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Locally available compost application in organic farms: 2-year effect on biological soil properties

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Abstract

Composting technologies have progressed parallel to the growing interest in recycling organic waste over recent decades, whilst in-field compost application requires technical improvement and more experience in order to optimize their effect according to the agro-environment and the type of crop which follow their incorporation into the soil. In response to compost application, biological soil features were assessed in field by adopting precision agricultural machinery and by limiting soil incorporation to a depth of 15 cm. A 2-year trial was carried out on two sites in the East Po valley (Northern Italy), an agricultural district which, in 2000, was classified as being on the verge of desertification, and where efforts to counteract soil organic matter decline have been underway for some decades. A green-waste compost produced in accordance with current national directives was applied in autumn 2019 and 2020 to two organic fields using precision farming machinery for compost spreading and conventional harrows for incorporation. Fields were divided into two large plots to compare the effect of compost treatment to an untreated control and were managed according to organic farming practices. Seven months after application, microbial biomass, assessed in terms of DNA, and 17 enzymatic activities were estimated by sampling root-explored soil at the vegetative stage of different seed crops for organic horticulture. A significant overall increase of biological soil activity was detected after the second application. The qualitative response varied slightly between the two sites: a higher impact of microbial biomass was observed in the site that was poorer in soil organic matter; whilst in the other, an increase of phosphatase activities contributed more to the general increase of biological activity. Findings show that, in those agricultural soils, an agronomic advantage from compost can be obtained only after repeated applications; furthermore, precision farming technologies facilitate compost application even in small, specialized farms such as those which hosted this trial.

Introduction

Soil organic matter decline of agricultural soils represents an environmental and agronomic issue worldwide, but is a central issue in southern Europe where the Mediterranean climate and land use are responsible for steady organic matter depletion (Zdruli *et al.*, 2004; Grilli *et al.*, 2021).

Although the European Commission has marked out soil desertification as a key issue and has engaged in a number of ways to counteract it, slowing down the depletion of soil organic matter and reversing this trend requires action on multiple levels. The latest and most incisive program undertaken by the European government is the European Green Deal whose goal is to reach climate neutrality within 2050 (European Commission, 2019). The European Green Deal aims at promoting efficient use of resources by moving to a clean and circular economy, restoring biodiversity and reducing pollution by taking a wide range of social and technological measures. It is a virtuous long-term program of land use whose main goals are the improvement of soil quality and the increase of C sequestration. Given that soil management at farm level plays a key role in improving land use, it is essential to involve farmers in this process. This goal is achievable by providing know-how and technical support to farmers, simultaneously assuring tangible benefits in the short-term to support them in their endeavors (Jat *et al.*, 2022). Increasing organic matter in intensively cultivated soils, where no tillage, crop residue incorporation or other low input techniques for improving C sequestration are often difficult (Paustian *et al.*, 2000), implies the application of exogenous organic matter (Moeskops *et al.*, 2012). Amongst these, composts are the organic soil amendments which most closely resemble animal manure. They derive from organic wastes of varying origin which, thanks to progressively more effective composting technologies able to speed and drive the fermentation process, are converted into stable products that are rich in humic matter (Liu *et al.*, 2022). Composts supply nutrients and improve soil water-holding capacity with undisputed importance in the environmental sustainability of agrifood production chains (Moran and Schupp, 2003; Almendro-Candel *et al.*, 2019; Bondì *et al.*, 2022). Nevertheless, the improvement of soil biological properties and related functions, such as suppressive activity toward soil-borne pathogens, an increase of nutrient bioavailability and promotion of plant growth, are the most frequently observed benefits of the compost use as an amendment (Hoitink *et al.*, 1997; Bonanomi *et al.*, 2010; Jakubus, 2016; Eden *et al.*, 2017). However, the increase of soil biological features following compost incorporation into the soil largely varies according to soil characteristics and management, environmental conditions as well as the origin and composition of waste material (Noble and Coventry, 2005; Franke-Whittle *et al.*, 2019; Jat *et al.*, 2022). Most studies concerning the increase of microbial-mediated soil functions (soil suppressiveness, plant growth promotion) after compost incorporation into the soil linked this improvement to the general increase of soil biological activity expressed in terms of microbial biomass, basal respiration or enzymatic activities (Lee *et al.*, 2004; Pérez-Piqueres *et al.*, 2006; De Corato, 2020). Much more rarely have beneficial functional increases, in response to compost amendment, been correlated to microbial populations added with composts. On the other hand, it has been widely reported in literature that commercial composts of varying origins can influence the native bacterial populations of the soil in different ways; in particular, some composts can increase antagonistic bacteria such as siderophore-producing bacteria (De Brito Alvarez *et al.*, 1995; Das Bibhutu and Dkhar, 2012).

Composting technologies have progressed parallel to a growing interest in organic waste recycling and reducing its environmental impact (Zheng *et al.*, 2020; de Nobile *et al.*, 2021; Chavez-Rico *et al.*, 2022). Nonetheless, limitations in logistics, organization and economic viability still affect the reuse of those materials in agriculture (Westerman and Bicudo, 2005). Another weakness in the recycling chain of organic waste in agriculture concerns the correct use of organic amendments at farm level. Further understanding for establishing long-term compost application plans for diverse pedological and climatic conditions, improving and updating techniques for mechanical incorporation of the compost into the soil, defining times between compost application and crop sowing to optimize physical, chemical and biological benefits would require a larger number of field experiments to maximize their benefits as a function of the different agro-environmental conditions in which composts are applied (Westerman and Bicudo, 2005).

Within this context, a 2-year field study to evaluate the impact of repeated massive compost applications to a series of soil biological features was carried out in an agricultural district of East Po valley (Emilia Romagna, Italy). This intensively cultivated area, which had formerly hosted fruit tree (particularly peach), has been evolving to field crops over the last 5–10 years and is currently the primary seed-producing district in Italy. The soil organic matter content here is lower than 2% (Zdruli *et al.*, 2004) and it is one of the Italian regions where the desertification rate is reported to have increased most in the early-2010s (Nickayin *et al.*, 2022). The main objectives of this field experiment were: (i) to evaluate the impact of a locally available compost suitable for organic farming; (ii) to verify the early boosting effect of compost application on biological soil features, as already reported in other environmental conditions (Kandeler *et al.*, 1999; Ros *et al.*, 2003); and (iii) to estimate whether compost incorporation to the surface layer of soil in fall, the commonly adopted practice in the area under study, can improve biological soil features for the subsequent crop cycle.

Materials and methods

Experimental sites

The study was carried out in large plots (about 4000 square meters) within two organic fields destined for the production of sowing seeds for horticulture. The fields belonged to two farms located in an agricultural district of East Po valley (Emilia Romagna) classified as ‘under desertification’ at the end of ‘90s (Zdruli *et al.*, 2004). Over recent decades, however, regional goverment and individual farmers have been endeavoring to counteract soil organic matter decline. Parallely, a shift by conventional farms toward organic farming was observed in this region which, combined with three others in southern Italy (Puglia, Sicily and Calabria), currently cover 51% of the entire national organic ground surface (Giovannini, 2021).

Two experimental fields were selected in two private organic farms at a distance of 44 km from one another and close to two towns: Forlì and Imola. These are also the names given to the experimental sites whose latitude and longitude are 44.228104 N, 12.101676E (Forlì) and 44.320897N, 11.656798E (Imola). The current climate of this agricultural district is classified as Cfa (humid subtropical climate: warm temperate, no dry season, hot summer) according to Köppen-Geiger climates (Peel *et al.*, 2007). The physical and chemical features of the soil are reported in Table 1 (Emilia Romagna Region, 2013). Both fields had been under organic management for over 20 years in accordance with guidelines for organic production in Italy and over the last decade both fields have frequently produced organic horticultural seeds for two local businesses specializing in worldwide seed distribution.

Compost features and soil amendment

The study was conducted over two growing seasons (2020 and 2021) in the Forlì and Imola experimental fields using a locally available compost called ‘Ammendante compostato verde’

Table 1. Main soil features for the upper layer 0–0.3 m in the two experimental site

	Forlì	Imola
Soil texture		
Sand (%)	5.31	25.52
Silt (%)	47.59	51.68
Clay (%)	47.10	22.80
SOM[a] (g kg^{-1} soil)	1.66	2.04
P_2O_5 (mg kg^{-1})	32.0	42.0
K_2O (mg kg^{-1})	542.0	159.0
pH	8.0	7.7
Classification[b]		
Forlì	mesic Uldertic Haplustepts fine, mixed, active	
Imola	mesic Udifluventic Haplustepts fine silty, mixed, superactive	

Data retrieved from the soil map of the Emilia Romagna Region (Emilia Romagna Region, 2013).

[a] Soil organic matter content evaluated at the beginning of the trial. Sampling time November 2019.

[b] Key to soil taxonomy (USDA 2010).

(EnomondoS.r.L, Faenza Italy). Compost origin was 100% green waste mainly represented by herbaceous and woody waste materials from mowing and pruning which are chopped and screened prior to the composting process. Composted material, after the 'curing' phase, which enables completion of the composting process, is finely sieved. The compost was suitable for organic farming according to the Italian legislation of 2010 (Mipaaf, 2010), developed from EU reg. (CE) n. 889/2008. The final product is a high-quality compost, with an average C/N and pH accounting for 13 and 7.5, respectively (the full list of the main standard features of this commercial compost are available at https://www.enomondo.it/wp-content/uploads/SCHEDE_ECONAT_2018_verde.pdf; last accessed on September 2022).

In agreement with the limits imposed by the European Nitrate Directive in 1991 (91/676/CEE) and further EU updates aiming at protecting water from nitrate contamination, in 2019 the regional regulation limited maximum nitrogen spread to 170 kg ha^{-1} *per annum*. In addition, regional guidelines for integrated production reduced the maximum dosage of nitrogen fertilization from 100 to 60 kg ha^{-1} in a single dose for herbaceous, horticultural and seed crops (Regione Emilia Romagna, 2018). Therefore, based on the average nitrogen compost concentration, a compost application dose of 24 t ha^{-1} per year, equal to a nitrogen dose application of 57.6 kg ha^{-1}, was defined. Compost was applied to 2000 m^2 (20 × 100 m) in each field, while a non-treated close field strip of identical dimensions was defined as control. Compost application in the two experimental fields was carried out on October 23, 2019 and October 8, 2020, through a local service agency that supplies farm machinery and specialized operators. In 2019, compost application was performed after seven dry days; whilst in 2020, it was performed after a week of rain with a cumulative rainfall of 74.5 mm (in 3 out of 7 days) in Forlì and 115.2 mm (in 4 out of 7 days) in Imola.

Spreading was carried out using a specific compost spreader with a rear centrifugal double disc system fed by an internal auger, a semi-automatic guidance system following the positive test described by Scarfone *et al.* (2021) and electronic control of the distributed dose. The RC 200 A model produced by Serri S.n.c (Predappio, FC, Italy) specifically for spreading waste material such as composts, solid separated digestate and animal manure, with a loading volume of 25 m^{3}and a total capacity of 20 t, was connected to a 116-kW, four-wheel drive tractor. The working width was set at 8 m, a 2-m overlap between parallel passages was maintained to ensure homogeneous distribution. The final compost layer in the surface of treated plots accounted for an average of 2.8 ± 0.8 cm in both experimental sites (Fig. 1). Deep compost incorporation was set at 15 cm to concentrate compost in the root-explored soil layer and to maximize its effects for the subsequent crop. Incorporation was carried out with a conventional rear mounted harrow with front discs and rigid rear elements, using a 66-kW four-wheel-drive tractor. The relevant control strips in each of the experimental sites underwent the same tillage.

Field measures

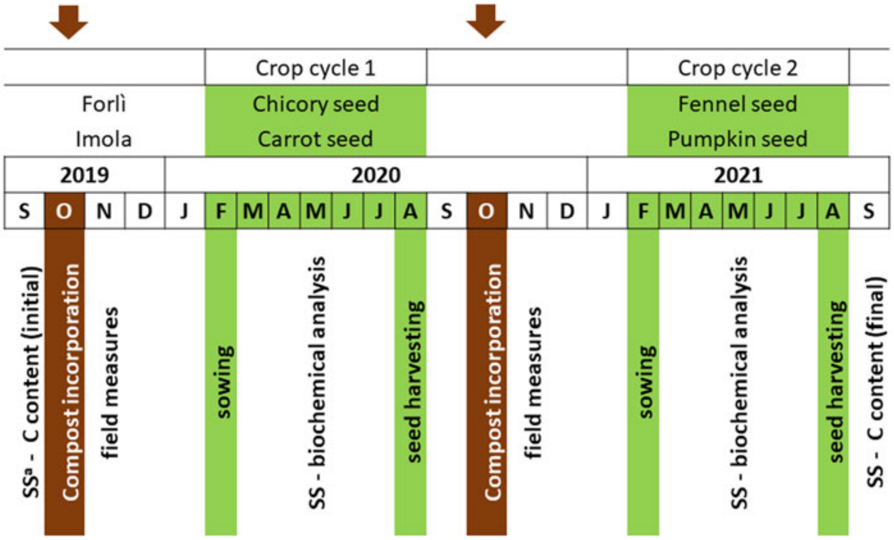
Timing of soil amendments with green-waste compost, crop cycles for organic seed production, soil sampling and measurement of the depth of compost incorporation into the soil over the 2-year trial period are shown in Figure 2. Deep compost incorporation into the soil was measured 15 days after soil amendment (Fig. 2). Two 50 × 50 cm holes were made to a depth of 30 cm in each of three sectors in the 2000-m^2 plots. Deep compost incorporation was measured in three sites (vertical planes) per hole to obtain a total of six values per plot. The average values of deep compost incorporation (Fig. 3) of each of the three sub-plots were considered as replicates.

Quality of compost distribution in the tilled layer was assessed through visual observation of photographs (6 per hole giving a total of 36 photos per plot) which were taken in three out of four vertical planes of each hole by placing a camera on the ground at the same distance from the vertical planes and orientation based on the position of the sun. Compost was clearly distinguishable from soil immediately after the incorporation thanks to its significantly darker color (Fig. 3).

Biochemical analysis

Soil for total organic carbon content (TOC) evaluation was sampled at the beginning and end of the trial, before the first compost incorporation into the soil in fall 2019 and after seed harvest in late summer 2021. Three 15 cm cores of bulk soil were collected at a depth of 15 cm from each of three subplots (660 m^2) into which each of the 2000 m^2 treated and untreated field strips had been divided. TOC was quantified through dry combustion using a TOC Vario Select analyzer (Elementar, Germany) (Vitti *et al.*, 2016); TOC of each replicate was the mean of three analyzed subsamples.

Fig. 1. Homogeneous distribution of the green compost surface layer before incorporation into the soil.



[a] SS Soil sampling

Fig. 2. Timing of compost soil amendment, soil sampling and main cultivation phases over two crop cycles (2019/2020 and 2020/2021) for organic seed production in Forli and Imola experimental sites. The brown arrows indicate compost application.

Soil sampling for biochemical analysis was carried out at the end of the vegetative stage of seed crops in May 2020 and May 2021 (Fig. 2). Composite soil samples were taken within each of three subplots as follows: three 15 cm cores were taken near three plants after removal of a 2-cm top layer, in order to obtain root-explored soil. Sampling sites were chosen in three different central rows of each of the three plots (replicates) of around 660 m^2 each. Soil subsamples of each replicate were joined and mixed by hand after root residues had been removed. These samples were air dried at room temperature for 24–48 h, 2 mm sieved and stored in sterile vials at -20°C in 50 g-aliquots.

Seventeen enzyme activities were quantified in soil extracts (Bardelli *et al.*, 2017). Briefly, enzymes were extracted by heteromolecular exchange using a 3% lysozyme solution (Cowie *et al.*, 2013). Enzyme activities were quantified in microplates using 4 methyl umbelliferyl (4-MUF) and 7 amino 4 methylcoumarine (7-AMS) fluorogenic conjugated substrates (BioSynth s.r.l. Rapolano Terme Siena, Italy). Acid phosphomonoesterase, α-glucosidase, arylsulfatase, β-glucosidase, β-galactosidase, chitinase, glucuronidase, xylosidase, inositol-phosphatase (phytase) and nonanoate-esterase were determined in 200 mM MES (morpholineptansulfonic acid) solution at pH 5.8. The activities of leucine aminopeptidase, arginine-aminopeptidase, serin-like protease, pyrophosphatase-phosphodiesterase and phosphodiesterase were determined in 200 mM tris–HCl solution at pH 7.5. Alkaline phosphomonoesterase activity was determined in 200 mM tris–HCl solution at pH 9.0. Data relating to enzymatic activity were expressed as nano moles of 4-MUF and 7-AMS per gram of dry soil and per hour (nmol 4-MUF g^{-1} h^{-1}). Finally, DNA-based quantitative (dsDNA) assessment of soil microbial biomass, expressed as μg of dsDNA per g of dry soil (μg dsDNA g^{-1}) was carried out as described by Fornasier *et al.* (2014).

Root health analysis

In the first year (2020), at this sampling time, six plants per replicate were taken for visual evaluation of root health which was

Fig. 3. Compost incorporation deep in the Imola experimental fields in the first (left) and second (right) year of the trial, respectively. Pictures were taken while measuring the depth of compost incorporation in early November 2019 and 2020.

associated to the analysis of root colonizing fungal communities as described by Manici *et al.* (2018). In this case, 12 0.3–0.5-root segments per plant were analyzed for a total of 216 segments per treatment (treated and non-treated control).

Statistical analysis

Data were analyzed using Statgraphics centurion version 18.1.01 software (Statgraphics Technologies, Inc. The Plains, Virginia, USA). Enzymatic activities were subjected to analysis of variance and mean separation test using Fisher's least significant difference (LSD) procedure was applied. The whole variables dataset was subjected to cluster analysis with the Nearest Neighbor method using Statgraphics 18 software, whilst PAST software version 3.24 (Hammer *et al.*, 2001) for analysis in paleoecology was used to carry out the SIMPER test to evaluate which biochemical variable was most responsible for the difference observed between the treatments (Clarke, 1993). In both cases Euclidean distance was adopted.

Results

Deep compost incorporation

Deep compost incorporation did not differ between replicates on either site or either year; as expected, it accounted for an average of about 15 cm (Table 2). Compost distribution was fairly homogeneous along the 15 cm top layer profile in the first year. In contrast, in October 2020, which had higher previous rainfall, compost accumulations in the lower layer of the profile were detected in 78 and 59% of the analyzed photographs taken at the Imola and Forlì sites, respectively (Fig. 3, 2020).

Biochemical soil features

Compost incorporation increased TOC in the 15 cm topsoil by 19 and 13% in the Forli and Imola field trials, respectively (Table 3). Biochemical soil features did not substantially vary between treated and non-treated plots in the first year. They did not show any variation in the Forlì site in 2020; whilst, in Imola, six over 18 analyzed variables differed from control at $P \leq 0.01$ and 0.05 (Table 4). Conversely in spring 2021, after two repeated compost treatment, microbial biomass and enzymatic activity overall resulted significantly increased as compared to control in the compost amended plots of both the experimental fields (Table 4). The impact of green-waste compost on biochemical features over a 2-year trial can be clearly inferred from the cluster analysis of 17 enzymatic activities and microbial biomass (sdDNA) using a multivariate analysis approach. The compost-treated (T) and -untreated (NT) plots in 2020, as well as the untreated plot in 2021 clustered together in both experimental sites with a distance which did not exceed 8 (Fig. 4). Conversely, the compost-treated plot after the second compost application (T, 2021) fell into a distinctly separate cluster in both sites (Fig. 4). The whole response of enzymatic activities together with that of microbial biomass (Fig. 5) clearly suggested that compost incorporation can induce an overall significant enhancement of biological property from the second year after its incorporation into the soil. Microbial biomass (dsDNA) values did not differ between amended (T) and unamended (NT) soil in the spring of 2020, whilst amended soil showed a significant

Table 2. Compost deep incorporation 15 days after field application in the two locations

	2019 (cm)	2020 (cm)
Forlì	$14.5 \pm 1.0^{\text{s.d.}}$	$15.0 \pm 1.1^{\text{s.d.}}$
Imola	$14.8 \pm 0.8^{\text{s.d.}}$	$15.2 \pm 1.26^{\text{s.d.}}$

s.d., standard deviation.

Table 3. Difference in total organic carbon (TOC) content in bulk soil (20 cm topsoil) in treated (T) and non-treated (NT) plots at end of the 2-year trial (Fig. 1)

	TOC	Increase
Treatment	g kg^{-1}	(%)
Forlì NT	9.70 b[a]	19
Forlì T	12.00 a	
Imola NT	12.48 b	13
Imola T	14.33 a	

[a] Means with different letters significantly differ according to LSD test ($P = 0.05$).

Table 4. Significance of difference for each of the biochemical parameters (dsDNA, soil DNA and 18 enzymatic activities) between treated and non-treated soil sampled in the root explored area of the seed crop following compost (Fig. 1)

	Forlì		Imola	
Variables	2020	2021	2020	2021
dsDNA	ns	****	ns	****
aryS	ns	*	ns	**
alfaG	ns	**	ns	ns
betaG	ns	ns	*	**
alfaGAL	ns	**	*	**
betaGAL	ns	*	*	ns
xilo	ns	**	ns	**
uroni	ns	**	ns	**
chit	ns	**	ns	**
CBZ	ns	**	ns	ns
leu	ns	**	ns	***
arginina	ns	**	ns	**
acP	ns	*	**	ns
bisP	ns	*	**	ns
piroP	ns	*	ns	****
alkP	ns	*	ns	****
inositP	ns	*	ns	*
nona	ns	*	ns	**

acP, acid phosphomonoesterase; alfaG, alpha-glucosidase; alfa_GAL, alpha-galattosidase; alkP, alkaline phosphomonoesterase; aryS, arylsulfatase; betaG, beta-glucosidse; beta_GAL, beta-galattosidase; bisP, phosphodiesterase; chit, chitinase; CBZ, serin-like protease; dsDNA, DNA-based quantitative assessment of soil microbial biomass; inositP, inositol-phosphatase (phytase); leu, leucine-aminopeptidase; argin, arginine-aminopeptidase; piroP, pirophosphate-phosphodiesterase; nona, nonanoate-esterase; uroni, glucuronidase; xilo, xilosidase.

P value: ****0.0001; ***0.001; **0.01; *0.05; ns, not significant.

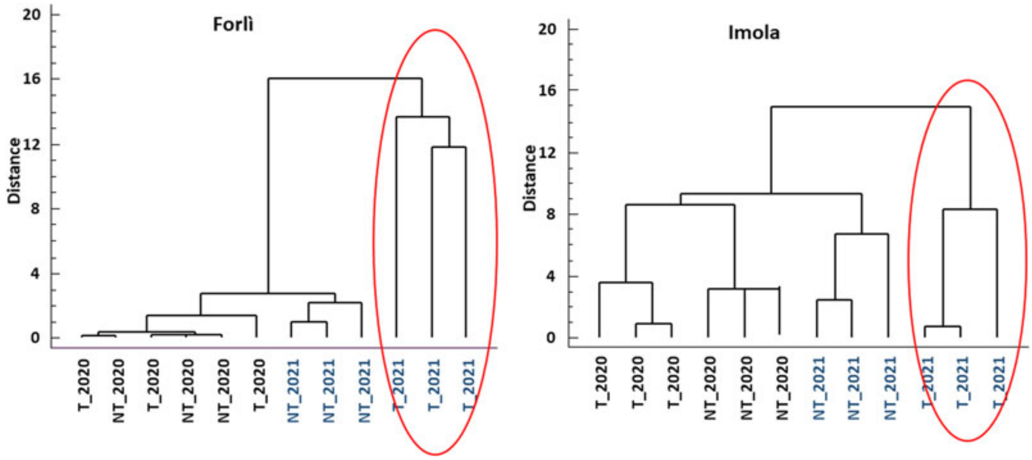


Fig. 4. Dendrogram inferred from 18 biochemical variables showing distance between compost treated (T) and non-treated (NT) soils in first (spring 2020) and second year (spring 2021). The red circle underlines that compost treated soil in the second year always clustered alone.

increase in dsDNA in 2021 (Fig. 5). Although the above results in biological features should be considered as a whole, SIMPER analysis allowed some considerations on the qualitative response in the two sites (Table 5). This analysis highlighted that five out of the 18 evaluated biochemical variables were primarily responsible for the changes observed in the cluster analysis (Fig. 4). In Forlì, the greater distance of the treated plot in 2021 (Fig. 4, Forlì) was mainly due to the variations of lipase-nonanoate-esterase (nano) (Fig. 6) and the increase of microbial biomass in terms of dsDNA (Fig. 5); whereas, in the Imola site, phosphatase activities (acP, alkP, bisP) other than nano (Fig. 6) most affected the general biological response in the compost treated soils across the 2-year trial (Fig. 4, Imola).

Root health

According to the root inspection of the seed crops at the end of vegetative stage, roots showed no discoloration, tip necrosis or any other tissue alteration in either treated or untreated plots. In line with this finding, no fungal agents of root rot complex such as *Pythium* spp., *Rhizoctoniasolani*, *Dactylonectria*spp, *Phoma* spp. and others were isolated from roots (Williamson-Benavides and Dhingra, 2021). Root colonization communities were represented by saprophytic microfungi such as *Mortierella*, *Acremonium* and *Moniliella*.

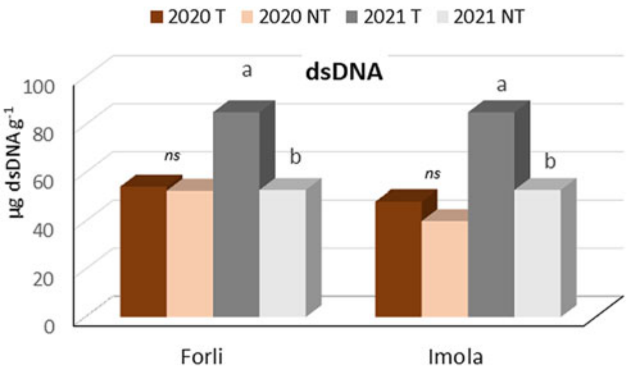


Fig. 5. Response of microbial biomass, in term of dsDNA, in the root explored area of the crops, 7 months after application. Compost treated (T) *vs* non treated soil (NT) in the springs 2020 and 2021. Means with different letters significantly differ according to LSD test ($P = 0.05$).

Discussion

The significant increase of microbial enzymatic activities observed in this study reflected the findings of several previous studies which report the increase of enzymatic activities as an early benefit in compost application (Kandeler *et al*., 1999; Ros *et al*., 2003; Jat *et al*., 2022). Nevertheless, this field trial gave a series of useful

Table 5. SIMPER analysis (percent similarity) showing which biochemical variable, among those (19) evaluated in 2020 and 2021, were primarily responsible for the observed difference between compost treated and non-treated control

	Forli	
Biochemical variables	Contribute (%)	Cumulative (%)
nona	38.06	38.06
dsDNA	20.27	58.33
alkP	17.88	76.21
argin	8.00	83.30
leu	4.00	87.31
All the others	12.7	12.69
	Imola	
	Contribute (%)	Cumulative (%)
nona	29.75	29.75
acP	19.73	49.48
alkP	15.71	65.19
dsDNA	7.081	72.27
bisP	6.693	78.96
All the others	21.04	21.04

arigin, arginine-aminopeptidase; acP, acid phosphomonoesterase; alkP, alkaline phosphomonoesterase; bisP, phosphodiesterase; dsDNA, DNA-based quantitative assessment of soil microbial biomass; leu, leucine-aminopeptidase; nona, lipase-nonanoate-esterase.

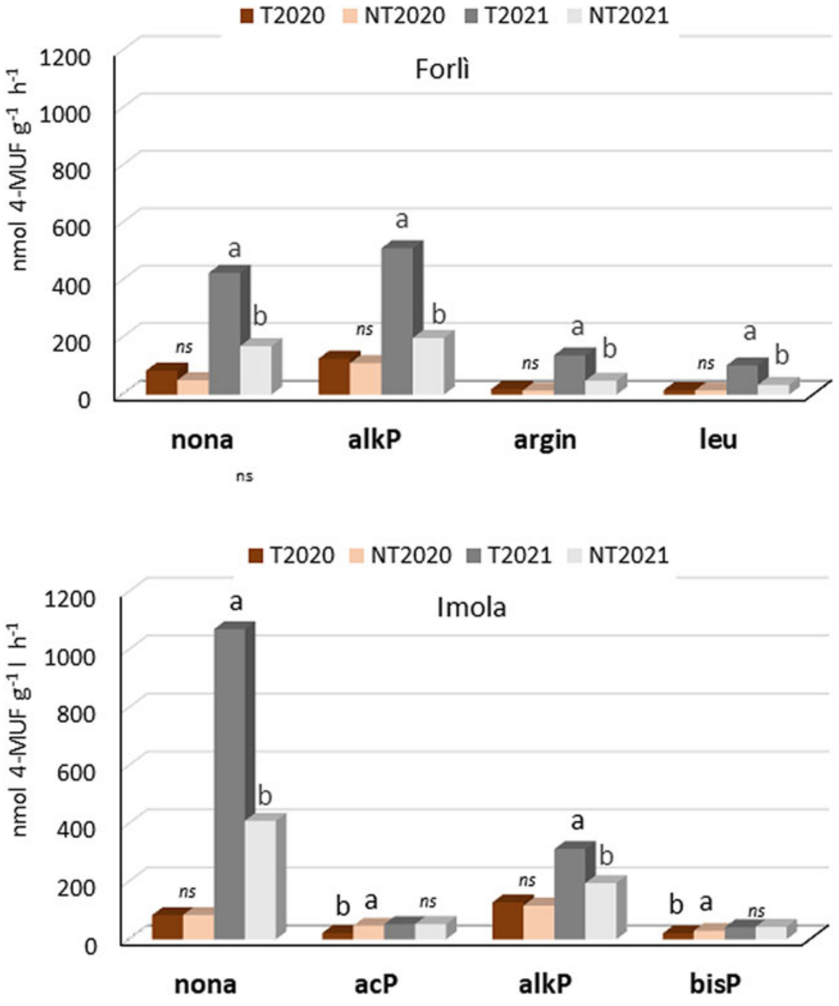


Fig. 6. Mean values of enzymatic activity which most affected the difference between amended and unamended treatments across the 2-year trial period. Compost treated (T) *vs* non treated soil (NT) in the springs 2020 and 2021. Means with different letters significantly differ according to LSD test ($P = 0.05$). ns, Not significant.

results for maximizing compost valorization within the process for counteracting soil organic matter decline in a long-term program, whilst simultaneously supporting agricultural productions in the present.

First, the booster effect of compost on soil biological activity was observed only after the second application. This increase was detected during the vegetative stage of the seed crop, namely 7 months after the second application suggesting that in order to reach agronomic advantage from compost, repeated applications in those cropping systems are required. This finding is consistent with those of a recent field survey on the impact of periodical use of digestates from biogas production in the same agricultural area, the Po valley in northern Italy (Manici *et al.*, 2020). In that study, experimental fields on a variety of sites across the Po valley showed a general increase in soil suppressiveness toward a series of fungal root pathogens of maize. The microbial components of that functional improvement (quantified in terms of nucleotide copies corresponding to functional bacterial populations such as *Pseudomonas* and actinomycetes) largely differed between the sites, thus showing that recycled organic masses affect soil native microbiome differently, but this always manifests as general increase in biological fertility (Manici *et al.*, 2020). In this study, soil microbial biomass, in terms of microbial DNA, and enzymatic activities increased in root-explored soil in response to a final C increase of about 15% in the 15 cm top layer. This cannot be considered a stable increase in soil organic matter, which instead can only be achieved with long-term fertilization (Mustafa *et al.*, 2022); however, the observed increase in microbial biomass and enzymatic activities in response to compost application suggests a carbon sequestration enhancement, which implies a progressive C build up parallel to an increase in soil microbial activity linked to a series of other advantages such as crop health and water retention improvements. In Mediterranean intensive cropping systems, these benefits can overcome those commonly expected from compost applications such as the increase of C, N and nutrient availability for the crop (Scotti *et al.*, 2015).

The most interesting finding of this study is the response times of soil biological activity in treated soil which confirms that microbial biomass increases at a faster rate than soil organic matter content (Tscherko *et al.*, 2003). From a qualitative point of view, interpreting functional meaning of single enzymatic activities in soil is complex. The greater compost effect on microbial biomass (dsDNA) in Forli compared to Imola (Table 4) indicates a major effect on the whole microbial biomass on this site, which may be explained by the lower soil organic matter content and, consequently, a more rapid response to the incorporation of exogenous organic matter in a poorer soil (Wang *et al.*, 2013). Conversely, on the Imola site, there was an overall increase of phosphatase activities (Table 4), which suggests a burst of P turnover. As those values were detected in the rot-explored area of the seed crops several months after compost incorporation, it is possible to conclude that compost application led to an increase of biological activity in soil during the following crop cycle and that soil features can qualitatively affect this response.

In this 2-year field trial, the agronomic benefits to crops could not be highlighted because seed production, as well as germinability, did not differ between treated and untreated soils over the 2 years of the trial (data provided by the seed companies and not shown). The absence of soil-borne fungal pathogens responsible for root development reduction in the experimental field may be one of the reasons for this result. Indeed, one of the first effects of the increase in biological activity is the reduction of root pathogen effects on the crop and the increase in soil suppressiveness, namely the natural ability of soil to control pathogens and increase plant growth (Weller *et al.*, 2002; Campos *et al.*, 2016). The soil health of the experimental fields was determined by their long-term organic management, which was, on the other hand, the primary reason for their selection by the seed companies to produce organic seeds.

The beneficial effects of compost incorporation into the 15-cm upper soil layer, obtained despite soil condition, were not as optimal in the second year as in the first. This might be due to a different water soil content dependent on the climatic trend which can change from year to year during the fall months when the treatment is commonly carried out. Incorporating compost into the 15-cm topsoil layer was established with the objective of combining efficiency, cost containment and sustainable adoption of modern technologies. The optimal compost distribution on the field surface is the basic requirement for homogeneous compost incorporation into the soil, especially when operating, as in this study, with limited volumes of amendments and small plots. The latter, however, are very frequent in intensive cropping systems such as those adopted for seed production, where this trial was carried out, and where the average surface per farm does not commonly exceed 6–8 ha. The adoption of an innovative compost spreader combined with an automatic guidance system guaranteed homogeneous spreading over a well-defined surface by avoiding overlapping in compost spreading on the field surface, thanks to perfectly parallel passages. In this way, high precision of spreading on the surface of the plot was reached which favored subsequent homogeneous compost incorporation into the soil profile using a simple conventional harrow. From a practical point of view, since automatically guided tractors are currently widespread in Italy, the adoption of this technology should not imply additional costs for farmers. Moreover, the compost spreader, such as the one used in this study, can be hired from local agricultural contractors who supply farm machinery and specialized operators or, increasingly frequently, they are provided by the compost producers themselves. They are more aware of the importance of supporting farmers in the transport and application of compost, which still represent a weak link when promoting the adoption of composts for organic fertilization of agricultural soils.

Conclusions

This study demonstrates that, in soils that are poor in organic matter, compost incorporation improves biological soil features from the first two applications with beneficial effects in the root-explored area of subsequent crops and activation of progressive C sequestration. These findings are consistent with previous studies and are in line with that which has been observed in soils repeatedly amended with digestate from biogas production in the same agro-environment. The precision agricultural technologies tested here for compost distribution, combined with incorporation into the soil to a limited depth of 15 cm, permitted greatly reduced operation costs by increasing the degree of precision in compost application. Considering that composts are the final segment in circular economy and availability of good quality composts to farmers is increasing, this study shows how much new technologies can facilitate their use even in small, specialized farms such as those included in this trial.

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