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Unveiling amending properties of biosolids from constructed wetland systems: a comparative study

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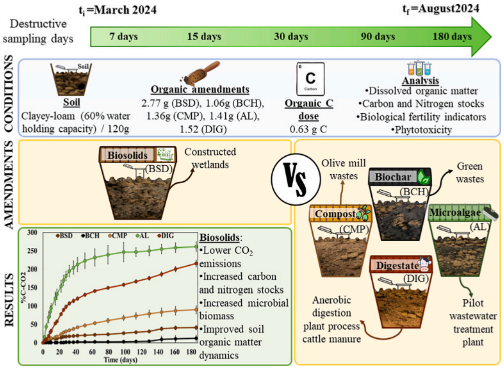
HIGHLIGHTS

- Constructed wetlands reduce reactive substances and enhance biosolids stabilization.
- The results of biosolids are comparable to amendments such as compost and biochar.
- Low-carbon mineralization was observed in the biosolids compared to other amendments.
- Carbon and nitrogen stocks increase with the application of biosolids over 6 months.
- Biosolids promote the increase of soil microbial biomass.

GRAPHICAL ABSTRACT



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ABSTRACT

Climate change and intensive farming have caused soil degradation and decreased organic carbon stocks. Current research focuses on restoring soil fertility, often through organic amendments. Biosolids stabilized in constructed wetlands (CWs) may serve as an applicable organic amendment, although limited literature exists on their properties. This study evaluated the effectiveness of biosolids as an organic amendment and compared them with other amendments such as biochar, compost, microalgae, and digestate. Over six months, a microcosm experiment with clay-loamy soil examined soil organic matter (SOM) dynamics, organic carbon and nitrogen distribution in pools, and microbial activity. Results indicated that biosolids positively affected SOM dynamics, with a low carbon mineralization rate (k: 0.006 d^{-1}). Biosolids significantly increased total organic carbon and total nitrogen stocks in the soil, reaching up to 18.1 $Mg{\cdot}ha^{-1}$ and 1.8 $Mg{\cdot}ha^{-1}$, respectively. They also enhanced stable and recalcitrant organic carbon fractions by up to 48 % and 57 %. Furthermore, biosolids improved soil biological fertility by boosting total enzymatic activity and microbial biomass carbon, which increased by 76 % compared to the unamended control at the end of the incubation. Overall, biosolids stabilized in CWs can be effective organic amendments, producing results comparable to other amendments.

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1. Introduction

Soil is a pillar of numerous Sustainable Development Goals (SDGs), i.e., SDGs 2, 6, 7, 13, and 15. Its health is essential for sustaining various ecosystem services and adapting to and mitigating the impact of climate change (Lal et al., 2021; Rodríguez-Espinosa et al., 2023). Soil health, which is influenced by factors such as soil organic matter (SOM) content and soil biodiversity, is critical for food and biomass production, as well as for the storage, filtration, and transformation of water, carbon, and nitrogen (Breure et al., 2018). SOM is a significant reservoir of soil organic carbon (SOC) and mediates the atmospheric CO_2 concentrations (Parmar et al., 2016). Estimates of SOC stored in a terrestrial ecosystem vary widely but are generally approximated to be between 1200 and 1600 Pg at depths of 0 to 10 cm (Antonelli et al., 2018). SOC comprises different pools, characterized by a variable biochemical reactivity determining their recalcitrance or availability for soil microbial activities (Mikutta et al., 2006).

Unfortunately, the Food and Agriculture Organization (FAO) estimates that nearly one-third of the world's soils are degraded (FAO., 2019) due to practices such as intensive agriculture and the overuse of chemical fertilizers, which lead to the deterioration of soil structure and biological fertility, as well as a reduction in SOC stocks (Wu et al., 2014). To mitigate soil degradation and preserve SOC stocks, applying organic amendments (OAs) is a management practice that enhances carbon sequestration (Ma et al., 2021). OAs improve soil fertility by enhancing physical (structure, aggregate stability, bulk density, and water retention), chemical (organic matter, nutrient content, cation exchange capacity, and pH), and biological (microbial biomass and enzymatic activities) properties (Garbowski et al., 2023; Rodríguez-Espinosa et al., 2023). Applying OAs also influences microbial community abundance and activity (Gutiérrez-Ginés et al., 2023). Soil biological fertility is vital for the nutrient cycle, and evaluating biological parameters provides evidence of soil stress or response to external factors, such as the application of OAs. This is mainly because OAs contain dissolved organic matter (DOM), which is recognized as the most active component of the soil organic matter (SOM) carbon pool (Haney et al., 2018). DOM includes a range of macromolecules, such as carbohydrates and proteins, as well as aliphatic and aromatic compounds (Zhang et al., 2022). In this context, DOM is a carbon-rich energy source for soil microorganisms. Although it is not the sole energy source, DOM is also considered a potential contributor to the stabilization of SOC.

In the context of the Circular Economy Strategy, there is a growing interest in recycling waste organic materials such as municipal organic wastes, biochar, green wastes, and biosolids as OAs. Biosolids, defined as sewage sludge obtained from wastewater treatment plants and subsequently treated in constructed wetlands (CWs), are often evaluated as amendments after undergoing stabilization processes like composting, anaerobic digestion, or pyrolysis (Eden et al., 2017). Many studies have shown that applying biosolids stabilized through treatments such as pyrolysis, composting, or anaerobic digestion improves soil biological fertility and carbon cycling (Truong and Marschner, 2018). Hechmi et al. (2020) found that applying biosolids increased the rate of organic matter degradation and carbon mineralization during the first 20 days of a three-month incubation. Biosolids application supports soil carbon sequestration, helping mitigate climate change by increasing soil carbon and reducing greenhouse gas emissions (Elgarahy et al., 2024). Similarly, Kizilkaya and Bayrakli (2005) observed improvements in organic matter and soil enzyme activity over a 30-day incubation period after applying 200 t·ha-1. Additionally, preliminary studies suggest that biosolids from constructed wetlands could serve as potential OAs or biofertilizers because of their nutrient content and ability to improve the soil's physical, chemical, and biological properties (Cano-Larrotta et al., 2024).

Several studies have addressed the impact of traditional OAs, biochar, compost, or treated sewage sludge on soil quality (Chen et al., 2018; Cooper et al., 2020; Giannetta et al., 2024; Kranz et al., 2020; Massaccesi et al., 2024; Vieira and Pazianotto, 2016). However, limited research has focused on using stabilized biosolids from constructed wetlands, especially as soil amendments in the Mediterranean region. Furthermore, there is a lack of studies that systematically compare different OAs, both conventional and emerging, and evaluate a comprehensive set of indicators, such as soil organic matter dynamics, dissolved organic matter quality, nutrient pools, and biological activity (Breza and Grandy, 2025; González et al., 2010). This gap is significant because the valorization of CW–derived biosolids could provide a sustainable and low-cost strategy to improve soil health in areas experiencing organic matter depletion and high fertilizer dependence. In this context, the objectives of this study were to assess the amending potential of biosolids and other OAs (biochar, compost, microalgae, and digestate) on: i) soil organic matter dynamics, ii) soil organic C and N pool stocks, and iii) soil microbial community activity. We hypothesized that biosolids from CWs could enhance soil quality by increasing SOM sequestration and soil biological fertility. Biosolids treated in CWs and the other OAs were evaluated in a six-month microcosm experiment using a clay-loamy soil to test this hypothesis.

2. Materials and methods

2.1. Soil and organic amendments

2.1.1. Soil sampling, preparation and characterization

The soil was collected from an agricultural field near Perugia (central Italy, N 43° 5′ 8″-E 12° 24′ 9″). The field is located about 400 m.a.s.l., with an average annual temperature of 24 °C and an average annual precipitation of 805 mm. Over the past 20 years, this soil has been used for cultivating olive trees and has not been treated with pesticides or fertilizers. According to the World Reference Base for Soil Resources (WRB), the soil was classified as a Cambisol. After evaluating the uniformity of the field's pedological conditions through several auger holes, 10 soil samples of approximately 1 kg each (from the Ap1 and Ap2 horizons, corresponding to 0–25 cm depth) were collected after removing grass and plant residues and then mixed to form a representative, homogeneous sample. An initial characterization was performed on air-dried soil sub-samples sieved at 2 mm before the amendment trials (see Table S1). The soil was classified as clayey-loam, comprising 6 % gross sand, 26 % fine sand, 38 % silt, and 30 % clay. Its properties included a pH of 7.8, an EC of 0.23 mS cm-1, a TOC content of 1.2 %, and a nitrogen content of 0.19 % (see Table S1). The soil was sieved through a 2-mm mesh for the amendment experiments, and the moisture content was adjusted to 60 % of the water-holding capacity (WHC) using deionized water.

2.1.2. Organic amendments sampling, preparation and characterization

The OAs used in this research included biosolids, microalgae, biochar, compost, and digestate. The biosolids were sourced from constructed wetlands and underwent a final stabilization period of six months. The heavy metal content was well below the threshold set by European regulations for using biosolids in agriculture, as detailed in Cano-Larrotta et al. (2024). The microalgae were obtained from a pilot plant associated with a wastewater treatment system, as described in Álvarez-González et al. (2022). The biochar, compost, and solid fraction of digestate were commercial products purchased at local fertilizer stores. Biochar was produced from the pyrolysis of green wastes, compost was obtained from olive tree pruning and olive mill wastes through thermophilic composting, whereas the solid fraction of digestate was derived by solid/liquid separation of digestate from a mesophilic anaerobic digestion plant treating cattle manure. The OAs were characterized in terms of total solids (%TS), volatile solids (%VS/%TS), pH (pH Meter Basic 20+, Crison Instruments, Barcelona, Spain), and EC (Ec Meter Basic 30+, Crison Instruments, Barcelona, Spain) (2:20 w/v ratio). Total organic C, total N, and the C/N ratio were analyzed using an elemental analyzer (MacroCUBE CNHS, Elementar Italia, Lomazzo, Italy). Dissolved organic matter (DOM) was determined from aqueous extracts following the methodology outlined by Li et al. (2009). Aqueous extracts were prepared by weighing 2 g of freeze-dried sample and dissolving it in 10 mL of deionized water, then agitating for 24 h, centrifuging at 5000 rpm for 10 min, and filtering through a 0.45 μm filter. Total reducing sugars (TRS) and total phenolic compounds (TPC) were measured on the aqueous extracts following the method of Massaccesi et al. (2013). DOM was characterized by determining Specific UV absorbance (SUVA254) according to the approach described by Zhang et al. (2022). Volatile fatty acids (VFAs) and alkalinity were analyzed based on the Standard Methods for the Examination of Water and Wastewater (APHA et al., 2017). Dried samples of OAs were examined using spectroscopic analysis to gain qualitative insights into their organic matter composition. Fourier Transform InfraRed (FT-IR) spectra were collected in total reflectance mode (ATR) using a Shimadzu IRAffinity-1S equipped with a Miracle Pike ATR device (Shimadzu Italia Srl, Milano, Italy), covering a wavenumber range of 4000–700 cm^{-1} with a resolution of 2 cm^{-1}. The collected FT-IR spectra were processed with Shimadzu LabSolutions IR Peak Software (Shimadzu Italia Srl, Milano, Italy).

For the amendment test, the OAs were freeze-dried, crushed, sieved through a 2 mm mesh, and then stored for the amendment trial.

2.2. *Experimental set-up*

Microcosms were set up using plastic pots to study the effects of different OAs over 180 days. Six treatments were tested: control (CTR) with no organic amendments, biosolids (BSD), biochar (BCH), compost (CMP), microalgae (AL), and digestate (DIG). The biosolids dose was based on the allowable sludge application rate in Italian agricultural soils, which is 15 $t \cdot ha^{-1}$ (Decreto Legislativo, 1992). Using this rate and the bulk density and soil profile data (Table S1), the biomass added to each microcosm was estimated at 2.77 $g \cdot microcosm^{-1}$. The required carbon addition was then calculated by considering the carbon content of the biosolids (Table 1), resulting in a total addition of 0.63 g C per microcosm. The applied mass for each treatment was determined based on its specific carbon content to ensure that all microcosms received the same carbon dose (0.63 g C). This approach met regulatory standards for biosolids and enabled direct comparison of how various OAs influence soil organic matter dynamics under consistent loading conditions.

For pots experiments, 120 g of soil prepared as described in Section 2.1.1 was placed in each plastic cylindrical pot (14 cm height, 8 cm diameter). The following amounts of dry matter (d.m.) were weighed out from each organic amendment: 2.77 g of BSD, 1.06 g of BCH, 1.36 g of CMP, 1.41 g of AL, and 1.52 g of DIG. The soil was then mixed with the amendments to create a homogeneous mixture. Each treatment was replicated four times. The pots were then sealed hermetically with screw caps and incubated in the dark at room temperature (20 °C). Soil moisture was maintained at 60 % of WHC by periodically measuring the weight of the pots to compensate for any water loss. All pots were opened weekly, and the soil was mixed to ensure proper aeration of the microcosms. Destructive microcosms were prepared for each treatment as described to allow sampling of soils at 7, 15, 30, and 90 days. At sampling time, destructive samples were divided into two portions: (i) the first was air-dried, crushed, and sieved at 0.5 mm for chemical analyses, whereas (ii) the second was frozen at −20 °C for biochemical analyses. On the last day of incubation (180 days), samples were collected and treated as described for intermediate samplings.

2.3. *Analytical procedures*

2.3.1. *Soil respiration*

Soil respiration was determined by measuring CO_2 evolution through titration over 180 days. CO_2 production was recorded daily for the first two weeks and then every two weeks due to decreased soil respiration. In short, the CO_2 released by the soil samples was captured in 2 M NaOH and titrated with 0.2 M HCl, using phenolphthalein as an indicator after precipitating carbonates with 3 mL of 20 % $BaCl_2$. Blank measurements, using soil incubated without OAs (i.e., CTR), were subtracted from each sample, and the results were expressed as a percentage of C mineralized. A first-order kinetic model was used to assess the kinetics of organic C mineralization from soils, as described by Mohanty et al. (2013) (Eq. (1)):

$$C_{min} = C_0 \times \left(1 - e^{-kt}\right) \tag{1}$$

where C_{min} is the cumulative mineralization (% C-CO_2) at time t, C_0 is the potentially mineralizable C, k (d^{-1}) is the apparent kinetic constant, and t (d) is the time. k was calculated by adjusting experimental data (C_{min}, t) and using non-linear regression (Microsoft Excel Software Solver, 2013).

2.3.2. *Dissolved organic matter extraction and characterization*

For extracting dissolved organic matter (DOM) from soils, 2 g of each soil sample was weighed and mixed with 10 mL of distilled water for 24 h (Solé-Bundó et al., 2017a). This water-based extraction method effectively retrieves bioavailable and water-soluble fractions of DOM, which are significant for short-term microbial and nutrient dynamics. This gentle, water-based approach is preferred over more aggressive methods, such as alkaline or saline solutions, because it better reflects the fraction of DOM soluble in water and bioavailable. This is especially important for microbial activity and the soil's short-term carbon cycle. Afterwards, the samples were centrifuged at 5000 r.p.m. for 10 min and filtered through 0.45 μm disc filters. The carbon content in the extracts (DOC) was measured by adapting the spectrophotometric method described by Li et al. (2009). Briefly, 1 mL of the filtered extract was

Table 1
Physiochemical properties of organic amendments obtained from sludge treatment wetlands, pyrolysis, composting, and wastewater treatment plants. $SUVA_{254}$: aromaticity. Results are reported on dry mass. Mean value ± SD, n = 3. Different letters indicate significant differences. <LOD: detection limit of the method. n.d: not determined.

Parameters	Units	Biosolids		Biochar		Compost		Microalgae		Digestate	
pH	–	5.9 ± 0.1	bc	9.8 ± 0	a	5.8 ± 0.1	c	6.7 ± 0	abc	9.4 ± 0	ab
Electrical conductivity	$mS \cdot cm^{-1}$	1.6 ± 0	b	0.31 ± 0.02	d	8.6 ± 0.3	a	1.1 ± 0	c	1.6 ± 0	b
Total solids	%	50 ± 0.9	ab	67 ± 2	ab	89 ± 1	a	12 ± 0	c	13 ± 1	bc
Volatile solids	%VS/%TS	47 ± 1	c	69 ± 0	bc	85 ± 1	a	81 ± 0	ab	83 ± 1	ab
Total organic C	%	23 ± 0	c	59 ± 4	a	46 ± 0	b	45 ± 0	b	41 ± 0	b
Total N	%	3.2 ± 0	c	1 ± 0	e	5.3 ± 0.1	b	7.5 ± 0	a	2.1 ± 0.1	d
C/N	–	7.04 ± 0.08	d	61 ± 1.6	a	8.7 ± 0.09	c	6 ± 0.02	e	19.4 ± 1.2	b
DOC (dissolved organic C)	$gC \cdot kg^{-1}$	0.8 ± 0.1	b	<LOD	–	1.8 ± 0.1	ab	14 ± 1.8	ab	14.9 ± 1.4	a
Total reducing sugars	$gGlucose \cdot kg^{-1}$	0.68 ± 0.01	ab	<LOD	–	1.98 ± 0	ab	2.1 ± 0.2	a	0.32 ± 0.03	b
Total phenolic compounds	$mgVanillicAcid \cdot kg^{-1}$	7.3 ± 0.4	c	<LOD	–	57.5 ± 0.97	bc	149 ± 4.6	ab	425 ± 16	a
$SUVA_{254}$	$L \cdot mg^{-1} \cdot m^{-1}$	0.04 ± 0	a	<LOD	–	0.03 ± 0	ab	0.002 ± 0	b	0.003 ± 0	ab
Volatile fatty acids	mg $CH_3COOH \cdot g^{-1}$	0.14 ± 0.07	bc	n.d	–	0.1 ± 0	c	10.1 ± 0	a	2.9 ± 0	ab
Alkalinity	mg $CaCO_3 \cdot g^{-1}$	0.2 ± 0	c	n.d	–	0.2 ± 0.03	bc	16 ± 1.7	ab	21.6 ± 0	a

transferred to a glass tube, followed by the addition of 2 mL of $K_2Cr_2O_7$. The mixture was vortexed, then 2 mL of 96 % H_2SO_4 was added, shaken, and incubated in a digester at 160 °C for 30 min. The absorbance was then measured at 400 nm and compared to a calibration curve prepared with increasing carbon concentrations (potassium phthalate).

Total phenolic compounds (TPC) and total reducing sugars (TRS) in the aqueous extracts were determined spectrophotometrically using a modified Folin-Ciocalteu method and the phenol reagent method as described by Massaccesi et al. (2013), respectively. TPC and TRS were expressed as $\text{mgC}\cdot\text{L}^{-1}$ against vanillic acid and glucose calibration curves.

SUVA254 was measured to evaluate the aromaticity of the aqueous extracts. SUVA254 was normalized based on the analyzed C concentration (C) (Zhang et al., 2022) (Eq. (2)):

$$\text{SUVA}_\lambda = \text{A}(254\ \text{nm})/\text{d}/\text{C} \tag{2}$$

where A(λ) is the absorbance at the wavelength of 254 nm, and d is the optical path of the cuvette (m). The results were expressed as $\text{L}\cdot\text{mg}^{-1}\cdot\text{m}^{-1}$.

2.3.3. C and N fractionation into pools

The fractionation of C and N into pools was conducted based on the method described by Mikutta et al. (2006) on the last destructive soil samples (180 days).

For separating the stable OC (SOC) fraction, 3 g of each air-dried sample underwent three treatment cycles with 30 mL of 6 wt% NaOCl (pH adjusted to 8) to remove labile OC (LOC). Each cycle lasted 6 h with agitation at room temperature. After each cycle, the samples were centrifuged at 5000 rpm for 10 min, and the supernatants discarded. The samples were washed with distilled water until no reaction with $AgNO_3$ indicated chlorine-free solutions. The residues were then dried in an oven at 60 °C. To isolate the recalcitrant OC (ROC) fraction, 2.25 g of NaOCl-treated samples were treated with 15 mL of 10 % HF to dissolve mineral components and associated mineral OC (MOC). The samples were agitated for 2 h, centrifuged at 5000 rpm for 10 min, and the supernatants discarded. The residues underwent five washes with distilled water and were dried in an oven at 60 °C. The C and N contents in the bulk soil and fractions were determined using an elemental analyzer (MacroCUBE CNHS, Elementar Italia, Lomazzo, Italy), after proper homogenization and removal of carbonates with 6 N HCl. LOC and MOC were calculated by subtracting TOC from SOC and SOC from ROC, respectively. Total N (TN), stable N (SN), labile N (LN), mineral-associated N (MN), and recalcitrant N (RN) were measured following the same procedure as for C. Finally, C stocks in the bulk soil and various fractions were calculated using the equation (Eq. (3)) (Massaccesi et al., 2018):

$$OC_{stock} = \text{OC} \times \text{BD} \times \text{d} \tag{3}$$

where OC stock represents soil organic carbon stock in $\text{Mg}\cdot\text{ha}^{-1}$, OC is the organic C content (%), BD is bulk density ($\text{g}\cdot\text{cm}^{-3}$), and d is the soil depth (10 cm). The calculation of OC stock was not corrected for coarse fragments because there were few (<1 %) in the soil at the studied sites. The same formula was used to determine the N stocks using the N concentrations in the bulk sample and fractions ($\text{mg}\cdot\text{g}^{-1}$).

2.3.4. Soil biochemical analysis

Microbial activity was measured using an enzymatic hydrolysis assay with fluorescein diacetate (FDA), following the method outlined by Sánchez-Monedero et al. (2008). Weighed 0.5 g of air-dried samples into Falcon tubes, combined them with 5 mL of 60 mM potassium phosphate buffer (pH 7.6), and added 0.1 mL of a stock FDA solution (200 $\text{mg}\cdot\text{mL}^{-1}$). The tubes were then incubated for 1 h at room temperature (25 °C). A blank was prepared for each sample by replacing the FDA with acetone. After incubation, the reaction was stopped by adding 5 mL of acetone, and the mixture was centrifuged at 5000 r.p.m. for 10 min. The supernatant was collected, and its absorbance was measured at 550 nm. Results were obtained by subtracting the absorbance of the blank, then comparing it to a calibration curve created with fluorescein, which was reported as mg $\text{fluorescein}\cdot\text{kg}^{-1}\cdot\text{h}^{-1}$.

Microbial biomass carbon (Bc) was measured using the fumigation-incubation method described by Vance et al. (2002). A 15 g moist soil sample was weighed, fumigated with chloroform, and then incubated in a vacuum in the dark for 24 h. After incubation, multiple extractions were performed with a vacuum pump to remove chloroform from the samples. The samples were then transferred into a 200 mL glass container, where 2 mL of NaOH was added in an Eppendorf tube. The containers were sealed and kept in the dark at 25 °C for 10 days. Meanwhile, non-fumigated samples were incubated as controls. After the 10 days, CO_2 production was determined by titration with HCl (see Section 2.3.1). Microbial biomass C was calculated using the following equation (Eq. (4)):

$$Bc = \frac{F_c}{k_c} \tag{4}$$

where F_c represents the difference in CO_2 production between the fumigated and non-fumigated samples, and k_c is a constant denoting the microbial C ratio that evolves as CO_2 over 10 days at 25 °C, set at 0.45.

2.4. Statistical analysis

The normality and homoscedasticity of the data were assessed using the Anderson-Darling test, and it was found that they did not meet the assumptions required for parametric analysis. A power transformation was applied to the original data to address this, and the residuals were examined to ensure normality (Montgomery and Merrill, 2017). After re-evaluating these properties, an ANOVA was performed, followed by mean comparisons among treatment groups using the Tukey test, which was used to analyze differences among treatments ($\alpha = 0.05$) for parameters such as basal respiration, dissolved organic carbon (DOC), and enzymatic activity, allowing control of the family-wise error rate. All statistical analyses were conducted using Minitab version 22.0.0.

3. Results and discussion

3.1. Characterization of OAs

3.1.1. Physicochemical characteristics

The physicochemical characterization of the OAs is shown in Table 1 and Fig. S1. From an agronomic perspective, pH values close to neutrality are ideal for enhancing soil fertility, as neutral pH improves nutrient availability. Biosolids exhibited a slightly acidic pH (5.9 ± 0.1), likely due to organic matter decomposition and the dewatering process during biosolids treatment in wetlands, as previously reported by Cano-Larrotta et al. (2024). Compost and microalgae also had pH values near neutrality (5.8 and 6.7, respectively), while biochar and digestate displayed alkaline pH levels (9.8 and 9.4, respectively), consistent with existing literature (Cooper et al., 2020) (Table 1).

Low EC values in soils can enhance soil properties, while high EC levels may cause soil salinization (Mpanga et al., 2023). In this study, biosolids, microalgae, and digestate had similar EC values (1.6 $\text{mS}\cdot\text{cm}^{-1}$, 1.1 $\text{mS}\cdot\text{cm}^{-1}$, and 1.6 $\text{mS}\cdot\text{cm}^{-1}$, respectively), whereas compost showed a high EC (8.6 mS cm^{-1}), likely because of the feedstock composition. Biochar was the only amendment with significantly lower conductivity (0.3 $\text{mS}\cdot\text{cm}^{-1}$). This could be due to the pyrolysis temperature and the specific feedstock used for biochar production (Hossain et al., 2011).

The total solids content varied among the amendments, with biosolids, biochar, and compost showing high %TS contents (i.e., 50 %, 67 %, and 89 %, respectively). In contrast, microalgae and digestate displayed lower TS values. Using OAs with high solid content offers advantages over liquid OAs, such as reduced volume, transportation costs, and lower runoff potential. Liquid amendments risk losing essential nutrients due to heavy rainfall or irrigation, while solid amendments adhere better to soil particles, facilitating a gradual nutrient release (Angelova et al., 2013).

Biochar, compost, microalgae, and digestate exhibited higher carbon contents (59 %, 46 %, 45 %, and 41 %, respectively) than biosolids (23 %). The lower carbon content of biosolids compared to other OAs may be due to the oxidation process during wastewater treatment. Additionally, the stabilization process of biosolids in CWs could have further affected the carbon content observed in this study (Mohseni et al., 2024). Regarding N content, biosolids showed a notable N content (3.2 %), which aligns with the literature (Cano-Larrotta et al., 2024). The other OAs analyzed showed increasing N contents following this order: biochar (2 %) < digestate (2.1 %) < compost (5.3 %) < microalgae (7.5 %). Among the OAs, compost and microalgae had the highest values, probably due to the feedstocks used for composting and abundant microbial biomass, respectively.

3.1.2. *Dissolved organic matter characterization*

The DOM content of the OAs decreased in the following order: digestate (14.9 $gC\cdot kg^{-1}$) > microalgae (14 $gC\cdot kg^{-1}$) > compost (1.8 $gC\cdot kg^{-1}$) > biosolids (0.8 $gC\cdot kg^{-1}$) (Table 1). Interestingly, biosolids showed the lowest DOC value, likely due to the oxidation of labile organic matter during their stabilization in CWs. Digestate and microalgae contained large amounts of labile organic matter, which is attributed to the absence of aerobic oxidation in their production processes (Cucina, 2023; Solé-Bundó et al., 2017b). Spectrophotometric analysis of DOC indicated that both microalgae and digestate DOC had low aromaticity (0.002 $L\cdot mg^{-1}\cdot m^{-1}$ and 0.003 $L\cdot mg^{-1}\cdot m^{-1}$, respectively), compared to biosolids and compost, which showed higher aromaticity (0.04 and 0.03 $L\cdot mg^{-1}\cdot m^{-1}$, respectively), as indicated by $SUVA_{254}$. These differences between biosolids/compost and microalgae/digestate likely result from the stabilization of organic matter during post-processing of biosolids in CWs and during compost curing. It is known that these processes increase the aromaticity of soluble organic matter, as described in Cucina (2023). Conversely, DOC in microalgae and digestate is characterized by lower aromaticity, which may lead to higher bioavailability of their DOM for microbial activity in soil (Solé-Bundó et al., 2017a).

Significant differences in TRS were observed among the OAs: microalgae showed the highest levels (2.1 g $Glucose\cdot kg^{-1}$), closely followed by compost (1.98 g $Glucose\cdot kg^{-1}$). In contrast, biosolids had a much lower TRS content (0.68 g $Glucose\cdot kg^{-1}$), while digestate exhibited the lowest value (0.32 g $Glucose\cdot kg^{-1}$). Conversely, TPC content displayed a different trend, with digestate leading at 425 mg Vanillic $acid\cdot kg^{-1}$, and biosolids showing the lowest concentration at 7.3 mg Vanillic $acid\cdot kg^{-1}$. The low TPC content in biosolids can be attributed to several factors, including oxidative degradation during wastewater treatment, the activity of specific microorganisms capable of breaking down phenolic compounds, and the stabilization processes that biosolids undergo (Kinney et al., 2006). Overall, the differences in both TRS and TPC content among the various OAs are mainly linked to their distinct origins and the inherent chemical composition of the biomasses used.

Regarding VFAs, biosolids showed similar values to compost (0.14 and 0.10 mg $CH_3COOH\cdot g^{-1}$, respectively), with both being roughly ten times lower than the VFA levels in microalgae and digestate (Cucina, 2023; Solé-Bundó et al., 2017a). The absence of aerobic oxidation during microalgae and digestate production likely explains these results; aerobic oxidation during composting and biosolid post-processing probably reduced VFAs in those materials. Managing VFAs benefits agriculture because they link to phytotoxicity effects (Cucina et al., 2021). Alkalinity also varied among the OAs, grouping them into two categories: biosolids/compost and microalgae/digestate. Biosolids and compost had low alkalinity (0.2 mg $CaCO_3\cdot g^{-1}$), about tenfold lower than microalgae and digestate (16 mg $CaCO_3\cdot g^{-1}$ and 21.6 mg$\cdot CaCO_3\cdot g^{-1}$, respectively). Alkalinity usually results from a basic pH buffer in the medium (i.e., ammonia-ammonium, carbonate-bicarbonate), which may explain the high values observed in microalgae and digestate.

3.1.3. *FT-IR analysis of OAs*

Finally, FT-IR analysis confirmed that the composition of biosolids and compost is similar in terms of organic matter content. As shown in Fig. S1 and Table S2, biosolids exhibited strong absorption bands at 3400–3200 cm^{-1} (-OH stretching vibration, related to carbohydrates), 1670 cm^{-1} (C═O stretching, linked to carbohydrates and esters), 1520 cm^{-1} (C—N bonds in amides, associated with proteins), and 1050 cm^{-1} (C—O bond stretching, related to carbohydrates) (Smidt and Meissl, 2007). The biosolids spectrum indicates that organic matter may include labile and recalcitrant organic compounds. The compost spectrum showed a similar absorption profile to that of biosolids but with more intense bands associated with carbohydrates, consistent with previous reports. Additionally, in the compost spectrum, other bands appeared, indicating the presence of polysaccharides and fats from plant sources (i. e., 2920–2850 cm^{-1}), as well as lignin structures likely originating from the bulking material (around 1200 cm^{-1}). Spectra of microalgae and digestate highlighted the same absorption bands as biosolids but with higher intensity, especially those linked to carbohydrates (3400–3200 cm^{-1}), proteins (1520 cm^{-1}), fats (2920–2850 cm^{-1}), and aromatic structures (1200 cm^{-1}), confirming greater nitrogen content. These spectra also revealed carbonates (900–870 cm^{-1}), aligning with the higher alkalinity observed in these two OAs. As expected, the biochar spectrum showed weak absorption bands due to the pyrolysis process, which produces low-intensity peaks at wavelengths characteristic of aromatic structures, based on the feedstock (green wastes) used to produce the biochar (Goglio et al., 2025).

3.2. *Impact of OAs on soil organic matter dynamics*

3.2.1. *Soil respiration and OAs mineralization in soil*

Table 2 and Fig. S2 show the cumulative production of C-CO_2 for each treatment. Overall, the order of cumulative C-CO_2 production over 180 days is: AL > DIG > CMP > BSD > BCH. Significant differences were found among the AL, DIG, and BCH treatments, while no statistical differences were observed between BSD and CMP.

Biosolids showed a high level of OM stability, as indicated by their slow C-mineralization over 180 days compared to microalgae and digestate (i.e., the kinetic constant k was 0.006 d^{-1} for biosolids and 0.046 and 0.0225 d^{-1} for microalgae and digestate, respectively). This slow mineralization rate may support long-term soil health and fertility benefits by enhancing the soil's ability to retain moisture, support microbial activity, and promote healthy root growth (Das et al., 2023). However, conducting field-scale experiments over longer periods is essential for responsible biosolids management and evaluating environmental variability. The slow mineralization rate of biosolids in soil could be related to its composition, including low levels of DOC, sugars, and VFAs (Table 1) and recalcitrant organic compounds (Fig. S1). It is well known that soluble organic compounds are the most reactive fraction of organic matter and are prone to rapid degradation in soil due

Table 2

Cumulative organic C mineralization (% C-CO2) and kinetic constant (k) from different types of organic amendments in clay-loam soil. BSD: biosolids, BCH: biochar, CMP: compost, AL: microalgae; DIG: digestate. Mean value ± SD, n = 3. Different letters in the same column indicate significant differences.

	Cumulative mineralization (% C-CO_2)	k (d^{-1})	R^2
BSD	41 ± 6c	0.006	0.957
BCH	12 ± 8d	0.00012	0.971
CMP	90 ± 11c	0.0125	0.997
AL	262 ± 13a	0.046	0.997
DIG	216 ± 9b	0.0225	0.982

to microbial activity (Cucina et al., 2021). These findings align with Bai et al. (2017), who reported that residual sludge increased soil OC by 60.4 %. Interestingly, soil biosolids mineralization (% $C\text{-}CO_2$) was similar to biochar (Table 2), confirming its inherent stability. Biochar has been widely studied as an organic amendment because of its unique properties, which improve soil biological fertility by stimulating microbial activity and promoting C immobilization within microbial biomass, thus helping prevent the degradation of soil organic carbon (SOC) (Massaccesi et al., 2024; Nogués et al., 2023). Although literature reports vary depending on composting feedstocks and operational parameters, the fertilizing properties of compost are generally recognized, as it enhances SOM content and soil fertility thanks to its stable organic fraction, which undergoes slow mineralization in soil (Cucina et al., 2018; Kranz et al., 2020). In this study, although no significant differences were observed between compost- and biosolids-amended soils regarding C mineralization ($P > 0.05$), compost mineralized faster than reported in previous research (e.g., k was 0.0125 d^{-1}, Table 2), achieving nearly complete C mineralization after 6 months of incubation. This may be due to incomplete maturation of the compost used in the experiments, as indicated by the high amount of soluble sugars detected in the compost (Table 1) and by the presence of absorption bands linked to immaturity in its FT-IR spectra (Fig. S1) (Smidt and Meissl, 2007).

In the present study, microalgae and digestate-treated soil showed increased respiration rates, ultimately leading to a priming effect (i.e., C mineralization was higher than 100 %, Table 2) (Simanjuntak et al., 2022). C mineralization exceeded 100 % after 15 and 32 days for microalgae and digestate, respectively, indicating strong microbial activity associated with these OAs (Fig. S2). The higher respiration rates of these OAs may be due to their high content of DOM, which was characterized by elevated levels of sugars and phenols, along with low aromaticity (Table 1). The rapid OM mineralization caused by microalgae and digestate treatments could potentially deplete the soil's organic matter reserves, leading to negative long-term effects. This quick mineralization may be linked to these specific organic biomasses not being stabilized before application. For example, the digestate used in this study is produced through anaerobic digestion, which does not produce stable or fully matured biomass (Cucina et al., 2018). Similarly, the microalgae were sourced from a pilot wastewater treatment system and did not undergo any stabilization process. Additionally, the high respiration rates observed may be due to a mixed culture of microalgae and bacteria (Álvarez-González et al., 2022), which show high activity levels consistent with the rapid OM mineralization observed in this study.

3.2.2. Dissolved soil organic matter characteristics and evolution

DOC concentration in amended and control soils over time (Fig. 1a) showed significant differences among treatments at four of the five sampling times (90 and 180-days incubation). No significant DOC differences ($P > 0.05$) were observed at the 30-day incubation.

When analyzing the temporal evolution of each treatment, statistical differences in the biosolids were observed at 7 days of incubation ($P > 0.05$), with a high DOC value associated with increased respiration rates (Fig. S2). The behavior of DOC concentration in the biosolids-amended soil was similar to that of biochar-amended soil, likely due to their prior stabilization in the CWs (Cano-Larrotta et al., 2024). Regarding compost-amended soil, statistical differences were observed at the 15-day incubation period, when it showed the highest DOC among all treatments ($P > 0.05$). For microalgae and digestate, results statistically higher than the control ($P > 0.05$) were recorded at 7, 15, and 90 days of incubation. From a comparative perspective, treatments with biosolids and biochar displayed lower DOC values than those using microalgae, compost, and digestate. This trend aligns with the DOC values observed in the OA's characterization (Fig. 1a; Table 1). The high DOC levels indicate that the organic matter from these amendments has not undergone extensive stabilization, as further evidenced by the high respiration rates associated with these biomasses. This increased respiration likely results from elevated microbial activity, promoting the hydrolysis of organic matter from the soil and the incorporated organic amendments (Yao et al., 2024).

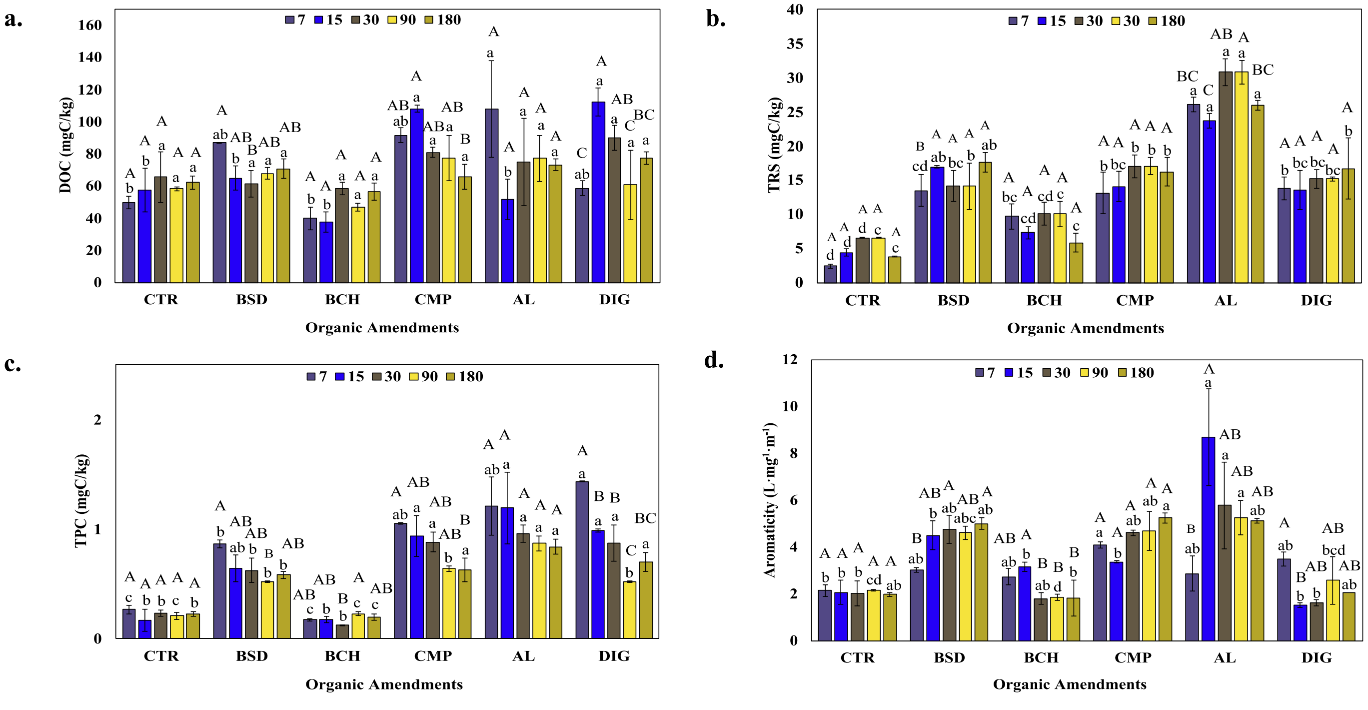

Fig. 1. Effect of organic amendments on the DOC, TRS, TPC, and Aromaticity concentration of clay-loam soil over various time intervals. a. DOC: dissolved organic carbon; b. TRS: total reducing sugars; c. TPC: total phenolic compounds, d. Aromaticity ($SUVA_{254}$). The error bars indicate the standard deviation of the mean ($n = 2$). Different letters indicate significant differences, lowercase letters indicate ANOVA results between treatments for each time point, capital letters indicate ANOVA results over time for each treatment.

TRS are considered a vital energy source for microorganisms in soil ecosystems. A high sugar content in organic amendments promotes the degradation of organic compounds, resulting in increased microbial activity (Antonious, 2016). The evolution of TRS over time in amended and control soils is shown in Fig. 1b, where significant treatment differences ($P > 0.05$) were observed. Levels of TRS in biosolids-amended soils were lower compared to those in microalgae and digestate-amended soils, matching the values found in biochar and compost-amended soils. This indicates that, although biosolids and biochar may not stimulate rapid microbial activity like microalgae and digestate (Fig. S2), they might contribute to the long-term stability of organic matter in soil. Interestingly, TRS showed a consistent increase over time across all treatments. While a decreasing trend might be expected due to TRS mineralization by soil microbes, the ongoing release of soluble sugars from organic matter hydrolysis likely helped maintain stable TRS levels in amended soils (Cucina et al., 2018).

Fig. 1c shows the TPC concentration across the different treatments tested. Significant differences among treatments were observed for each amendment ($P > 0.05$), especially during the first 7 days, with notable variations among biosolids, biochar, and digestate. The soil amended with digestate exhibited the highest TPC content ($1.44\ \text{mgC}\cdot\text{kg}^{-1}$) at this sampling point, likely due to the high TPC concentration in the digestate (Table 1). Soluble TPC in the digestate may result from partial anaerobic degradation of feedstock components containing aromatic organic carbon (i.e., lignin) (Fermoso et al., 2018). However, TPC levels in soil generally decreased over time, except in biochar-amended soil. This decline may be due to soil microbial activity, which biodegraded and incorporated TPC into soil organic matter, as Sierra et al. (2007) described, with phenolic compounds playing a key role in synthesizing humic substances.

Finally, Fig. 1d shows the aromaticity of dissolved organic matter (DOM) in the different treatments studied during incubation. Statistically significant differences were observed between the treatments for each incubation time and within each treatment ($P < 0.05$). Significant changes over time were noted in biosolids-amended soil, except at the 7-day sampling point. Notably, biosolids maintained a high level of DOM aromaticity, similar to compost-amended soil, which is an important feature influencing the overall quality of DOM (Soria et al., 2021). Interestingly, aromatic compounds added to soil with biosolids appeared more stable and less susceptible to biodegradation compared to those with microalgae and digestate (Solé-Bundó et al., 2017a). The upward trend of DOM aromaticity in biosolids-amended soil may stem from the natural tendency of DOM's aromatic compounds to concentrate as organic matter matures. This maturation improves the quality of DOM, making it more effective at enhancing soil health and fertility, as Jaffrain et al. (2007) suggested. Furthermore, the increase in aromaticity has important implications for the soil's ability to absorb organic contaminants. As highlighted by De Paolis and Kukkonen (1997), the presence of aromatic compounds can boost this absorption capacity, potentially reducing the mobility of pollutants and improving the overall ecological function of the soil system.

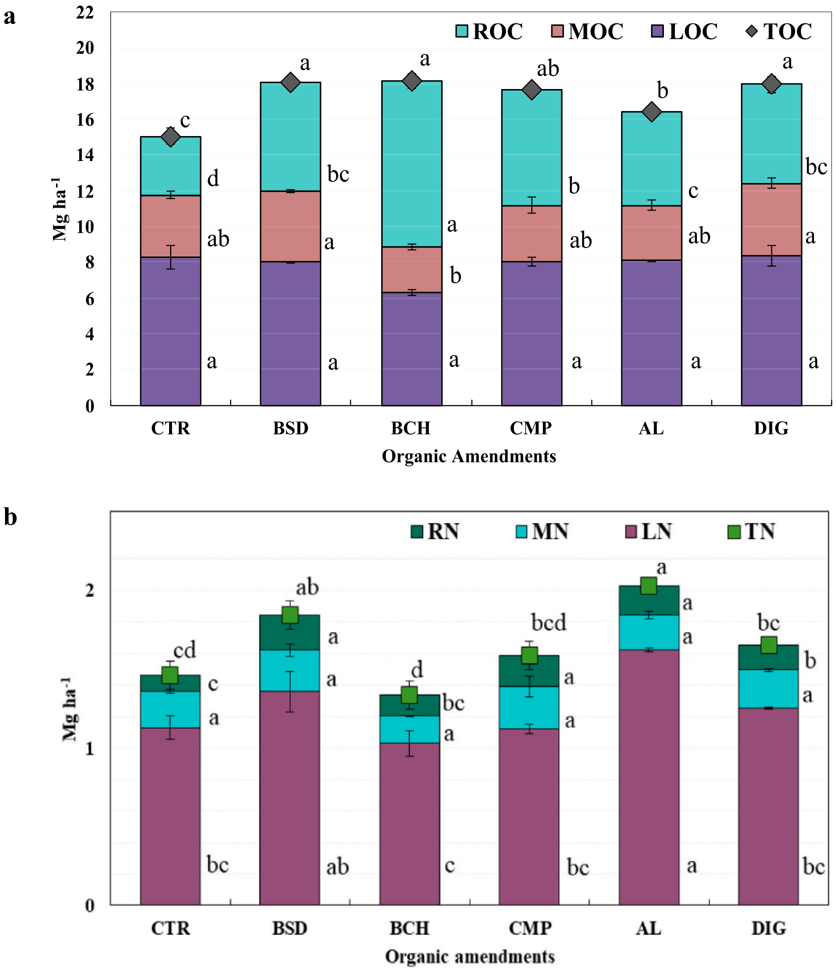

Fig. 2. Effect of applying OAs to a clay loam soil on carbon (a) and nitrogen (b) stocks after 6-months from amendments application. ROC: recalcitrant organic carbon; MOC: mineral-associated organic carbon; LOC: labile organic carbon; TOC: total organic carbon; RN: recalcitrant nitrogen; MN: mineral-associated nitrogen; LN: labile nitrogen; TN: total nitrogen. Error bars indicate the standard deviation of the mean, n = 2. Different letters indicate significant differences.

3.2.3. Effects of OAs on soil organic C and N stocks

The analysis of carbon and nitrogen stocks after applying various OAs is crucial for understanding their ability to improve soil carbon and nitrogen reserves.

Fig. 2a shows C stocks after 6 months of incubation with various OAs. A significant increase in TOC stock was observed in soils amended with biosolids, biochar, and digestate compared to the control, which had a TOC stock value of 15 Mg·ha^{-1}. The TOC stock values for soils amended with biosolids, biochar, and digestate were 18.1 Mg·ha^{-1}, 18.2 Mg·ha^{-1}, and 18 Mg·ha^{-1}, respectively, demonstrating a statistically significant increase over the control ($P < 0.05$). Notably, biosolids may have provided a long-term benefit due to the recalcitrant nature of organic matter, balancing labile and stable carbon (see Section 3.1), which enhanced soil carbon sequestration over time.

Regarding OC fractions, biosolids-amended soil showed higher SOC and ROC stocks than the control, with values of 10.1 Mg·ha^{-1} (+ 48 %) and 5.8 Mg·ha^{-1} (+ 57 %), respectively. In this study, biosolids increased soil carbon stocks to a level comparable with compost and digestate, which are widely recognized as effective organic soil amendments. Interestingly, microalgae application to the soil induced limited OC stock enhancement in the treated soil, likely due to the strong priming effect observed during the six-month incubation period (Section 3.2.1). Finally, biochar resulted in the most significant increase in stable organic carbon stocks (i.e., SOC and ROC), as expected based on the literature, confirming its potential for long-term carbon sequestration. Although literature suggests that a high C/N ratio in the OAs is particularly effective in boosting SOC reserves (Truong and Marschner, 2018), in this study, the C/N of the tested OAs was not directly correlated with the OC stock results. Specifically, the increase in stable organic OC followed the order: biochar > biosolid > compost > digestate > microalgae, whilst the C/N ratio decreased in the order: biochar > digestate > compost > biosolids > microalgae (Table 1). Therefore, the positive effects observed with biosolids application might be due to the high stability of biosolids' organic matter, as previously discussed in Section 3.1. MOC was the least affected among all OC pools, with only biosolids and digestate slightly increasing MOC stocks compared to the control. This may be influenced by several factors, primarily the experiment's duration and soil chemical properties. Literature reports that forming organic matter-clay complexes associated with the MOC pool takes longer after OAs application (Giannetta et al., 2024). Additionally, the formation of these complexes depends on the soil's mineralogical properties; soils rich in Al- and Fe-hydroxides/oxides are more prone to form complexes with exogenous organic matter applied with OAs (Cucina et al., 2025).

Results align with the literature, where biosolids affect SOC through direct C addition (Badewa et al., 2023). Antonelli et al. (2018) demonstrated that anaerobically digested biosolids increase C levels and pools for up to 13 years due to high organic matter. Lin et al. (2024) found that using composted biosolids resulted in short-term gains in carbon reserves compared to alkaline-treated and anaerobically digested biosolids.

Fig. 2b shows the stocks of different N fractions after 6 months of incubation. Significant differences in nitrogen stocks were observed among the treatment groups. Notably, total nitrogen (TN) stocks were substantially higher in biosolids and microalgae, with recorded values of 1.8 Mg·ha^{-1} and 2.0 Mg·ha^{-1}, respectively. This increase in TN stock associated with biosolids and microalgae was due to their high N content (Table 1), which enriched the soil. Accordingly, previous studies have identified these OAs as effective biofertilizers, capable of replacing inorganic fertilizers while improving soil fertility (Álvarez-González et al., 2022; Cano-Larrotta et al., 2024). When examining the stock of labile nitrogen (LN), it is noteworthy that its levels remained consistent across soils amended with biosolids, compost, and digestate, and they did not differ from the control soils. Conversely, biochar showed lower LN levels, with a value of 0.5 Mg·ha^{-1}, which was expected because nutrients in biochar are mostly in non-bioavailable forms (Clagnan et al., 2023). Microalgae, however, differed from the control, showing the highest LN level at 1.0 Mg·ha^{-1}, indicating their potential as a dynamic nitrogen source for plant uptake. This may be because of the high bioavailability of N in microalgae biomass, as previously reported in the literature (Solé-Bundó et al., 2017a). Regarding stable nitrogen (SN) stock, which acts as an important soil reserve, significant differences were found among soils amended with biosolids, compost, and digestate ($P < 0.05$), all of which showed increased SN stocks compared to the control. This suggests that biosolids increased immediate nitrogen availability and may support longer-term nitrogen retention in the soil. Finally, notable differences emerged in mineral-associated nitrogen (MN) and recalcitrant nitrogen (RN) stocks, especially in soils amended with biosolids and compost, which exhibited high MN values. While all amendments increased RN levels, biochar-amended soil maintained the same values as the control, indicating it may not enhance this form of nitrogen as effectively as the other OAs. Considering that excessive input of labile N might lead to its loss through leaching and emissions (Silveira and Kohmann, 2020), results from applying biosolids to soil are promising from an agronomic perspective. Biosolids application increased all N stocks in soil, highlighting its potential to support short- and long-term N needs for soil microorganisms and plants, while reducing environmental concerns related to N losses.

3.3. Effects of OAs on soil biological fertility indicators

Biological properties of soil, primarily soil microbial biomass carbon (Bc) and enzymatic activities, are vital for nutrient cycling within the soil ecosystem. These parameters are key to understanding soil quality and evaluating biological stress in various management practices (Dhanker et al., 2021). In this study, we found that enzymatic activity (measured through FDA hydrolysis activity) related to the OAs generally showed higher levels compared to the control treatment ($P < 0.05$) (Fig. 3). The hydrolysis of FDA is commonly used as a proxy to assess soil microbial activity (Adam and Duncan, 2001), since the hydrolytic process involves enzymes like esterases, proteases, and lipases, which are essential for decomposing soil organic matter (Sánchez-Monedero et al., 2008).

The application of biosolids increased enzymatic activity, reaching hydrolysis rates of 32 mg Fluoresceine kg^{-1}·h^{-1} during the first 7 days of incubation. Although this activity dropped to 21 mg Fluoresceine kg^{-1}·h^{-1} by day 15, it then increased again, stabilizing at 25 mg Fluoresceine kg^{-1}·h^{-1} from days 30 to 90, and reaching 33 mg Fluoresceine kg^{-1}·h^{-1} by day 180 (Fig. 3). This overall boost in enzymatic activity can be attributed to the readily biodegradable organic matter and nutrients in biosolids, which encouraged microbial growth within the soil matrix and enhanced enzymatic activity (Cucina et al., 2018). Several studies have shown that applying residual sludge or high-quality biosolids can promote soil microbiological activity and biochemical indicators. These include microbial biomass and enzymatic activity, which are closely linked to biogeochemical cycles that affect plant productivity (Araújo et al., 2016). Notably, Vieira and Pazianotto (2016) reported lower enzymatic activity after applying anaerobically digested sewage sludge compared to the values recorded in this study. In contrast, Silva et al. (2014) found that a dose of 5 t·ha^{-1} resulted in FDA enzymatic activity of 23.7 kg^{-1}·h^{-1}, similar to the findings of this study. Regarding other treatments, soils amended with digestate and microalgae showed the highest enzymatic activity at 7 days of incubation, with values of 56 mg Fluoresceine kg^{-1}·h^{-1} and 30 mg Fluoresceine kg^{-1}·h^{-1}, respectively—both exceeding the activity in biosolids-amended soil (Fig. 3). The elevated enzymatic activity observed in the digestate and microalgae treatments coincided with the highest respiration rates (see Fig. 1S). Compost also maintained high enzymatic activity in soil, behaving similarly to the biosolids treatment.

Bc is a helpful indicator of the soil's microbiological status and the possible effects of soil management practices on crop productivity (Jedidi et al., 2004). This study showed that *Bc* in soils treated with all

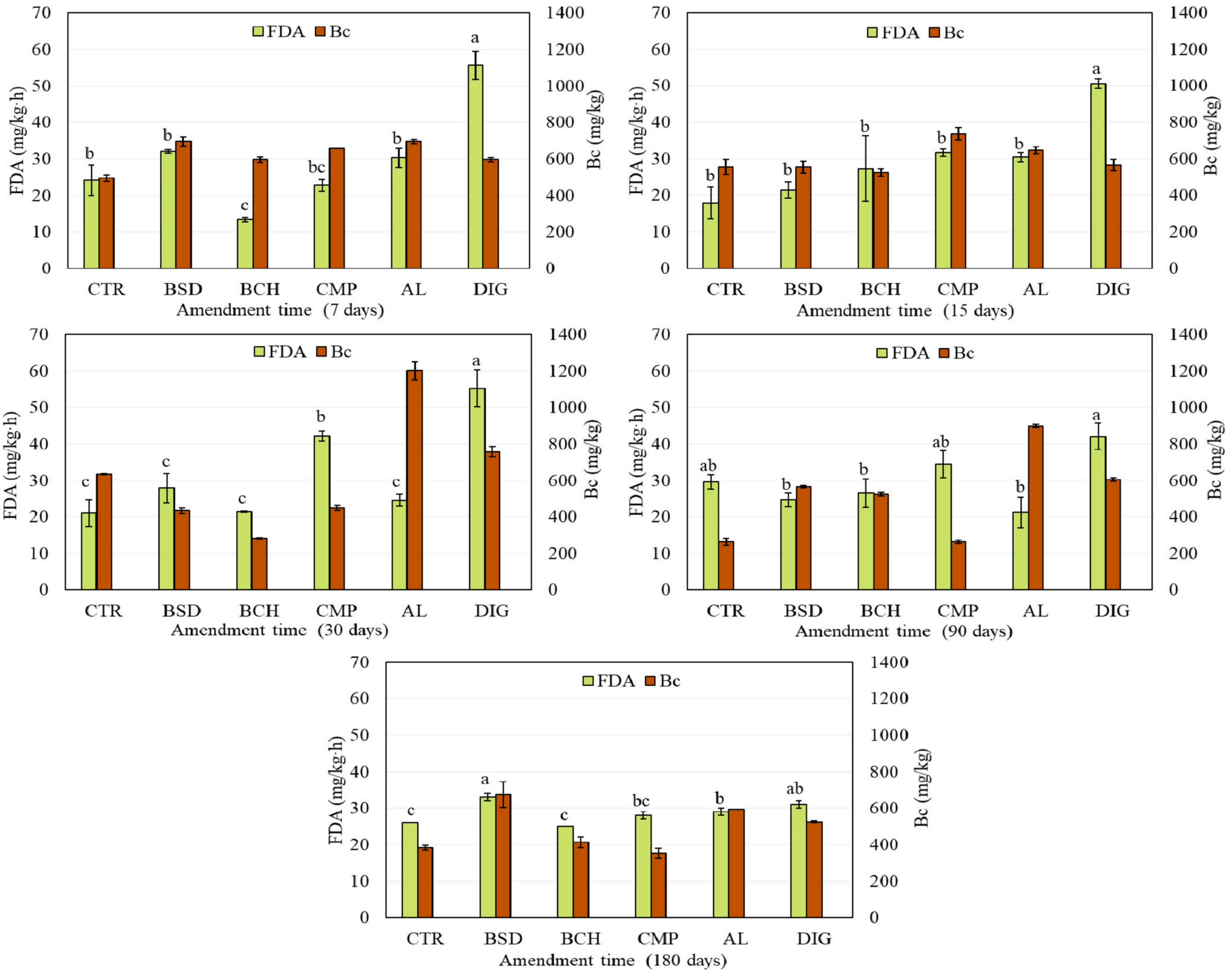

Fig. 3. Fluoresceine diacetate hydrolysis activity (FDA) and microbial biomass carbon (*Bc*) in amendments applied to a clay loam soil. The different letters represent significant differences between treatments for each amendment day. Results are expressed on dry matter. Mean value ± SD, n = 3.

examined OAs significantly exceeded that of the unamended control soil after only 7 days of incubation. The treatment with biosolids increased *Bc* compared to the control soil, with fluctuations at day 30, leading to a final increase of +76 % at day 180. Multiple studies have shown that using sewage sludge as an amendment significantly influences *Bc* in soil ecosystems, depending on sludge stability, soil type, and climatic conditions (Zoghlami et al., 2016). For example, Fernández et al. (2009) noted that the instability of the sludge used in their study could be a key reason for the observed outcomes. In contrast, Pavan Fernandes et al. (2005) found that applying secondary sewage sludge significantly increased microbial biomass carbon in the surface soil layer, up to 10 cm deep. *Bc* rose from 99 mgC·kg^{-1} to 809 mgC·kg^{-1}. In the subsurface layer (10–20 cm), *Bc* ranged from 71 mgC·kg^{-1} to 577 mgC·kg^{-1}. These findings are similar to those in the current study, further supporting that well-managed amendments, such as stabilized biosolids, can substantially boost microbial activity and biomass in agricultural soils. The microalgae treatment showed a high *Bc* (1200 mgC·kg^{-1}) after 30 days of incubation. This elevated microbial biomass reflects the enzyme activity observed in this study, which matches the increased respiration rate linked to this type of OA. This significant rise was likely due to the synergistic interactions within a mixed culture of microalgae and bacteria in the treatment (Table 2). Additionally, digestate caused a quick increase in *Bc* compared to the control soil. In contrast, compost and biochar did not effectively enhance microbial biomass carbon. After an initial rise, compost could not sustain the microbial population, leading to a decline in *Bc* after day 30.

Overall, biosolids application to the soil affected soil biological fertility indicators like those of compost and digestate, indicating a good potential for improving key biological functions of soil.

These findings indicate that the specific physicochemical and molecular characteristics of OAs, such as the C/N ratio, aromaticity index, hydrophobicity, and molecular size, are essential for shaping short- and long-term soil response. This underscores the importance of selecting OAs based not only on their nutrient content but also on their molecular complexity and potential for transformation within the soil environment.

However, several limitations must be considered, such as the incubation period of the experiment, which was conducted under controlled laboratory conditions, making it not fully representative of the complexity of the field environment, including plant-soil interactions, climatic variations, and diverse microbial communities. Furthermore, although the 180-day incubation period provided insights into medium-term processes, examining its effect on the dynamics of recalcitrant organic fractions that may decompose over extended periods is important. Therefore, it is recommended that future field trials be conducted over extended periods to validate these findings and explore the environmental and agronomic implications of using various OAs under real-world agricultural conditions.

Additionally, the specific effects observed from the biosolids can be attributed to their stabilization through constructed wetlands, which leads to a low carbon mineralization rate (k: 0.006^{-1}), significant accumulation of stable carbon reserves (TOC, SOC, ROC), and nitrogen (TN, SN, MN), as well as microbial biomass and activity. These features distinguish biosolids from more labile amendments such as microalgae and digestate, which cause rapid carbon loss and short-term microbial stimulation, placing biosolids alongside more stable materials such as compost and biochar. To better understand the biological and chemical mechanisms behind these differences, future research should consider approaches such as sequencing microbial communities, enzymatic profiling, and fractionating the organic matter specific to each compound.

4. Conclusions

This study demonstrated that stabilized biosolids in constructed wetlands significantly improved soil quality by promoting the sequestration of soil organic matter and enhancing biological fertility. Furthermore, biosolids showed results comparable to those of biochar and compost across all parameters analyzed, confirming their amending properties and the potential of constructed wetlands to reduce reactive organic substances while stabilizing biosolids effectively. Although this study indicated that biosolids increased microbial biomass, microbial diversity analysis will be necessary for a deeper understanding of potential shifts in community composition. Finally, to validate the beneficial effects of biosolids as an organic amendment in soil environments, conducting both short- and long-term experiments at a real field scale, using various soil types in different climatic conditions, will be essential. These findings provide important insights to guide future research on biosolids reuse strategies and promote sustainable organic waste management in agriculture. For example, evaluating the long-term buildup of trace contaminants and their potential for plant uptake is crucial to ensure biosolids safety, including assessments of crop performance and metal bioavailability. Additionally, these insights can help shape environmental policies to improve soil health and support circular nutrient use.

CRediT authorship contribution statement

Ana Cano-Larrotta: Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation. **Luisa Massaccesi:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization. **Enrica Uggetti:** Writing – review & editing, Supervision, Investigation, Conceptualization. **Mirko Cucina:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2025.180365.

Data availability

Data will be made available on request.

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