Alma Mater Studiorum Università di Bologna Archivio istituzionale della ricerca

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

This is the final peer-reviewed author's accepted manuscript (postprint) of the following publication:

Published Version:

Mazzon, M., Cavani, L., Ciavatta, C., Campanelli, G., Burgio, G., Marzadori, C. (2021). Conventional versus organic management: application of simple and complex indexes to assess soil quality. AGRICULTURE, ECOSYSTEMS & ENVIRONMENT, 322(1 December 2021), 1-10 [10.1016/j.agee.2021.107673].

Availability:

This version is available at: https://hdl.handle.net/11585/832924 since: 2021-09-21

Published:

DOI: http://doi.org/10.1016/j.agee.2021.107673

Terms of use:

Some rights reserved. The terms and conditions for the reuse of this version of the manuscript are specified in the publishing policy. For all terms of use and more information see the publisher's website.

This item was downloaded from IRIS Università di Bologna (https://cris.unibo.it/). When citing, please refer to the published version.

(Article begins on next page)

- 1 Conventional versus organic management: application of simple and complex indexes to assess
- 2 soil quality
- 3 Martina Mazzon¹*, Luciano Cavani¹, Claudio Ciavatta¹, Gabriele Campanelli², Giovanni Burgio¹, Claudio
- 4 Marzadori¹
- 5 Department of Agricultural and Food Sciences Alma Mater Studiorum University of Bologna, Bologna (BO), Italy
- ⁶ Council for Agricultural Research and Economics Research Centre for Vegetable and Ornamental Crops (CREA-OF),
- 7 Monsampolo del Tronto (AP), Italy
- 8 *Corresponding author: Martina Mazzon, martina.mazzon2@unibo.it

10 Abstract

Organic management aims to maintain and/or enhance the soil quality by adopting crop rotation, reduced tillage, and application of organic fertilizers. Several studies highlight the advantages of organic management in terms of soil quality and soil fertility, the key themes in sustainable agronomy. The aim of this study was to understand whether there were differences in soil quality between two different approaches of organic management. Soil quality was assessed using a set of biochemical parameters individually or in combination, in simple and complex indexes, respectively. Soil samples were procured from a long-term field experiment area located in Monsanpolo del Tronto (Central Italy) in which conventional (Conv), and two different organic managements (Org1, organic traditional; Org2, organic agroecological) were followed, and were assessed for soil quality. Results from individual soil properties, simple and complex indexes highlighted that Conv and Org1 were similar in terms of acquisition activity indicating that both mineral and organic fertilizers supply C, N, and P equally to agro-ecosystems. However, in Org2, despite theoretical imbalances in terms of C:N:P, microbial activity efficiently filled this imbalance without affecting soil organic carbon content. The indexes showed a positive metabolic response and a higher soil quality in both the organically managed plots, indicating that organic 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 Keywords: 30 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 Specific hydrolytic enzyme activities increased in the conventional management 36 Soil quality indexes highlight differences between the two organic managements 37

Soil quality indexes help in understanding the soil biochemical processes

Introduction

39

40

41

42

43

44

45

46

47

48

49

50

51

52

53

54

55

56

57

58

59

60

61

62

63

64

Agriculture is moving from conventional to more sustainable soil management with a 102% increase in organic managed areas from 1999 to 2019 globally (IFOAM and FiBL, 2021) and a 46% increase from 2012 to 2019 in the European Union (Eurostat, 2020). Indeed, intensive tillage leads to degradation of ecosystems through soil erosion, water contamination, deforestation, desertification and, consequently, to loss of productivity (Edwards et al., 1993; Garini et al., 2017). In contrast, sustainable agriculture is based on integrated systems of agricultural production with minimum dependence upon high inputs of energy, that should maintain – or only slightly decrease – productivity and protect the environment from soil and food contamination, preserve ecological diversity, and maintain and/or improve soil quality and fertility (Edwards et al., 1993; Francis and Wezel, 2015). Therefore, the major challenge is to identify safe and secure methods to produce food, while reducing the negative impacts of agriculture on the environment (Dupré et al., 2017). Organic management aims to achieve the required yield while maintaining soil quality by the adoption of crop rotation with cover crops, reduced tillage (Hartmann et al., 2015; Tilman et al., 2002), and the application of organic fertilizers such as animal and green manures, and off-farm organic wastes (Drinkwater et al., 1995). Moreover, organic agriculture contributes to global food supply (Luo et al., 2019; Sandhu et al., 2010) although the yields obtained are usually lower than in conventional agriculture (Bonanomi et al., 2016; De Ponti et al., 2012). Soil quality and fertility not only affect productivity and mineral nutrition, but also determine the biological equilibrium and regulation of agroecosystems (Lemanceau et al., 2014). Several studies have been conducted to compare the effects of organic and conventional management strategies on soil quality and fertility. Results from studies conducted in Central Italy and Spain (temperate areas characterized by different soil textures and pH range between 6.8-7.8) highlight that in both arable and orchard systems, organic agriculture, characterized by the application of compost, manure, or crop residues, induced a higher soil organic carbon (SOC) content (10-50%) and higher microbial (enzymatic) activity (~30%) compared to conventional agriculture (Baldi 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 (qCO2, 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

65

66

67

68

69

70

71

72

73

74

75

76

77

78

79

80

81

82

83

84

85

86

87

88

89

depends on many soil reactions and properties (Gil-Sotres et al., 2005; Nannipieri et al., 2002; Nannipieri et al., 2018). Gil-Sotres et al. (2005) delineated three approaches in using soil biochemical parameters to estimate soil quality: (i) individual properties, (ii) simple indexes derived from the relationship between two individual properties, and (iii) complex indexes derived from the combinations of different properties or on the basis of statistical procedures. Individual properties could be, for example, SOC, microbial biomass, nitrogen mineralization, soil respiration, and dehydrogenase activity. However, individual properties could return contradictory results, and being the expression of specific soil processes, they cannot reflect many of the reactions which determine soil quality. Therefore, simple indexes could overcome the constraints of individual properties and fulfil the requirements of a good indicator (Gil-Sotres et al., 2005). The four most frequently used simple indexes are the specific enzyme activities (the ratio of soil enzymatic activities to the microbial biomass), the metabolic quotient (the ratio of soil basal respiration to the soil microbial biomass, qCO₂), the microbial quotient (the ratio of microbial C to the total organic C), and the metabolic index (the ratio of dehydrogenase activity to the extractable organic C, MI). Nevertheless, simple indexes possess limitations in terms of soil quality definition, as they do not have reference levels, and their responses are not always easy to relate to specific soil modification processes. Complex indexes evaluate the soil quality by combining different biochemical properties such as the biological fertility index, the enzymatic activity number, the lignocellulosic factor of Sinsabaugh et al. (1994), and the geometric mean of enzyme activities. Other complex indexes are obtained by applying statistical techniques to combine the biochemical properties (Gil-Sotres et al., 2005) and thus, the use of complex expressions or statistical procedures can appropriately describe the complexity of soil systems. In this study, as complex indexes, we focused on the ecosystem ratios of C:N, C:P and N:P acquisition activity and the soil management assessment framework (SMAF). The ecosystem ratios are given by the natural logarithm of enzyme activities involved in C, N, and P cycles, and are a measure of the enzymatic resources directed to the acquisition of organic P and organic N relative to C (Sinsabaugh et al., 2008). The soil management assessment framework (hereafter in the text SQI - Soil Quality Index) aims to evaluate the soil quality using a minimum dataset chosen from a larger dataset that includes physical,

91

92

93

94

95

96

97

98

99

100

101

102

103

104

105

106

107

108

109

110

111

112

113

114

115

chemical, and biological soil properties (Andrews and Carroll, 2001). We chose these two complex indexes as they satisfy the majority of the characteristics for a good indicator: they measure one or more soil functions, are sensitive and respond quickly to changes in soil management, and are easy to obtain and interpret. The use of biochemical indexes to assess soil quality is not novel, although their practical application in field experiments is more recent. However, Bunemann et al. (2018) stressed that an overall SQI, even if desirable, must be cautiously used to assess soil quality only in relation to specific soil functions. In addition, the sensitivity of selected indexes to evaluate the soil quality in different ecosystems and the ability to discriminate between different soil management systems is debatable. Nowadays, for example, different organic management systems have been developed, and we need affordable procedures to evaluate their strengths and weaknesses. In light of the above, a long-term field experiment in a Mediterranean area (Marche Region, Italy), with conventional and two different organic managements, was performed to understand whether there are differences in soil quality between the different management strategies. Specifically, we asked: i) Do the organic managements always lead to better soil quality compared to the conventional management? ii) Do the soil biochemical indexes differ between the two organic management approaches? iii) Can soil quality indexes help us to understand the soil biochemical processes under different organic management strategies?

135

136

137

138

139

140

141

117

118

119

120

121

122

123

124

125

126

127

128

129

130

131

132

133

134

Material and Methods

Area of study

The investigations were conducted at the Council for Agricultural Research and Economics – Research Centre for Vegetable and Ornamental Crops (CREA-OF) in Monsampolo del Tronto, Marche Region, (latitude 42°53' N, longitude 13°48' E). The climate of the locality is classified as thermo-Mediterranean, 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 m² 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.

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_{Olsen}) was determined according to Olsen method (Olsen et al., 1954) using a spectrophotometer (Jasko V-530 UV/VIS Spectrophotometer; JASCO Corporation, Japan) at $\lambda = 882$ nm. P_{Olsen} content was expressed as g kg_{ds}⁻¹. Soil basal respiration rate (SBR) was determined according to Isermeyer (1952) and expressed as mg of C-CO₂ produced per kg of dry soil during the incubation time (mg C-CO₂ kg_{ds}⁻¹ h⁻¹).

176

177

178

179

180

181

182

183

184

185

186

187

188

189

190

191

192

193

168

169

170

171

172

173

174

175

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 λ 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 nmol MUF gds⁻¹ h⁻¹. Measurements for the following oxidative enzymes were performed spectrophotometrically (Jasko V-530 UV/VIS Spectrophotometer; JASCO Corporation, Japan). Dehydrogenase activity (Dehy) was measured as described by Von Mersi and Sehinner (1991). Released iodo-nitrotetrazolium formazan (INTF) was measured at $\lambda = 464$ nm and the activity was expressed as nmol INTF g_{ds}⁻¹ 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⁺), was measured at $\lambda = 420$ nm. The activity was calculated using a molar extinction coefficient of 18460 L mol⁻¹ cm⁻¹ and expressed as μmol ABTS⁺ g_{ds}⁻¹ min⁻¹. Tyrosinase activity (Tyr) was measured as described by Sinsabaugh et al. (1999). The absorbance was measured at $\lambda = 475$ nm, and the activity was calculated using a molar extinction coefficient of 3700 L mol⁻¹ cm⁻¹ and expressed as μ mol DOPA g_{ds}^{-1} h⁻¹.

Simple soil quality indexes

The following simple indexes were determined: the metabolic quotient (qCO₂), calculated by dividing the SBR by the MBC and expressed as mg C-CO₂ gc⁻¹, represents the quantity of substrate mineralized per unit of microbial biomass, per unit of time (Gil-Sotres et al., 2005), and indicates the usage efficiency of C sources (Anderson and Domsch, 1993; Bastida et al., 2008); the metabolic index (MI), obtained by dividing the Dehy by the DOC and expressed as µmol INTF gc⁻¹ h⁻¹ (Masciandaro et al., 1998), connects the possible C availability for microbial metabolism with the microbial activity (Bastida et al., 2008); and the specific soil enzymatic activities (Gil-Sotres et al., 2005; Kandeler and Eder, 1993; Trasar-Cepeda et al., 2008), calculated by dividing the enzymatic activity values with the MBC; an increase may be due to more enzyme production, more release of enzymes immobilized in clays or humic colloids (Kandeler and Eder, 1993), or an increase in substrate available for enzymatic activity (Bastida et al., 2008).

Complex soil quality indexes

Complex soil quality indexes as ecosystem ratios and SQI were calculated. The ecosystem ratios were determined using the natural logarithm of the soil enzymatic activities involved in C, N, and P cycles, thus determining the C:N (C:N_{enz}), C:P (C:P_{enz}) and N:P (N:P_{enz}) acquisition activity (Sinsabaugh et al., 2008); for example, the C:N_{enz} was obtained by dividing the $\ln(\beta glu + \alpha glu + \beta cel + \beta xyl)$ by the $\ln(NAG)$. The SQI was determined using a process (Fig. 1) that included i) indicator selection, ii) indicator scoring and iii) integration of the scores into the index. Briefly, the minimum dataset (MDS) was selected from the principal comonent analysis (PCA), in which the principal components (PC) with eigenvalues ≥ 1 and the properties with highest loadings were assumed to best represent the system. The correlation was then used to reduce the redundancy between parameters and only the parameters that did not correlate 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)

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

Statistical analysis

Data were analyzed with the Kruskal-Wallis nonparametric test (α < 0.05) and multiple comparisons were performed using Dunn's test (α < 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).

236 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_{Olsen} (Table 2).

Soil enzymatic activities

The activities of β-glucosidase, β-xylosidase, β-cellobiosidase and N-acetyl-β-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 α-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 qCO₂ decreased while the MI increased in the order Conv - Org1 - Org2 (Table 3), with Conv showing +21% in the qCO₂ and -38% in the MI values with respect to the average mean of Org1 and Org2. The specific hydrolytic enzyme activities (Table 3) of β -glucosidase, α -glucosidase, N-acetyl- β -glucosaminidase, and β -xylosidase were lower in Org2, while phosphomonoesterase showed no differences between the managements, and β -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%,

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

Principal Component Analysis

PCA was performed on C and N pools, available P, and specific enzyme activities for the three management strategies considered. The first two PCs accounted for 42% and 20% of the total variance, respectively (Fig. 4). Conv was distinct from both Org1 and Org2, and characterized by different parameters. Moreover, a slight clustering was observed between the two organic managements. However, focusing on the comparison between Conv and Org, the latter was characterized by SOC, TN, P_{Olsen} , microbial biomass (MBC and MBN), and specific activities of Lac, PME, β -glu, and β -cel, while the specific activities of Tyr and α -glu defined the former.

Discussion

Conventional vs traditional organic management (Org1)

Soil chemical and biochemical parameters were significantly affected by the different management strategies. As observed in previous studies (Baldi et al., 2018; García-Ruiz et al., 2008; Marinari et al., 2006; Mazzon et al., 2018; Plaza et al., 2012), also in this work SOC content increased with the organic management following the trend Conv (< Org2) < Org1. This difference between Conv and Org1 was mainly because Org1 management involves the addition of organic fertilizers, which could increase SOC content. This was previously observed also in other studies where manure increased SOC content by 35–50% compared to mineral fertilization (Giacometti et al., 2014; Plaza et al., 2012). In the Conv-managed plot, where the lowest SOC content was measured, we observed an increase in qCO₂ and a decrease in MI, highlighting that the microbial community was under stressed (Balota et al., 2004; Braman et al., 2016; Laudicina et al., 2011) and less efficient conditions (Masciandaro et al., 1988; Saviozzi et al., 2001). Therefore, it is likely that the mineral fertilizer application in Conv management promoted the microbial

activity, but with a low usage efficiency of C compounds, thus impacting the SOC pool (Braman et al., 2016). The organic management exhibited lower qCO₂ and higher MI; similar results were obtained by Fließbach et al. (2007) who observed lower qCO₂ in organically managed soils, and Saviozzi et al. (2001) who found higher MI values in forest and grassland soils than in arable fields. Thus, organic management could have resulted in soil conditions that favor microbial activity, which have better energy (i.e., carbon) efficiency, thereby favoring SOC accumulation (Fließbach et al., 2007; Saviozzi et al., 2001). On the other hand, both the organic and mineral fertilizers applied in Org1 and Conv-managed plots, respectively, led to an increase in the available pools of C and N. This increase would lead to the supposition that similar hydrolytic enzyme activities linked to C and N cycles (β-glu, α-glu, NAG, β-xyl, and β-cel) would be observed in Org1 and Conv. It is noteworthy that both organic management (with compost application) and N fertilization could increase the enzyme activities involved in C and N cycles (Bowles et al., 2014; García-Ruiz et al., 2008; Stursova and Sinsabaugh, 2008). However, only Org1 resulted in higher enzymatic activities, probably owing to the input of organic fertilizers (Baldi et al., 2018; García-Ruiz et al., 2008; Marinari et al., 2006). Nevertheless, the lower MBC in Conv makes the comparison of the enzymatic activities difficult, as their changes could be due to both the management and/or the MBC content. For this reason, it is more appropriate to consider the specific enzyme activities (Gil-Sotres et al., 2005; Mazzon et al., 2018) which gives the enzymatic activity per unit of MBC, thus highlighting the differences owing to management strategies. Indeed, the specific hydrolytic enzyme activities involved in C and N cycles (β-glu, α-glu, NAG, and β-xyl) showed similar values (generally higher than those measured under the Org2 management) in Conv and Org1, confirming that both mineral N fertilization and compost addition induced the production/activation of those activities. Similar to the specific hydrolytic enzyme activities, also Lac and Dehy oxidative specific activities showed no differences between Conv and Org1 managements and only Tyr specific activity resulted significantly higher in the Conv-managed plots. Soil oxidative enzyme activities determination is really important as these enzymes play a crucial role in soil nutrient cycles as they can oxidize phenolic compounds and degrade humic substances with the release of C and nutrients (Piotrowska-Dlugosz, 2014; Sinsabaugh,

298

299

300

301

302

303

304

305

306

307

308

309

310

311

312

313

314

315

316

317

318

319

320

321

322

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 (β -glu, α -glu, NAG, β -xyl, and β -cel) and Tyr activity were positively correlated with the qCO₂ (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:Penz and on the N:Penz 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 (qCO₂) 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

346

347

348

349

345

324

325

326

327

328

329

330

331

332

333

334

335

336

337

338

339

340

341

342

343

344

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

Additionally, also the PCA clearly distinguished the Conv management from the organic management.

Org2 (Conv < Org2 < Org1). The differences observed between the two organic managements could be linked to the input of C into the soil; indeed, while Org1 management involves the addition of organic fertilizers, Org2 relies purely on the intercropping system (sampled here with faba bean) and, as previously observed in other studies (Giacometti et al., 2014; Plaza et al., 2012), while manure could increase SOC content by 35-50%, crop residues increased it only by 10-19%. We already stated that probably the mineral fertilizer application in Conv management promoted microbial activity, but with a low C use efficiency (high qCO₂ and low MI), thus impacting on the SOC content (Braman et al., 2016). Similar to Org1, also the Org2 showed lower qCO2 and higher MI, indicating that also organic agroecological management could enhance soil conditions that favor both microbial activity and SOC accumulation (Fließbach et al., 2007; Saviozzi et al., 2001). Therefore, both Org1 and Org2 induced favorable conditions for microbial biomass, confirming that the adoption of organic management, independently from the strategy chosen, could effectively have positive effects on this component (Baldi et al., 2018; Lemanceau et al., 2014; Plaza et al., 2012). On the other hand, the Org2 management led to lower soil DOC and TDN content than those in the Org1-managed plots. Hence, while organic fertilization could contribute to available C and N content to the same extent as mineral fertilization, intercropping and crop residues incorporation not. However, the hydrolytic enzyme activities involved in C and N cycles (β-glu, α-glu, NAG, β-xyl, and β-cel) resulted to be lower in Org2 than in Org1 and Conv, and these differences increased considering the specific enzyme activities since the MBC in the Org2 was higher than that in Conv and Org1. Therefore, the lower C and N availability (i.e. presence of less quantity of substrate that could be directly mineralized or immobilize) reduced the hydrolytic enzyme activity probably favoring other activities (i.e. the oxidative enzyme activities). Among the oxidative enzyme activities, Dehy and Lac activities were higher in Org2, while Tyr activity was higher in Conv. The increased Lac activity in Org2 could be the microbial response to the low TDN content measured in these soil samples because, as suggested by Sinsabaugh (2010), N availability is one of the main factors controlling soil phenol oxidase activity, such as Lac (Fig. 5). Indeed, the soil

350

351

352

353

354

355

356

357

358

359

360

361

362

363

364

365

366

367

368

369

370

371

372

373

374

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:Nenz) 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:Nenz (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:Penz and the N:Penz 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 (qCO₂) 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

376

377

378

379

380

381

382

383

384

385

386

387

388

389

390

391

392

393

394

395

396

397

398

399

400

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:Nenz, and SQI. However, Org1 and Org2 differed in terms of soil processes, as shown by C:Penz, N:Penz, 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
- 424 Cavani: formal analysis, writing, review and editing
- 425 Ciavatta: supervision, review and editing
- 426 Campanelli: conceptualization, methodology
- 427 Burgio: supervision, funding acquisition

428	Marzadori: supervision, funding acquisition
429	
430	Funding sources
431	This research was supported by the ALMAIDEA Grant Senior of the University of Bologna.
432	
433	References
434	Anderson, T.H., Domsch, K.H., 1993. The metabolic quotient for CO2 (qCO2) as a specific activity
435	parameter to assess the effects of environmental conditions, such as ph, on the microbial biomass
436	of forest soils. Soil Biol. Biochem. 25, 393–395. https://doi.org/10.1016/0038-0717(93)90140-7
437	Andrews, S.S., Carroll, R., 2001. Designing a Soil Quality Assessment Tool for Sustainable. Ecol. Appl.
438	11, 1573–1585.
439	Andrews, S.S., Karlen, D., Cambardella, C.A., 2004. The Soil Management Assessment Framework: A
440	Quantitative Soil Quality Evaluation Method. Soil Sci. Soc. Am. J 68, 1945–1962.
441	Andrews, S.S., Karlen, D.L., Mitchell, J.P., 2002. A comparison of soil quality indexing methods for
442	vegetable production systems in Northern California. Agric. Ecosyst. Environ. 90, 25-45.
443	https://doi.org/10.1016/S0167-8809(01)00174-8
444	Askari, M.S., Holden, N.M., 2015. Quantitative soil quality indexing of temperate arable management
445	systems. Soil Tillage Res. 150, 57–67. https://doi.org/10.1016/j.still.2015.01.010
446	Baldi, E., Cavani, L., Margon, A., Quartieri, M., Sorrenti, G., Marzadori, C., Toselli, M., 2018. Effect of
447	compost application on the dynamics of carbon in a nectarine orchard ecosystem. Sci. Total
448	Environ. 637-638, 918-925. https://doi.org/10.1016/j.scitotenv.2018.05.093
449	Balota, E.L., Kanashiro, M., Filho, A.C., Andrade, D.S., Dick, R.P., 2004. Soil Enzyme Activities Under
450	Long-Term Tillage and Crop Rotation Systems in Subtropical Agro-Ecosystems 300-306.
451	Bastida, F., Zsolnay, A., Hernández, T., García, C., 2008. Past, present and future of soil quality indices:
452	A biological perspective. Geoderma 147, 159–171.
453	https://doi.org/10.1016/j.geoderma.2008.08.007

- Bonanomi, G., De Filippis, F., Cesarano, G., La Storia, A., Ercolini, D., Scala, F., 2016. Organic
- farming induces changes in soil microbiota that affect agro-ecosystem functions. Soil Biol.
- 456 Biochem. 103, 327–336. https://doi.org/10.1016/j.soilbio.2016.09.005
- Bowles, T.M., Acosta-Martínez, V., Calderón, F., Jackson, L.E., 2014. Soil enzyme activities, microbial
- communities, and carbon and nitrogen availability in organic agroecosystems across an intensively-
- 459 managed agricultural landscape. Soil Biol. Biochem. 68, 252–262.
- 460 https://doi.org/10.1016/j.soilbio.2013.10.004
- Braman, S., Tenuta, M., Entz, M.H., 2016. Selected soil biological parameters measured in the 19th year
- of a long term organic-conventional comparison study in Canada. Agric. Ecosyst. Environ. 233,
- 463 343–351. https://doi.org/10.1016/j.agee.2016.09.035
- Bünemann, E.K., Bongiorno, G., Bai, Z., Creamer, R.E., Deyn, G. De, Goede, R. De, Fleskens, L.,
- Geissen, V., Kuyper, T.W., Mäder, P., Pulleman, M., Sukkel, W., Willem, J., Groenigen, V.,
- Brussaard, L., 2018. Soil quality A critical review. Soil Biol. Biochem. 120, 105–125.
- 467 https://doi.org/10.1016/j.soilbio.2018.01.030
- 468 Campanelli, G., Canali, S., 2012. Crop Production and Environmental Effects in Conventional and
- Organic Vegetable Farming Systems: The Case of a Long-Term Experiment in Mediterranean
- 470 Conditions (Central Italy). J. Sustain. Agric. 36, 599–619.
- 471 https://doi.org/10.1080/10440046.2011.646351
- 472 Canali, S., Campanelli, G., Ciaccia, C., Leteo, F., Testani, E., Montemurro, F., 2013. Conservation
- tillage strategy based on the roller crimper technology for weed control in Mediterranean vegetable
- organic cropping systems. Eur. J. Agron. 50, 11–18. https://doi.org/10.1016/j.eja.2013.05.001
- Chavarria, D.N., Pérez-Brandan, C., Serri, D.L., Meriles, J.M., Restovich, S.B., Andriulo, A.E.,
- 476 Jacquelin, L., Vargas-Gil, S., 2018. Response of soil microbial communities to agroecological
- versus conventional systems of extensive agriculture. Agric. Ecosyst. Environ. 264, 1–8.
- 478 https://doi.org/10.1016/j.agee.2018.05.008
- 479 De Ponti, T., Rijk, B., Van Ittersum, M.K., 2012. The crop yield gap between organic and conventional

- 480 agriculture. Agric. Syst. 108, 1–9. https://doi.org/10.1016/j.agsy.2011.12.004
- 481 Drinkwater, L.E., Letourneau, D.K., Workneh, F., Van Bruggen, A.H., Shennan, C., 1995.
- Fundamental differences between conventional and organic tomato agroecosystems in California.
- 483 Ecol. Appl. 5, 1098–1112. https://doi.org/10.2307/2269357
- Dupré, M., Michels, T., Le Gal, P.Y., 2017. Diverse dynamics in agroecological transitions on fruit tree
- 485 farms. Eur. J. Agron. 90, 23–33. https://doi.org/10.1016/j.eja.2017.07.002
- 486 Edwards, C.A., Grove, T.L., Harwood, R.R., Pierce Colfer, C.J., 1993. The role of agroecology and
- integrated farming systems in agricultural sustainability. Agric. Ecosyst. Environ. 46, 99–121.
- 488 https://doi.org/10.1016/0167-8809(93)90017-J
- 489 Eurostat, 2020. Organic farming statistics -Statistics Explained.
- 490 Fließbach, A., Oberholzer, H.R., Gunst, L., Mäder, P., 2007. Soil organic matter and biological soil
- 491 quality indicators after 21 years of organic and conventional farming. Agric. Ecosyst. Environ.
- 492 118, 273–284. https://doi.org/10.1016/j.agee.2006.05.022
- 493 Floch, C., Alarcon-Gutiérrez, E., Criquet, S., 2007. ABTS assay of phenol oxidase activity in soil. J.
- 494 Microbiol. Methods 71, 319–324. https://doi.org/10.1016/j.mimet.2007.09.020
- 495 Francis, C.A., Wezel, A., 2015. Agroecology and Agricultural Change, Second Edi. ed, International
- 496 Encyclopedia of the Social & Behavioral Sciences: Second Edition. Elsevier.
- 497 https://doi.org/10.1016/B978-0-08-097086-8.91026-2
- 498 García-Ruiz, R., Ochoa, V., Hinojosa, M.B., Carreira, J.A., 2008. Suitability of enzyme activities for the
- 499 monitoring of soil quality improvement in organic agricultural systems. Soil Biol. Biochem. 40,
- 500 2137–2145. https://doi.org/10.1016/j.soilbio.2008.03.023
- Garini, C.S., Vanwindekens, F., Scholberg, J.M.S., Wezel, A., Groot, J.C.J., 2017. Drivers of adoption of
- agroecological practices for winegrowers and influence from policies in the province of Trento,
- 503 Italy. Land use policy 68, 200–211. https://doi.org/10.1016/j.landusepol.2017.07.048
- Giacometti, C., Cavani, L., Baldoni, G., Ciavatta, C., Marzadori, C., Kandeler, E., 2014. Microplate-
- scale fluorometric soil enzyme assays as tools to assess soil quality in a long-term agricultural field

- experiment. Appl. Soil Ecol. 75, 80–85. https://doi.org/10.1016/j.apsoil.2013.10.009
- Giacometti, C., Mazzon, M., Cavani, L., Triberti, L., Baldoni, G., Ciavatta, C., Marzadori, C., 2021.
- Rotation and Fertilization Effects on Soil Quality and Yields in a Long Term Field Experiment.
- Agronomy.
- 510 Gil-Sotres, F., Trasar-Cepeda, C., Leirós, M.C., Seoane, S., 2005. Different approaches to evaluating soil
- quality using biochemical properties. Soil Biol. Biochem. 37, 877–887.
- 512 https://doi.org/10.1016/j.soilbio.2004.10.003
- Hartmann, M., Frey, B., Mayer, J., Mäder, P., Widmer, F., 2015. Distinct soil microbial diversity under
- long-term organic and conventional farming. ISME J. 9, 1177–1194.
- 515 https://doi.org/10.1038/ismej.2014.210
- 516 IFOAM, O.I., FiBL, R.I. of O.A., 2021. The World of Organic Agriculture Statistics and emerging
- 517 trends 2021.
- Isermeyer, H., 1952. Estimation of soil respiration in closed jars, in: Alef, K., Nannipieri, P. (Eds.),
- Methods in Applied Soil Microbiology and Biochemistry. London, pp. 214–216.
- Kandeler, E., Eder, G., 1993. Effect of cattle slurry in grassland on microbial biomass and on activities
- of various enzymes. Biol. Fertil. Soils 16, 249–254. https://doi.org/10.1007/BF00369300
- Kwiatkowski, C.A., Harasim, E., Feledyn-Szewczyk, B., Antonkiewicz, J., 2020. Enzymatic activity of
- loess soil in organic and conventional farming systems. Agric. 10, 1–14.
- 524 https://doi.org/10.3390/agriculture10040135
- Laudicina, V.A., 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.
- 527 https://doi.org/10.1007/s00374-010-0502-8
- Lemanceau, P., Maron, P.A., Mazurier, S., Mougel, C., Pivato, B., Plassart, P., Ranjard, L., Revellin, C.,
- Tardy, V., Wipf, D., 2014. Understanding and managing soil biodiversity: a major challenge in
- agroecology. Agron. Sustain. Dev. 35, 67–81. https://doi.org/10.1007/s13593-014-0247-0
- Luo, Z., Eady, S., Sharma, B., Grant, T., Liu, D.L., Cowie, A., Farquharson, R., Simmons, A., Crawford,

- D., Searle, R., Moore, A., 2019. Mapping future soil carbon change and its uncertainty in
- croplands using simple surrogates of a complex farming system model. Geoderma 337, 311–321.
- https://doi.org/10.1016/j.geoderma.2018.09.041
- Marinari, S., Mancinelli, R., Campiglia, E., Grego, S., 2006. Chemical and biological indicators of soil
- quality in organic and conventional farming systems in Central Italy. Ecol. Indic. 6, 701–711.
- 537 https://doi.org/10.1016/j.ecolind.2005.08.029
- Masciandaro, G., Ceccanti, B., Gallardo-Lancho, J.F., 1998. Organic matter properties in cultivated
- versus set-aside arable soils. Agric. Ecosyst. Environ. 67, 267–274.
- 540 https://doi.org/10.1016/S0167-8809(97)00124-2
- Mazzon, M., Cavani, L., Margon, A., Sorrenti, G., Ciavatta, C., Marzadori, C., 2018. Changes in soil
- 542 phenol oxidase activities due to long-term application of compost and mineral N in a walnut
- orchard. Geoderma 316. https://doi.org/10.1016/j.geoderma.2017.12.009
- Moorhead, Daryl L. and Sinsabaugh, R.L., 2006. Mosaic Patterns of Thermal Stress in the Rocky
- Intertidal Zone: Implications for Climate Change. Ecol. Monogr. 76, 151–174.
- 546 https://doi.org/10.1890/0012-9615(2006)076
- 547 Muñoz-Rojas, M., 2018. Soil quality indicators: critical tools in ecosystem restoration. Curr. Opin.
- 548 Environ. Sci. Heal. 5, 47–52. https://doi.org/10.1016/j.coesh.2018.04.007
- Nannipieri, P., Kandeler, E., Ruggiero, P., 2002. Enzyme activities and microbiological and biochemical
- processes in soil., in: Burns, R.G., Dick, R.P. (Eds.), Enzymes in the Environment: Activity,
- Ecology, and Applications. CRC Press, pp. 1–33.
- Nannipieri, P., Trasar-cepeda, C., Dick, R.P., 2018. Soil enzyme activity: a brief history and
- biochemistry as a basis for appropriate interpretations and meta-analysis 11–19.
- 554 https://doi.org/10.1007/s00374-017-1245-6
- Olsen, S.R., Cole, C. V, Watandbe, F., Dean, L., 1954. Estimation of Available Phosphorus in Soil by
- Extraction with sodium Bicarbonate. J. Chem. Inf. Model. 53, 1689–1699.
- 557 https://doi.org/10.1017/CBO9781107415324.004

- Piotrowska-Długosz, A., 2014. Enzymes and soil fertility, in: Enzymes in Agricultural Sciences;
- Gianfreda, L Rao, M, Eds.; OMICS Group EBooks.
- Plaza, C., Gollany, H.T., Baldoni, G., Polo, A., Ciavatta, C., 2012. Predicting long-term organic carbon
- dynamics in organically amended soils using the CQESTR model. J. Soils Sediments 12, 486–493.
- 562 https://doi.org/10.1007/s11368-012-0477-1
- R Core Team, 2020. R: A language and environment for statistical computing.
- Roldán, A., Salinas-García, J.R., Alguacil, M.M., Díaz, E., Caravaca, F., 2005. Soil enzyme activities
- suggest advantages of conservation tillage practices in sorghum cultivation under subtropical
- conditions. Geoderma 129, 178–185. https://doi.org/10.1016/j.geoderma.2004.12.042
- 567 Sandhu, H.S., Wratten, S.D., Cullen, R., 2010. Organic agriculture and ecosystem services. Environ. Sci.
- Policy 13, 1–7. https://doi.org/10.1016/j.envsci.2009.11.002
- Saviozzi, A., Levi-Minzi, R., Cardelli, R., Riffaldi, R., 2001. A comparison of soil quality in adjacent
- 570 cultivated, forest and native grassland soils. Plant Soil 233, 251–259.
- 571 https://doi.org/10.1023/A:1010526209076
- 572 Sinsabaugh, R.L., 2010. Phenol oxidase, peroxidase and organic matter dynamics of soil. Soil Biol.
- 573 Biochem. 42, 391–404. https://doi.org/10.1016/j.soilbio.2009.10.014
- 574 Sinsabaugh, R.L., Klug, M.J., Collins, H.P., Yeager, P.E., Peterson, S.O., 1999. Characterizing soil
- microbial communities, in: Robertson, G.P., Bledsoe, C.S., Coleman, D.C., Sollins, P. (Eds.),
- 576 Standard Soil Methods for Long-Term Ecological Research; Robertson, G. P., Bledsoe, C. S.,
- Coleman, D. C., Sollins, P., Eds.; Oxford University Press, New York., Characterizing Soil
- 578 Microbial Communities. Oxford University Press, pp. 318–348.
- 579 Sinsabaugh, R.L., Lauber, C.L., Weintraub, M.N., Ahmed, B., Allison, S.D., Crenshaw, C., Contosta,
- A.R., Cusack, D., Frey, S., Gallo, M.E., Gartner, T.B., Hobbie, S.E., Holland, K., Keeler, B.L.,
- Powers, J.S., Stursova, M., Takacs-Vesbach, C., Waldrop, M.P., Wallenstein, M.D., Zak, D.R.,
- Zeglin, L.H., 2008. Stoichiometry of soil enzyme activity at global scale. Ecol. Lett. 11, 1252–1264.
- 583 https://doi.org/10.1111/j.1461-0248.2008.01245.x

- 584 Sinsabaugh, R.L., Moorhead, D.L., Linkins, A.E., 1994. The enzymic basis of plant litter
- decomposition: emergence of an ecological process. Appl. Soil Ecol. 1, 97–111.
- 586 https://doi.org/10.1016/0929-1393(94)90030-2
- 587 Sinsabaugh, R.L., Shah, J.J.F., 2011. Ecoenzymatic stoichiometry of recalcitrant organic matter
- decomposition: The growth rate hypothesis in reverse. Biogeochemistry 102, 31–43.
- 589 https://doi.org/10.1007/s10533-010-9482-x
- 590 Stursova, M., Sinsabaugh, R.L., 2008. Stabilization of oxidative enzymes in desert soil may limit organic
- matter accumulation. Soil Biol. Biochem. 40, 550–553.
- 592 https://doi.org/10.1016/j.soilbio.2007.09.002
- Tilman, D., Cassman, K.G., Matson, P.A., Naylor, R., Polasky, S., 2002. Agricultural sustainability and
- intensive production practices. Nature 418, 671–677.
- 595 https://doi.org/https://doi.org/10.1038/nature01014
- Toselli, M., Baldi, E., Cavani, L., Mazzon, M., Quartieri, M., Sorrenti, G., Marzadori, C., 2019. Soil-
- plant nitrogen pools in nectarine orchard in response to long-term compost application. Sci. Total
- 598 Environ. 671, 10–18. https://doi.org/10.1016/j.scitotenv.2019.03.241
- 599 Trasar-Cepeda, C., Leirós, M.C., Gil-Sotres, F., 2008. Hydrolytic enzyme activities in agricultural and
- forest soils. Some implications for their use as indicators of soil quality. Soil Biol. Biochem. 40,
- 601 2146–2155. https://doi.org/10.1016/j.soilbio.2008.03.015
- 602 USDA Soil Taxonomy, 2006. A Basic System of Soil Classification for Making and Interpreting Soil
- Surveys, Second ed. Washington, DC.
- Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial
- 605 biomass C. Soil Eiol. Biochem 19, 703–707. https://doi.org/10.1016/0038-0717(87)90052-6
- Von Mersi, W., Sehinner, F., 1991. Biology and Fertility ~ Soil s An improved and accurate method for
- determining the dehydrogenase activity of soils with iodonitrotetrazolium chloride, Biol Fertil
- Soils.
- Wallenstein, M.D., Haddix, M.L., Lee, D.D., Conant, R.T., Paul, E.A., 2012. A litter-slurry technique

610	elucidates the key role of enzyme production and microbial dynamics in temperature sensitivity of
611	organic matter decomposition. Soil Biol. Biochem. 47, 18–26.
612	https://doi.org/10.1016/j.soilbio.2011.12.009
613	Zuber, S.M., Villamil, M.B., 2016. Meta-analysis approach to assess effect of tillage on microbial
614	biomass and enzyme activities. Soil Biol. Biochem. 97, 176-187.
615	https://doi.org/10.1016/j.soilbio.2016.03.011
616	