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Conventional versus organic management: application of simple and complex indexes to assess soil quality

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1 **Conventional *versus* organic management: application of simple and complex indexes to assess**  
2 **soil quality**

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9

10 **Abstract**

11 Organic management aims to maintain and/or enhance the soil quality by adopting crop rotation, reduced  
12 tillage, and application of organic fertilizers. Several studies highlight the advantages of organic  
13 management in terms of soil quality and soil fertility, the key themes in sustainable agronomy. The aim  
14 of this study was to understand whether there were differences in soil quality between two different  
15 approaches of organic management. Soil quality was assessed using a set of biochemical parameters  
16 individually or in combination, in simple and complex indexes, respectively. Soil samples were procured  
17 from a long-term field experiment area located in Monsanpolo del Tronto (Central Italy) in which  
18 conventional (Conv), and two different organic managements (Org1, organic traditional; Org2, organic  
19 agroecological) were followed, and were assessed for soil quality. Results from individual soil properties,  
20 simple and complex indexes highlighted that Conv and Org1 were similar in terms of acquisition activity  
21 indicating that both mineral and organic fertilizers supply C, N, and P equally to agro-ecosystems.  
22 However, in Org2, despite theoretical imbalances in terms of C:N:P, microbial activity efficiently filled  
23 this imbalance without affecting soil organic carbon content. The indexes showed a positive metabolic  
24 response and a higher soil quality in both the organically managed plots, indicating that organic  
25 management, independent of the agronomic approach, maintains the soil quality despite the differences

26 in biochemical processes. Moreover, simple and complex soil quality indexes were sensitive enough to  
27 discriminate the two organic management strategies.

28

29

30 *Keywords:*

31 soil functionality; agronomic management; strip cropping; enzyme activities; biochemical indexes

32

33 *Highlights:*

- 34 • Conventional and organic management effects on soil quality were evaluated
- 35 • Soil organic C and microbial biomass were enhanced by the organic managements
- 36 • Specific hydrolytic enzyme activities increased in the conventional management
- 37 • Soil quality indexes highlight differences between the two organic managements
- 38 • Soil quality indexes help in understanding the soil biochemical processes

## 39    **Introduction**

40    Agriculture is moving from conventional to more sustainable soil management with a 102% increase in  
41    organic managed areas from 1999 to 2019 globally (IFOAM and FiBL, 2021) and a 46% increase from  
42    2012 to 2019 in the European Union (Eurostat, 2020). Indeed, intensive tillage leads to degradation of  
43    ecosystems through soil erosion, water contamination, deforestation, desertification and, consequently,  
44    to loss of productivity (Edwards et al., 1993; Garini et al., 2017). In contrast, sustainable agriculture is  
45    based on integrated systems of agricultural production with minimum dependence upon high inputs of  
46    energy, that should maintain – or only slightly decrease – productivity and protect the environment from  
47    soil and food contamination, preserve ecological diversity, and maintain and/or improve soil quality and  
48    fertility (Edwards et al., 1993; Francis and Wezel, 2015). Therefore, the major challenge is to identify safe  
49    and secure methods to produce food, while reducing the negative impacts of agriculture on the  
50    environment (Dupré et al., 2017).

51    Organic management aims to achieve the required yield while maintaining soil quality by the adoption of  
52    crop rotation with cover crops, reduced tillage (Hartmann et al., 2015; Tilman et al., 2002), and the  
53    application of organic fertilizers such as animal and green manures, and off-farm organic wastes  
54    (Drinkwater et al., 1995). Moreover, organic agriculture contributes to global food supply (Luo et al.,  
55    2019; Sandhu et al., 2010) although the yields obtained are usually lower than in conventional agriculture  
56    (Bonanomi et al., 2016; De Ponti et al., 2012). Soil quality and fertility not only affect productivity and  
57    mineral nutrition, but also determine the biological equilibrium and regulation of agroecosystems  
58    (Lemanceau et al., 2014). Several studies have been conducted to compare the effects of organic and  
59    conventional management strategies on soil quality and fertility. Results from studies conducted in  
60    Central Italy and Spain (temperate areas characterized by different soil textures and pH range between  
61    6.8–7.8) highlight that in both arable and orchard systems, organic agriculture, characterized by the  
62    application of compost, manure, or crop residues, induced a higher soil organic carbon (SOC) content  
63    (10–50%) and higher microbial (enzymatic) activity (~30%) compared to conventional agriculture (Baldi  
64    et al., 2018; García-Ruiz et al., 2008; Marinari et al., 2006; Mazzon et al., 2018; Plaza et al., 2012). Braman

et al. (2016), in a study conducted in Canada on a clay soil with a pH of 7.4, and Chavarria et al. (2018), in a study conducted in Argentina on a silt loam soil with a pH of 5.8, demonstrated lower values of the soil metabolic quotient ( $qCO_2$ , a metabolic efficiency index determined by the ratio between soil respiration and microbial biomass carbon content) in organic management, thus suggesting a high efficiency of using organic carbon (C) sources under this management. Kwiatkowski et al. (2020), in an experiment comparing organic and conventional management on a silt loam soil with a pH of 6.5 in Poland, found higher SOC and total nitrogen (TN) contents as well as a higher soil pH in the organic system compared to the conventional one. Moreover, worldwide studies and meta-analyses comparing organic and conventional managements (Drinkwater et al., 1995; Luo et al., 2019), including analysis of soil tillage intensity (Laudicina et al., 2011; Roldán et al., 2005), and of N dynamics from mineral and organic fertilization (Toselli et al., 2019), highlight the advantages that organic management provides in term of sustainability, soil quality and soil fertility.

Several soil chemical and biochemical indicators are useful in determining the soil quality: chemical (pH, organic C, total N, and available P), physical (texture, bulk density, and water retention) and biological (microbial activity and biomass) (Bünemann et al., 2018). However, it is noteworthy that soils react slowly to changes in management; therefore, in assessing soil quality, the identification of an appropriate and sensitive set of soil attributes is an important step (Bünemann et al., 2018). For example, physical soil properties are certainly important in soil quality determination but they are considered “slow-changes” indicators, while biochemical properties are considered “dynamic” indicators connected to soil functionality and dynamics in relation with the nutrient acquisition processes (Bünemann et al., 2018; Muñoz-Rojas, 2018). Within the biochemical indicators, the soil enzymatic activities are considered to be sensitive and early indicators of changes in soil quality (Gil-Sotres et al., 2005; Nannipieri et al., 2018, 2002; Sinsabaugh et al., 2008). Indeed, they are natural catalysts for many important soil processes, such as organic matter decomposition, nutrient release, molecular N fixation, and C, N, and other major nutrient cycles (Balota et al., 2004; Kwiatkowski et al., 2020; Wallenstein et al., 2012). Nonetheless, it is inappropriate to consider a single enzymatic activity as an index of soil quality or soil fertility, which

91 depends on many soil reactions and properties (Gil-Sotres et al., 2005; Nannipieri et al., 2002; Nannipieri  
92 et al., 2018). Gil-Sotres et al. (2005) delineated three approaches in using soil biochemical parameters to  
93 estimate soil quality: (i) individual properties, (ii) simple indexes derived from the relationship between  
94 two individual properties, and (iii) complex indexes derived from the combinations of different properties  
95 or on the basis of statistical procedures. Individual properties could be, for example, SOC, microbial  
96 biomass, nitrogen mineralization, soil respiration, and dehydrogenase activity. However, individual  
97 properties could return contradictory results, and being the expression of specific soil processes, they  
98 cannot reflect many of the reactions which determine soil quality. Therefore, simple indexes could  
99 overcome the constraints of individual properties and fulfil the requirements of a good indicator (Gil-  
100 Sotres et al., 2005). The four most frequently used simple indexes are the specific enzyme activities (the  
101 ratio of soil enzymatic activities to the microbial biomass), the metabolic quotient (the ratio of soil basal  
102 respiration to the soil microbial biomass,  $qCO_2$ ), the microbial quotient (the ratio of microbial C to the  
103 total organic C), and the metabolic index (the ratio of dehydrogenase activity to the extractable organic  
104 C, MI). Nevertheless, simple indexes possess limitations in terms of soil quality definition, as they do not  
105 have reference levels, and their responses are not always easy to relate to specific soil modification  
106 processes. Complex indexes evaluate the soil quality by combining different biochemical properties such  
107 as the biological fertility index, the enzymatic activity number, the lignocellulosic factor of Sinsabaugh et  
108 al. (1994), and the geometric mean of enzyme activities. Other complex indexes are obtained by applying  
109 statistical techniques to combine the biochemical properties (Gil-Sotres et al., 2005) and thus, the use of  
110 complex expressions or statistical procedures can appropriately describe the complexity of soil systems.  
111 In this study, as complex indexes, we focused on the ecosystem ratios of C:N, C:P and N:P acquisition  
112 activity and the soil management assessment framework (SMAF). The ecosystem ratios are given by the  
113 natural logarithm of enzyme activities involved in C, N, and P cycles, and are a measure of the enzymatic  
114 resources directed to the acquisition of organic P and organic N relative to C (Sinsabaugh et al., 2008).  
115 The soil management assessment framework (hereafter in the text SQI – Soil Quality Index) aims to  
116 evaluate the soil quality using a minimum dataset chosen from a larger dataset that includes physical,

117 chemical, and biological soil properties (Andrews and Carroll, 2001). We chose these two complex  
118 indexes as they satisfy the majority of the characteristics for a good indicator: they measure one or more  
119 soil functions, are sensitive and respond quickly to changes in soil management, and are easy to obtain  
120 and interpret.

121 The use of biochemical indexes to assess soil quality is not novel, although their practical application in  
122 field experiments is more recent. However, Bunemann et al. (2018) stressed that an overall SQI, even if  
123 desirable, must be cautiously used to assess soil quality only in relation to specific soil functions. In  
124 addition, the sensitivity of selected indexes to evaluate the soil quality in different ecosystems and the  
125 ability to discriminate between different soil management systems is debatable. Nowadays, for example,  
126 different organic management systems have been developed, and we need affordable procedures to  
127 evaluate their strengths and weaknesses.

128 In light of the above, a long-term field experiment in a Mediterranean area (Marche Region, Italy), with  
129 conventional and two different organic managements, was performed to understand whether there are  
130 differences in soil quality between the different management strategies. Specifically, we asked: *i)* Do the  
131 organic managements always lead to better soil quality compared to the conventional management? *ii)*  
132 Do the soil biochemical indexes differ between the two organic management approaches? *iii)* Can soil  
133 quality indexes help us to understand the soil biochemical processes under different organic management  
134 strategies?

135

## 136 **Material and Methods**

### 137 **Area of study**

138 The investigations were conducted at the Council for Agricultural Research and Economics – Research  
139 Centre for Vegetable and Ornamental Crops (CREA-OF) in Monsampolo del Tronto, Marche Region,  
140 (latitude 42°53' N, longitude 13°48' E). The climate of the locality is classified as thermo-Mediterranean,  
141 characterized by mild–cool winters and hot summers with cumulative annual precipitation and mean

annual temperature (in 2018) of 789 mm and 15.7 °C, respectively. The soil is classified as Typic Calcixerepts fine-loamy, mixed thermic (USDA, 1996) (USDA Soil Taxonomy, 2006).

The experimental design included three experimental areas: two were within an organic field managed since 2001, according to European legislation for organic farming, and one was within a neighboring conventional field. Three agronomic management strategies for tomatoes (*Solanum lycopersicum* L.) were studied: Org1 (organic traditional), Org2 (organic agroecological) and Conv (conventional).

Descriptions of the agronomic practices for each management are provided in Table 1. The Org2 management is part of the MOnsampolo VEgetable (MOVE) organic long-term field experiment and is characterized by a four-year crop rotation (Campanelli and Canali, 2012). In particular, the in-line roller-crimper technique (Canali et al., 2013) was used for flattening strips of faba bean (*Vicia faba* L.) after fresh pod harvest. In this way, a conservation tilling strategy and strip cropping cultivation of tomato for the fresh market and faba bean for dry grain harvest was realized. The measurements were conducted on a surface area of 176 m<sup>2</sup> for each experiment. In June 2018, from the plot of each management, during tomato-growing season, soil samples from the top 20 cm soil profile were taken along the tomato rows, for a total of 24 soil samples (8 per plot). In each plot, the external rows were not sampled to avoid border effects owing to neighboring crops. Fresh samples were immediately kept in a cooler for transportation to the laboratory where they were sieved to 2 mm; roots and plant residues were carefully removed using forceps. Samples were then homogenized and divided into two aliquots: one was air-dried and the other was stored in plastic bags at 4 °C.

161

## 162 **Soil chemical and biochemical analysis**

Soil pH was measured in ultrapure water (ISO 10390, 2005). SOC and total N (TN) were analyzed using an elemental analyzer (Flash 2000; Thermo Fisher Scientific, USA). Soil microbial biomass C (MBC) and N (MBN) were determined using the chloroform-fumigation extraction method (Vance et al., 1987), and the potassium sulfate extracts were analyzed using an elemental analyzer (TOC - TN Hypertoc; Shimadzu Corp., Kyoto, Japan). MBC was calculated as the difference in organic C between the fumigated and



unfumigated soil extracts. The organic C content of the unfumigated extracts was used as an estimation of soil extractable C (DOC, dissolved organic C); similarly, MBN and soil extractable N (TDN, total dissolved N) were calculated.

Available phosphorous ( $P_{\text{Olsen}}$ ) was determined according to Olsen method (Olsen et al., 1954) using a spectrophotometer (Jasco V-530 UV/VIS Spectrophotometer; JASCO Corporation, Japan) at  $\lambda = 882$  nm.  $P_{\text{Olsen}}$  content was expressed as  $\text{g kg}_{\text{ds}}^{-1}$ . Soil basal respiration rate (SBR) was determined according to Isermeyer (1952) and expressed as mg of C-CO<sub>2</sub> produced per kg of dry soil during the incubation time ( $\text{mg C-CO}_2 \text{ kg}_{\text{ds}}^{-1} \text{ h}^{-1}$ ).

#### **Soil enzymatic activities**

The activities of eight extracellular enzymes and one intracellular enzyme were measured (Table S1). Six extracellular hydrolytic enzyme activities were determined using fluorogenic 4-methylumbelliferyl (MUF) conjugates according to Giacometti et al. (2014) in the equivalent of 2 g of dried soil. The 96-well microplates were incubated in the dark at 30 °C and fluorescence was measured using a microplate fluorometer (Infinite® 200; TECAN, Männedorf, Switzerland) with  $\lambda$  at 365 nm and 450 nm for excitation and emission, respectively. The microplates were shaken for 5 s before each reading and measurements were recorded immediately after the plate setup and at intervals of 30 min, for a duration of 3.5 h. The activity was expressed as  $\text{nmol MUF g}_{\text{ds}}^{-1} \text{ h}^{-1}$ . Measurements for the following oxidative enzymes were performed spectrophotometrically (Jasco V-530 UV/VIS Spectrophotometer; JASCO Corporation, Japan).

Dehydrogenase activity (Dehy) was measured as described by Von Mersi and Schinner (1991). Released iodo-nitrotetrazolium formazan (INTF) was measured at  $\lambda = 464$  nm and the activity was expressed as  $\text{nmol INTF g}_{\text{ds}}^{-1} \text{ h}^{-1}$ . Laccase activity (Lac) was determined as described by Floch et al. (2007), and the reaction product, 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS<sup>+</sup>), was measured at  $\lambda = 420$  nm. The activity was calculated using a molar extinction coefficient of  $18460 \text{ L mol}^{-1} \text{ cm}^{-1}$  and expressed as  $\mu\text{mol ABTS}^+ \text{ g}_{\text{ds}}^{-1} \text{ min}^{-1}$ . Tyrosinase activity (Tyr) was measured as described by Sinsabaugh et al.

194 (1999). The absorbance was measured at  $\lambda = 475$  nm, and the activity was calculated using a molar  
195 extinction coefficient of  $3700 \text{ L mol}^{-1} \text{ cm}^{-1}$  and expressed as  $\mu\text{mol DOPA g}_{\text{ds}}^{-1} \text{ h}^{-1}$ .

196

### 197 **Simple soil quality indexes**

198 The following simple indexes were determined: the metabolic quotient ( $q\text{CO}_2$ ), calculated by dividing the  
199 SBR by the MBC and expressed as  $\text{mg C-CO}_2 \text{ g}_C^{-1}$ , represents the quantity of substrate mineralized per  
200 unit of microbial biomass, per unit of time (Gil-Sotres et al., 2005), and indicates the usage efficiency of  
201 C sources (Anderson and Domsch, 1993; Bastida et al., 2008); the metabolic index (MI), obtained by  
202 dividing the Dehy by the DOC and expressed as  $\mu\text{mol INTF g}_C^{-1} \text{ h}^{-1}$  (Masciandaro et al., 1998), connects  
203 the possible C availability for microbial metabolism with the microbial activity (Bastida et al., 2008); and  
204 the specific soil enzymatic activities (Gil-Sotres et al., 2005; Kandeler and Eder, 1993; Trasar-Cepeda et  
205 al., 2008), calculated by dividing the enzymatic activity values with the MBC; an increase may be due to  
206 more enzyme production, more release of enzymes immobilized in clays or humic colloids (Kandeler and  
207 Eder, 1993), or an increase in substrate available for enzymatic activity (Bastida et al., 2008).

208

### 209 **Complex soil quality indexes**

210 Complex soil quality indexes as ecosystem ratios and SQI were calculated. The ecosystem ratios were  
211 determined using the natural logarithm of the soil enzymatic activities involved in C, N, and P cycles,  
212 thus determining the C:N ( $\text{C:N}_{\text{enz}}$ ), C:P ( $\text{C:P}_{\text{enz}}$ ) and N:P ( $\text{N:P}_{\text{enz}}$ ) acquisition activity (Sinsabaugh et al.,  
213 2008); for example, the  $\text{C:N}_{\text{enz}}$  was obtained by dividing the  $\ln(\beta_{\text{glu}} + \alpha_{\text{glu}} + \beta_{\text{cel}} + \beta_{\text{xyl}})$  by the  $\ln(\text{NAG})$ .  
214 The SQI was determined using a process (Fig. 1) that included i) indicator selection, ii) indicator scoring  
215 and iii) integration of the scores into the index. Briefly, the minimum dataset (MDS) was selected from  
216 the principal component analysis (PCA), in which the principal components (PC) with eigenvalues  $\geq 1$   
217 and the properties with highest loadings were assumed to best represent the system. The correlation was  
218 then used to reduce the redundancy between parameters and only the parameters that did not correlate  
219 with each other were selected. Each selected parameter was standardized to a value between 0 and 1 using

functions such as “more is better”, “less is better”, or “optimum” depending on the variables. Specific enzymatic activities were included and standardized using the function “less is better”. Finally, SQI was calculated using the “weighted additive” equation (Eq. 1) that comprises the sum of the scores ( $S_i$ ) of the MDS multiplied by the amount of variation ( $W_i$ ) in the corresponding PC. In general, higher SQI values correspond to higher soil quality (Andrews et al., 2002, 2004; Andrews and Carroll, 2001; Askari and Holden, 2015). (Andrews et al., 2004, 2002; Andrews and Carroll, 2001; Askari and Holden, 2015)

$$SQI = \sum_{i=1}^n W_i S_i \quad (\text{Eq. 1})$$

## Statistical analysis

Data were analyzed with the Kruskal-Wallis nonparametric test ( $\alpha < 0.05$ ) and multiple comparisons were performed using Dunn’s test ( $\alpha < 0.05$ ). PCA was performed as a multivariate analysis of data (C and N pools, available P, and enzymatic activities) using the “princomp” function. PCA was performed (C and N pools, available P, and specific enzymatic activities) as the first step for the determination of SQI. Finally, Pearson correlation was conducted on all the considered parameters and indicators (Table S2). All statistical analyses were performed using R software (R Core Team, 2020).

## Results

### Soil chemical and biochemical analysis

Soil pH showed a slight increase over time (it was 7.8 in 2001) and was significantly affected by management, with a slight increase in Org2 (Table 2). The SOC and TN content (Table 2) showed the same trend with a significant increase in the order Conv < Org2 < Org1. SOC and TN increased by 15–37% and 10–37%, respectively, in Org1 compared to Conv and Org2 management. Both Conv and Org1 promoted DOC and TDN content (+16% and +42%, respectively) compared to Org2 management (Table 2). The DOC:TDN ratio (Table 2) showed values increasing in the order Org1 < Conv < Org2. In contrast, MBC (Table 2) was enhanced in Org2 (+29%) and Org1 (+23%) compared to Conv. Although a similar trend was observed for MBN (+19% and +10% with Org2 and Org1, respectively),

the differences were not statistically significant ( $p$ -value = 0.570) (Table 2). No significant differences ( $p$ -value > 0.05) were observed in MBC:MBN, SBR, and  $P_{\text{Olsen}}$  (Table 2).

#### **Soil enzymatic activities**

The activities of  $\beta$ -glucosidase,  $\beta$ -xylosidase,  $\beta$ -cellobiosidase and N-acetyl- $\beta$ -glucosaminidase were significantly higher (with  $p$ -value < 0.05) in Org1 (+35%, +28%, +57%, and +33%, respectively) (Fig. 2). A similar trend was observed for  $\alpha$ -glucosidase activity (+15%), but the differences were not statistically significant ( $p$ -value = 0.444) (Fig. 2B). Phosphomonoesterase activity significantly ( $p$ -value = 0.002) increased in both Org1 (+26%) and Org2 (+36%) management compared to Conv (Fig. 2F). Dehydrogenase activity (Fig. 2G) was higher in Org2 (+15% compared to Org1 and +36% compared to Conv). A similar trend was observed for laccase activity (Fig. 2H) which increased by 18% and 48% in Org2 compared to Org1 and Conv, respectively. However, tyrosinase activity (Fig. 2I) was higher in Conv (+23% compared to Org1 and +14% compared to Org2).

#### **Simple soil quality indexes**

The  $q\text{CO}_2$  decreased while the MI increased in the order Conv - Org1 - Org2 (Table 3), with Conv showing +21% in the  $q\text{CO}_2$  and -38% in the MI values with respect to the average mean of Org1 and Org2. The specific hydrolytic enzyme activities (Table 3) of  $\beta$ -glucosidase,  $\alpha$ -glucosidase, N-acetyl- $\beta$ -glucosaminidase, and  $\beta$ -xylosidase were lower in Org2, while phosphomonoesterase showed no differences between the managements, and  $\beta$ -cellobiosidase showed lower activity for both Conv and Org2 management. Among the oxidative specific enzymatic activities, only tyrosinase activity was significantly ( $p$ -value = 0.034) different between managements, with higher values in Conv (+65%).

#### **Complex soil quality indexes**

The C:N ecosystem ratio (Fig. 3A) increased in the order Conv < Org1 < Org2, while the C:P and N:P ecosystem ratios (Fig. 3B, 3C) showed the opposite result, with lower values in Org2 (-11% and -18%,

272 respectively). The SQI (Fig. 3D) was characterized by a minimum dataset composed of specific activities  
273 of NAG and Dehy, TN, TDN and MI. The final index highlighted the lower SQI in Conv, with no  
274 significant differences between Org1 and Org2.

275

## 276 **Principal Component Analysis**

277 PCA was performed on C and N pools, available P, and specific enzyme activities for the three  
278 management strategies considered. The first two PCs accounted for 42% and 20% of the total variance,  
279 respectively (Fig. 4). Conv was distinct from both Org1 and Org2, and characterized by different  
280 parameters. Moreover, a slight clustering was observed between the two organic managements. However,  
281 focusing on the comparison between Conv and Org, the latter was characterized by SOC, TN, P<sub>Olsen</sub>,  
282 microbial biomass (MBC and MBN), and specific activities of Lac, PME,  $\beta$ -glu, and  $\beta$ -cel, while the  
283 specific activities of Tyr and  $\alpha$ -glu defined the former.

284

## 285 **Discussion**

### 286 **Conventional vs traditional organic management (Org1)**

287 Soil chemical and biochemical parameters were significantly affected by the different management  
288 strategies. As observed in previous studies (Baldi et al., 2018; García-Ruiz et al., 2008; Marinari et al.,  
289 2006; Mazzon et al., 2018; Plaza et al., 2012), also in this work SOC content increased with the organic  
290 management following the trend Conv (< Org2) < Org1. This difference between Conv and Org1 was  
291 mainly because Org1 management involves the addition of organic fertilizers, which could increase SOC  
292 content. This was previously observed also in other studies where manure increased SOC content by 35–  
293 50% compared to mineral fertilization (Giacometti et al., 2014; Plaza et al., 2012). In the Conv-managed  
294 plot, where the lowest SOC content was measured, we observed an increase in qCO<sub>2</sub> and a decrease in  
295 MI, highlighting that the microbial community was under stressed (Balota et al., 2004; Braman et al.,  
296 2016; Laudicina et al., 2011) and less efficient conditions (Masciandaro et al., 1988; Saviozzi et al., 2001).  
297 Therefore, it is likely that the mineral fertilizer application in Conv management promoted the microbial

298 activity, but with a low usage efficiency of C compounds, thus impacting the SOC pool (Braman et al.,  
 299 2016). The organic management exhibited lower  $qCO_2$  and higher MI; similar results were obtained by  
 300 Fließbach et al. (2007) who observed lower  $qCO_2$  in organically managed soils, and Saviozzi et al. (2001)  
 301 who found higher MI values in forest and grassland soils than in arable fields. Thus, organic management  
 302 could have resulted in soil conditions that favor microbial activity, which have better energy (i.e., carbon)  
 303 efficiency, thereby favoring SOC accumulation (Fließbach et al., 2007; Saviozzi et al., 2001).  
 304 On the other hand, both the organic and mineral fertilizers applied in Org1 and Conv-managed plots,  
 305 respectively, led to an increase in the available pools of C and N. This increase would lead to the  
 306 supposition that similar hydrolytic enzyme activities linked to C and N cycles ( $\beta$ -glu,  $\alpha$ -glu, NAG,  $\beta$ -xyl,  
 307 and  $\beta$ -cel) would be observed in Org1 and Conv. It is noteworthy that both organic management (with  
 308 compost application) and N fertilization could increase the enzyme activities involved in C and N cycles  
 309 (Bowles et al., 2014; García-Ruiz et al., 2008; Stursova and Sinsabaugh, 2008). However, only Org1  
 310 resulted in higher enzymatic activities, probably owing to the input of organic fertilizers (Baldi et al., 2018;  
 311 García-Ruiz et al., 2008; Marinari et al., 2006). Nevertheless, the lower MBC in Conv makes the  
 312 comparison of the enzymatic activities difficult, as their changes could be due to both the management  
 313 and/or the MBC content. For this reason, it is more appropriate to consider the specific enzyme activities  
 314 (Gil-Sotres et al., 2005; Mazzon et al., 2018) which gives the enzymatic activity per unit of MBC, thus  
 315 highlighting the differences owing to management strategies. Indeed, the specific hydrolytic enzyme  
 316 activities involved in C and N cycles ( $\beta$ -glu,  $\alpha$ -glu, NAG, and  $\beta$ -xyl) showed similar values (generally  
 317 higher than those measured under the Org2 management) in Conv and Org1, confirming that both  
 318 mineral N fertilization and compost addition induced the production/activation of those activities.  
 319 Similar to the specific hydrolytic enzyme activities, also Lac and Dehy oxidative specific activities showed  
 320 no differences between Conv and Org1 managements and only Tyr specific activity resulted significantly  
 321 higher in the Conv-managed plots. Soil oxidative enzyme activities determination is really important as  
 322 these enzymes play a crucial role in soil nutrient cycles as they can oxidize phenolic compounds and  
 323 degrade humic substances with the release of C and nutrients (Piotrowska-Długosz, 2014; Sinsabaugh,

2010) (Piotrowska-Długosz, 2014; Sinsabaugh, 2010). The increased Tyr activity with Conv management indicates the need for the microbial biomass to recover nutrients (C and N) from the stabilized organic matter. Both the hydrolytic enzymes activities ( $\beta$ -glu,  $\alpha$ -glu, NAG,  $\beta$ -xyl, and  $\beta$ -cel) and Tyr activity were positively correlated with the  $qCO_2$  (Table 4), emphasizing the strong linkage between enzyme activities and microbial metabolic efficiency. These two simple soil quality indexes represented two key points of the same soil biochemical process, linking the recovery and the efficient use of nutrients and indicating that in the Conv-managed plots the microbial community was less efficient and mainly focused on the activation of the oxidative enzymes. Thus, if the individual utility of the simple soil quality indexes as soil quality indicators is considered weak (Gil-Sotres et al., 2005), a combined evaluation can strengthen their significance in terms of biochemical processes and soil quality assessment.

The available P content and the  $PME_{MBC}$  activity did not show significant differences between Conv and Org1 management, suggesting that the management did not impact the P cycle. This finds support on the  $C:P_{enz}$  and on the  $N:P_{enz}$  ecosystem ratios which showed comparable values with Conv and Org1, indicating that the hydrolytic activity was not preferentially focused on the acquisition of P compared to C or N (Giacometti et al., 2021; Sinsabaugh et al., 2008). Together, the simple and complex indexes delineate a consistent picture. Conv and Org1 were similar in terms of acquisition activity (specific enzyme activities and ecosystem ratios), indicating that both organic and mineral fertilizers, in this context, were able to supply C, N, and P equally to agroecosystems. However, they differ in terms of microbial metabolic response with the metabolic quotient ( $qCO_2$ ) and index (MI) that showed a positive metabolic response and a higher soil quality with Org1. This becomes evident with the SQI which showed lower values with the Conv management, indicating that it led to a lower overall soil quality. Additionally, also the PCA clearly distinguished the Conv management from the organic management.

#### **The organic agroecological management (Org2)**

As previously observed comparing Conv and Org1 managements, SOC content resulted to be higher also in Org2 with respect to Conv, and significant differences were observed also between Org1 and

350 Org2 (Conv < Org2 < Org1). The differences observed between the two organic managements could be  
 351 linked to the input of C into the soil; indeed, while Org1 management involves the addition of organic  
 352 fertilizers, Org2 relies purely on the intercropping system (sampled here with faba bean) and, as  
 353 previously observed in other studies (Giacometti et al., 2014; Plaza et al., 2012), while manure could  
 354 increase SOC content by 35–50%, crop residues increased it only by 10–19%. We already stated that  
 355 probably the mineral fertilizer application in Conv management promoted microbial activity, but with a  
 356 low C use efficiency (high  $qCO_2$  and low MI), thus impacting on the SOC content (Braman et al., 2016).  
 357 Similar to Org1, also the Org2 showed lower  $qCO_2$  and higher MI, indicating that also organic  
 358 agroecological management could enhance soil conditions that favor both microbial activity and SOC  
 359 accumulation (Fließbach et al., 2007; Saviozzi et al., 2001). Therefore, both Org1 and Org2 induced  
 360 favorable conditions for microbial biomass, confirming that the adoption of organic management,  
 361 independently from the strategy chosen, could effectively have positive effects on this component (Baldi  
 362 et al., 2018; Lemanceau et al., 2014; Plaza et al., 2012).  
 363 On the other hand, the Org2 management led to lower soil DOC and TDN content than those in the  
 364 Org1-managed plots. Hence, while organic fertilization could contribute to available C and N content to  
 365 the same extent as mineral fertilization, intercropping and crop residues incorporation not. However, the  
 366 hydrolytic enzyme activities involved in C and N cycles ( $\beta$ -glu,  $\alpha$ -glu, NAG,  $\beta$ -xyl, and  $\beta$ -cel) resulted to  
 367 be lower in Org2 than in Org1 and Conv, and these differences increased considering the specific enzyme  
 368 activities since the MBC in the Org2 was higher than that in Conv and Org1. Therefore, the lower C and  
 369 N availability (i.e. presence of less quantity of substrate that could be directly mineralized or immobilize)  
 370 reduced the hydrolytic enzyme activity probably favoring other activities (i.e. the oxidative enzyme  
 371 activities).  
 372 Among the oxidative enzyme activities, Dehy and Lac activities were higher in Org2, while Tyr activity  
 373 was higher in Conv. The increased Lac activity in Org2 could be the microbial response to the low TDN  
 374 content measured in these soil samples because, as suggested by Sinsabaugh (2010), N availability is one  
 375 of the main factors controlling soil phenol oxidase activity, such as Lac (Fig. 5). Indeed, the soil



DOC:TDN ratio was higher in Org2, supporting the previous explanation for the increased Lac activity (Fig. 5). Similarly, the  $C:N_{enz}$  ecosystem ratio was higher in Org2, indicating the requirement of higher hydrolytic enzyme activity to recover C than N (Giacometti et al., 2021; Sinsabaugh et al., 2008). The two  $C:N$  ratios (soil and enzymatic) appear to be contradictory, as the first (DOC:TDN) suggests a greater requirement to recover N than C, while the second ( $C:N_{enz}$ ) showed a higher activity related to C acquisition. The link between these two contrasting results could be explained by the increased Lac activity (Fig. 5). While the increase in Lac activity was due to the reduced TDN content in Org2, there was a positive correlation between Lac activity and the  $C:N_{enz}$  (Table 4), suggesting a link between these two parameters. Thus, it could be assumed that the low TDN content in Org2 induced Lac activity, which initiated the degradation of faba bean residues and proceeded through the hydrolytic enzyme activities involved in the C cycle (Moorhead and Sinsabaugh, 2006; Sinsabaugh and Shah, 2011), leading to a higher  $C:N_{enz}$  ratio (Fig. 5). Therefore, the two contrasting parameters were, in fact, the result of the same biochemical process.

The available P content did not show significant differences among the three management strategies, and the same was observed for the  $PME_{MBC}$ . This suggests that neither Conv nor Org managements did not significantly impact the P cycle, and that probably they covered the crop and soil microbial biomass P requirements. Nonetheless, the  $C:P_{enz}$  and the  $N:P_{enz}$  ecosystem ratios showed that the enzymatic activities were mainly focused on P acquisition activity than C and N recovery only in Org2 (Giacometti et al., 2021; Sinsabaugh et al., 2008), thus indicating a stoichiometric P imbalance only in Org2.

Therefore, the acquisition activity in Org2 was influenced by the insufficient N (higher DOC:TDN) and P (lower  $C:P_{enz}$  and  $N:P_{enz}$ ) availabilities. However, the metabolic quotient ( $qCO_2$ ) and index (MI), and the SQI showed a positive metabolic response and a higher soil quality not only with Org1 but also with Org2. This indicates that even if theoretical deficiencies or imbalances in terms of C:N:P were identified in Org2, the microbial activity was efficient and functioned not only to fill these imbalances but also to maintain adequate soil quality without affecting the SOC. Indeed, it is noteworthy that SOC content is somehow “the core” of the biochemical soil quality indicators, and in this study, SOC and calculated

indexes (simple and complex) seem to indicate the same outcome: both the organic managements were able to maintain the soil quality despite the differences in biochemical processes. Additionally, PCA clearly distinguished the Conv management from both the two organic managements, corroborating that different approaches to organic management bring equal benefits in terms of soil quality compared to a conventional agronomic approach.

## **Conclusions**

All tested managements affected the soil quality, although the two organic managements, independent of the approach, improved the soil quality compared to the conventional management. Both organic management strategies induced higher MBC, SOC, and TN content, and increased activities of PME, Dehy and Lac. Simple and complex indexes indicated that Org1 and Org2 promoted higher MI, C:N<sub>enz</sub>, and SQI. However, Org1 and Org2 differed in terms of soil processes, as shown by C:P<sub>enz</sub>, N:P<sub>enz</sub>, DOC:TDN, and Lac activity.

This study lays the foundations for the simultaneous use of multiple SQIs in order to consider both the final outputs and the processes that determine them. In fact, the SQIs considered here were able to discriminate not only Conv and Org management strategies but also the two different organic management strategies (Org1 and Org2). Further research comparing different organic management approaches is needed to identify the most appropriate sustainable management and to implement the use of SQIs at the local level in generating a parameter dataset for different soils and crops.

## **CRedit authorship contribution statement**

Mazzon: investigation, formal analysis, writing, review and editing

Cavani: formal analysis, writing, review and editing

Ciavatta: supervision, review and editing

Campanelli: conceptualization, methodology

Burgio: supervision, funding acquisition

428 Marzadori: supervision, funding acquisition

429

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