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Comparing the effects of soil fauna on litter decomposition and organic matter turnover in sustainably and conventionally managed olive orchards

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ABSTRACT

Soils and crops in Mediterranean agrosystems are vulnerable to climate change and environmental stresses, and they will be more and more in the next future. In this scenario, soil organic matter (SOM) plays a crucial role and its level is principally determined by the continuous physical and chemical action of soil fauna. While the importance of microorganisms in fruit agrosystems has been extensively and recently highlighted, the role of soil fauna - and particularly of macrofauna - to ecosystem services has been often overlooked. On this basis, the aim of this study was to characterize and compare C/N dynamics and other soil physicochemical parameters, soil macrofauna abundance, bioturbation and litter/SOM decomposition indices in a Mediterranean olive (*Olea europaea* L.) orchard subjected to two different soil management systems (namely sustainable, S_{mng}, and conventional, C_{mng}) for 18 years. The adoption of the S_{mng} system significantly increased almost three times the abundance of earthworms and two times that of other soil macrofauna. Bioturbation due to soil fauna and roots was significantly higher in the S_{mng} system, and this caused a significantly faster SOM decomposition measured both in 90-day incubated local litter bags (decomposition constant = 0.092 and 0.072 in the S_{mng} and C_{mng} system, respectively) and in tea bags (decomposition rate constant = 0.018 and 0.010 in the S_{mng} and C_{mng} system, respectively). Soil C and N dynamics were also affected by different soil management. The results highlighted that the soil chemical quality of the S_{mng} system is the result of the higher abundance and activity of soil fauna, in terms of enhanced litter decomposition and bioturbation. From the general analysis of the data obtained, it emerged that the role of soil fauna should be seriously taken into account in land management strategies not exclusively oriented to fruit yield and quality, but also to soil fertility restoration.

1. Introduction

Soils and crops in Mediterranean agrosystems are vulnerable to climate change and environmental stresses, and they will be more and more in the next future (IPCC, 2019). Mediterranean fruit orchards are endangered by an increasing water shortage often because of changes in rainfall frequency and distribution, and rise of soil aridity and desertification, with resulting low levels of soil organic matter (SOM) and contents of macro- and micronutrients, both essential for plant growth (Palese et al., 2015; Pascazio et al., 2018; Sofo et al., 2019). This triggers a detrimental vicious circle which ultimately leads to an increase in using mineral fertilizers and pesticides as external inputs (EASAC, 2018; Silva et al., 2019), and in frequent soil tillage, increasing SOM losses by erosion and runoff (Costantini et al., 2018). To change this vicious circle, practices based on increased carbon inputs are required to facilitate sustainable use and conservation of soils (Bhogal et al., 2009).

In Mediterranean areas, for the natural lack of resources (particularly soil and water), conventional fruit production is going to be economically and environmentally disadvantageous. On the other side, fruit organic farming is not always self-sustaining and durable, because it does not always guarantee the nutrient balance optimal for plant growth and cannot cover the increasing fruit demand (Palese et al., 2009; Pergola et al., 2013; Sofo et al., 2014). For avoiding this dilemma, the fruit production systems should be directed towards the principles of sustainable and/or conservation agriculture (Xiloyannis et al., 2015). Several studies showed that fruit orchards could contribute to face and adapt to climate change through the application of sustainable practices (no- or minimum soil tillage, use of organic fertilizers, guided irrigation and recycling of polygenic carbon sources)

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aimed at improving soil fertility, increasing SOM levels and, consequently, atmospheric CO_2 removal (Lal, 2004).

In this scenario, SOM, especially its stabilized fraction (humus), plays a crucial role. The amount and types of SOM are principally determined by the continuous physical and chemical action of soil organisms, as soil fauna takes part to SOM shredding, transformation and decomposition, in cooperation with microorganisms (Matson et al., 1997; Six et al., 2004). Where SOM mineralization rates are high and the low SOM levels negatively affect the stability of soil aggregates, soil fauna-SOM interactions are of particular relevance in determining soil physical, chemical and microbiological fertility (Giller et al., 1997; FAO, 2017; Totsche et al., 2018). Soil fauna is also responsible for incorporating SOM into the soil profile by bioturbation, where it becomes available to the soil microbial community or is protected against mineralization by mixing it with soil inorganic particles (Wiesmeier et al., 2019).

Among soil fauna, earthworms are terrestrial invertebrates that can be used as bio-indicators in the assessment of environmental characteristics and management variations in agrosystems. These detritivore organisms are essential for the soil nutrient cycles (especially of C and N) and contribute to keep soils well-drained and aired (Paoletti et al., 2010). Species, number and biomass of soil fauna are affected by agricultural practices (Paoletti et al., 1998; Castro et al., 2019). Particularly, due to their strict linkage with soil, earthworms are strongly affected by soil tillage, and by the addition of mineral fertilizers and pesticides (Paoletti et al., 1998).

The role and function of soil fauna – and particularly of macrofauna – to ecosystem services in productive orchards have been often overlooked (FAO, 2017), while it should be seriously taken into account in land management strategies oriented not only to fruit production but also to soil fertility restoration. From an ecological point of view, the relationships between local changes (e.g., soil fauna/microorganisms) and global effects (e.g., soil quality/fertility, environmental issues, global change) – the so-called "local to global" concept – aims particularly important in fruit groves, whose products are a relevant source of income for many farmers operating in the Mediterranean area and could deliver ecosystem services, if properly managed (Pergola et al., 2013; Mininni et al., 2018).

In this research, olive (*Olea europaea* L.), a typical and widely spread Mediterranean fruit crop, has been chosen for its multifunctional role (e.g., agricultural, economic, environmental, social, cultural) (Sofo et al., 2014, Mininni et al., 2018). On such a basis, the aim of this study was to characterize and compare soil physicochemical properties, soil fauna presence and effects, and litter/SOM decomposition in a mature Mediterranean olive orchard subjected to two different soil management systems (sustainable, S_{mng}, and conventional, C_{mng}) for a long term of 18 years. We hypothesize that the better soil chemical quality deriving from the sustainable agronomic practices adopted (S_{mng} system) could be the result of the higher abundance and activity of soil fauna, in terms of enhanced litter decomposition and bioturbation.

2. Materials and methods

2.1. Experimental site, orchard management and soil sampling

The trial was carried out 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 is characterized by a semi-arid climate, with an annual rainfall of 558 mm (mean 1995–2017) and a mean annual temperature of 16.0 °C. The soil is a sandy loam, a Haplic Calcisol, according to the World Reference Base for Soil Resources, with sediment as parental material.

Half of the orchard (1 ha) has been managed using sustainable/conservation agricultural practices for 18 years (2000–2018) (sustainable management, S_{mng}). Trees were drip-irrigated from March to October (2850 m^3 ha^{-1} $year^{-1}$) with urban wastewater. The average values of organic C, N, P and K contained in the treated wastewater were 124, 54, 3 and 50 kg ha^{-1} $year^{-1}$, respectively. 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 mowed twice a year. Grasses and pruning residues were shredded and left along the row as mulch. An integrative amount of 40 kg ha^{-1} $year^{-1}$ of $N\text{-}NO_3^-$ 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 conducted with locally conventional management (C_{mng}) for 18 years (2000–2018), according to the practices usually adopted by farmers in the region (Table S1). The C_{mng} was rainfed and 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. 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 kg ha^{-1} $year^{-1}$).

In June 2018, soil sampling was performed in the inter-row area of both the systems (S_{mng} and C_{mng}). The same type of position was chosen for the analyses described in the following paragraphs. Soil sub-samples were picked in 10 points in the inter-row area (4 m far from each tree) at different soil depths (0–5, 5–10 and 10–20 cm) for chemical analysis. The 10 sub-samples were taken in proximity and pooled on site to constitute a composite soil sample of about 1 kg. For both the soil management systems (S_{mng} and C_{mng}), five composite samples ($n = 5$) were prepared. This sampling technique allowed to minimize spatial variability, according to Sofo et al. (2019). After removing visible crop residues, the soil composite samples were immediately stored in sterilized plastic bags at 4 °C for chemical measurements, and subsequently analyzed within 10 days.

2.2. Soil physicochemical 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, 5–10 and 15–20 cm), total organic carbon (TOC), total carbonates, 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 (model Basic 20®; Crison Instruments SA, Barcelona, Spain) in distilled water using a suspension 1:2.5 soil to liquid phase ratio. Total organic carbon (TOC) was determined by Walkley and Black method by oxidation at 170 °C with potassium dichromate ($K_2Cr_2O_7$) in presence of sulfuric acid (H_2SO_4), and the excess $K_2Cr_2O_7$ was measured by Möhr salt titration (Pansu and Gautheyrou, 2006). Total carbonates were measured by calcimetry using hydrochloric acid and calculating the volume of released carbon dioxide at controlled temperature and pressure, according to Pansu and Gautheyrou (2006). Total inorganic carbon (TIC) was estimated stechiometrically by the values of total carbonates. Total carbon (TC) was calculated by adding the values of TOC and TIC from each soil. Total nitrogen (TN) was measured by the Kjeldahl method. Bulk density at soil depths of 0–5, 5–10 and 15–20 cm was measured using volumetric rings (5 cm of internal Ø), according to Pansu and Gautheyrou (2006).

2.3. Sampling and preserving soil macrofauna

2.3.1. Hand-sorting in the field

Vegetation was cut from sampling spots near those chosen for chemical analyses ($n = 5$) in both S_{mng} and C_{mng} systems. The litter layer was transferred onto a plastic sheet and the earthworms and any

other clearly visible macrofauna (length > 10 mm) manually sorted. With the help of a wooden frame, a 25 × 25 × 25-cm deep soil block was excavated with a flat-blade spade cutting along edges rapidly first, without cutting inside block. The soil block was placed on a large plastic sheet, sorting through soil manually, removing all earthworms and other fauna and placing them in labeled 50 mL glass containers.

Earthworms and other fauna were separated and the vessel for the latter was filled with 70% ethanol to avoid any predation. To avoid mortality, the containers were placed in a cool box containing ice blocks and away from sunlight until being processed in the lab. Each replicate sample was kept separate throughout the sorting process.

2.3.2. Chemical expelling in the field

Chemical expelling is a dynamic and behavioral method where the worms are encouraged to leave the soil so that they can be collected from the soil surface, and it supplements the hand-sorting, in particular for anecic species. Mustard oil solution was previously prepared in the lab mixing 2 mL (=2.04 g) allyl isothiocyanate (no. 37,743–0; Sigma-Aldrich, Saint Louis, MI, USA) into 40 mL isopropanol (2-propanol) in small glass bottles easily transported to the field in cool boxes. Just before application in the field, this mixture was added to 20 L tap water in drums and mixed vigorously. Freshly prepared dilute mustard oil solution was added in each of the 25 × 25 × 25 cm pit excavated for hand-sorting using a watering can reach maximum saturation of sampling spot. The solution (about 5–10 L for each pit, depending on soil conditions and water infiltration) was poured slowly and avoiding the runoff. The expelled earthworms and any other clearly visible macrofauna (length > 10 mm) were collected with forceps from inside pits as they emerged, removing worms once they left their burrows completely. The duration of the operation for each pit was approximately 15–20 min.

The collected worms and any other macrofauna (length > 10 mm) were transferred to containers with clean tap water to rinse off the irritant. Soon after, they were placed in separate labeled glass containers, that were kept away from the sunlight and placed in a cool box to avoid mortality and decomposition until they were processed in the lab. The rinsing and dark, cool storage was important to avoid mortality. Once the worms had stopped emerging, water on the soil surface was poured. Each sample was kept separate throughout the sorting process. The removed soil was finally put back in the soil pit and the spot was left in a tidy state. Each sample of worms was rinsed with tap water and blotted on paper towels to measure the fresh weight (Fig. S2a).

2.4. Bioturbation mesh bag experiment

2.4.1. Mesh bags preparation

Bole white (kaolin clay), technical grade and washed (n. MFCD00062311; VWR International, Dublin, Ireland) and sand, quartz, crystalline, washed, Ø 0.6–1.2 mm (no. GERB5464; VWR International) were oven-dried at 105 °C overnight before use. A mixture of 75% sand with 25% kaolin (3:1; w/w) was prepared. Aliquots of this mixture (450 g sand and 150 g kaolin) were poured with a funnel into plastic bottles for transport in the field site. Each aliquot was enough for filling one mesh bag.

2.4.2. Field installation mesh bags

On 20 June 2017, 5 cm diameter spots, near those chosen for chemical and macrofauna analyses, were marked for installing mesh bags. Litter and cut off vegetation were removed and collected in a plastic box. Any vegetation and surface litter of the excavated core was cut, any macrofauna sorted out manually and set free, and the plant matter and surface litter retained. For each spot, two pits (5 cm diameter, 20 cm deep), arranged in pairs in close proximity (for meshes with and without holes for macrofauna access; Fig. S1) were excavated with an auger. The uppermost rooted horizon was discarded and the rest of the soil temporarily collected in a box, after removing larger roots.

The preparation of the mesh bags has been inspired by the work of Cui et al. (2016). Two types of mesh bags were prepared. In the former, the mesh size of 1 mm allowed microorganisms and smaller fauna to enter the bags but excluded macrofauna, as the net was continuous and uninterrupted. In the latter, the mesh was interrupted in the central part at an interval of 5 cm with holes having Ø = 10 mm for allowing also macrofauna access. Each mesh bag was inserted into the pit and the 3:1 (w/w) sand/clay mixture was poured through a funnel inside the mesh bag, making sure that no material got lost, especially from the bags with holes, and that the mesh cylinder was in good contact with the surrounding soil. At the end of the filling, the plant matter and surface litter from each soil core were applied on top of the sand/clay core before closing the bags. As the mesh was longer than 20 cm, it was bent horizontally to the side, folding it twice at the end and fixed it at the surface with a wire. Then, approximately 100 mL of tap water were poured on the surface of the core. The pH quickly adjusted to the surroundings upon watering and subsequent rainfall. Any gap between the cylinder and surrounding soil was filled with part of the excavated soil. Finally, the excavation site was covered with surrounding litter and the locations of each site marked and geo-referred.

2.4.3. Sampling mesh bags

The excavation of the cores took place one after year after mesh bags installation (21 June 2018) (Fig. S2b). After making sure that the soil was really wet before excavating the cores for avoiding sand and kaolin losses, the mesh cores were located and any litter removed. A long knife was used to cut a ring around the core at a few cm distant from the mesh, excavating adjacent to one side of this ring with a spade a soil block (about 20 × 20 × 20 cm), in order to easily access the core with its surrounding soil. Then, surrounding roots were cut off, and the spade inserted well underneath > 20 cm. Then, the cores were carefully lifted and placed inside a tray, the surrounding soil was carefully removed, and roots were cut off directly at the mesh surface using a knife, and the cleaned cores were put onto a plastic sheet.

The top part of the mesh was cut off and then the mesh was cut and opened vertically along the core from top to the bottom and spread to both sides. The length of the organic horizon on top was measured and photographed to extrapolate details and peculiarities. Any sand/clay fallen outside the mesh bags was ignored. The core was subdivided into 0–5, 5–10, 10–20 cm layers. These were put separately using shovel and hands into labeled plastic containers for keeping their structure during transport and stored in a cool place.

2.4.4. Biogenic structures extraction

All the other core layers were hand-sorted for evaluating root ingrown and soil fauna specimens (abundance index: 0 = none; 1 = few; 2 = many). Then, the larger biogenic structures (i.e. aggregates and soil including any organic trace, not the pure white clay/sand mixture) were separated from the sand/kaolin.

After air-drying, the biogenic structures were separated manually for each layer and let dry in a desiccator until mass constancy for recording dry mass. The contribution of macrofauna alone to soil bioturbation was evaluated by subtracting, for each layer, the dry weight of the biogenic structures from the mesh bags with holes from the respective values from the mesh bags without holes, and expressing $(1 - x)$ as a percentage.

2.5. Tea and local litter decomposition

2.5.1. Tea bags installation and final sampling

On the 5 February 2018, one green tea bag (*Camelia sinensis*; n. EAN 87 10,908 90,359 5; Lipton) and one rooibos tea (red tea) bag (*Aspalanthus linearis*; n. EAN 87 22,700 18,843 8; Lipton Unilever, Glascow, UK) were air-dried, weighted (including bag, cord and label) and inserted 15 cm apart each other at 10 cm soil depth using a planting spade at each spot ($n = 5$) of both S_{mng} and C_{mng} systems. The chosen

spots were located near those chosen for the other above-cited analyses. The tea bags used were of the non-woven type. The string and the label were left above the ground to facilitate subsequent retrieval. The pits were closed using the same removed soil, and the positions marked and geo-referred.

The tea bags were retrieved after 90 days on 6 April 2018 (Fig. S2d). Soil parts and roots were removed and the tea bags oven-dried at 70 °C for 48 h and then placed in a desiccator until reaching a constant weight. After drying, the soil attached to the surface of tea bags was carefully removed with hands and a small brush, and final weight (including bag and cord, but not the label) was recorded.

It is important to remark that the sites where all the three types of bags (mesh, litter and tea bags) were placed in the C_{mng} system, were bordered with red and white striped ribbon to avoid interference and disturbance due to the soil tillage practices, with consequent mesh tearing and/or destruction.

2.5.2. Local litter bags preparation, installation and final sampling

Litter bags with a size of 20 × 16 cm were prepared using indecomposable tulle tissue and filled with litter sampled, pooled and mixed on site, sun-dried and then placed in a desiccator until constant weight. Tissue and dry litter weight were recorded. The litter was mainly composed of dry olive leaves and twigs at different stages of decomposition. The bag mesh size of 1 mm allowed microorganisms and small mesofauna to enter the bags but excluded macrofauna. Litter was taken from the S_{mng} systems and dried in a desiccator until reaching a constant weight before using it for filling the litter bags. On 10 October 2017, the local litter bags were buried at 10 cm depth in spots near those chosen for the other above-cited analyses for both S_{mng} and C_{mng} systems. After digging, the excavated soil was used to cover the pits and the locations marked and geo-referred.

After one year (11 October 2018), the local litter bags were retrieved after making sure that the soil was really wet and any litter removed (Fig. S2c). Then, surrounding roots were cut off and the surrounding soil was carefully removed, and roots were cut off directly at the mesh surface using small forceps, and the cleaned litter bags were put onto a plastic sheet. Then the local litter bags were oven-dried at 70 °C and then placed in a desiccator until reaching a constant weight, that was measured. A similar experiment using smaller 10 × 8 cm litter bags was carried out starting from 5 February 2018 until 6 April 2018 (90-day period).

2.5.3. Calculation of decomposition index

From the weight differences calculated from both green and red tea bags, two main indices were calculated according to the model of Keuskamp et al. (2013), namely the decomposition rate constant (k) and the stabilization factor (S) (i.e. the inhibiting effect of environmental conditions on the decomposition of the labile fraction). While k can only be estimated from the early stages of decomposition (i.e., from red tea data after three months), S is related to the limit value and is estimable after most of the labile material (i.e., green tea) decomposed. Both k and S were calculated following these equations:

$$X_t = ae^{-kt} + (1 - a) \quad (1)$$

where X_t is the weight after the incubation time t in days (=90 in this case), a is the labile fraction (green tea), and $(1 - a)$ is the recalcitrant fraction (red tea) of the litter.

$$S = 1 - (a_g/H_g) \quad (2)$$

where a_g is the decomposable fraction of green tea and H_g is the hydrolyzable fraction of green tea.

The decomposable fraction of red tea (a_r) was calculated from the hydrolyzable fraction of red tea (H_r) and the stabilization factor S:

$$a_r = H_r(1 - S) \quad (3)$$

With X_t of the red tea (Xr_t) and a_r known, k can be calculated using the eqn. (1).

The difference between the initial and post-incubation total weights of the local litter bags were used for calculating the mass loss due to litter decomposition due to microorganisms and mesofauna. The decomposition constant (z) was calculated using a single exponential decay model (Harmon et al., 1999):

$$Xl_t = Xl_0 e^{-zt} \quad (4)$$

where t is the time in years (=1 in this case), Xl_0 is the initial amount of litter, Xl_t is the remaining amount of local litter after time t and z is the decomposition constant.

2.6. Statistical analysis

The statistical analysis of the soil chemical data, macrofauna presence, soil bioturbation and litter/tea decomposition 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 a factor. Means were separated according to Fisher's LSD test at $p \leq 0.05$. In this experiment, five analytical replicates for each treatment from the five independent composite soil samples ($n = 5$) were considered.

3. Results

3.1. Soil physico-chemical analysis

The profiles of soil total organic carbon (SOC) and soil total nitrogen (STN) in the two management systems, and particularly in the topsoil (0–5 cm), were considerably different (Table 1). Here, SOC levels were significantly higher ($p \leq 0.05$) in the S_{mng} system, compared to the C_{mng} one, while the differences in SOC levels were not significant in 5–10 cm layer, and reversed in the 10–20 cm one (Table 1). The contribution of soil inorganic carbon (SIC) as the fraction of soil total

Table 1
Soil total organic carbon (SOC), total inorganic carbon (SIC), total carbon (STC), total nitrogen (STN), SOC/STN ratio, pH and bulk density in soils of the sustainable (S_{mng}) and conventional (C_{mng}) systems measured at different depths. Each value represents the mean (± SD) from composite soil samples ($n = 5$). The values followed by different letters are statistically different ($p \leq 0.05$) within columns. nd = not detected.

Soil system	Soil depth (cm)	SOC (g kg^{-1})	SIC (g kg^{-1})	STC (g kg^{-1})	STN (g kg^{-1})	SOC/STN	pH	Bulk density (g cm^{-3})
Sustainable (S_{mng})	Litter	43.38 ± 0.95 a	nd	43.38 ± 0.95 a	5.90 ± 0.50 a	73.53 ± 19.02 a	–	–
	0–5	24.19 ± 0.76b	2.44 ± 0.32c	26.62 ± 1.88b	2.38 ± 0.19b	10.17 ± 0.12c	7.61 ± 0.03 e	1.13 ± 0.03c
	5–10	9.25 ± 0.60 d	5.26 ± 0.59b	14.51 ± 0.76 cd	1.80 ± 0.91c	6.02 ± 0.27 e	7.70 ± 0.06c	1.22 ± 0.05 bc
	10–20	5.84 ± 0.12 e	6.35 ± 0.45 a	12.19 ± 0.41 d	1.03 ± 0.26c	5.88 ± 0.33 e	7.80 ± 0.03 bc	1.29 ± 0.02b
Conventional (C_{mng})	0–5	11.99 ± 0.46c	4.54 ± 0.38 bc	16.52 ± 0.83c	1.10 ± 0.22c	11.23 ± 0.06b	7.85 ± 0.04b	1.20 ± 0.03 bc
	5–10	11.42 ± 0.65 cd	4.47 ± 0.54 bc	15.88 ± 0.52c	1.20 ± 0.07c	9.51 ± 0.29 d	7.91 ± 0.05 a	1.38 ± 0.05 a
	10–20	9.66 ± 0.16 d	6.86 ± 0.34 a	16.52 ± 0.37c	1.12 ± 0.06c	8.65 ± 0.32 d	8.08 ± 0.05 a	1.45 ± 0.06 a

Table 2
Number, total weight and mean weight of (left) earthworms and (right) other macrofauna in soils of the sustainable (S_{mng}) and conventional (C_{mng}) systems. Each value represents the mean (± SD, except for total weight) from five soil samples (n = 5). The values of macrofauna number and mean weight followed by different letters are statistically different ($p \leq 0.05$) within columns.

	Earthworms			Other macrofauna		
Soil system	Number	Total weight (g)	Mean weight (g)	Number	Total weight (g)	Mean weight (g)
Sustainable (S_{mng})	7 ± 1 a	4.011 ± 0.702 a	0.540 ± 0.043 a	5 ± 2 a	0.552 ± 0.038 a	0.109 ± 0.016 a
Conventional (C_{mng})	3 ± 1b	1.397 ± 0.334b	0.523 ± 0.096 a	3 ± 1b	0.252 ± 0.072b	0.075 ± 0.013b

carbon (STC) was higher with increasing soil depths, but no significant differences were found between the two soil systems (Table 1). The levels of STN were significantly higher in the S_{mng} system at 0–5 cm, while the differences in the remaining soil depths and soil systems were not statistically significant (Table 1). The SOC/STN ratios in both the soil systems were significantly different between the 0–5 cm soil layer and the other two depths and were statistically higher in the S_{mng} system (Table 1). In the litter of the S_{mng} system, the values of SOC and STN were 43.38 g kg^{-1} and 5.90 g kg^{-1}, while no litter was found in the C_{mng} system due to the soil management adopted (Table 1). In the S_{mng} system, the values of soil pH and bulk density increased with rising soil depth and were significantly lower from those of the C_{mng} system (Table 1).

3.2. Soil macrofauna and bioturbation

Both the number (7 ± 1 specimens) and total weight (4.011 ± 0.702 g) of the collected earthworms were higher in the S_{mng} system, compared to the C_{mng} values (3 ± 1 specimens and 1.397 ± 0.334 g, respectively) (Table 2). Similar trends were found for other macrofauna specimens' number and total weight (Table 2). The mean weight of earthworms was not statistically different between the two soil management systems, while that of other macrofauna was higher in the S_{mng} system (Table 2).

The weight of the biogenic structures in the mesh bags with holes (access to macrofauna) was significantly higher in the S_{mng} system than in the C_{mng} one, with differences marked in the 0–5 cm soil depth (10.058 and 3.952 g in the S_{mng} and C_{mng} systems, respectively) (Table 3). The same trend was found in the mesh bags without holes (access to smaller fauna only) (3.710 and 1.392 g in the S_{mng} and C_{mng} systems, respectively, at 0–5 cm soil depth). At the deepest soil depths (5–10 and 10–20 cm) bioturbation was very low compared to the 0–5 cm soil layer (Table 3).

In both the mesh bags with and without holes of the S_{mng} system, the abundance index for roots and soil fauna was higher in the topsoil (0–5 cm) and generally decreased with increasing soil depth (Table 3). In the C_{mng} system, the same indices were equal to 0 for both roots and fauna in all the soil layers and mesh bag type, whereas they were = 1 in the 0–5 cm soil layer for the mesh bags without holes (Table 3).

3.3. Tea and local litter decomposition

The weight differences of the tea inside the two types of the tea bags (green and red), allowed to calculate the decomposition indices (Table 4). Among these, the fractions of remaining green and red tea (Wr_t and Wr_t, respectively) were lower in the S_{mng} system (Table 4). The stabilization factor (S) resulted to be significantly higher in the C_{mng} system (0.670 vs 0.585), while the decomposition rate constant (k) showed a reverse trend (0.018 in the S_{mng} system and 0.010 in the C_{mng} one) (Table 4).

The fraction of remaining local litter (Xl_t) in the bags kept in the soil for one year was significantly higher in the C_{mng} system (0.847) than in the S_{mng} one (0.626) (Table 5). Regarding the litter decomposition constant (z; Eq. (4)), it resulted to be 0.515 in the S_{mng} system and 0.168 in the C_{mng} system, being significantly different at $p \leq 0.05$ (Table 5). Similar trends of local litter bags decomposition parameters, but with higher remaining litter and lower z values were found in the bags kept in the soil for 90 days (Table 5).

4. Discussion

Several studies highlight that the adoption of a sustainable orchard management (S_{mng}; Table S1) cause higher sequestration of atmospheric CO_2 in the soil, tree biomass and litter, enhancing SOC stock and soil microbial biodiversity (Palese et al., 2015; Pascazio et al., 2018; Sofo et al., 2019).

4.1. Bioturbation

Bioturbation, defined as the reworking of soils and sediments by animals or plants, including burrowing, ingestion and defecation of sediment grains, is a primary driver of soil biodiversity and it has a profound effect on soil quality, fertility and ecology in agrosystems (Richards et al., 2011; Breuning-Madsen et al., 2017; Piron et al., 2017; Yu et al., 2017; Tuma et al., 2019). Here, bioturbation due to soil fauna, evaluated by weighting the biogenic structures in the mesh bags after one year (Fig. S1 and Fig. S2), resulted to be higher in the S_{mng} system (Table 3).

The significantly higher number of soil faunal specimens detected in the S_{mng} system (Table 2) caused significant differences in bioturbation

Table 3
Weight of biogenic structures in the mesh bags with and without holes in soils of the sustainable (S_{mng}) and conventional (C_{mng}) systems measured at different depths. Each value represents the mean (± SD) from five mesh bags (n = 5). The values of dry weight and macrofauna bioturbation followed by different letters are statistically different ($p \leq 0.05$) within columns.

		With holes			Without holes		
Soil system	**Mesh depth (cm)**	**Dry weight (g)**	**Abundance index - roots**	**Abundance index - fauna**	**Dry weight (g)**	**Abundance index - roots**	**Abundance index - fauna**
Sustainable (S_{mng})	0–5	10.058 ± 2.702 a	2	2	3.710 ± 1.098 a	2	2
	5–10	1.739 ± 0.481b	1	0	0.434 ± 0.150 bc	0	1
	10–20	0.916 ± 0.325c	0	0	0.634 ± 0.153 bc	0	0
Conventional (C_{mng})	0–5	3.952 ± 0.815b	0	0	1.392 ± 0.272b	1	1
	5–10	0.316 ± 0.065 cd	0	0	0.118 ± 0.032c	0	0
	10–20	0.184 ± 0.026 d	0	0	0.148 ± 0.051c	0	0

Table 4
Initial and final weight of green and red tea, fraction of remaining green tea (Xg_t), fraction of remaining red tea (Xr_t), stabilisation factor (S) and decomposition rate constant (k) from the green tea and red tea bags kept for 90 days in soils of the sustainable (S_{mng}) and conventional (C_{mng}) systems. Each value represents the mean (± SD) from five pairs (green tea/red tea) of bags ($n = 5$). The values (excepting initial dry weights) followed by different letters are statistically different ($p \leq 0.05$) within columns.

Soil system	Initial weightgreen tea (g)	Final weight green tea (g)	Fraction of remaining green tea - Xg_t	Initial weight red tea (g)	Final weightred tea (g)	Fraction of remaining red tea - Xr_t	Stabilisation factor - S	Decomposition rate constant - k
Sustainable (S_{mng})	1.365 ± 0.021 a	0.888 ± 0.050b	0.651 ± 0.036b	1.985 ± 0.060 a	1.631 ± 0.040 a	0.822 ± 0.012b	0.585 ± 0.013b	0.018 ± 0.002 a
Conventional (C_{mng})	1.391 ± 0.006 a	1.005 ± 0.035 a	0.722 ± 0.028 a	1.918 ± 0.053 a	1.708 ± 0.035b	0.891 ± 0.039 a	0.670 ± 0.023 a	0.010 ± 0.002b

Table 5
Initial and final dry weight, fraction of remaining local litter (Xl_t), and decomposition constant (z) from the local litter bags kept (left) for one year and (right) for 90 days in soils of the sustainable (S_{mng}) and conventional (C_{mng}) systems. Each value represents the mean (± SD) from five litter bags ($n = 5$). The values followed by different letters are statistically different ($p \leq 0.05$) within columns.

	One year				90 days			
Soil system	Initial dry weight (g)	Final dry weight (g)	Fraction of remaining local litter (Xl_t)	Decomposition constant - z	Initial dry weight (g)	Final dry weight (g)	Fraction of remaining local litter (Xl_t)	Decomposition constant - z
Sustainable (S_{mng})	27.931 ± 4.102 a	17.002 ± 4.431b	0.626 ± 0.074b	0.515 ± 0.160 a	6.464 ± 2.549 a	5.907 ± 0.924b	0.912 ± 0.005b	0.092 ± 0.006 a
Conventional (C_{mng})	24.880 ± 2.455 a	21.027 ± 0.692 a	0.847 ± 0.054 a	0.168 ± 0.064b	8.521 ± 2.215 a	7.938 ± 0.586 a	0.931 ± 0.004 a	0.072 ± 0.004b

at all three soil depths, compared to C_{mng} system (Table 3). This suggests that soil fauna of the C_{mng} system was disturbed by the agronomic practices adopted and/or by the lack of soil nutrients and scarcer microbial populations they feed on (Brussaard et al., 2007; Sofo et al., 2019). Similar results were found in other studies (Paoletti et al., 1998; Brévault et al., 2007; Errouissi et al., 2011; Ashworth et al., 2017; Jiang et al., 2018; Melman et al., 2019; Castro et al., 2019), where a no-till strategy was reported to improve soil macrofauna abundance and composition. The beneficial effect of sustainable/organic farming on the biological activity of orchard soils, including soil fauna, was also demonstrated by Walmsley and Cerdà (2017). In contrast, Bucholz et al. (2017) pointed out that the influence of periodical tillage on soil biota in commercial vineyards was not always detrimental, and that plant biomass and soil parameters were also important factors to be taken into account.

Sorting of soil worms, directly removed from the soil manually and by chemical expelling (Fig. S2a) is of fundamental importance for defining soil biological fertility, in turn, connected to soil chemical and physical parameters (Six et al. 2004; Totsche et al. 2018.). This technique should be accompanied by soil chemical analyses focused on C and N dynamics (Buchholz et al., 2017; Pant et al., 2017; Sofo et al., 2019). The results of Table 1 showed that sustainable soil management (S_{mng}) caused reduced soil compaction (lower values of bulk density) and a decrease in soil pH, likely due to increased microbial and root activity. The S_{mng} system had high contents of SOC and STN in the litter and in the topsoil (0–5 cm), that are at the same time cause and effect of more abundant soil fauna and plants (Ashworth et al., 2017; Buchholz et al., 2017; Melman et al., 2019). These results were confirmed by the higher indices of root and fauna abundance in the S_{mng} system (Table 3). Finally, SOC and STN contents in the S_{mng} system (Table 1) were roughly proportional to the levels of bioturbation (Table 3), whereas in the C_{mng} system there was a kind of uniformity of both SOC and STN among soil depths (Table 1), likely due to soil tillage (Table S1).

4.2. Soil fauna and degrading microorganisms

From the general picture described so far, a more dynamic soil ecology was found in the S_{mng} system, where soil fauna was expected to enhance crop-residues decay processes. Measuring heterotrophic decomposition indices for soil litter is useful for many purposes, such as for evaluating the nutrient return to the soil and soil carbon stores. The classic method to study decomposition at the soil surface involves the use of local litter bags (Fig. S2c) (Kim, 2007; Domìnguez et al., 2010; Frouz et al., 2015).

The higher decomposition rate of the litter of the S_{mng} system (Table 4) was likely affected by the enhanced activity of both detritivore fauna (Tables 2 and 3) and decomposing microorganisms (Sofo et al., 2019). These two groups of soil organisms have a key role in the movement of organic matter in the soil, and in soil aggregate stabilization and turnover (Kim, 2007), as reflected by the higher values of SOC and STN in the topsoil of the S_{mng} system (Table 1). In a recent review, Frouz (2018) highlighted that microbial decomposition of the litter is most of the cases higher when soil macrofauna has access to litter bag contents. This is partly due to litter fragmentation and chemical modification in the gut of macrofaunal organisms, to the resulting increase in microbial activity in feces, and to the reduction in C/N ratio (Frouz, 2018). A similar effect is caused by the physical (root growth and penetration) and chemical (root exudates and rhizospheric microbes) action of the grasses (Domìnguez et al., 2010; Palese et al., 2015). In our case, spontaneous weeds and grasses covered the S_{mng} soils (Table S1) and their roots were abundant in the mesh bags of the S_{mng} system (Table 3). The other two indices of the higher biological activity of the S_{mng} soils were the lower pH and SOC/STN ratio (Table 1), likely due to microbial, faunal and grass roots (Ashworth et al., 2017; Frouz, 2018; Pascazio et al., 2018).

An innovative and standardized way to measure litter decomposition is the tea bag index (TBI) method, that measures the decay of plant material by using two types of tea bags (green and red tea) as standard plant material (Keuskamp et al., 2013; Didion et al., 2016). This method has been successfully used for evaluating early-stage litter decomposition across nine biomes, and it was found that tea decomposition resulted to be affected firstly by litter type and then by land use, soil temperature and soil moisture (Djukic et al., 2018). Contrary to the experiment with local litter bags, here tea was not only used as a standard litter. Green tea decomposed faster than red tea. Indeed, the fraction of remaining green tea (Xg_t) was lower than the fraction of remaining red tea (Xr_t) in both the soil systems (Table 4). It was possible to determine how much of the labile fraction of the material was decomposed and how much was stabilized (S) (Eq. (2)). This value was significantly lower in the S_{mng} system, so showing a higher and further decomposition of the labile fraction, compared to the C_{mng} system (Table 4). On the other side, red tea decomposed much slower, so after three months it can be considered being still in the first phase of decomposition (Sarneel and Veen, 2017). In the C_{mng} system, the higher fraction of Xr_t caused a lower initial decomposition rate (k) (Eqs. (1) and (3)), with a slower decomposition, compared to the S_{mng} system (Table 4). The values of the two main indices of the tea bag experiment, i.e. S and k (Table 4), indicated that the decomposition of the labile fraction of the litter was faster (k) and higher (S) in the S_{mng} system, likely because of the contribution of soil fauna activity and grass cover (Tables 2–3 and S1).

The local litter used in this study was mainly composed of recalcitrant organic matter (mostly olive prunings and leaves and few species of grass, with a SOC/STN = 73.53; Table 1). Compared to tea, the labile fraction of local litter was lower, as suggested by the fraction of remaining local litter (Xl_t) after 90 days (Table 5), which was higher than both Xg_t and Xr_t of the tea bags (Table 4). The degree of decomposition (z) of the local litter kept in the soil both for one year and for 90 days was significantly higher in the S_{mng} system (Table 5), confirming the same trend observed for tea bags (Table 4).

4.3. Conclusions

From the analysis of the data obtained, the S_{mng} system increased macrofauna abundance (Table 2) and bioturbation (Table 3), with repercussions on SOC decomposition both in litter and tea bags (Tables 4 and 5). Higher microbial and faunal biodiversity in agrosystems leads to greater stability and multi-functionality (Giller et al. 1997; Sofo et al. 2019; Wu and Wang, 2019). From a productive point of view, in soil fauna-plant interactions both the animal and the plant profit from each other, and these interactions could play an important role in fruit growing, positively affecting plant status, water and nutrient uptake and improving product quality (Brussaard et al., 2007).

In view of circular economy principles and to capitalize on the natural potential of soils, strategies have to be developed for sustainable land-use practices that optimize nutrient and energy use. This will reduce SOM decline, soil erosion and soil degradation but also promote ecosystem services and foster biodiversity, with consequent benefits to the whole agrosystem stability and its resilience against biotic and abiotic factors.

Declaration of competing 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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2020.114393.

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

Practice	S_{mng}		C_{mng}
Soil tillage	No tillage. Spontaneous weeds and grasses mowed at least twice a year. Crop residues were cut and left on the ground as mulch.		Tillage (milling at 10 cm soil depth) performed 2-3 times per year in order to keep the soil bare.
Fertilization	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 $N\text{-}NO_3^-$ was distributed in the early spring.		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	Guided drip irrigation (6 self-compensating drippers per tree delivering 8 L h^{-1}) with treated municipal wastewater. The irrigation was 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).		No irrigation (about 35 m^3 rainfall $plant^{-1}$ $year^{-1}$).
Pruning	Light winter pruning was performed each year in order to reach vegetative-reproductive balance of trees. Pruning material was cut and left on the ground as mulch.		Heavy pruning carried out every two years. Pruned residues burned out of the olive grove.

Figure S1. The mesh bags with holes for macrofauna access used in the experiment.

Figure S2. (a) Earthworms, (b) mesh bags, (c) local litter bags, and (d) tea bags recovered from the soils studied in the experiment.