proteobacteria; c: Alphaproteobacteria; o: Rhizobiales; f: Bradyrhizobiaceae; g: Bradyrhizobium; s: pachyrhizi. Free N-fixing – (Cyanobacteria) 1FNF: k: Bacteria; p: Cyanobacteria; c: Oscillatorioophycidae; o: Chroococcales; f: Phormidiaceae; g: Microcoleus; s: vaginatus; 2FNF: k: Bacteria; p: Cyanobacteria; c: Synechococcophycidae; o: Synechococcales; f: Acaryochloridaceae; g: Acaryochloris; 3FNF: k: Bacteria; p: Cyanobacteria; c: Nostocophycidae; o: Nostocales; f: Nostocaceae; g: Nostoc; (Other bacteria) 4FNF: k: Bacteria; p: Actinobacteria; c: Actinobacteria; o: Actinomycetales; f: Frankiaceae; g: Frankia; 5FNF: k: Bacteria; p: Proteobacteria; c: Alphaproteobacteria; o: Rhodospirillales; f: Rhodospirillaceae; g: Azospirillum; 6FNF: k: Bacteria; p: Proteobacteria; c: Alphaproteobacteria; o: Rhodospirillales; f: Rhodospirillaceae; g: Phaespirillum; s: fulvum; 7FNF: k: Bacteria; p: Proteobacteria; c: Alphaproteobacteria; o: Rhodospirillales; f: Rhodospirillaceae; 8FNF: k: Bacteria; p: Proteobacteria; c: Alphaproteobacteria; o: Rhodospirillales; f: Acetobacteraceae; g: Acidisphaera; 9FNF: k: Bacteria; p: Proteobacteria; c: Alphaproteobacteria; o: Rhodospirillales; f: Acetobacteraceae; g: Roseomonas; 10FNF: k: Bacteria; p: Proteobacteria; c: Alphaproteobacteria; o: Rhodospirillales; f: Acetobacteraceae.

free N-fixing bacteria (higher in the C_{mng}) and symbiont N-fixing bacteria (higher in the S_{mng}) (Table S2 and Fig. 3). This discrepancy between the two systems was likely due to the presence of the dense root systems of cover crops in the S_{mng} (Paese et al., 2014), including many spontaneous Fabaceae, whose root nodules host Rhizobiales N-fixing bacteria (Robertson and Groffman, 2015). On the other side, the C_{mng} relies on a N-fixation mainly carried out by free N-fixing bacteria, including Cyanobacteria, as the continuous mechanical disturbance of this plot did not allow the presence of spontaneous Fabaceae, with their root-associated N-fixing bacteria. In the C_{mng} , soil TN in the 0–30 cm soil layer was significantly lower and the C/N higher than in the S_{mng} (Table 3). Considering that the average C/N value of the biomass of N-cycling bacteria calculated from the literature (de Vries and Shade, 2013, 2018; Van Groenigen et al., 2015; and references within) is 7.86, the soil C/N in the S_{mng} (7.69) was very close to the optimum for bacterial growth and can be the cause of the enhanced N-turnover observed in this system (Table S2 and Fig. 3).

From the general analysis of the data obtained by the study of soil profiles, C and N dynamics, and the genetic and functional diversity of soil bacteria, it emerged that a sustainable orchard management caused an improvement in soil organic matter, whose positive effects were reflected on soil N-cycling bacteria, without affecting total bacterial metabolic activity and diversity. Even if there is substantial diversity regarding olive orchard management among the Mediterranean countries (Pergola et al., 2013), the determination of set of standards and common operating principles based on scientific knowledge is urgent, and they should be adopted to design and adjust the local soil management practices in order to increase microbiological diversity. The results obtained could be important for prediction of the impacts of environmental perturbations on key functions carried out by the soil microbiome and will promote the development of new approaches for optimizing soil carbon cycling, managing nutrient transport, and

sustaining and improving crop yield and product quality.

Finally, the benefits we have recorded in the soil have significant and functional consequences on human health (de Vries and Wallenstein, 2017; Zanella et al., 2018d) and also positive effects on climate warming (Caitlin et al., 2017; Ferrarini et al., 2018; Minasny et al., 2018). We really hope that this article could help to understand how important is the soil as a living matrix capable of recycling biological structures and transforming them into future biodiversity. We would like to have a ‘more biological’ soil, the only basis for a healthier world.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.apsoil.2018.12.022>.

References

Bacon, J.R., Hudson, G., 2001. A flexible methodology for the characterisation of soils: a case study of the heavy metal status of a site at Dornach. *Sci. Total Environ.* 264,

- 153–162. [https://doi.org/10.1016/S0048-9697\(00\)00617-3](https://doi.org/10.1016/S0048-9697(00)00617-3).
- Bünemann, E.K., Bongiorno, G., Bai, Z., Creamer, R.E., De Deyn, G., de Goede, R., Fleskens, L., Geissen, V., Kuyper, T.W., Mäder, P., Pulleman, M., Sukkel, W., van Groenigen, J.W., Brussaard, L., 2018. Soil quality – a critical review. *Soil Biol. Biochem.* 120, 105–125. <https://doi.org/10.1016/j.soilbio.2018.01.030>.
- Caitlin, E., Hicks, P., Castanha, C., Porras, R.C., Torn, M.S., 2017. The whole-soil carbon flux in response to warming. *Science* 355, 1420–1423. <https://doi.org/10.1126/science.aal1319>.
- Celano, G., Palese, A.M., Ciucci, A., Martorella, E., Vignozzi, N., Xiloyannis, C., 2011. Evaluation of soil water content in tilled and cover-cropped olive orchards by the geoelectrical technique. *Geoderma* 163, 163–170. <https://doi.org/10.1016/j.geoderma.2011.03.012>.
- de Vries, F.T., Shade, A., 2013. Controls on soil microbial community stability under climate change. *Front. Microbiol.* 4, 1–16. <https://doi.org/10.3389/fmicb.2013.00265>.
- de Vries, F.T., Wallenstein, M.D., 2017. Below-ground connections underlying above-ground food production: a framework for optimising ecological connections in the rhizosphere. *J. Ecol.* 105, 913–920. <https://doi.org/10.1111/1365-2745.12783>.
- Enwall, K., Nyberg, K., Bertilsson, S., Cederlund, H., Stenström, J., Hallin, S., 2007. Long-term impact of fertilization on activity and composition of bacterial communities and metabolic guilds in agricultural soil. *Soil Biol. Biochem.* 39, 106–115. <https://doi.org/10.1016/j.soilbio.2006.06.015>.
- FAO, 2006. Guidelines for Soil Description, fourth ed. Food and Agriculture Organization of the United Nations, Rome. <http://www.fao.org/3/a-a0541e.pdf> (accessed 15 December 2018).
- Ferrarini, A., Bini, C., Amaducci, S., 2018. Soil and ecosystem services: current knowledge and evidences from Italian case studies. *Appl. Soil Ecol.* 123, 693–698. <https://doi.org/10.1016/j.apsoil.2017.06.031>.
- Jansson, J.K., Hofmockel, K.S., 2018. The soil microbiome — from metagenomics to metaproteomics. *Curr. Opin. Microbiol.* 43, 162–168. <https://doi.org/10.1016/j.mib.2018.01.013>.
- Jeanbille, M., Buée, M., Bach, C., Cébron, A., Frey-Klett, P., Turpault, M.P., Uroz, S., 2016. Soil parameters drive the structure, diversity and metabolic potentials of the bacterial communities across temperate beech forest soil sequences. *Microb. Ecol.* 71, 482–493. <https://doi.org/10.1007/s00248-015-0669-5>.
- Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., Glöckner, F.O., 2013. Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. *Nucl. Acids Res.* 41, e1. <https://doi.org/10.1093/nar/gks808>.
- Lakshmanan, V., Selvaraj, G., Bais, H.P., 2014. Functional soil microbiome: belowground solutions to an aboveground problem. *Plant Physiol.* 166, 689–700. <https://doi.org/10.1104/pp.114.245811>.
- Li, Z., Zhao, B., Olk, D.C., Jia, Z., Mao, J., Cai, Y., Zhang, J., 2018. Contributions of residue-C and -N to plant growth and soil organic matter pools under planted and unplanted conditions. *Soil Biol. Biochem.* 120, 91–104. <https://doi.org/10.1016/j.soilbio.2018.02.005>.
- Minasny, B., Arrouays, D., McBratney, A.B., Angers, D.A., Chambers, A., Chaplot, V., Chen, Z.S., Cheng, K., Das, B.S., Field, D.J., Gimona, A., Hedley, C., Hong, S.Y., Mandal, B., Malone, B.P., Marchant, B.P., Martin, M., McConkey, B.G., Mulder, V.L., O'Rourke, S., Richer-de-Forges, A.C., Odeh, I., Padarian, J., Paustian, K., Pan, G., Poggio, L., Savin, I., Stolbovov, V., Stockmann, U., Sulaeman, Y., Tsui, C.C., Vågen, T.G., van Wesemael, B., Winowiecki, L., 2018. Rejoinder to Comments on Minasny et al., 2017 Soil carbon 4 per mille. *Geoderma* 292, 59–86. <https://doi.org/10.1016/j.geoderma.2017.05.026>.
- Montanaro, G., Celano, G., Dichio, B., Xiloyannis, C., 2010. Effects of soil-protecting agricultural practices on soil organic carbon and productivity in fruit tree orchards. *Land Degrad. Dev.* 21, 132–138. <https://doi.org/10.1002/ldr.917>.
- Montanaro, G., Dichio, B., Briccoli Bati, C., Xiloyannis, C., 2012. Soil management affects carbon dynamics and yield in a Mediterranean peach orchard. *Agric. Ecosyst. Environ.* 161, 46–54. <https://doi.org/10.1016/j.agee.2012.07.020>.
- Montanaro, G., Xiloyannis, C., Nuzzo, V., Dichio, B., 2017. Orchard management, soil organic carbon and ecosystem services in Mediterranean fruit tree crops. *Sci. Hortic. (Amsterdam)* 217, 92–101. <https://doi.org/10.1016/j.scienta.2017.01.012>.
- Mooshammer, M., Wanek, W., Hämmerle, I., Fuchslueger, L., Hofhansl, F., Knoltsch, A., Schneckner, J., Takriti, M., Watzka, M., Wild, B., Keiblinger, K.M., Zechmeister-Boltenstern, S., Richter, A., 2014. Adjustment of microbial nitrogen use efficiency to carbon: Nitrogen imbalances regulates soil nitrogen cycling. *Nat. Commun.* 5, 1–7. <https://doi.org/10.1038/ncomms4694>.
- Palese, A.M., Pasquale, V., Celano, G., Figliuolo, G., Masi, S., Xiloyannis, C., 2009. Agriculture, Ecosystems and Environment Irrigation of olive groves in Southern Italy with treated municipal wastewater: Effects on microbiological quality of soil and fruits. 129, 43–51. doi: 10.1016/j.agee.2008.07.003.
- Palese, A.M., Ringersma, J., Baartman, J.E.M., Peters, P., Xiloyannis, C., 2015. Runoff and sediment yield of tilled and spontaneous grass-covered olive groves grown on sloping land. *Soil Res.* 53, 542–552. <https://doi.org/10.1071/SR14350>.
- Palese, A.M., Vignozzi, N., Celano, G., Agnelli, A.E., Pagliani, M., Xiloyannis, C., 2014. Influence of soil management on soil physical characteristics and water storage in a mature rainfed olive orchard. *Soil Tillage Res.* 144, 96–109. <https://doi.org/10.1016/j.still.2014.07.010>.
- Pansu, M., Gautheyrou, J., 2006. Handbook of soil analysis: mineralogical, organic and inorganic methods. In: *Handbook of Soil Analysis: Mineralogical, Organic and Inorganic Methods*. <https://doi.org/10.1007/978-3-540-31211-6>.
- Pascasio, S., Crecchio, C., Scagliola, M., Mininni, A.N., Dichio, B., Xiloyannis, C., Sofo, A., 2018. Microbial-based soil quality indicators in irrigated and rainfed soil portions of Mediterranean olive and peach orchards under sustainable management. *Agric. Water Manage.* 195, 172–179. <https://doi.org/10.1016/j.agwat.2017.10.014>.
- Pergola, M., Favia, M., Palese, A.M., Perretti, B., Xiloyannis, C., Celano, G., 2013. Alternative management for olive orchards grown in semi-arid environments: an energy, economic and environmental analysis. *Sci. Hortic. (Amsterdam)* 162, 380–386. <https://doi.org/10.1016/j.scienta.2013.08.031>.
- Robertson, G.P., Groffman, P.M., 2015. Nitrogen Transformations. In: Paul, E.A. (Ed.), *Soil Microbiology, Ecology and Biochemistry*, fourth ed. Academic Press, Burlington, MA, USA, pp. 421–446. <https://doi.org/10.1016/B978-0-12-415955-6.00014-1>.
- Sofa, A., Ciarfaglia, A., Scopa, A., Camele, I., Curci, M., Crecchio, C., Xiloyannis, C., Palese, A.M., 2014a. Soil microbial diversity and activity in a Mediterranean olive orchard using sustainable agricultural practices. *Soil Use Manage.* 30, 160–167. <https://doi.org/10.1111/sum.12097>.
- Sofa, A., Elshafie, H.S., Scopa, A., Mang, S.M., Camele, I., 2018. Impact of airborne zinc pollution on the antimicrobial activity of olive oil and the microbial metabolic profiles of Zn-contaminated soils in an Italian olive orchard. *J. Trace Elem. Med. Biol.* 49, 276–284. <https://doi.org/10.1016/j.jtemb.2018.02.017>.
- Sofa, A., Palese, A.M., Casacchia, T., Celano, G., Ricciuti, P., Curci, M., Crecchio, C., Xiloyannis, C., 2010. Genetic, functional, and metabolic responses of soil microbiota in a sustainable olive orchard. *Soil Sci.* 175, 81–88. <https://doi.org/10.1097/SS.0b013e3181ce8a27>.
- Sofa, A., Palese, A.M., Casacchia, T., Xiloyannis, C., 2014b. Sustainable soil management in olive orchards: effects on telluric microorganisms. In: Parvaiz, S., Rasool, S. (Eds.), *Emerging Technol. Manage. Crop Stress Tolerance*. Academic Press, San Diego, CA, USA, pp. 471–484. <https://doi.org/10.1016/B978-0-12-800875-1.00020-X>.
- Tian, G., Vose, J.M., Coleman, D.C., Geron, C.D., Walker, J.T., 2004. Evaluation of the effectiveness of riparian zone restoration in the southern Appalachians by assessing soil microbial populations. *Appl. Soil Ecol.* 26, 63–68. <https://doi.org/10.1016/j.apsoil.2003.10.003>.
- Van Groenigen, J.W., Huygens, D., Boeckx, P., Kuyper, T.W., Lubbers, I.M., Rütting, T., Groffman, P.M., 2015. The soil n cycle: New insights and key challenges. *Soil* 1, 235–256. <https://doi.org/10.5194/soil-1-235-2015>.
- Zanella, A., Ponge, J.F., Jabiol, B., Sartori, G., Kolb, E., Gobat, J.M., Bayon, R.C. Le, Aubert, M., Waal, R. De, Delft, B. Van, Vacca, A., Serra, G., Chersich, S., Andreetta, A., Cools, N., Englisch, M., Hager, H., Katzensteiner, K., Brêthes, A., Nicola, C. De, Testi, A., Bernier, N., Graefe, U., Juilleret, J., Banas, D., Garlato, A., Obber, S., Galvan, P., Zampedri, R., Frizzera, L., Tomasi, M., Menardi, R., Fontanella, F., Filoso, C., Dibona, R., Bolzonella, C., Pizzeghello, D., Carletti, P., Langohr, R., Cattaneo, D., Nardi, S., Nicolini, G., Viola, F., 2018a. Humusica 1, article 4: terrestrial humus systems and forms — specific terms and diagnostic horizons. *Appl. Soil Ecol.* 122, 56–74. <https://doi.org/10.1016/j.apsoil.2017.07.005>.
- Zanella, A., Ponge, J.F., Jabiol, B., Sartori, G., Kolb, E., Le Bayon, R.C., Gobat, J.M., Aubert, M., Waal, R. De, Delft, B. Van, Vacca, A., Serra, G., Chersich, S., Andreetta, A., Kölli, R., Brun, J.J., Cools, N., Englisch, M., Hager, H., Katzensteiner, K., Brêthes, A., De Nicola, C., Testi, A., Bernier, N., Graefe, U., Wolf, U., Juilleret, J., Garlato, A., Obber, S., Galvan, P., Zampedri, R., Frizzera, L., Tomasi, M., Banas, D., Bureau, F., Tatti, D., Salmon, S., Menardi, R., Fontanella, F., Carraro, V., Pizzeghello, D., Concheri, G., Squartini, A., Cattaneo, D., Scatollin, L., Nardi, S., Nicolini, G., Viola, F., 2018b. Humusica 1, article 5: Terrestrial humus systems and forms — keys of classification of humus systems and forms. *Soil Ecol. Appl.* 122, 75–86. <https://doi.org/10.1016/j.apsoil.2017.06.012>.
- Zanella, A., Ponge, J.F., Topoliantz, S., Bernier, N., Juilleret, J., 2018c. Humusica 2, Article 15: agro humus systems and forms. *Appl. Soil Ecol.* 122, 204–219. <https://doi.org/10.1016/j.apsoil.2017.10.011>.
- Zanella, A., Geisen, S., Ponge, J.-F., Jagers, G., Benbrook, C., Dilli, T., Vacca, A., Kwiatkowska-Malina, J., Aubert, M., Fusaro, S., De Nobili, M., Lomolino, G., Gomiero, T., 2018d. Humusica 2, article 17: techno humus systems and global change — three crucial questions. *Appl. Soil Ecol.* 122, 237–253. <https://doi.org/10.1016/j.apsoil.2017.10.010>.
- Zhang, X., Xu, S., Li, C., Zhao, L., Feng, H., Yue, G., Ren, Z., Cheng, G., 2014. The soil carbon/nitrogen ratio and moisture affect microbial community structures in alkaline permafrost-affected soils with different vegetation types on the Tibetan plateau. *Res. Microbiol.* 165, 128–139. <https://doi.org/10.1016/j.resmic.2014.01.002>.

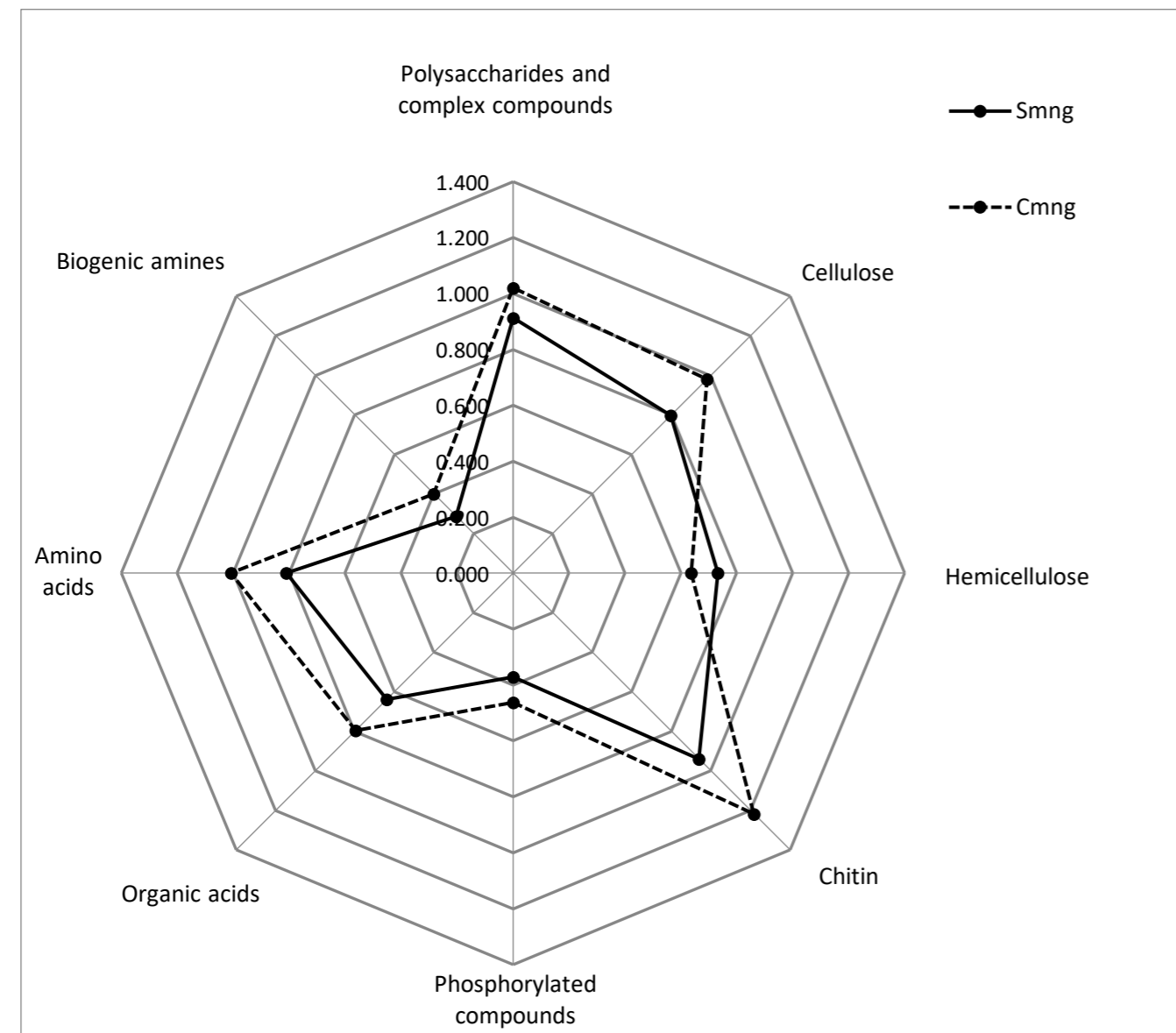
Supplementary Table 1. Agricultural practices adopted in the sustainable (S_{mng}) and in the conventional (C_{mng}) systems.

Practice	S_{mng}	C_{mng}
Soil tillage	<ul style="list-style-type: none"> No tillage. Spontaneous weeds and grasses mowed at least twice a year. 	<ul style="list-style-type: none"> Tillage (milling at 10 cm soil depth) performed 2-3 times per year in order to keep the soil bare.
Fertilization	<ul style="list-style-type: none"> Guided fertilization: fertigation based on a nutrient balance approach which takes into account nutrient input (by wastewater), output (by yield), and recycling/immobilisation in the grove system (by pruned material, senescent leaves, cover crops). The average values of organic C, N, P and K contained in the treated wastewater were 124, 54, 3 and 50 kg ha⁻¹ year⁻¹. An integrative amount of 40 kg ha⁻¹ year⁻¹ of N-NO₃⁻ was distributed in the early spring. 	<ul style="list-style-type: none"> Mineral fertilization carried out empirically once per year in early spring by using granular product applied to the soil (NPK 20-10-10 fertilizer at doses ranging from 300 to 500 kg ha⁻¹ year⁻¹).
Irrigation	<ul style="list-style-type: none"> Guided drip irrigation with treated municipal wastewater based on crop evapotranspiration calculated according to FAO equation: $ET_c = K_r \times K_c \times ET_o$ (K_r = reduction coefficient; K_c = crop coefficient; ET_o = potential evapotranspiration) - (6 self-compensating drippers per tree delivering 8 L h⁻¹). 	<ul style="list-style-type: none"> No irrigation (about 35 m³ rainfall plant⁻¹ year⁻¹).
Pruning	<ul style="list-style-type: none"> Light winter pruning performed each year in order to reach vegetative-reproductive balance of trees. Pruning material cut and left on the ground as mulch. 	<ul style="list-style-type: none"> Heavy pruning carried out every two years. Pruned residues burned out of the olive grove.

Supplementary Table 3. Relative abundances of the bacterial operational taxonomical units (OTUs) of N-cycling bacteria that were statistically different in soils from the sustainable (S_{mng}) and the conventional (C_{mng}) systems. Only the OTUs with a statistical difference ($p \leq 0.05$) between S_{mng} and C_{mng} were considered. k: kingdom; p: phylum; c: class; o: order; f: family; g: genus; s: species.

Type	Taxonomy	Relative abundance	
		S_{mng}	C_{mng}
Free N-fixing	k: Bacteria; p: Proteobacteria; c: Alphaproteobacteria; o: Rhodospirillales; f: Acetobacteraceae	11.67	1.00
	k: Bacteria; p: Proteobacteria; c: Alphaproteobacteria; o: Rhodospirillales; f: Acetobacteraceae; g: Roseomonas	97.00	301.33
	k: Bacteria; p: Proteobacteria; c: Alphaproteobacteria; o: Rhodospirillales; f: Acetobacteraceae; g: Acidisphaera	12.33	1.67
	k: Bacteria; p: Proteobacteria; c: Alphaproteobacteria; o: Rhodospirillales; f: Rhodospirillaceae	1.67	3.33
	k: Bacteria; p: Proteobacteria; c: Alphaproteobacteria; o: Rhodospirillales; f: Rhodospirillaceae; g: Phaeospirillum; s: fulvum	0.00	1.67
	k: Bacteria; p: Proteobacteria; c: Alphaproteobacteria; o: Rhodospirillales; f: Rhodospirillaceae; g: Azospirillum	1.67	0.00
	k: Bacteria; p: Actinobacteria; c: Actinobacteria; o: Actinomycetales; f: Frankiaceae; g: Frankia	3.67	8.33
	k: Bacteria; p: Cyanobacteria; c: Nostocophycideae; o: Nostocales; f: Nostocaceae; g: Nostoc	0.00	8.67
	k: Bacteria; p: Cyanobacteria; c: Synechococcophycideae; o: Synechococcales; f: Acaryochloridaceae; g: Acaryochloris	0.00	2.00
	k: Bacteria; p: Cyanobacteria; c: Oscillatoriothycideae; o: Chroococcales; f: Phormidiaceae; g: Microcoleus; s: vaginatus	0.00	22.33
Total free N-fixing	116.33	349.33	
Symbiotic N-fixing	k: Bacteria; p: Proteobacteria; c: Alphaproteobacteria; o: Rhizobiales; f: Bradyrhizobiaceae; g: Bradyrhizobium; s: pachyrhizi	1.67	0.00
	k: Bacteria; p: Proteobacteria; c: Alphaproteobacteria; o: Rhizobiales; f: Bradyrhizobiaceae; g: Bradyrhizobium; s: elkanii	452.00	193.00
	k: Bacteria; p: Proteobacteria; c: Alphaproteobacteria; o: Rhizobiales; f: Rhizobiaceae; g: Sinorhizobium; s: meliloti	1.67	6.67
	k: Bacteria; p: Proteobacteria; c: Alphaproteobacteria; o: Rhizobiales; f: Phyllobacteriaceae; g: Mesorhizobium	38.00	18.33
Total symbiotic N-fixing	493.33	218.00	
Total N-fixing	609.67	567.33	
Denitrifying	k: Bacteria; p: Proteobacteria; c: Betaproteobacteria; o: Burkholderiales; f: Alcaligenaceae; g: Denitrobacter	2.33	0.00
	k: Bacteria; p: Proteobacteria; c: Gammaproteobacteria; o: Pseudomonadales; f: Pseudomonadaceae; g: Pseudomonas; s: corrugata	23.00	11.33
	Total denitrifying	25.33	11.33
Nitrifying	k: Archaea; p: Crenarchaeota; c: Thaumarchaeota; o: Nitrososphaerales; f: Nitrososphaeraceae; g: Nitrososphaera	4.33	1.33
	k: Bacteria; p: Proteobacteria; c: Betaproteobacteria; o: Nitrosomonadales; f: Nitrosomonadaceae; g: Nitrosovibrio; s: tenuis	20.33	4.67
	k: Bacteria; p: Nitrospirae; c: Nitrospira; o: Nitrospirales; f: Nitrospiraceae; g: Nitrospira	16.67	9.67
Total nitrifying	41.33	15.67	

Supplementary Figure S1. Radar diagrams of bacterial AWCD calculated for all the principal classes of carbon substrates, identified by Biolog® 96-well Eco-Microplates™, in soils from the sustainable (S_{mng} , continuous line) and the conventional (C_{mng} , dashed line) systems. Means ($n = 3$) with the * are statistically different ($p \leq 0.05$).



Classes of carbon substrates	S_{mng}		C_{mng}	
	AWCD	SD	AWCD	SD
Polysaccharides and complex compounds	0.913	0.130	1.020	0.156
Cellulose	0.797	0.212	0.982	0.192
Hemicellulose	0.733	0.167	0.636	0.084
Chitin	0.940	0.338	1.217	0.091
Phosphorylated compounds	0.371	0.294	0.463	0.196
Organic acids	0.638	0.113	0.797	0.096
Amino acids	0.810	0.130	1.008	0.110
Biogenic amines	0.287	0.145	0.400	0.130