Bioindicators and nutrient availability through whole soil profile under orange groves after long-term different organic fertilizations

Eristanna Palazzolo¹ · Vito Armando Laudicina¹ · Giancarlo Roccuzzo² · Maria Allegra³ · Biagio Torrisi³ · Anna Micalizzi¹ · Luigi Badalucco¹

© Springer Nature Switzerland AG 2019

Abstract

We investigated long-term (18 years) effects of three organic (cow manure (CM), poultry manure (PM), compost from agro-industry orange wastes (OW)) and one inorganic fertilization (IF) on various soil biological indicators (microbial biomass C, soil respiration, total phospholipid fatty acids (PLFAs), total bacteria, Gram-positive and Gram-negative bacteria and fungi) and nutrient contents (total and extractable organic C, total and mineral N, available P and K) along the profile of a Typic Haplustept under orange Mediterranean orchards. All fertilizers were added on the same N content basis (190 kg N ha⁻¹ per year). Variables related to carbon cycling gradually worsened with depth, regardless of treatment, but at any soil depth they improved according to IF < PM < OW < CM. Similarly, mineral nutrient availability decreased with increasing soil depth, according to CM > OW > PM > IF. Organic manures favoured Gram-negative bacteria, especially between 35 and 55 cm depth, whereas Gram-positive bacteria flourished better under inorganic fertilization up to 55 cm. Organic fertilizers increased microbial biomass C, compared with IF, with CM being the most efficient, i.e. inducing the greatest C use efficiency, in turn coupled with a greater amount of fungi compared to bacteria. This is crucial from the environmental viewpoint, since fungi better contribute to soil C storage. PLFAs signatures of microbial communities reflected the differences in resources availability determined by soil depth and fertilizer type; indeed, fungi and Gram-negative bacteria responded more promptly to nutrient contents than other microbial groups. Oligotrophic conditions in deeper soil horizons increased the metabolic quotient, an indicator of stress conditions.

Keywords Farmyard manure · Orange waste compost · Mineral fertilization · Subsoil carbon · Bioindicators · Semiarid Mediterranean climate

1 Introduction

Soils are usually added with organic fertilizers to improve their physical, chemical and biological properties, thus optimizing plant growth and preserving soil conservation [1, 2]. In contrast to inorganic fertilizers, nutrients immobilized into organic fertilizers must be mineralized by soil micro-organisms before becoming available to plants through root absorption. The long-term organic

management of agricultural soils positively influences soil properties since the addition of organic fertilizers promotes the organic matter accumulation in soil by favouring C pools with slower turnover times [3–5]. On the contrary, the addition of readily decomposable organic substrates may also trigger off the decomposition of native soil organic matter through a priming effect devoted to reduce the N deficiency for microbial biomass [1, 6, 7].

✉ Luigi Badalucco, luigi.badalucco@unipa.it | ¹Department of Agricultural, Food and Forestry Sciences, University of Palermo, Viale delle Scienze, Building 4, 90128 Palermo, Italy. ²Research Centre for Olive, Citrus and Tree Fruit, Council for Agricultural Research and Economics (CREA), Via la Canapona 1 bis, 47121 Forlì, Forlì, Italy. ³Research Centre for Olive, Citrus and Tree Fruit, Council for Agricultural Research and Economics (CREA), Corso Savoia 190, 95024 Acireale, Italy.

Although it is well established that long-term (> 10 years) organic fertilization enhances soil organic matter (SOM) and nutrients availability in topsoil (generally less than the first 30 cm) [8, 9], the impact of organic fertilizers along the soil profile is still controversial and not widely known [10, 11]. Organic fertilizers applied to topsoil can affect carbon and nutrients availability of deeper soil horizons through transport of dissolved organic matter. Therefore, to understand how organic fertilizers affect SOM dynamics and nutrient availability also in deeper soil horizons, it is of great importance, especially for those crops whose roots extend down more than 30 cm, such as tree crops, with subsequent increase in rhizodepositions [12].

With regard to inorganic N fertilization, it may have either positive or negative effects not only on soil organic C accumulation but also on its fractions [13–15]. On the one hand, N fertilization has proven to increase the SOC through increasing biomass production and hence C inputs to the soil [16]. On the other hand, Khan et al. [17] and Mulvaney et al. [18] suggested that the loss of SOC from soils may occur in response to synthetic N fertilizers as they promote the decomposition of native SOM and crop residues [19].

The effects of organic fertilizers on microbial community composition and activities are even less known, especially in deeper horizons [20, 21]. Farmyard manure and inorganic fertilizers can have both positive and negative effects on microbial biomass and activities [22]. Generally, organic manure markedly increases soil microbial biomass carbon (MBC), microbial quotient (MQ), i.e. MBC/SOC ratio and also soil respiration (SR) [3]. MBC increases due to rapid immobilization of simple organic substrates held within manure, while SR increases to their rapid mineralization [11].

Deep soils have a high potential in storing carbon [23] and, therefore, any agricultural management stimulating or repressing microbial activity, biomass and composition in subsoils impacts C and nutrients dynamics because micro-organisms drive the turnover of SOM [24]. Soil microbial communities are not uniformly dispersed throughout soil profile, but reflect patches of available resources [25, 26]. Organic substrates and microbial biomass and activity, generally, decline with increasing soil depth [27, 28]. However, when soil quality indicators were normalized to microbial biomass, specific enzyme activities showed either similar values throughout soil profile or even increased with depth [24].

As the agronomical impact of long-term organic fertilization along the soil profile is not well known so far, especially under orchards in semiarid Mediterranean environment, the aim of this study was to investigate the effects of three long-term (18 years) organic fertilizations

(cow manure, poultry manure and compost from agro-industry orange wastes) on various SOM pools, nutrients availability and microbial community composition along the profile of a Typic Haplustept under orange orchards in semiarid Mediterranean conditions. An inorganically (NPK) fertilized orange orchard soil profile was also investigated for comparison. We hypothesized that: (1) the impact of long-term organic fertilization on chemical, biochemical and microbial soil properties would vary with both fertilizer type and soil depth, especially in the deeper soil horizons (below 30 cm) and (2) the interaction between fertilizer type and soil depth would uncouple SOM pools and microbial groups abundance.

2 Materials and methods

2.1 Study site, experimental design and soil sampling

The study was performed in an experimental site designed for orange orchards (37° 20' 22.84" N; 14° 53' 32.02" E) of Italian Council for Research in Agriculture (CREA, Catania province, Sicily, Italy). Average annual rainfall and air temperature during last 25 years were 350 mm and 18.7 °C, respectively. The climate is typical Mediterranean (Köppen–Geiger's classification). Since 1995, orchards have been annually fertilized inorganically (IF) or organically with: 4-month-aged solid cow manure (CM) from dairy; 7-month-aged solid poultry manure (PM); and 5-month-aged compost (OW) from orange juice processing wastes (75%) and garden cleaning (25%). Agro-industrial wastes consisted of about 60% peel, 30% pulp and 10% pips, while garden cleaning contained triturated pruning residues and mown grasses. All fertilizations were calibrated to the same N inputs (190 kg N per hectare); due to the different chemical nature of fertilizers, added amounts of C, P and K varied among fertilizations. Table 1 shows main chemical properties of organic fertilizers, while Table 2 shows annual C, N, P and K inputs for all fertilizations.

Three randomized orchards (each 50 m × 30 m) for each treatment were selected (12 in total); then, five equidistant plots (each 2 m × 2 m) were diagonally marked off for each orchard to totally explore it. A soil profile was opened in spring 2013 in one-by-five plots of each orchard. According to the sequence of genetic horizons [29] of the respective opened profile, a single soil sample, constituted by five subsamples (one for each plot), was collected for each horizon and for each orchard ($n = 3$, four treatments, five horizons for each profile, sixty soil samples in total). In the plots used for opening the profiles, soil subsamples were collected by a shovel, while in the other four plots for each treatment, the soil subsamples were collected by an auger,

Table 1 Chemical composition of applied aged farmyard manures and compost from orange wastes. Data are expressed on dry weight basis (24 h at 105 °C)

Fertilizer	TOC (%)	TN (%)	TP (%)	TK (%)	RH (%)	TOC/TN
Poultry manure (PM)	35.5	4.0	0.81	1.04	17	8.9
Orange waste compost (OW)	37.0	2.1	0.44	1.65	40	17.6
Cow manure (CM)	29.0	1.5	0.52	0.88	30	19.3

TOC total organic carbon; TN total nitrogen; TP total phosphorous; TK total potassium; RH relative humidity on wet weight basis

Table 2 Amounts (kg ha⁻¹) of nutrients supplied each year in the four treatments. Data are expressed on dry weight basis (24 h at 105 °C) of fertilizer

Fertilizer	TOC	TN	TP	TK
Inorganic (IF)	–	190	25.5	66.4
Poultry manure (PM)	1550	190	46.9	78.9
Orange waste compost (OW)	2760	190	12.2	41.5
Cow manure (CM)	1780	190	48.9	99.6

TOC total organic carbon; TN total nitrogen; TP total phosphorous; TK total potassium

Soil profile field description [29–31]

Horizon	Depth cm	Boundary	Moist color	Structure	Roots	Consistence dry	moist
rh1A	0–12	Clear	5YR 4/3	sbk, me, 1	f, m, 2	sh	fr
maA	12–35	Diffuse	7.5YR 4/4	abk, me, 2	f, m, 3	mh	sh
AB	35–55	Diffuse	7.5YR 4/4	abk, me, 2	m, c, 2	mh	mh
Bw	55–75	Gradual	7.5YR 5/3	ma	c, m, 1	h	mh
BC	75–90	Gradual	7.5YR 5/2	ma	c, 1	mh	mh
C	>90	Gradual	7.5YR 5/2	ma		mh	mh

Fig. 1 Soil profile field description. Soil profile opened in the cow manure treatment. Structure: *abk* angular blocky, *sbk* subangular blocky, *ma* massive; 1 moderate, 2 strong; *f* fine, *me* medium. Roots: 1 few, 2 common, 3 many; *f* fine, *m* medium, *c* coarse. Consistence: *sh* slightly hard, *mh* moderately hard, *h* hard, *fr* friable

correspondingly to the depth of genetic horizons of the opened profile. Figure 1 shows the field description of the soil profile of one-by-three orchards of CM treatment. Soil profiles were opened, and samples were collected 1 year

after the last fertilizer application (spring 2012) to minimize effects of supplied fresh nutrients.

Field-moist soil samples were divided into two aliquots, one freshly sieved at 4 mm, rewetted up to 50% of water holding capacity (WHC) and stored at 4 °C in scantily sealed polyethylene bottles prior to soil biochemical analyses, while the second air-dried, sieved (< 2 mm) and stored in sealed polyethylene bottles at 4 °C prior to soil chemical analyses.

2.2 Physical and chemical analyses of soil samples

Physical and chemical soil properties were determined using standard, internationally recognized methods. Bulk density (BD) was determined from undisturbed samples using a steel cylinder (5 cm diameter, 5 cm height); texture (sand, 2–0.02 mm; silt, 0.02–0.002 mm; clay, < 0.002 mm) by pipette method after shaking slurries for 2 h in (NaPO₃)₆ and Na₂CO₃, without removing organic matter; soil pH by glass membrane electrode in distilled water (soil/water ratio 1:2.5 (w/v)); total carbonates content by the Dietrich–Fruehling's calcimeter; soil electrical conductivity (EC) in distilled water (soil/water ratio 1:5 (w/v)); cation exchange capacity (CEC) by Mehlich's method using BaCl₂ and triethanolamine at pH 8.2 as extracting solution; total organic C (TOC) and total N (TN) by wet digestion (H₂SO₄)—oxidation (K₂Cr₂O₇) and Kjeldahl digestion, respectively; soil available P according to Olsen et al. [30]; and soil available K as sum of water extractable potassium (10 g soil:50 mL distilled water) and exchangeable potassium (2 g of soil:25 mL BaCl₂–triethanolamine at pH 8.2).

2.3 Determination of soil biochemical properties and main microbial groups

Microbial biomass C (MBC) was determined by fumigation–extraction method [31]; it corresponded to the difference between organic C extracted from fumigated and unfumigated samples, multiplied by 2.64. The concentration of K₂SO₄-extractable C from unfumigated soil was a proxy of available C [32]. Ammonium and nitrate concentrations

were also determined on soil K_2SO_4 extracts by indophenol blue and chromotropic acid colorimetric methods, respectively. Microbial quotient (MQ) was calculated as the percentage of TOC present as MBC.

Soil respiration (SR) corresponded to cumulative CO_2 evolved from incubated soil (24 h, 50% WHC, 25 °C) and metabolic quotient (qCO_2), i.e. specific respiration, was calculated as $mg\ CO_2-C\ h^{-1}\ g^{-1}\ MBC$ [24].

PLFAs were extracted and analysed according to modified Bligh and Dyer's method [33]. Nonadecanoic acid methyl ester was the internal standard for FAMES quantification. Peaks were identified by comparison with retention times of known standards (Supelco BAME mix 47080-U; Supelco 37 Component FAME mix 47885-U). FAs with less than 14 or more than 19 carbon atoms were neglected as originating from non-microbial sources. The FAs i15:0, a15:0, 15:0, i16:0, i17:0, 17:0, cy17:0, 18:1 ω 7, cy19:0 represented bacterial biomass, while 18:2 ω 6,9 corresponded to fungal biomass [34]. The FAs i15:0, a15:0, i16:0, i17:0 represented Gram-positive bacteria while 18:1 ω 7, cy17:0 and cy19:0 Gram-negative bacteria [35].

2.4 Statistical analyses

Data were expressed on oven-dry weight soil basis (105 °C, 24 h). As the experimental site was homogeneous pedogenically, i.e. the opened four profiles (one for each treatment) resulted highly comparable as all showed the same five horizons (Fig. 1), we opted for not considering the soil depth as a main experimental factor, thus excluding the two-way ANOVA. Consequently, significant differences in soil properties at the same genetic horizon (depth) induced by different fertilizers or among genetic horizons within the same soil profile (fertilizer) were assessed by one-way ANOVA (fertilizer type or genetic horizon as single factors, respectively) followed by Tukey's HSD post hoc test ($p < 0.05$). Normal distribution and variance homogeneity of data were checked by Kolmogorov–Smirnov goodness of fit and Levene's tests, respectively. Statistical analyses were run using Statgraphics 15.0.

3 Results

3.1 Physical and chemical properties of soil

The sequence of genetic horizons (Ap (rhiA+maA)–AB–Bw–BC–C) was determined following both Zanella et al. [36, 37] and Soil Survey Staff [29], and it was the same throughout all soil profiles within the experimental site. For example, rhiA horizon in CM treatment was 12 cm in depth, and it had a sub-angular blocky structure and was well permeated by roots, mainly belonging to

the spontaneous herbaceous vegetation (Fig. 1). The maA horizon (12–35 cm) had an angular blocky structure and was permeated by few to some roots belonging to both herbaceous vegetation and orange trees. The AB horizon (35–55 cm) showed features similar to those of maA horizon, except for the roots belonging to only orange trees. The thickness of Bw horizon was 20 cm, i.e. from 55 to 75 cm of depth, had some orange roots but less than the overlying horizon. The BC horizon (75–90 cm) had a massive soil structure, and only scant roots were present, becoming even more massive below 90 cm (C horizon; Fig. 1).

Soil bulk density (BD) significantly increased with depth, averaging from $1.06\ g\ cm^{-3}$ in Ap horizon to 1.15 in C horizons, but did not differ among treatments regardless of depth (data not shown). Soil texture shifted from sand-silty and clay-sandy to sandy along profiles. Soil EC was generally the least in rhiA horizon and, like BD, was not affected by fertilizers type (Table 3). Total carbonates generally increased with soil depth. Soil pH also generally slightly increased with soil depth and was not significantly affected by treatments. Cation exchange capacity among treatments was highest in CM and OW while, among horizons, highest always in maA or AB ones (Table 3).

Regardless of the fertilizers supplied, C and N pools as well as available P and K decreased by increasing soil depth. Topsoils (Ap horizons) fertilized with IF and PM had TOC contents about 25% lower than soils fertilized with OW and CM. By increasing soil depth, such a difference among treatments diminished, but they were still significant (Fig. 2a). Extractable organic C (EOC), similar to TOC, increased always as $IF < PM < OW < CM$ also in the deepest horizons ($> 55\ cm$) (Fig. 2b). Remarkably, the EOC ratio between IF (the poorest in EOC) and CM (the richest in EOC) treatments decreased with soil depth. Indeed, in Ap horizon, it was 0.44, whereas in Bw horizon it lowered to 0.15 (Fig. 2b).

Similar to TOC, TN gradually decreased with depth, regardless of fertilizers, and it was 25% higher with CM but in Ap horizon only (Fig. 3a). Mineral N (NH_4^+ plus NO_3^-) content was always higher with organic fertilizers, compared to IF, and also decreased with soil depth. Remarkably, mineral N content in Ap horizon with CM more than doubled that with IF (Fig. 3b).

In the topsoils, total organic C to total N ratio (C/N) ranged from 13 to 19 and was the lowest in IF treatment and the highest in OW. In the subsoils, such a ratio did not show a clear trend among treatments, except at AB horizon which was the lowest for PM. At lower depths, the C/N ratio did not change appreciably among treatments (Fig. 4). Available P in soil fertilized with CM and PM was in average more than twice as much as with IF and OW (Fig. 5a) also in the deepest horizons. Available K in the

Table 3 Edaphic properties of soils fertilized with four different fertilizers (IF, inorganic fertilizer; PM, poultry manure; OC, orange waste compost; CM, cow manure)

Fertilizer	Horizon	Sand %	Silt %	Clay %	PH	EC dS m ⁻¹	TC g kg ⁻¹	CEC cmol ₍₊₎ kg ⁻¹
IF	rhiA	63 Aa	19 ABab	18 Aa	7.8 Ba	0.7 Ca	4.5 ABb	18 Bc
IF	maA	63 Aa	19 ABa	18 Ab	8.1 ABa	1.2 ABa	3.6 Bb	26 Ab
IF	AB	63 Aa	25 Aa	12 ABb	8.1 AB	0.9 BCb	2.7 Bb	20 ABc
IF	Bw	73 Ba	15 Bab	12 ABb	8.4 Aa	1.3 Aa	7.6 Aa	16 BCb
IF	BC	70 Bb	20 ABa	10 Bb	8.5 Aa	1.4 Aa	8.1 Aa	12 Ca
PM	rhiA	57 Aab	21 Aa	22 Aa	7.8 ABa	1.0 ABa	2.7 Bb	15 Bc
PM	maA	58 Aa	21 Aa	21 Aab	7.9 ABa	1.4 Aa	4.5 ABb	23 Ab
PM	AB	63 Ba	13 Bb	24 Aa	7.7 Ba	0.7 Bb	3.3 Bb	18 ABc
PM	Bw	73 Ca	6 Bc	21 Aa	8.3 Aa	1.2 Aa	7.1 Aa	17 ABb
PM	BC	69 BCb	10 Bbc	21 Aa	8.4 Aa	1.3 Aa	7.8 Aa	15 Ba
OW	rhiA	63 Aa	15 ABb	22 Aa	7.8Ba	0.9 Ba	2.7 Cc	26 Bb
OW	maA	63 Aa	10 Bb	27 Aa	7.9 ABa	1.4 Aa	2.7 Cc	36 Aa
OW	AB	60 Aa	18 ABab	22 Aa	8.0 AB	1.7 Aa	5.7 BCbc	42 Aa
OW	Bw	68 Ba	20 Aa	12 Bb	8.2 Aa	1.3 Aa	8.2 ABab	29 Ba
OW	BC	70 Bb	15ABab	15 Bab	8.5 Aa	1.3 Aa	11.1 Aa	13 Cba
CM	rhiA	53 Ab	25 Aa	22 ABa	8.0 Aa	0.9 Ba	2.7 Cc	34 Ba
CM	maA	58 Aa	15 Bab	27 Aa	8.1 Aa	1.4 Aa	3.6 BCc	41 Aa
CM	AB	58 Aa	20 ABa	22 ABa	8.1 Aa	1.4 Aa	6.3 Bb	31 BCb
CM	Bw	68 Ba	10 BCbc	22 ABa	8.3 Aa	1.3 Aa	13.4 Aa	25 CDa
CM	BC	78 Ca	5 Cc	17 Bab	8.4 Aa	0.9 Bb	15.2 Aa	19 Da

Different upper-case letters indicate significant differences at $P < 0.05$ among genetic horizons (depths) within the same soil profile; different lower-case letters indicate significant differences at $P < 0.05$ among soil profiles (fertilizers) at the same depth

Sand $0.02 < \phi < 2$ mm; silt $0.002 < \phi < 0.02$ mm; clay $0.002 < \phi$; pH in water (1:2.5, soil:water); EC electrical conductivity in soil extract (1:5, soil:water); TC total carbonates; CEC cation exchange capacity

upper two horizons did not show a clear trend among treatments, whereas in the deepest one (> 55 cm), it was the highest in CM and OW treatments and the lowest in PM (Fig. 5b).

3.2 Biochemical soil properties

Soil MBC was always the highest in Ap horizon and, regardless of depth, decreased as $CM > OW > PM > IF$ (Table 4); moreover, it showed larger differences than TOC with regard to fertilizer type. MBC content strongly decreased with increasing soil depth; indeed, it at any horizon was roughly twice over the horizon immediately below (Table 4). SR in Ap horizon (Table 4) behaved as MBC, with CM and OW higher than PM and IF. Also SR strongly decreased with depth, regardless of fertilizer, and reached comparable values among treatments in the deeper three horizons (Table 4). MQ as well decreased with depth, regardless of fertilizer; for example, at Bw horizon, it was almost half compared to rhiA horizon (Table 4). Across the

three upper horizons, CM induced the highest MQ, while IF the lowest, with PM and OW showing intermediate values.

The trend of qCO_2 was inversely related to that of MQ; indeed, regardless of fertilizer, it increased with soil depth, and regardless of depth, it increased as $CM < PM \sim OW < IF$ (Table 4).

3.3 Microbial community structure

Total PLFAs content resembled that of MBC as, regardless of fertilizers, it decreased with soil depth, whereas irrespective of depth it was the highest with CM and the lowest with IF (Table 5). Soils fertilized with PM and OW showed intermediate values.

The percentage of bacteria was higher than that of fungi (Table 5), and except for the BC horizon of PM treatment, it did not change along the soil profiles. Among topsoils, the highest bacteria abundance occurred for IF treatment (42.1%). Contrarily to total bacteria, percentages of Gram-positive and Gram-negative bacteria, and consequently their ratio, changed along soil profiles, with the first ones increasing with depth, especially in PM

Fig. 2 Total organic C (**A**) and extractable organic (**B**) of soils fertilized with four different fertilizers (inorganic fertilizer, NPK; poultry manure; orange waste compost; cow manure). Different upper-case letters indicate significant differences at $P < 0.05$ among genetic horizons (depths) within the same soil profile (fertilizer); different lower-case letters indicate significant differences at $P < 0.05$ among soil profiles (fertilizers) at the same horizon (depth)

treatment (Table 5). Differently, Gram-negative bacteria decreased with depth, except for PM. Consequently, the ratio Gram-positive to Gram-negative bacteria increased with depth, irrespective of fertilizer. The greatest shift of Gram-positive to Gram-negative bacteria ratio among fertilizers occurred in Ap (rhIA+maA) horizons, where it was the highest in IF treatment and the lowest in CM one (Table 5).

Fungi abundance significantly strongly decreased with depth in all treatments. Irrespective of depth, CM induced the highest % of fungi, PM and OW intermediate %, while IF the lowest %. As changes in total bacteria were much less pronounced than fungi, the trend of fungi/bacteria ratio (F/B) reflected that of fungi (Table 5).

4 Discussion

4.1 Shift in chemical soil properties and in organic C content

The sequence of genetic horizons among orchard plots was similar, thus suggesting that 18 years of different

Fig. 3 Total (**A**) and mineral (**B**, extractable ammonium plus nitrate) nitrogen of soils fertilized with four different fertilizers (inorganic fertilizer, NPK; poultry manure; orange waste compost; cow manure). Different upper-case letters indicate significant differences at $P < 0.05$ among genetic horizons (depths) within the same soil profile (fertilizer); different lower-case letters indicate significant differences at $P < 0.05$ among soil profiles (fertilizers) at the same horizon (depth)

Fig. 4 Total organic C (TOC) to total N ratio of soils fertilized with four different fertilizers (inorganic fertilizer, NPK; poultry manure; orange waste compost; cow manure). Different upper-case letters indicate significant differences at $P < 0.05$ among genetic horizons (depths) within the same soil profile (fertilizer); different lower-case letters indicate significant differences at $P < 0.05$ among soil profiles (fertilizers) at the same horizon (depth)

Fig. 5 Available phosphorous (**A**) and potassium (**B**) of soils fertilized with four different fertilizers (inorganic fertilizer, NPK; poultry manure; orange waste compost; cow manure). Different upper-case letters indicate significant differences at $P < 0.05$ among genetic horizons (depths) within the same soil profile (fertilizer); different lower-case letters indicate significant differences at $P < 0.05$ among soil profiles (fertilizers) at the same horizon (depth)

fertilizations did not alter the pedogenesis, probably due to climatic conditions that speed up the mineralization of organic amendments. Due to its morphological characteristics assessed during field survey and based on its physical and chemical properties, the soil was classified as Typic Haplustept [29].

Soil pH was not affected by fertilizers; it increased with soil depth, likely due to the concomitant increase in total carbonates. As the impact on soil reaction by fertilizers was likely the highest in topsoils, the carbonates content was adequate to buffer any acidification or alkalization process driven by fertilizers.

CEC is a soil chemical parameter rarely investigated in similar studies, although it plays a key role in soil nutrient retention. The highest CEC values in CM and OW treatments, also down to 75 cm of depth, suggest that such organic fertilizers could enhance the nutrient retention capacity of soils through the soil profile mainly explored by tree roots. Moreover, since soil texture did not show significant differences among horizons at the same depth, it suggests that CEC increase can be ascribed to a greater humification degree of soil organic matter in soil fertilized with CM and OW.

Soils organically fertilized, as expected in similar environments [38], had higher TOC and EOC than with IF. This likely depended on many concurrent causes: 1) direct C supply by organic fertilizers; 2) enhanced SOM mineralization rate by microbial activity triggered off with inorganic N supply [7]; and 3) presumed increase in soil aggregates stability by organic matter supplied. Indeed, mineral fertilization generally reduces soil aggregates stability and, therefore, the protection of SOM, especially in microaggregates [39, 40]. Instead, organic fertilizers usually improve aggregates stability, thus physically protecting organic C from microbial mineralization [41]. However, organic fertilizers here did not affect TOC content equally. The greater TOC increase with OW and CM, compared to PM and IF, mainly resulted from the highest absolute amounts of TOC supplied since 1995 and calibrated to add the same TN amount (190 kg N ha^{-1} , Table 2), what caused the highest C/N for OW and CM. Thus, organic substrates in OW and CM likely were decomposed by micro-organisms more slowly than in PM, because the lower the C/N of organic substrates the higher their quality, i.e. the assimilation rate [3]. Here, after nearly 20-year supply of organic fertilizers with different C/N, the consequent soil C/N only in maA horizon reflected that of organic fertilizers, while in rhiA and deeper horizons, the soil C/N mostly depended on soil spatial variability (Fig. 4). Thus, in fertilization studies, results about soil C/N should be extended to the whole soil profile.

Interestingly, whereas the effects of organic fertilizers on TOC were detectable only down to 35 cm of soil, their impact on EOC persisted also down to the deepest horizons, being CM and OW more effective than PM and IF in increasing the labile organic C pool in the roots zone explored by orange trees and improving the fruit yield and quality through soil microbial biomass mediation [22]. That extractable C is an early chemical indicator of C changes along the soil profiles better than TOC has been already reported [32].

4.2 Shift in soil nutrients

Understanding the macronutrient distribution after different fertilizations in the whole zone explored by roots is crucial for proper crop fertilization, especially if root structures expand up to subsoil. Such understanding and proper estimation led to an efficient supply of fertilizers so it is increasing nutrient uptake and reducing nutrient leaching [22].

With regard to total N, the relatively small significant differences among treatments were expected since it was yearly supplied at the same rate ($190 \text{ kg ha}^{-1} \text{ y}^{-1}$). In contrast, mineral N was higher in topsoil (Ap horizon) up to 75 cm depth for CM and PM compared to IF and OW,

Table 4 Biochemical properties of soils fertilized with four different fertilizers (IF, inorganic fertilizer; PM, poultry manure; OC, orange waste compost; CM, cow manure)

Fertilizer	Horizon	MBC mg kg ⁻¹	Soil respiration mg CO ₂ -C g ⁻¹ d ⁻¹	MBC/TOC %	QCO ₂ mg CO ₂ -C g ⁻¹ MBC h ⁻¹
IF	rhiA	450 Ad	20.0 Ac	2.2 Ac	1.8 Da
IF	maA	181 Bc	12.4 Bb	1.1 Bc	2.9 CDa
IF	AB	94 Cc	7.8 Cb	0.7 Bd	3.5 Ca
IF	Bw	63 CDb	7.1 CDa	0.8 Bb	4.8 Ba
IF	BC	30 Da	5.2 Ca	1.1 Ba	7.4 Aa
PM	rhiA	580 Ac	22.3 Abc	2.7 Ab	1.6 Cab
PM	maA	246 Bc	13.2 Bb	1.6 Bb	2.2 BCab
PM	AB	128 Cb	8.9 BCab	1.0 CDc	2.9 Bab
PM	Bw	91 Ca	6.9 CDa	1.4 Ca	3.2 Bb
PM	BC	28 Da	4.8 Da	0.8 Da	7.1 Aa
OW	rhiA	732 Ab	24.1 Aab	2.5 Ab	1.4 Db
OW	maA	338 Bb	14.3 Bb	1.8 Bb	1.8 CDb
OW	AB	190 Ca	9.3 Cab	1.2 Cb	2.0 Cb
OW	Bw	106 Da	6.9 CDa	1.2 Cab	2.7 Bb
OW	BC	37 Ea	4.2 Da	0.7 Ca	5.0 Aab
CM	rhiA	844 Aa	25.8 Aa	3.1 Aa	1.3 Dc
CM	maA	485 Ba	17.3 Ba	2.3 Ba	1.5 CDb
CM	AB	198 Ca	9.7 Ca	1.6 Ca	2.0 Cb
CM	Bw	104 Da	6.9 CDa	1.4 CDa	2.8 Bb
CM	BC	41 Ea	4.2 Da	1.2 Da	4.3 Ab

Different upper-case letters indicate significant differences at $P < 0.05$ among genetic horizons within the same soil profile (fertilizer); different lower-case letters indicate significant differences at $P < 0.05$ among soil profiles (fertilizers) at the same depth

Table 5 Total PLFAs (nmoles FA g⁻¹ dry soil), main microbial groups (% of total ELFAs) and their ratio in soils fertilized with four different fertilizers (IF, inorganic fertilizer; PM, poultry manure; OC, orange waste compost; CM, cow manure)

Fertilizer	Horizon	Total PLFAs	Bacteria	Fungi	Bacteria G+	Bacteria G–	Bacteria G+/G–	Fungi/Bacteria
IF	rhiA	189.5 Ac	42.1 Aa	10.1 Ac	21.0 Ba	19.2 Aa	1.10 Ba	0.24 Ac
IF	maA	130.7 Bb	38.9 Aa	8.0 Bb	20.2 Ba	17.3 Aa	1.18 Ba	0.21 ABb
IF	AB	75.0 Cc	42.7 Aa	6.6 Bab	27.5 Aa	11.7 Ba	2.37 Aa	0.16 BCb
IF	Bw	40.4 Cb	37.4 Ab	3.5 Ca	24.9 Ab	12.9 Ba	2.01 Aa	0.10 Ca
PM	BC	230.9 Ab	33.0 Cc	15.6 Ab	15.3 Cbc	17.0 Aa	0.92 Cb	0.47 Ab
PM	rhiA	159.9 Bab	36.3 BCa	7.9 Bb	18.0 BCab	17.7 Aa	1.03 BCa	0.22 Bab
PM	maA	108.3 Cb	38.4 Bab	4.1 Cb	22.2 Bb	17.4 Aa	1.33 Bb	0.11 Cb
PM	AB	37.4 Db	48.6 Aa	5.3 Ca	36.4 Aa	18.1 Aa	2.05 Aa	0.11 Ca
OW	Bw	248.7 Ab	36.2 Ab	15.2 Ab	16.3 Cb	20.9 Aa	0.78 Cbc	0.42 Ab
OW	BC	150.0 Bab	37.7 Aa	8.1 Bb	18.4 BCab	19.2 ABa	0.97 BCa	0.22 Bab
OW	rhiA	116.9 Bab	37.5 Ab	8.4 Ba	20.0 Bc	17.2 Ba	1.18 Bb	0.23 Ba
OW	maA	51.2 Cab	37.6 Ab	3.4 Ca	26.6 Aab	11.9 Ca	2.31 Aa	0.09 Ca
CM	AB	274.6 Aa	35.6 Ab	20.3 Aa	14.5 Cc	21.8 Aa	0.66 Cc	0.57 Aa
CM	Bw	188.4 Ba	36.1 Aa	11.1 Ba	17.5 Bcb	18.5 ABa	0.95 BCa	0.31 Ba
CM	BC	137.7 Ba	36.6 Ab	8.9 Ba	20.2 Bbc	16.7 BCa	1.22 Bb	0.24 Ba
CM	rhiA	86.7 Ca	37.1 Ab	4.8 Ca	25.4 Ab	13.7 Ca	2.02 Aa	0.13 Ca

Different upper-case letters indicate significant differences at $P < 0.05$ among genetic horizons (depths) within the same soil profile (fertilizer); different lower-case letters indicate significant differences at $P < 0.05$ among soil profiles (fertilizers) at the same horizon (depth)

although much more up to 35 cm. This is important for both nutritional and environmental viewpoints. Indeed, based on origin and chemical nature of the organic fertilizers, their N mineralization rate likely decreased as $CM > PM > OW$. This since CM caused the highest soil EOC and available P, while OW caused much less soil available P, compared to CM and PM (Fig. 5). Remarkably, as the C/N of PM (8.7) was the closest to soil microbial biomass C/N, likely the proper amount of inorganic N to be immobilized was achieved even with a relatively slow PM mineralization rate. Certainly, inorganic N from IF was the most promptly used by plant and micro-organisms and/or easily leached from soil since its content was the lowest at any depth. The available P, as it essentially derives from the mineralization of organic P, showed a trend similar to that of inorganic N, which in turn derives from mineralization of organic N. Finally, the K availability reflected that of total K content, especially in topsoils.

4.3 Shift in biochemical soil properties

Microbial biomass acts as both labile nutrient reservoir and catalyst for organic matter decomposition; thus, its dynamics helps to understand soil nutrient fluxes, especially in the root zone of organically fertilized soils [12]. MBC and SR, as expected, were much higher in organically fertilized plots in Ap and AB horizons, compared to IF, while in deepest horizons this difference among fertilizers vanished. This is because organic fertilizers can stimulate soil micro-organisms by improving soil physical characteristics and by furnishing readily available sources of major macronutrients [22].

Since soil BD was affected only by depth and not by fertilizer type, the increases in MBC and SR depended likely on the amount and quality of available nutrients from organic fertilizers. Indeed, the highest increases in both MBC and SR occurred with CM since its C/N was the farthest, among other organic fertilizers, from the putative soil microbial biomass average C/N, i.e. 10 [42], thus suggesting a higher mineralization rate of added CM for a proper supply of N and P to be immobilized [43, 44]. Moreover, the highest EOC content in CM, i.e. readily available organic substrate, may explain its highest soil MBC.

Both MQ and qCO_2 were affected differently by the four fertilizers, with qCO_2 generally increasing with soil depth, whereas MQ decreasing. MQ is considered a more sensitive soil health indicator than either MBC or TOC individually [45]. They speculated that the higher the MQ, the higher the efficiency of organic matter utilization for microbial growth, due to a higher quality of organic substrates. That the highest MQ occurred with CM confirmed its highest quality. Specific respiration (qCO_2) is considered inversely related to stresses in microbial communities [24]. The

microbial metabolic efficiency is supposed to be reflected by their qCO_2 . Lower qCO_2 values with organic fertilizers compared to IF ($CM < OW < PM < IF$), coupled with concomitantly higher MQ values ($CM > OW > PM > IF$), indicate that organic fertilizers energetically favoured microbial communities able to shift more to organic immobilization than mineralization [46]. In other words, with mineral fertilization, the organic substrates were more diverted towards catabolic than anabolic processes, leading to reduced MBC in the long term and thus to lower MQ. Also Böhme et al. [43] found lower qCO_2 under manure than mineral NPK fertilization, similar to Badalucco et al. [3], who observed lower qCO_2 with organic managements as compared to conventional intensive ones.

The qCO_2 increase with depth indicated that the carbon use efficiency was lower in deeper horizons, where oligotrophic conditions generally occur [24]. Thus, when more organic substrates must be catabolized to CO_2 to provide needed nutrients, then less organic substrates can be incorporated inside the microbial biomass [46].

4.4 Shift in abundance of microbial groups

Both fertilizer type and soil depth significantly affected the content of total PLFAs, whose pattern resembled that of MBC, and the abundance of main microbial groups. Indeed, regardless of fertilizers, PLFAs decreased with depth, whereas at any depth, PLFAs generally followed the abundance order $CM > OW > PM > IF$, i.e. higher with organic than inorganic fertilization [44]. Such patterns, in turn, reflected the availability of both organic and inorganic nutrients determined by fertilizers along the soil profiles.

Main microbial groups were also affected by soil depth and fertilizers, thus confirming that surface microbial communities are larger and more diverse compared to sub-surface communities [47]. Similarly, vertical distribution of Gram-negative and Gram-positive bacteria agreed with the previous observations, where microbial communities generally shifted from greater Gram-negative dominance in topsoil to greater Gram-positive dominance in subsoil [27].

Fungal abundance and F/B ratio too, that decreased with soil depth, agreed with similar studies [25, 48]. Since some soil edaphic properties (texture, pH, electrical conductivity and total carbonates) changed little along the four profiles, the microbial community composition was likely driven by both inorganic and organic nutrients availability that, regardless of soil depth, decreased as $CM > OW > PM > IF$. Likewise, Griffiths et al. [48] have also found higher fungi and Gram-negative bacteria and lower Gram-positive bacteria with increased organic C availability. Similarly, Zhong et al. [43] found that organic manures

favoured the Gram-negative bacteria, whereas Gram-positive bacteria flourished better under inorganic fertilization. This is because, generally, Gram-positive bacteria are more able than Gram-negative bacteria in using more complex carbon sources like older SOM, which is more common in deeper soil horizons [49].

The PLFAs signatures of microbial communities reflected the differences in resources availability determined by soil depth and fertilizer type and indicated that fungi and Gram-negative bacteria responded more promptly to nutrient content than other microbial groups. The above considerations agree with patterns of qCO_2 that increased with depth, i.e. by decreasing both nutrients availability and fungal PLFAs. Fungi are well known to have higher C use efficiency [50].

5 Conclusions

Long-term addition of organic fertilizers compared to IF had strong chemical and biological effects on a Typic Haplustept, and even some differences occurred between the use of animal (poultry or cow) and composted orange wastes organic fertilizers. The changes observed in some chemical properties, mainly organic C pools, mineral N and CEC up to 75 cm soil depth, suggest that studies aimed at investigating the impact of fertilization should be extended to the depth explored by plant roots, i.e. generally the whole soil profile.

Soil micro-organisms were stimulated by both the amount and quality of available nutrients supplied by organic fertilizers that, in turn, determined the extent of C use efficiency (low metabolic and high microbial quotients). In this sense, cow manure was the most efficient among the three organic fertilizers tested. The greatest C use efficiency with cow manure was also coupled with a greater amount of fungi that, as widely reported in the literature, use organic C substrates more efficiently than bacteria. Such a shift of microbial community composition coupled with oligotrophic conditions in deeper soil horizons, in turn, determined the patterns of metabolic quotient that increased with depth. The latter issue is of great environmental importance because if on the one hand organic fertilizers contribute to increase organic C in soils, on the other hand fungi better contribute to its storage.

Furthermore, the PLFAs signatures of microbial communities reflected the differences in resources availability determined by soil depth and fertilizer type and indicated that fungi and Gram-negative bacteria responded more promptly to nutrient contents than other microbial groups [51]. Finally, although it was not an aim of this

study, it was confirmed that all soil bioindicators related to C and N cycling were much more sensitive than total C and pools, even down to 1-meter depth.

Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

References

1. Cesarano G, De Filippis F, La Stora A, Scala F, Bonanomi G (2017) Organic amendment type and application frequency affect crop yields, soil fertility and microbiome composition. *Appl Soil Ecol* 120:254–264
2. Rahman MT, Zhu QH, Zhang ZB, Zhou H, Peng X (2017) The roles of organic amendments and microbial community in the improvement of soil structure of a Vertisol. *Appl Soil Ecol* 111:84–93
3. Badalucco L, Rao M, Colombo C, Palumbo G, Laudicina VA, Gianfreda L (2010) Reversing agriculture from intensive to sustainable improves soil quality in a semiarid South Italy soil. *Biol Fertil Soil* 46:481–489
4. Laudicina VA, Badalucco L, Palazzolo E (2011) Effects of compost input and tillage intensity on soil microbial biomass and activity under Mediterranean conditions. *Biol Fertil Soils* 47:63–70
5. Tian J, Lou Y, Gao Y, Fang H, Liu S, Xu M, Blagodatskaya E, Kuzyakov Y (2017) Response of soil organic matter fractions and composition of microbial community to long-term organic and mineral fertilization. *Biol Fertil Soils* 53:523–532
6. Chen L, Zhang J-B, Zhao B-Z, Xin X-L, Zhao J-H (2014) Carbon mineralization and microbial attributes in straw-amended soils as affected by moisture level. *Pedosphere* 24:167–177
7. Kätterer T, Börjesson G, Kirchmann H (2014) Changes in organic carbon in topsoil and subsoil and microbial community composition caused by repeated additions of organic amendments and N fertilisation in a long-term field experiment in Sweden. *Agr Ecosyst Environ* 189:110–118
8. Diacono M, Montemurro F (2010) Long-term effects of organic amendments on soil fertility: a review. *Agron Sustain Dev* 30:401–422
9. Bastida F, Selevsek N, Torres IF, Hernández T, García C (2015) Soil restoration with organic amendments: linking cellular functionality and ecosystem processes. *Sci Rep* 5:15550
10. Fontaine S, Barot S, Barré S, Bdioui P, Mary N, Rumpel C (2007) Stability of organic carbon in deep soil layers controlled by fresh carbon supply. *Nature* 450:277–280
11. Jacinthe P-A, Shukla MK, Ikemura Y (2011) Carbon pools and soil biochemical properties in manure-based organic farming systems of semi-arid New Mexico. *Soil Use Manag* 27:453–463
12. Paterson E (2003) Importance of rhizodeposition in the coupling of plant and microbial productivity. *Eur J Soil Sci* 54:741–750
13. Neff JC, Townsend AR, Gleixner G, Lehman SJ, Turnbull J, Bowman WD (2002) Variable effects of nitrogen additions on the stability and turnover of soil carbon. *Nature* 419:915–917
14. Huang S, Zhang WJ, Yu XC, Huang QR (2010) Effects of long-term fertilization on corn productivity and its sustainability in an Ultisol of southern China. *Agric Ecosyst Environ* 138:44–50

15. Laudicina VA, Barbera V, Gristina L, Badalucco L (2012) Management practices to preserve soil organic matter in semiarid Mediterranean environments. In: Björklund PA et al (eds) *Soil organic matter: ecology, environmental impact and management*. Nova Science Publishers, Inc., New York, pp 39–61
16. Lou Y, Xu M, Wang W, Sun X, Liang C (2011) Soil organic carbon fractions and management index after 20 yr of manure and fertilizer application for greenhouse vegetables. *Soil Use Manag* 27:163–169
17. Khan SA, Mulvaney RL, Ellsworth TR, Boast CW (2007) The myth of nitrogen fertilization for soil carbon sequestration. *J Environ Qual* 36:1821–1832
18. Mulvaney RL, Khan SA, Ellsworth TR (2009) Synthetic nitrogen fertilizers deplete soil nitrogen: a global dilemma for sustainable cereal production. *J Environ Qual* 38:2295–2314
19. Benbi DK, Brar K, Toor AS, Sharma S (2015) Sensitivity of labile soil organic carbon pools to long-term fertilizer, straw and manure management in rice–wheat system. *Pedosphere* 25:534–545
20. Yu HY, Ding WX, Luo JF, Donnison A, Zhang JB (2012) Long-term effect of compost and inorganic fertilizer on activities of carbon-cycle enzymes in aggregates of an intensively cultivated sandy loam. *Soil Use Manag* 28:347–360
21. Geisseler D, Scow KM (2014) Long-term effects of mineral fertilizers on soil microorganisms—a review. *Soil Biol Biochem* 75:54–63
22. Liu E, Yan C, Mei X, He W, Bing SH, Ding L, Liu Q, Liu S, Fan T (2010) Long-term effect of chemical fertilizer, straw, and manure on soil chemical and biological properties in northwest China. *Geoderma* 158:173–180
23. Fontaine S, Barot S (2005) Size and functional diversity of microbe populations control plant persistence and long-term soil carbon accumulation. *Ecol Lett* 8:1075–1087
24. Laudicina VA, Palazzolo E, Piotrowska-Długosz A, Badalucco L (2016) Soil profile dismantlement by land levelling and deep tillage damages soil functioning but not quality. *Appl Soil Ecol* 107:298–306
25. Fierer N, Schimel JP, Holden PA (2003) Variations in microbial community composition through two soil depth profiles. *Soil Biol Biochem* 35:167–176
26. Gelsomino A, Petrovičová B, Vecchio G, Laudicina VA, Badalucco L (2013) Chemical, biochemical and microbial diversity through a Pachic Humudept profile in a temperate upland grassland. *Agrochimica* 67:214–232
27. Blume E, Bischoff M, Reichert J, Moorman T, Konopka A, Turco R (2002) Surface and subsurface microbial biomass, community structure and metabolic activity as a function of soil depth and season. *Appl Soil Ecol* 592:1–11
28. Fang C, Moncrieff JB (2005) The variation of soil microbial respiration with depth in relation to soil carbon composition. *Plant Soil* 268:243–253
29. Soil Survey Staff (2015) *Illustrated guide to soil taxonomy*, version 2. U.S. Department of Agriculture, Natural Resources Conservation Service, National Soil Survey Center, Lincoln, Nebraska
30. Olsen S, Cole C, Watanabe F, Dean L (1954) Estimation of available phosphorus in soils by extraction with sodium bicarbonate. *USDA Circular Nr 939*, Washington, DC. pp 1–19
31. Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass C. *Soil Biol Biochem* 19:703–707
32. Laudicina VA, Palazzolo E, Badalucco L (2013) Natural organic compounds in soil solution: potential role as soil quality indicators. *Curr Org Chem* 17:2991–2997
33. Wu Y, Ding N, Wang G, Xu J, Wu J, Brookes PC (2009) Effects of different soil weights, storage times and extraction methods on soil phospholipid fatty acid analyses. *Geoderma* 150:171–178
34. Frostegård A, Bååth E (1996) The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. *Biol Fertil Soils* 22:59–65
35. Zelles L (1997) Phospholipid fatty acid profiles in selected members of soil microbial communities. *Chemosphere* 35:275–294
36. Zanella A, Ponge J-F, Fritz I, Pietrasiak N, Matteodo M, Nadporozhskaya M, Juilleret J, Tatti D, Le Bayon R-C, Rothschild L, Mancinelli R (2018) *Humusica 2*, article 13: para humus systems and forms. *Appl Soil Ecol* 122:181–199
37. Zanella A, Ponge J-F, Jabiol B, Sartori G, Kolb E, Gobat J-M, Le Bayon R-C, Aubert M, De Waal R, Van Delft B, Vacca A, Serra G, Chersich S, Andreetta A, Cools N, Englisch M, Hager H, Katzensteiner K, Brêthes A, De Nicola C, 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 (2018) *Humusica 1*, article 4: terrestrial humus systems and forms—specific terms and diagnostic horizons. *Appl Soil Ecol* 122:56–74
38. Melero S, Ruiz Porras JC, Herencia JF, Madejón E (2006) Chemical and biochemical properties in a silty loam soil under conventional and organic management. *Soil Tillage Res* 90:162–170
39. Herencia JF, Garcia-Galavis PA, Maqueda C (2011) Long-term effect of organic and mineral fertilization on soil physical properties under greenhouse and outdoor management practices. *Pedosphere* 21:443–453
40. Mäder P, Fließbach A, Dubois D, Gunst L, Fried P, Niggli U (2002) Soil fertility and biodiversity in organic farming. *Science* 296:1694–1697
41. Canali S, Di Bartolomeo E, Trinchera A, Nisini L, Tittarelli F, Intrigliolo F, Rocuzzo G, Calabretta ML (2009) Effect of different management strategies on soil quality of citrus orchards in Southern Italy. *Soil Use Manag* 25:34–42
42. Cleveland CC, Liptzin D (2007) C:N: P stoichiometry in soil: is there a “Redfield ratio” for the microbial biomass? *Biogeochemistry* 85:235–252
43. Böhme L, Langer U, Böhme F (2005) Microbial biomass, enzyme activities and microbial community structure in two European long-term field experiments. *Agric Ecosyst Environ* 109:141–152
44. Zhong W, Gu T, Wang W, Zhang B, Lin X, Huang Q, Shen W (2010) The effects of mineral fertilizer and organic manure on soil microbial community and diversity. *Plant Soil* 326:511–522
45. Anderson JPE, Domsch KH (1989) Ratios of microbial biomass carbon to total carbon in arable soils. *Soil Biol Biochem* 21:471–479
46. Dilly O (2005) Microbial energetics in soils. In: Buscot F et al (eds) *Microorganisms in soils: roles in genesis and functions*. Springer, Berlin, pp 123–138
47. Fritze H, Pietikainen J, Pennanen T (2000) Distribution of microbial biomass and phospholipid fatty acids in Podzol profiles under coniferous forest. *Eur J Soil Sci* 51:565–573
48. Griffiths B, Ritz K, Ebbelwhite N, Dobson G (1999) Soil microbial community structure: effects of substrate loading rates. *Soil Biol Biochem* 31:145–153
49. Kramer C, Gleixner G (2008) Soil organic matter in soil depth profiles: distinct carbon preferences of microbial groups during carbon transformation. *Soil Biol Biochem* 40:425–433
50. Six J, Frey SD, Thiet RK, Batten KM (2006) Bacterial and fungal contributions to carbon sequestration in agroecosystems. *Soil Sci Soc Am J* 70:555–569
51. de Menezes AB, Richardson AE, Thrall PH (2017) Linking fungal–bacterial co-occurrences to soil ecosystem function. *Curr Opin Microbiol* 37:135–141

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.