



## RESEARCH ARTICLE

# Long-term organic management: Mitigating land use intensity drawbacks and enhancing soil microbial redundancy

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## Abstract

**Background:** Soils under organic farming systems exhibit better quality and higher biological activity than conventional systems. Manure addition, especially coupled with reduced or no tillage, significantly enhances microbial biomass and activity by improving soil physical properties and providing carbon (C) and nitrogen (N) sources. While several studies have examined the effects of transitioning from conventional to organic farming on soil chemistry and biochemistry, limited research has explored the influence of land use variations on soil fertility within long-term organic farming systems.

**Aims:** Therefore, the aim of this study was to assess how three different land uses—pasture, vegetable crops, and orchard—affected soil fertility under a long-term organic farming system.

**Methods:** Soil samples were collected from the 0 to 15 cm layer of plots used for pasture, vegetable crops and orchard, being the latter cover cropped with legumes, and analyzed to determine chemical and biochemical soil parameters.

**Results:** Contrary to expectations, high land use intensity (vegetable crops and orchard soils) resulted in increased soil organic C and total N, compared to low intensity (pasture). Such an increase was ascribed to farmyard manure addition that counteracted the negative impact of tillage. Consequently, microbial biomass C and activity also increased. The greatest availability of organic substrates favored bacteria, particularly gram-positive strains, shaping the microbial community. However, despite changes of microbial biomass and of the main microbial groups, microbial activity was only slightly affected, suggesting high functional redundancy of microorganisms in long-term organic farming soil.

**Conclusions:** Results suggested that if land use intensification provides for organic supply, its negative impact on soil fertility may be mitigated.

## KEYWORDS

bacteria, enzyme activities, fungi, microbial activity, microbial biomass, organic farming

## 1 | INTRODUCTION

The overall soil fertility relies on the integration of physical, chemical, and biochemical properties. While physical and chemical soil proper-

ties mainly affect the nutrient availability, biochemical soil properties are more sensitive to external inputs being linked to the living microbial community inhabiting soil (Laudicina, Dennis et al., 2012; Maurya et al., 2020). Moreover, soil microorganisms are crucial for many soil

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processes and functions, such as organic matter decomposition, nutrient cycling, soil structure, and pollutants degradation (Pulleman et al., 2012). The biochemical parameters most used as biological indicators of soil fertility are soil microbial biomass carbon (MBC), phospholipid fatty acids (PLFAs), microbial respiration, and enzyme activities (Laudicina, Dennis, et al., 2012; Schloter et al., 2003). Both MBC and PLFAs reflect the size of soil microbial community (Li et al., 2018), and, in addition, the latter provides information about the relative importance of main microbial groups (Joergensen, 2022). On the other hand, microbial respiration and enzyme activities reflect the whole metabolic activity of soil microorganisms (Dilly et al., 2007). As essential catalysts for recycling organic matter, soil enzymes play a crucial role in soil fertility and agronomic production. In fact, quantitative and qualitative changes in soil enzymes may indirectly regulate the nutrient availability (Kanté et al., 2021). However, the combination of single bioindicators with more complex indicators, such as microbial quotient (Q<sub>micr</sub>), that is, the ratio between MBC and soil organic C (SOC), is likely more reliable for a better evaluation of the whole soil fertility (Anderson, 2003).

Organic farming is the only sustainable agricultural system that is legally defined within the EU. Indeed, crop and livestock products sold as organic must be certified as such under European Community Regulation 2092/91 and 1804/99. Organic farming systems essentially rely on the control of both content and quality of soil organic matter (SOM) to improve all soil properties, in order to sustain crop production (Watson et al., 2002). Indeed, the enhancement, or at least the maintenance, of soil fertility is one of the pivotal benefits of organic farming systems (Barnwal et al., 2021). Soils under organic farming systems usually exhibit better quality and higher biological activity than conventional farming systems (Krause et al., 2022). The soil manuring considerably stimulates microbial biomass and activity by improving soil chemico-physical properties and supplying easily assimilable C and nitrogen (N) sources (Ferreira et al., 2021; Ren et al., 2019). Generally, organic management of agricultural soils, that is, the addition of organic fertilizers, also promotes the organic matter accumulation by enhancing the C pools with slower turnover times (Badalucco et al., 2010; Laudicina et al., 2011). Through a meta-analysis including 149 pairwise comparisons originating from different climatic zones and experimental duration ranging from 3 to more than 100 years, Lori et al. (2017) showed that organic systems had 32% to 84% greater MBC and N, total phospholipid fatty-acids, and dehydrogenase, urease, and protease activities than conventional systems. Moreover, they found that differences in microbial size and activity between organic and conventional farming systems varied as a function of land use (arable, orchards, and grassland), plant life cycle (annual and perennial), and climatic zone.

Many studies have been carried out to evaluate the impact of shifting from conventional to organic farming systems on soil chemical and biochemical properties, but to our knowledge, few studies, if any, have investigated the response of soil fertility to different land use under long-term organic farming systems. Fromm et al. (1993) reported that biological soil fertility was more influenced by soil use and management practices (pasture vs. arable) than soil type, probably due to the relevant impact of tillage. In fact, several authors have demonstrated how conservation tillage or no tillage leads to increased soil microbial

biomass and diversity (Badagliacca et al., 2021; Cookson et al., 2008; Laudicina et al., 2011).

The aim of this study was to assess how three different land uses—pasture, vegetable crops, and orchard—affect soil fertility as a whole under a long-term organic farming system. It is hypothesized that soil chemical fertility (e.g., SOC and total N [TN]) decreases as the intensity of land use increases (pasture < orchard < vegetable crops). However, being the investigated soil under long-term organic management, it is also hypothesized that, though changes in chemical fertility might anyway occur, microbial biomass and its activity could be resistant to land use changes due to the continuous supply of organic substrates.

## 2 | MATERIALS AND METHODS

### 2.1 | Study area and treatments setup

The study was carried out on an organic farm (province of Latina, Lazio region, Italy).

Since the end of the 1950s and for about 30 years, soil has been left uncultivated or, in few cases, cultivated with conventional management. In 1998, the farm was converted to organic management and was divided into three equal parts, each one destined to a different land use: (1) pasture with cereals and legumes in rotation and a cattle load of about 50 sheep per hectare (thereafter identified as pasture, no tillage, no irrigation); (2) vegetable garden in continuous cycle with production of various vegetables (thereafter identified as garden, three-four tillage per year up to a depth of 12 cm, biomass removal, drip irrigation, organic amendment of 15 t ha<sup>-1</sup> y<sup>-1</sup>); (3) grassed plum orchard (thereafter identified as orchard, two-three tillage per year up to a depth of 15 cm, legume cover crop incorporation, drip irrigation, organic amendment of 15 t ha<sup>-1</sup> y<sup>-1</sup>). The organic amendment consisted of farmyard manure prepared by mixing ovine manures with plant residues and left in the open air for about 6 months. Each ton of compost has added to the soil, in average, 148.0, 7.9, and 0.44 kg ha<sup>-1</sup> y<sup>-1</sup> of organic C, TN, and P, respectively. Tillage consisted of harrowing both in the garden and the orchard. Soil sampling was carried out in April 1998, prior to the conversion to organic farming, and in May 2019. Soil samples were analyzed to determine the main chemical and biochemical properties. In May 2019, field-moist soil samples were collected from the topsoil (0–15 cm depth), and three samples (each consisting of five sub-samples) were taken for each land use.

### 2.2 | Soil chemical analyses

Soils samples were sieved at 2 mm and divided into two aliquots. The first one was rewetted up to 50% of water holding capacity (WHC) and pre-incubated at 25°C for 7 days before performing right after soil biochemical analyses. The second aliquot was air-dried and stored in sealed polyethylene bottles at 4°C before being used for soil chemical analyses. Soil texture (sand, 2–0.02 mm; silt, 0.02–0.002 mm; clay, <0.002 mm) was determined by pipette method (Gee & Bauder, 1986). Soil pH was measured in distilled water (actual acidity) or in 1 M KCl

solution (potential acidity) using a soil/solution ratio of 1:2.5 (w/v) and a glass membrane electrode. SOC and TN were determined on milled soil samples by the Walkley–Black dichromate oxidation method and the Kjeldahl method (Bremner, 1996), respectively. Cation exchange capacity was determined with ammonium acetate solution buffered at pH 7.0 (Sumner & Miller, 1996).

## 2.3 | Soil biochemical analyses

PLFAs of microbial cell membranes were extracted and analyzed according to the modified Bligh and Dyer's method as reported by Frostegård et al. (1993). Lipids were extracted with a mixture of chloroform, methanol, and a buffer solution (1:2:0.8 v/v/v), then separated on a chromatographic column into neutral lipids, glycolipids, and phospholipids. Then the latter were methylated and the resulting methylated fatty acids (FAMES) separated and quantified by gas chromatography. Nonadecanoic acid methyl ester was used as 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). Fatty acids (FAs) with less than 14 or more than 19 C atoms were neglected as originating from non-microbial sources. The FAs 15:0, i15:0, a15:0, i16:0, 16:1 $\omega$ 7, 17:0, i17:0, a17:0, 18:1 $\omega$ 7, cy19:0 represented the bacterial biomass, while 18:2 $\omega$ 6,9 corresponded to the fungal biomass and 16:1 $\omega$ 5 represented mycorrhizae (Joergensen, 2022). The FAs i15:0, a15:0, i16:0, i17:0; a 17:0 represented gram-positive bacteria (bacG+), while 16:1 $\omega$ 7, 18:1 $\omega$ 7, and cy19:0 gram-negative bacteria (bacG-). The abundance of each main microbial groups was expressed as the percentage of total FAs.

According to Bailey et al. (2002), MBC was calculated by applying the following relationship:

$$\text{mgC}_{\text{flush}} \text{kg}^{-1} \text{drysoil} = 2.4 \times (\text{nmolPLFAg}^{-1} \text{drysoil}) + 46.2, \quad (1)$$

and multiplying the calculated  $\text{C}_{\text{flush}}$  by 2.64 being the latter conversion factor proposed by Vance et al. (1987) with the  $\text{CHCl}_3$  fumigation-extraction method.

The  $\text{Q}_{\text{micr}}$  was calculated as the percentage of SOC present as MBC (MBC/SOC %; Anderson, 2003).

Soil respiration was determined by quantifying the  $\text{CO}_2$  evolved from the soil during 28 days of incubation. Briefly, 7 g of each soil sample moistened at 50% of WHC were placed in a 50-mL plastic syringe at 22°C, and after 1, 3, 7, 10, 15, 20, and 28 days of incubation, 1 mL was taken from the headspace of the syringe and  $\text{CO}_2$  determination was performed using a gas chromatograph equipped with a thermal conductivity detector (TraceGC, Thermo Fisher Scientific, s.r.l.).

Soil  $\beta$ -glucosidase, arylsulfatase, and phosphatase activities were determined by measuring the *p*-nitrophenol released after incubation of the properly buffered soil with, as substrates, *p*-nitrophenyl glucoside (Eivazi & Tabatabai, 1988), *p*-nitrophenyl sulphate (Tabatabai & Bremner, 1970), and *p*-nitrophenyl phosphate (Eivazi & Tabatabai, 1977; Tabatabai & Bremner, 1969), respectively. Used buffers were the

modified universal buffer (MUB) at pH 6.0, the acetate buffer at pH 5.8, and the MUB at the soil pH (in water). Protease was determined by quantifying the tyrosine released, during a controlled incubation (60 min at 50°C), from a soil aliquot to which sodium caseinate as substrate and a properly buffered solution (0.1 M Tris buffer at pH 8.1) were added (Ladd & Butler, 1972). Urease activity was determined by monitoring the ammonium release from a soil sample treated with urea as a substrate and incubated with borate buffer at pH 10.0 (Kandeler & Gerber, 1988). Triphenyltetrazolium chloride was used as a substrate (Thalmann, 1968), buffered with TRIS-HCl at pH 7.4 for dehydrogenase determination.

## 2.4 | Statistical analysis

Reported data are arithmetic means of three field-replicated soil samples for each land use and are expressed on an oven-dry basis (105°C). Before performing parametric statistical analyses, normal distribution and variance homogeneity of the data were checked by Kolmogorov–Smirnov goodness of fit and Levene's tests, respectively.

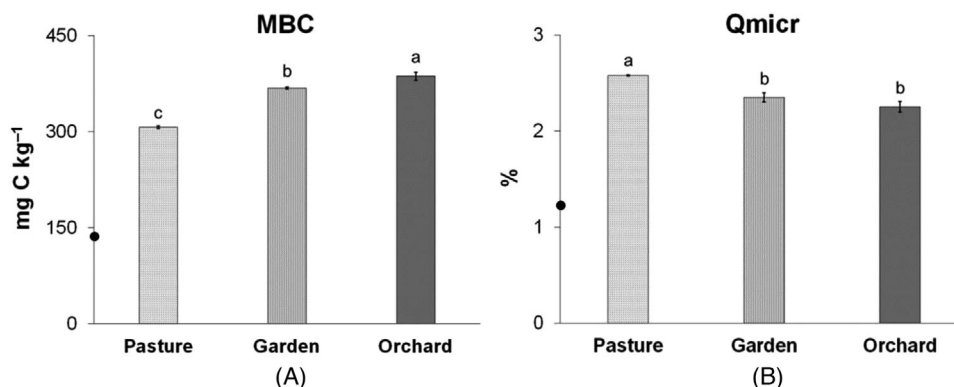
Soil data were analyzed with PROC GLIMMIX in SAS 9.2 (SAS Institute Inc.) testing treatment as a fixed factor and replicates as a random effect. When a treatment effect was detected, data were compared using Tukey's HSD (Honestly Significant Difference) test at the 5% probability level ( $p < 0.05$ ).

## 3 | RESULTS

The following soil characteristics, determined at the beginning of the experiment (year 1998) were observed: sandy-loam texture, actual acidity 6.9 (in water) and potential acidity 5.7 (in 1 M KCl solution), 0.6  $\text{dS m}^{-1}$  electrical conductivity, 10.5  $\text{g kg}^{-1}$  total organic C, 1.6  $\text{g kg}^{-1}$  TN, 141.7  $\text{mg kg}^{-1}$  soil microbial biomass, and 1.3%  $\text{Q}_{\text{micr}}$ .

Soil texture was always sandy loam, regardless of land use (data not shown). Both actual and potential soil pH values measured before conversion to organic farming were higher than pH values observed in soils after 21 years of different organic land use (Table 1; Table S1). The actual soil reaction was weakly acidic and showed the highest pH value (6.5) in the garden soil. As expected, values of potential soil reaction were rather lower than that of actual reaction and showed the lowest pH value in orchard soil. Also, electrical conductivity was slightly affected by land use and showed the highest value in garden soil (Table 1; Table S1). However, on average, no significant differences were recorded between the measurements taken before and after conversion to organic farming.

After 21 years of conversion to organic farming, there was an average increase of 42% in SOC. SOC ranged from 11.9 to 17.2  $\text{g kg}^{-1}$  and showed the highest values under orchard and the lowest in pasture. Also, TN, which ranged from 1.9 to 2.9  $\text{g kg}^{-1}$ , resembled the SOC pattern (Tables 1 and S1), increasing after organic conversion by an average of 56%.



**FIGURE 1** Soil microbial biomass carbon (MBC) (a) and microbial quotient (Qmicr) (b) of soil subjected to different land uses. The red dots on y-axis indicate the MBC and Qmicr values of soil at T0 (April 1998). Error bars indicate standard deviation ( $n = 3$ ). Different letters indicate significant differences at  $p < 0.05$ .

**TABLE 1** Chemical properties of soil subjected to different land use.

Land use	pH(H <sub>2</sub> O)	pH(KCl)	EC (dS m <sup>-1</sup> )	SOC (g kg <sup>-1</sup> )	TN (g kg <sup>-1</sup> )
Pasture	6.2 b	5.3 a	0.4 b	11.9 c	1.9 b
Garden	6.5 a	5.1 a	0.7 a	15.7 b	2.9 a
Orchard	6.3 ab	4.9 a	0.5 ab	17.2 a	2.7 ab

Note: Values represent the mean and standard deviation of three samples ( $n = 3$ ).

Abbreviations: EC, electrical conductivity; pH(H<sub>2</sub>O), pH in distilled water; pH(KCl), pH in 1 M KCl solution; SOC, soil organic carbon; TN, total nitrogen.

Different letters within the same column indicate significant differences at  $p < 0.05$ .

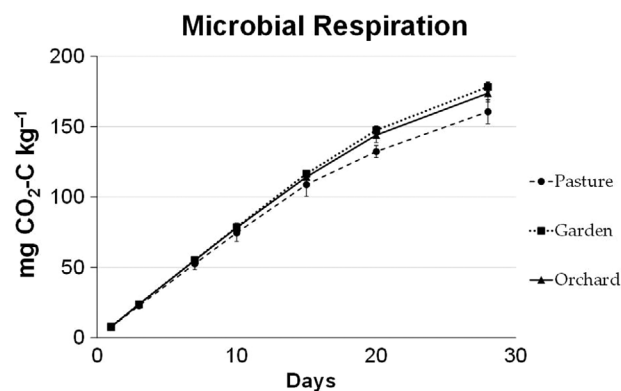
### 3.1 | Soil MBC and main microbial groups

Soil MBC ranged from 307 to 387 mg C kg<sup>-1</sup> and decreased according to the following order: orchard > garden > pasture (Figure 1a). The Qmicr showed significantly ( $p < 0.05$ ) higher values in pasture (2.6%) than in garden and orchard soils (2.3%, on average), between which no significant differences occurred (Figure 1b). Both MBC and Qmicr more than doubled after conversion to organic farming (Figure 1).

Regarding the main microbial groups, the percentage of fungi and bacteria were not affected by land use, although the ratio of fungi to bacteria was the lowest in pasture, compared to cultivated soils (Table 2; Table S2). bacG+ were the highest in the orchard and gram-negative in the garden. Additionally, the bacG+/G- ratio was the highest in the orchard, whereas no difference occurred between the soils of the remaining two land uses. Mycorrhizae exhibited the highest percentage in pasture and orchard soils.

### 3.2 | Soil microbial activity

Microbial respiration rate did not show significant differences among land uses except on the 20th day of incubation when the pasture soil showed significantly lower values than the other land uses. Toward



**FIGURE 2** Microbial respiration (CO<sub>2</sub> emission) during 28 days of incubation of soils under three different land uses (pasture, garden, orchard). Error bars indicate standard deviation ( $n = 3$ ).

the end of the incubation, however, microbial respiration was slightly higher in agricultural soils than in pasture soil, although not significantly (Figure 2).

Except for  $\beta$ -glucosidase, which showed a significant lower value in soil under pasture and urease, with a higher value in garden soil, all remaining enzyme activities did not show significant differences among land uses (Table 3; Table S3).

## 4 | DISCUSSIONS

### 4.1 | Land use effect on chemical indicators of soil fertility

One of the major benefits of organic cultivation to soil is to increase organic matter content, thus improving soil structure and fertility. SOC and TN increased after 21 years of organic management in all the three land uses, which agrees with previous findings (i.e., Crystal-Ornelas et al., 2021; Tong et al., 2022).

Land uses affected soil fertility, but contrarily to what was hypothesized, the higher land use intensities (garden and orchard) increased

**TABLE 2** Total phospholipid fatty acids (PLFAs; nmol FA g<sup>-1</sup>) and main microbial groups (% of total PLFA) determined in soil subjected to different land uses.

Land use	Total PLFAs (nmol g <sup>-1</sup> )	Bacteria (%)	Fungi (%)	BacG+ (%)	BacG- (%)	Mycorrhizae (%)	Fungi/bacteria	BacG+/G-
Pasture	31.6 c	79.1 a	13.4 a	22.1 c	52.7 b	7.5 a	0.17 a	0.42 b
Garden	40.7 b	84.8 a	10.6 a	24.8 b	56.5 a	4.6 b	0.12 b	0.44 b
Orchard	44.0 a	82.0 a	11 a	28.1 a	50.7 b	7.1 a	0.14 ab	0.55 a

Note: Values represent the mean and standard deviation of three samples ( $n = 3$ ).

Abbreviations: bacG-, gram-negative bacteria; bacG+, gram-positive bacteria.

Different letters within the same column indicate significant differences at  $p < 0.05$ .

**TABLE 3** Enzymatic activities of soil in response to different land uses.

Land use	$\beta$ -glucosidase (mg pNP kg <sup>-1</sup> h <sup>-1</sup> )	Dehydrogenase (mg TPF kg <sup>-1</sup> 24h <sup>-1</sup> )	Phosphatase (mg pNP kg <sup>-1</sup> h <sup>-1</sup> )	Arylsulfatase (mg pNP kg <sup>-1</sup> h <sup>-1</sup> )	Urease (mg NH <sub>4</sub> <sup>+</sup> -N h <sup>-1</sup> )	Protease (mg Tyr kg <sup>-1</sup> h <sup>-1</sup> )
Pasture	46 b	0.29 a	303 a	19 a	1.3 b	1.9 a
Garden	59 a	0.37 a	282 a	22 a	3.1 a	2.1 a
Orchard	52 a	0.26 a	276 a	20 a	1.2 b	1.5 a

Note: Values represent the mean and standard deviation of three samples ( $n = 3$ ).

Abbreviations: pNP, *p*-nitrophenol; TPF, 3-triphenyl formazan; Tyr, tyrosine.

Different letters within a column indicate significant differences at  $p < 0.05$ .

both soil SOC and TN content, compared to pasture. Changes of SOC as a function of the investigated land uses may depend on many different factors such as tillage, cover crops, organic amendment, crop growing duration, and biomass removal (Giacometti et al., 2013; Laudicina et al., 2011; Li et al., 2018; Lori et al., 2017).

The soil under pasture was expected to show higher SOC and TN content, compared to cultivated soils, as it did not provide for crop removal and soil tillage but benefited from direct manure addition by sheep and abundant rhizodepositions from grasses (Conant et al., 2003; Schipper et al., 2014). However, the continuous presence of sheep on the same soil coupled with the absence of the relevant supply of organic amendment, except for the direct manure addition by sheep, probably was the cause of the decrease of SOC and TN. Thus, such findings suggested that long-term pasture with cereals and legumes in rotation with a cattle load of about 50 sheep per hectare, although in absence of tillage, seemed to decrease soil chemical fertility.

On the other hand, the higher SOC and TN in cultivated soils, compared to pasture soil, may be ascribed to, besides the continuous supply of the organic amendment, also to legume cover crop incorporation, thus counteracting the negative effect of tillage and yield removal. The positive effect of the supply of organic amendment on SOC and TN agreed with the findings of many studies carried out in similar environments (Giacometti et al., 2013; Montiel-Rozas et al., 2018). However, the slightly lower amount of SOC in garden soil, compared to orchard soil, may be ascribed to the greater number of tillages applied to the first soil, compared to the latter. Indeed, it is well known that tillage speeds up the mineralization of SOM by disrupting soil aggregates, increasing soil aeration, and favoring the substrates' accessibility to

microorganisms (Laudicina et al., 2015; Six et al., 2000). Similar findings are reported by Laudicina et al. (2011), who found that even maintaining constant the amount of organic input, high tillage intensity causes a decrease in the amount of SOC and TN. However, also irrigation might have contributed to the increase in SOC. Indeed, in semiarid agricultural systems, irrigation may increase SOM due to higher biomass production (Núñez et al., 2022).

Land use also affected soil potential (exchangeable) acidity. Indeed, the highest differences between soil pH values in water and KCl occurred for cultivated soils (1.4 vs. 0.9) and were proportional to SOC contents. In general, pH decreased in all land uses, compared to values measured in soil, before conversion to organic farming.

Soil acidification correlated to the SOC increase could be due to the formation of soluble complexes between humic acids and non-acidic cations (Ca<sup>2+</sup>, Mg<sup>2+</sup>), which were easily leached, or to the dissociation of the acid functional groups of humic and fulvic acids with consequent release of H<sup>+</sup> ions (Brady & Weil, 2017). The increase in soil acidity by increasing SOC agrees with Guckland et al. (2009) who found that an increase of SOM causes a decrease of pH values due to the contribution of organic acids. Also, Huang et al. (2009), studying 886 soils, demonstrated a significant negative correlation between organic matter content and soil pH.

## 4.2 | Land use effect on biochemical indicators of soil fertility

After conversion to organic farming, both MBC and Qmicr were more than doubled. This suggests that the MBC and Qmicr increased significantly due to the shift to organic farming practices as already

widely demonstrated by several authors (i.e., Lori et al., 2017; Peltoniemi et al., 2021; Santos et al., 2012).

MBC resembled SOC patterns, being the lowest in pasture and the highest in orchard and garden soil. The responsiveness of MBC to farmyard manure addition agrees with previous studies (e.g., E. Liu et al., 2010; Palazzolo et al., 2019) and may be ascribed to the stimulation of soil microorganisms due to the improvement of soil physical characteristics and the supply of readily available sources of major macronutrients. Such behavior suggested that the supply of farmyard manure counteracted the negative impact of tillage on MBC. Moreover, also irrigation might regulate MBC. Indeed, Z.-X. Liu et al. (2015) reported that organic manure application with moderate irrigation could increase the quantity of the soil microbial biomass and microbial diversity. The increase of MBC is fundamental as it represents a source of labile nutrients and a catalyst for organic matter mineralization (Laudicina, Dennis, et al., 2012). However, contrarily to MBC, the  $Q_{micr}$  was the highest in pasture and the lowest in orchard and garden, thus suggesting a better SOC use efficiency by microbial biomass in soil with the lowest land use intensity. The lower SOM quality in cultivated soils, compared to pasture one, can be ascribed to the effect of tillage. Indeed, as reported by Laudicina et al. (2015), tillage favors the degradation of aliphatic groups of molecules such as lipids, FAs, alkanes, and alkenes, leading to a parallel increase of aromatic groups (phenols and lignin monomers and alkyl-aromatics and N components; cf. Schnitzer et al., 2006). These, being recalcitrant to microbial assimilation, do not allow a proportional increase of MBC (Hättenschwiler & Vitousek, 2000). Such a hypothesis is confirmed by soil respiration data. Indeed, soil respiration showed a similar pattern among the land uses but, toward the end of the incubation (after 20 days), it was slightly lower in pasture soil, compared to agricultural ones. This suggests that tillage, by disrupting soil aggregates, increases the accessibility of organic substrates to microbial biomass, thus increasing  $CO_2$  emission (Laudicina, Barbera, et al., 2012). However,  $Q_{micr}$ , regardless of land use, ranged between 2.3% and 2.6%, thereby suggesting an overall good balance between MBC and SOC (Jenkinson & Ladd, 1981).

The fungi/bacteria ratio decreased in agricultural soils probably due to the high availability of organic substrates following the addition of farmyard manure that favors the growth of bacteria over fungi. Moreover, tillage also favors bacteria over fungi since, by disrupting soil aggregates, it determines high accessibility to organic substrates (Badagliacca et al., 2021; Six et al., 2000). Such findings agree with those of Giacometti et al. (2013) and Marschner et al. (2003) who found high bacterial biomass and  $bacG+$  in manure-amended soils. In addition, the increase of this microbial group copies the increase in SOC concentration in both agricultural soils and, as postulated by Fanin et al. (2019), can be linked to their oligotrophic metabolism, which prefers soil-derived C formed by more recalcitrant organic and stable C compounds. The effect of tillage on microbial groups was evident also considering the relative abundance of mycorrhizae. Indeed, they were higher in pasture and orchard soils, compared to garden one, that is, in those soils less tilled. This finding is reasonable since tillage contributes to suppression of fungal hyphae, and hence fungal growth, by intensive soil perturbation (Laudicina et al., 2016; Wang et al., 2017).

The changes in microbial biomass and in the main microbial groups were not accompanied by changes in functionality, except for  $\beta$ -glucosidase and urease activity, thus suggesting a high functional redundancy of the soil microbial community. Functional redundancy is a common phenomenon in microbial communities since a wide range of taxa may be able to perform similar metabolic functions, and as a result, compositional changes in the microbial community do not always affect ecosystem processes (Louca et al., 2018). Such finding, which agrees with Watzinger et al. (2023) and Zhang et al. (2023), may be ascribed to the greater buffering capacity in terms of organic matter and nutrient availability of agricultural soils.

Enzymes are the vital activators in life processes; likewise, in the soil, they are known to play a substantial role in maintaining soil health and quality. The enzymatic activity in the soil is mainly of microbial origin, being derived from intracellular, cell-associated, or secreted enzymes. Urease activity in soils is influenced by many factors. These include cropping history, organic matter content of the soil, N availability, soil depth, soil amendments, heavy metals, and environmental factors such as temperatures (Acosta-Martínez et al., 2003; Tabatabai, 1977). Among all the factors potentially affecting urease, probably in this study, it was affected by the higher N uptake by vegetables in the garden land use, as well as by continuous tillage that aerating frequently the soil stimulated the turnover of microbial biomass, with consequent higher secretion of the urease.

## 5 | CONCLUSION

In general, after 21 years, the conversion to organic management had a positive impact on both chemical and biochemical soil fertility in this study. Three different land use under long-term organic farming were investigated.

Based on the putative average crop yields throughout the 21 years of organic management, pasture was considered a land use at low intensity, plum orchard cover cropped with legume at medium intensity and vegetable garden at high intensity. Despite to what was expected, by increasing land use, intensity soil chemical and biochemical parameters were improved. Indeed, SOC, TN, and MBC increased as land use intensity increased. Such an improvement was ascribed to the supply of farmyard manure to soil that counteracted the negative impact of tillage on soil fertility. On the other hand, the supply of organic substrates shaped the main microbial groups by favoring the bacterial biomass, mainly bacteria gram-positive. Despite the observed changes of MBC and of the main microbial groups, microbial activity was slightly affected, thus suggesting high functional redundancy of microbial community in soil under long-term organic farming. Overall, results suggested that if land use intensification provides for organic supply, its negative impact on soil fertility may be mitigated.

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## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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