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Vermifiltration and sustainable agriculture: unveiling the soil health-boosting potential of liquid waste vermicompost

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Vermifiltration is a promising technique that can help recover nutrients from wastewater for further use in agriculture. We conducted a field experiment to assess the effectiveness of vermicompost produced from the vermifiltration of liquid waste (manure and food production waste) and how it can affect the soil health and yield of a squash crop. We tested the effect of three rates of vermicompost (low, medium, and high) applied over two consecutive years and measured physical, chemical, and biological soil health indicators, squash yield, and nutritional status. The results showed that the use of vermicompost, especially at a high rate, increased total soil carbon, total nitrogen, potentially mineralizable nitrogen, and particulate organic matter, as well as the activity of C-N-P cycling enzymes, as compared to a control with only inorganic fertilization. The yield of the squash crop remained stable, while the crop nutritional value improved as the levels of boron and copper in the treated squash increased. These findings indicate an improvement in soil health after the use of vermicompost. Overall, results strongly support using this type of vermicompost as a sustainable management approach to recycle nutrients and enhance soil health.

KEYWORDS

agricultural wastewater, vermifiltration, vermicompost, soil organic matter, microbial activity, micro and macro nutrients

1 Introduction

Agricultural and agro-industrial activities have grown considerably over the last few decades, leading to substantial environmental pollution (Martinez-Burgos et al., 2021; De Rooij et al., 2024). These activities generate large amounts of wastewater with high concentrations of organic matter and nutrients, especially nitrogen compounds (Simas et al., 2019). Inappropriate disposal of this liquid waste can cause soil acidification, nitrate leaching to groundwater, eutrophication of surface waters, emissions of greenhouse gasses, and unpleasant odors (Lazcano et al., 2016; Köninger et al., 2021; Zhang et al., 2022; Rocha et al., 2024).

By treating these wastes through vermifiltration process, farmers can reduce their demand for high-quality water (Sarkar et al., 2006; Lahlou et al., 2021), recover nutrients from wastewater that can be used for crop production (Kenneth et al., 2023), and reduce greenhouse gas emissions (Dore et al., 2022). Indeed, vermifiltration is an eco-friendly and cost-effective biological aerobic process that can be used for secondary wastewater treatment using epigenic earthworms (Pasha et al., 2018; Singh et al., 2019). Earthworms ingest and burrow through the filter media, producing mucus and casting that alter the properties of the biofilm. This, in turn, increases the microbial population and activity, resulting in a significant reduction in biological oxygen demand (up to 90%), chemical oxygen demand (between 80 and 90%), and total dissolved solids (up to 90%) (Sinha et al., 2008).

Using vermicompost from vermifiltration treatment in agriculture could increase the attractiveness and efficacy of vermifiltration as a recycling strategy, reducing the environmental impact of liquid organic wastes in the livestock farming industry and agroindustries. This approach aligns with the principles of a circular economy, developing effective wastewater recycling processes and sustainable soil management strategies.

Vermicompost produced after vermifiltration is rich in plant-available nutrients (Xing et al., 2005; Kumar et al., 2015; Dey Chowdhury et al., 2022), indicating its potential use as an organic amendment to improve soil health and crop production. Additionally, vermicomposting and vermifiltration represent two distinct methods of earthworm-mediated waste management, each with its own unique processes and applications (Enebe and Erasmus, 2023; Saapi et al., 2024). These distinctions may introduce specific physico-chemical and microbial characteristics, ultimately impacting the final composition and functionality of the vermicompost produced (A'ali et al., 2017; Rynk et al., 2022; Stehouwer et al., 2022; Luo et al., 2023). Therefore, it is crucial to investigate whether these distinctions lead to differential impacts on soil health, nutrient availability, and plant growth.

The current body of literature primarily focuses on applying vermicompost derived from treating solid organic waste materials in agricultural practices. This type of vermicompost has previously been observed to enhance plant growth (Lazcano et al., 2009; Song et al., 2022), increase soil microbial biomass and diversity (Danish Toor et al., 2024), act as a source of macro-and micro-nutrients (Nurhidayati et al., 2016; Raza et al., 2021), and support soil carbon sequestration (Ngo et al., 2012, 2014; Naikwade, 2019). However, there is a need for more information about the use of vermicompost produced from liquid waste treatment in agriculture.

Furthermore, soils in arid and semi-arid regions are characterized by poor soil health, low nutrient content, high soil pH, low microbial biomass and activity, and limited vegetation cover. The intense use of chemical fertilizers, monoculture farming, and overall expansion of land use worsens the state of soil health in those regions (Ayangbenro and Babalola, 2021), affecting negatively agricultural production and favoring land degradation process (Fernández-Ugalde et al., 2011). Hence, using nutrient-rich organic amendments such as vermicompost can be a sustainable soil management practice that can help improve soil health and restore degraded lands. To our knowledge, there is a lack of studies on the impact of vermicompost resulting from vermifiltration on soil health. Specifically, carbon pools, enzymatic activities, macro and micronutrients on soil and crops.

In this context, this study aimed to investigate the impact of vermicompost obtained from the vermifiltration of wastewater on physicochemical soil properties, nutrient cycling, enzymatic activities, and carbon sequestration potential, as well as on the yield and nutrient status of summer squash, a widely cultivated crop in nearly all regions of California giving its adaptability to arid and semi-arid climate. We hypothesized that applying the vermifiltration byproduct to the soil would stimulate microbial activity and nutrient cycling, providing valuable soil nutrients and enriching soil organic matter. Furthermore, we expected that its application could increase particulate and mineral-associated organic matter, ultimately contributing to carbon sequestration and improved crop yield and quality.

2 Materials and methods

2.1 Study site

We conducted a field experiment on a commercial farm in Winters, California, United States, in 2021 and 2022. The area has a semi-arid climate with an average annual precipitation of 557 mm, an average maximum temperature of 24.6°C, and an average minimum temperature of 9.3°C (Western Regional Climate Center, n.d.). The soil in the field is classified as Rincon silty clay loam, and the baseline top 15 cm soil has a pH of 6.47 ± 0.05, total %N of 0.15 ± 0.01, total %C of 1.28 ± 0.07, and EC of 759.42 ± 19.82 µS cm^{-1}. The field is subjected to a crop-rotating system, with tomatoes planted in 2021 and summer squash (*Cucurbita pepo*), the '*Ananashyi*' variety planted in 2022.

2.2 Characterization of the organic amendment

We employed vermicompost generated as a by-product of the vermifiltration system. The vermifiltration system is operated by Biofiltro Inc. The system consisted of a rectangular concrete enclosure (49 m × 11 m × 1.5 m) filled with 1 meter of filtering material (woodchips) and earthworms (*Eisenia andrei*) in the top 30 cm. It has a 30 cm deep drainage area at the bottom vented to the outside by PVC exhaust pipes (15 cm in diameter) for passive air exchange (Dore et al., 2022). The sprinkler system applied influent for 2 min to the vermifilter surface every 30 min. The applied influent percolated through the vermifilter to the underlying drainage space and drained under gravity in about 4 h. The vermifilter material, comprising mature vermicompost and the residual filtering material, is typically extracted from the system every 18 months. It was stored in piles and used for soil application.

Due to the availability of the organic waste feedstock and output rates of the vermifiltration system, it was not possible to harvest enough of the same material for two consecutive years. Therefore, we used vermicompost produced from two different feedstock materials in each year. In the initial year of the study (2021), pepper plant processing wastewater served as the feedstock, while dairy manure wastewater was utilized in the subsequent year (2022). In both cases, woodchips were utilized as the filtering material. Characteristics of the vermicompost used in the study are outlined in (Table 1). To uphold the experiment's consistency, application rates to the soil were

TABLE 1 Physicochemical characteristics of the vermicompost extracted from the vermifiltration system analyzed before application to the field.

	Vermicompost (pepper plant)	vermicompost (dairy manure)
N-NO_3^- (mg g^{-1} dw)	0.378	0.237
N-NH_4^+ (mg g^{-1} dw)	0.170	0.406
Inorganic N (mg g^{-1} dw)	0.548	0.643
Total N (%) dw	2.02	1.31
Organic N (mg g^{-1} dw)	19.65	12.50
C (%)	42	38.63
C/N	20.79	29.39
BD[1] Wet (g cm^{-3})	0.48	0.51
BD[1] Dry (g cm^{-3} dw)	0.17	0.15

[1]BD, bulk density.

determined based on the available nitrogen content of the vermicompost, maintaining uniformity across both study years.

2.3 Experimental design and management

We conducted a field experiment to test the impact of vermicompost, used as an organic amendment, on both soil health and crop yield. The field experiment consisted of four treatments arranged in a randomized complete block design with three replications (Figure 1). The treatments were (1) control with no vermicompost applied, (2) low rate of vermicompost application (LV, 18 kg ha^{-1} N), (3) medium rate of vermicompost application (MV, 28.8 Kg ha^{-1} N), and (4) high rate of vermicompost application (HV, 54 kg ha^{-1} N). The application rate was based on the available nitrogen content of the vermicompost applied and summarized in Table 2.

The application of vermicompost was performed manually after the beds were established and prior to spring planting. We evenly raked the soil to mix in the material within each bed while the areas between the beds remained free of any amendment. Each treatment was applied to three 8 m × 8 m plots containing eight beds, resulting in 12 experimental plots distributed in the field. Buffer zones of six beds were set between plots to avoid disturbances. All treatments were provided with standard mineral fertilizer to supplement the plants with essential nutrients during their growth. This mineral fertilization was applied through subsurface drip irrigation (20 cm), consisting of 100 kg ha^{-1} of 32% N of urea and ammonium nitrate.

2.4 Laboratory analyses

2.4.1 Determination of soil health indicators

This study aimed to investigate the cumulative effect of vermicompost application on soil health. Therefore, we collected soil samples at the end of the second year. In September 2022, after the summer squash harvest, we collected three soil cores from each experimental plot at 0–15 cm depth. The samples were homogenized and composited into one sample per plot and then moist sieved to 8 mm to remove plant matter and organic debris. The soil samples were then stored at 4°C until further use.

Nitrate (NO_3^--N) and ammonium (NH_4^+-N) concentrations in the soil samples were determined colorimetrically in a soil extract prepared with 8 g of fresh soil using 0.5 M of K_2SO_4 (Miranda et al., 2001; Doane and Horwath, 2003). Another 8 g subsample was taken to measure potentially mineralizable nitrogen (PMN) in the soil samples. Briefly, 10 mL of water was added to the soil, and then the solution was purged with N_2 gas and incubated for 7 days at 37°C. A soil extract was subsequently prepared using 0.67 M of K_2SO_4, and NH_4^+-N was determined colorimetrically in the soil extracts. The difference between NH_4^+-N in the incubated and non-incubated samples was the PMN.

To measure the microbial biomass carbon (MBC), a 6 g subsample was exposed to chloroform for 24 h and then extracted using a solution of 30 mL of 0.5 K_2SO_4. Another 6 g subsample was used to prepare a non-fumigated extract. Dissolved organic carbon concentrations were determined by UV-persulfate oxidation (Teledyne-Tekmar Fusion), and the MBC was calculated as the difference between the fumigated and non-fumigated samples (Horwath and Paul, 1994). The soil pH and electrical conductivity (EC) were determined in soil slurries [1:2 soil to Deionized water (DI) water] using a pH/EC meter (Mettler Toledo, Columbus, OH, United States).

A high-throughput microplate assay method was used to measure the potential activity of carbon cycle related enzymes (**α**-Glucosidase, β-Glucosidase, Cellulase, Xylosidase) and nitrogen cycle-related enzymes (Leucine aminopeptidase, *N*-Acetyl-glucosaminidase) and phosphate cycle enzyme (Phosphatase) as described in Bell et al. (2013). Briefly, 2.75 g of moist soil was mixed with 91 milliliters of a 50-millimolar solution of Sodium Acetate buffer to prepare the soil slurry. The 4-methylumbelliferone (MUB) standard was used for all enzymes except the LAP enzyme, where we used the 7-Amino-4-methylcoumarin (MUC) standard. Two deep 96-well plates were utilized for the two standards, while one plate was dedicated to the enzyme substrates. Each corresponding well in the plates was pipetted with 200 μL of the standards or the substrates, followed by adding 800 μL of soil slurry to all wells in the three plates. Next, the three plates were placed in a dark incubator for 3 h. Afterward, they were centrifuged for 3 min at approximately 2,900 x *g*. Then, 250 μL was carefully transferred from each well of the incubated deep well plates to the corresponding wells in clear 96-well plates to be read using a microplate reader (BioTek synergy HTX multi-mode reader) (Excitation Wavelength = 365 nm, Emission Wavelength = 450 nm).

Soil respiration was determined after 24 h of soil incubation in 227.3 mL glass jars. After determining soil water holding capacity, DI water was added to 10 g of 2 mm sieved air-dried soil to reach 60% of water holding capacity. The jars were airtight and incubated in the dark for 24 h. Another set of jars was used to set up standards with known concentrations of CO_2. The soil respiration was estimated through the CO_2 evolved from the samples measured with a continuous flow LICOR 850 IRGA CO_2/H_2O analyzer (LI-COR Environmental, Lincoln Nebraska, United States). To determine the concentration of active C in soil, we measured Pyruvate oxidase/carboxylase (POXC) using the colorimetric protocol described in Weil et al. (2003). Briefly, we mixed 2.5 g of air-dried soil samples with 0.2 M potassium permanganate solution and measured the resulting solution's absorbance at 550 nm using a microplate reader (BioTek synergy HTX multi-mode reader).

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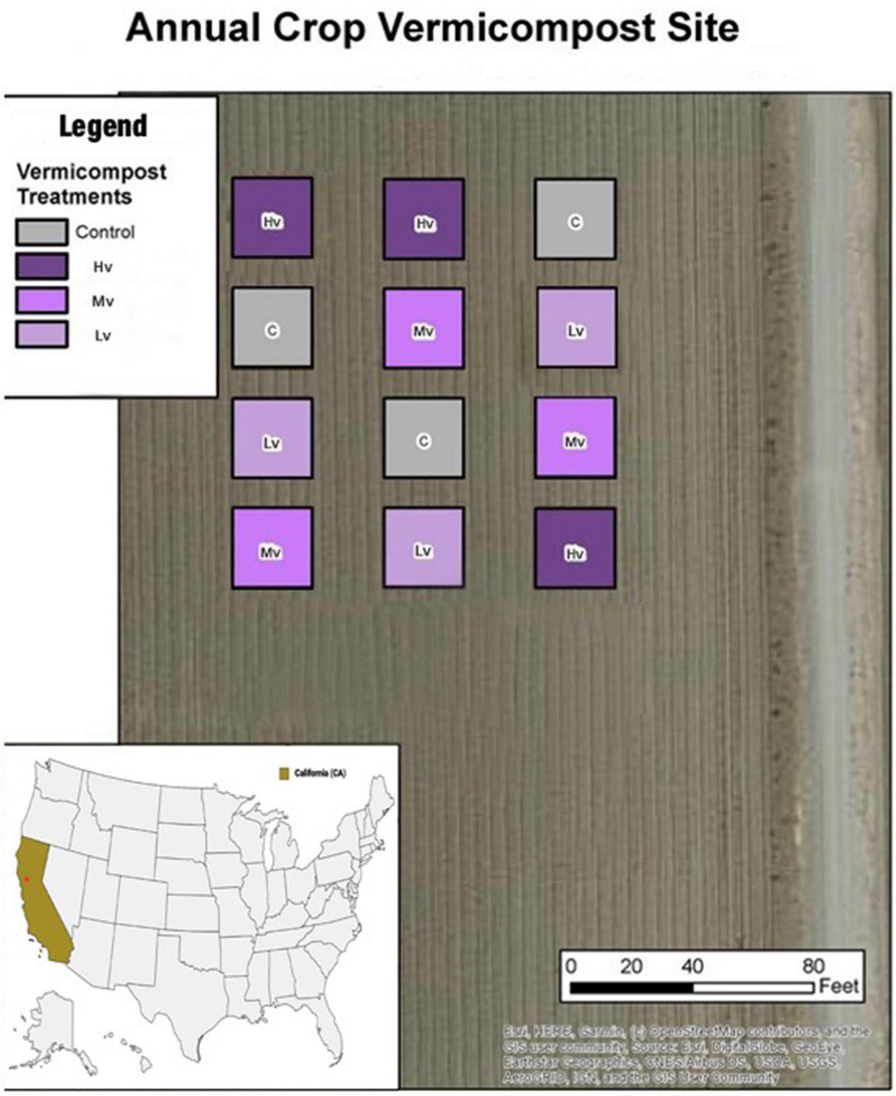

FIGURE 1
Aerial view of the experimental design, LV stands for low rate of vermicompost, MV stands for vermicompost at a medium rate, and HV stands for vermicompost at a high rate.

TABLE 2 The rate of vermicompost applied to the soil each year.

		Target available N (kg ha^{-1})	vermicompost applied (dry ton ha^{-1})
2021	Low rate (LV)	18	2,8
	Medium rate (MV)	28.8	4.48
	High rate (HV)	54.0	8.39
2022	Low rate (LV)	18	4.22
	Medium rate (MV)	28.8	6.56
	High rate (HV)	54.0	12.20

Total soil C (%) and N (%) were determined by dry combustion in an elemental analyzer (Costech analytical technologies Inc. model ECS 4010).

To measure particulate organic matter (POM) and mineral-associated organic matter (MAOM), 10 g subsamples were taken from each plot and mixed with 30 mL of 5 g/L sodium hexametaphosphate. The mixture was shaken for 15 h on a reciprocal shaker and then passed through a 53-μm sieve. The material that remained on the sieve after rinsing with water several times was identified as POM, whereas the soil slurry that passed through the sieve was identified as

TABLE 3 Variation in soil physicochemical properties after vermicompost application.

	Control	LV	MV	HV	*P*-value
pH	7.29 ± 0.16	7.42 ± 0.21	7.43 ± 0.26	7.54 ± 0.15	0.5
EC (µs cm-1)	345 ± 115	259 ± 62.5	280 ± 60.4	276 ± 83.3	0.6
Bulk density (g cm $^{-3}$)	1.07 ± 0.05	1.04 ± 0.07	1.14 ± 0.14	1.09 ± 0.08	0.6
TIN (kg ha^{-1})	47.1 ± 29.2	38.1 ± 18	38.1 ± 18	27.3 ± 8.39	0.4
PMN (kg ha^{-1})	18.1 ± 4.37 b	20.1 ± 7.68 b	27.8 ± 12.8 ab	51.6 ± 17.5 a	0.02

LV stands for vermicompost at a low rate, MV stands for vermicompost at a medium rate, and HV stands for vermicompost at a high rate. TIN stands for total inorganic nitrogen, PMN stands for potentially mineralizable nitrogen. Different letters indicate a significant difference between treatments according to the Tukey test results.

MAOM. Both parts were then dried overnight at 50°C and ground with a mortar and pestle. Afterward, the samples were analyzed for total organic C and total Kjeldahl N (Cambardella and Elliott, 1992).

Soil available phosphorus was extracted by adding 50.0 mL of 0.5 M $NaHCO_3$ (pH = 8.5) to 2.50 g soil. The mixture was shaken for 30 min and filtered. 40 µL aliquot of soil $NaHCO_3$ extract was mixed with 20 µL MA reagent in each well of the 96-well microplate and shaken for 1 min, then 140 µL aliquot of deionized water was added. Absorbance was read at 700 nm using a microplate reader, and soil Olsen-P concentrations were calculated based on the standard curve (Song et al., 2019).

Soil samples were sent to the UC DAVIS Analytical Lab to measure the potential availability of soil micronutrients; Zn, Mn, Cu, and Fe using the diethylenetriaminepentaacetic acid (DTPA) extraction method (Lindsay and Norvell, 1978).

2.4.2 Crop performance indicators

At harvest, the yield of the squash crop (kg ha^{-1}) was measured by randomly harvesting 6 healthy plants from each plot. All the squash resulting from the harvest of six plants was weighed in the field and then taken to the lab. Squashes from each batch were cut horizontally, and one-half of each squash was mixed into a puree and frozen; the samples were then freeze-dried and ground to a powder. Samples were sent to the UC Davis analytical lab to analyze macro and micronutrients. The concentration of B, Ca, Cu, Fe, Mg, Mn, P, K, Na, S, and Zn were analyzed in a 5 g sample using the nitric acid/hydrogen peroxide microwave digestion and determination by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES) (Meyer and Keliher, 1992; Sah and Miller, 1992). The total nitrogen in the squash was measured using the combustion method (*AOAC Official Method 972.43*, 2006; *AOAC Official Method 990.03*, 2005).

2.5 Data analysis

All statistical analyses were done using R studio statistical software (v.2023.03.0+386). We conducted a one-way ANOVA to determine if there was a significant treatment effect on the soil and plant variables measured in this experiment. To ensure the accuracy of our analysis, we transformed any variables that did not follow a normal distribution using box-cox transformations. In the case of significant treatment effects, we followed up with a Tukey *Post Hoc* test to evaluate the treatment effects further. We reported our data as mean values or treatment differences, followed by the standard error. We used a *p*-value of less than 0.05 to determine statistical significance.

3 Results

3.1 Effects of the vermicompost treatments on soil health indicators

According to Table 3, the soil pH ranged between 7.29 and 7.54 and generally increased with vermicompost application, but the change was not significant ($p > 0.05$). The soil EC ranged between 259 and 345 µs cm^{-1}, with a tendency to decrease when vermicompost was applied. However, the variation was not significant ($p > 0.05$). As for the soil bulk density, its values ranged between 1.04 and 1.14 g cm^3 and did not change significantly after applying the vermicompost treatments compared to the control ($p > 0.05$). Applying vermicompost with different rates did not affect the total inorganic nitrogen in the soil ($p > 0.05$). However, the amount of PMN increased significantly in the HV treatment compared to the control and LV ($p < 0.05$).

The total carbon content in the soil at harvest was 37.14% higher in the HV treatment than in the control (Figure 2A). The total carbon was 21.42 and 15.00% higher in the MV and LV treatments, respectively, compared to the control, but these differences were not significant (Figure 2A). The total nitrogen in the HV treatment increased significantly by 24.66% compared to the control. The total nitrogen also increased in the two other treatments (MV, LV) compared to the control, but the difference was not significant (Figure 2B).

The vermicompost application had a significant effect on particulate organic carbon (POC) ($p < 0.05$). The low vermicompost treatment showed no significant change in POC compared to the control. In contrast, the MV and the HV treatment increased the POC by 40.82 and 93.02%, respectively, compared to the control, with the increase in HV treatment being significant (Figure 2C). The particulate organic nitrogen (PON) exhibited the same trend as the POC; According to ANOVA, the use of vermicompost had a significant effect on PON ($p < 0.05$). The LV treatment maintained the same level of PON as the control, whereas the MV and HV treatments increased the PON by 31.67 and 93.12% compared to the control. However, the Tukey test revealed that the difference was significant only when comparing the HV treatment to the control (Figure 2D). Vermicompost application did not have any significant effect ($p > 0.05$) on the mineral-associated organic carbon (MAOC) and nitrogen (MAON) ($p > 0.05$) (Figures 2E,F).

According to Table 4, the available P on soil samples varied between 0.57 and 0.90 mg kg^{-1}. Similarly, the concentration of Zn varied between 2.40 and 2.93 mg kg^{-1} across treatments, while the amount of Cu on soil samples ranged between 6.09 and 7.03 mg kg^{-1}.

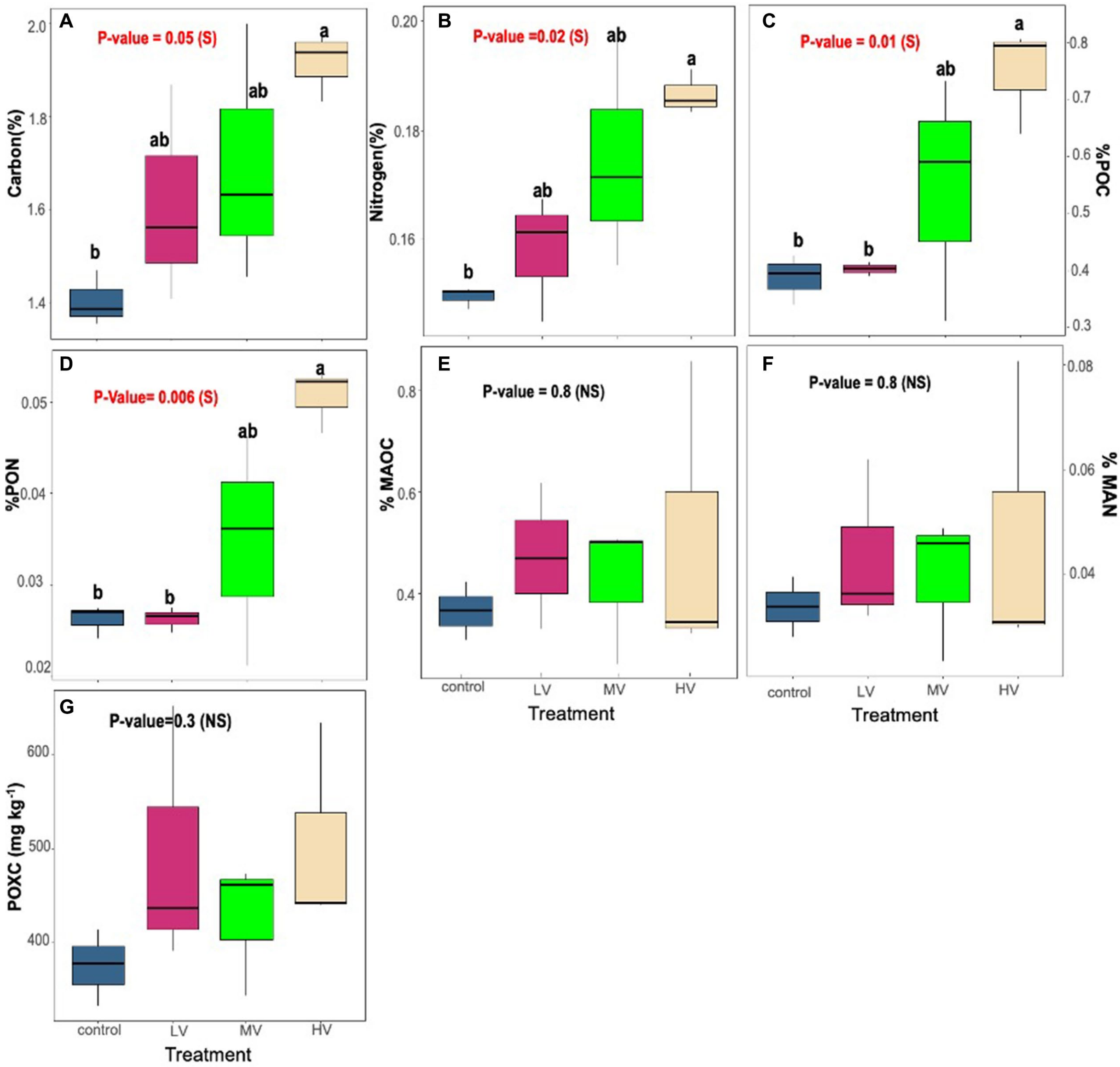

FIGURE 2
(A) Total carbon, **(B)** total nitrogen, **(C)** particulate organic carbon (POC), **(D)** particulate organic N (PON), **(E)** mineral associated organic C (MAOC), **(F)** mineral associated organic N (MAN), **(G)** active carbon POXC, under different treatments. LV stands for low rate of vermicompost, MV stands for vermicompost at a medium rate, and HV stands for vermicompost at a high rate. NS stands for non-significant when the *p*-value is higher than 0.05, and S means a significant effect when the $p < 0.05$. Different letters indicate a significant difference between treatments according to the Tukey test results.

Additionally, the available Fe concentration fell between 10.6 and 11.7 $mg\,kg^{-1}$.

Although the MV treatment tended to exhibit higher nutrient content for all measured nutrients in the soil samples, these differences were not found to be statistically significant according to ANOVA tests.

3.2 Microbial biomass and activity

Applying vermicompost with different rates did not affect POXC concentration (Figure 2G), the soil microbial biomass carbon, nor the mineralizable carbon ($p > 0.05$) (Figures 3A,B).

The ANOVA analysis indicated that the treatment type had a significant effect on the activity of β-glucosidase (Figure 4B) and cellulase (Figure 4C), where the HV treatment had the highest enzymatic activity among the four treatments. The activity of **α**-Glucosidase and Xylosidase showed the same trend where the enzymatic activity increased with an increasing application rate of the vermicompost, with the HV treatment having the highest enzymatic activity. However, the difference was not significant ($p > 0.05$ for both enzymes) (Figures 4A,D). The enzymatic activity related to the N cycle tended to be higher on the HV treatment, especially for the *N*-Acetyl-glucosaminidase enzymes ($p > 0.05$) (Figures 4E,F). The ANOVA analysis revealed that the treatment type significantly affected the phosphatase activity ($p < 0.05$), with the HV treatment having the highest enzymatic activity compared to the other treatments (389 ± 30.2 nmol activity/g dry soil/h) (Figure 4G).

3.3 Effects of the treatments on crop performance

The squash yield ranged between 6.78 and 8.25 tons ha^{-1} and did not change significantly after the application of the vermicompost treatments as compared to the control without vermicompost ($p > 0.05$) (Figure 5).

Table 5 summarizes the changes in squash macro and micronutrient content across treatments. The concentration of macronutrients (N, K, Ca, Mg, P, and S) remained consistent across different treatments. Similarly, the application of vermicompost did not affect the concentration of micronutrients Zn, Fe, and Mn. However, the treatments did have a significant impact on the B concentration ($p = 0.05$). Specifically, the B concentration increased by 14.07, 31.98, and 6.6% in LV, MV, and HV treatments compared to the control. The use of vermicompost also had a noticeable impact on the concentration of Cu ($p < 0.05$). In the HV treatment, the concentration of copper decreased by 9.59% compared to the control. In contrast, in the LV and MV treatments, the concentration of copper increased by 36.52 and 47.90%, respectively, compared to the control.

4 Discussion

In contrast to existing research primarily centered on evaluating the efficiency of vermifiltration, our study breaks new ground by exploring the potential of its byproduct, vermicompost. Focusing on the impact of vermicompost on soil health and crop performance, we have established compelling evidence supporting its use as an efficient organic amendment. When applied at a rate equivalent to 54 kg N ha^{-1} it resulted in improved soil health compared to lower rates and control groups. This was manifested by increased carbon and nitrogen content, (mostly as POM), and enhanced enzymatic activity. It also supported similar crop yields as the control treatment with only inorganic N fertilizer. Our findings demonstrate the validity of the vermifiltration process to recycle agro-industrial wastewater to close the cycle of nutrients in agricultural systems while supporting soil health and sustainable soil management in arid and semi-arid regions.

TABLE 4 Variation in soil nutrient concentrations across treatments at harvest time.

	Control	LV	MV	HV	*P*-value
P (mg kg^{-1})	0.59 ± 0.23	0.57 ± 0.21	0.90 ± 0.22	0.75 ± 0.22	0.3
Zn (mg kg^{-1})	2.63 ± 0.1	2.8 ± 0.34	2.93 ± 0.05	2.4 ± 0.7	0.5
Mn (mg kg^{-1})	30.4 ± 0.63	31.5 ± 1.4	30.3 ± 1.16	26.4 ± 7.42	0.4
Cu (mg kg^{-1})	6.93 ± 0.32	6.56 ± 0.32	7.03 ± 0.20	6.09 ± 1.15	0.3
Fe (mg kg^{-1})	10.9 ± 1.63	11.7 ± 2.66	11.6 ± 1.28	10.6 ± 3.16	0.9

LV stands for vermicompost at low rate, MV stands for vermicompost at a medium rate, and HV stands for vermicompost at a high rate.

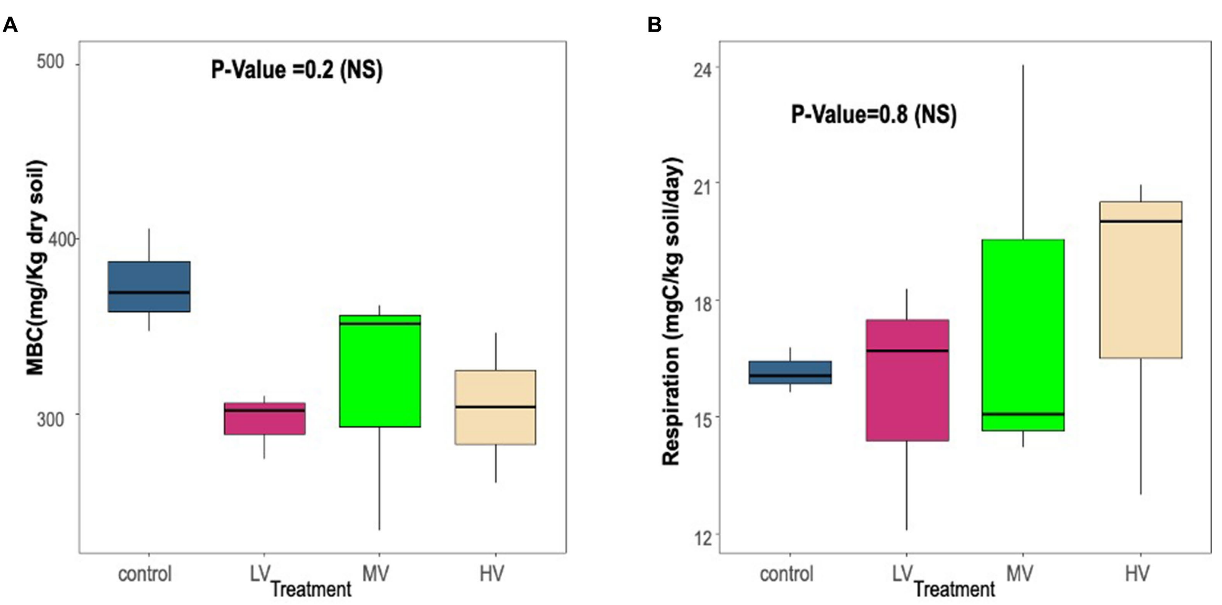

FIGURE 3
(A) microbial biomass carbon, **(B)**; respiration in different treatments at harvest time. LV stands for low rate of vermicompost, MV stands for vermicompost at a medium rate, and HV stands for vermicompost at a high rate. NS stands for non-significant when the *p*-value is higher than 0.05, and S means a significant effect when the $p < 0.05$.

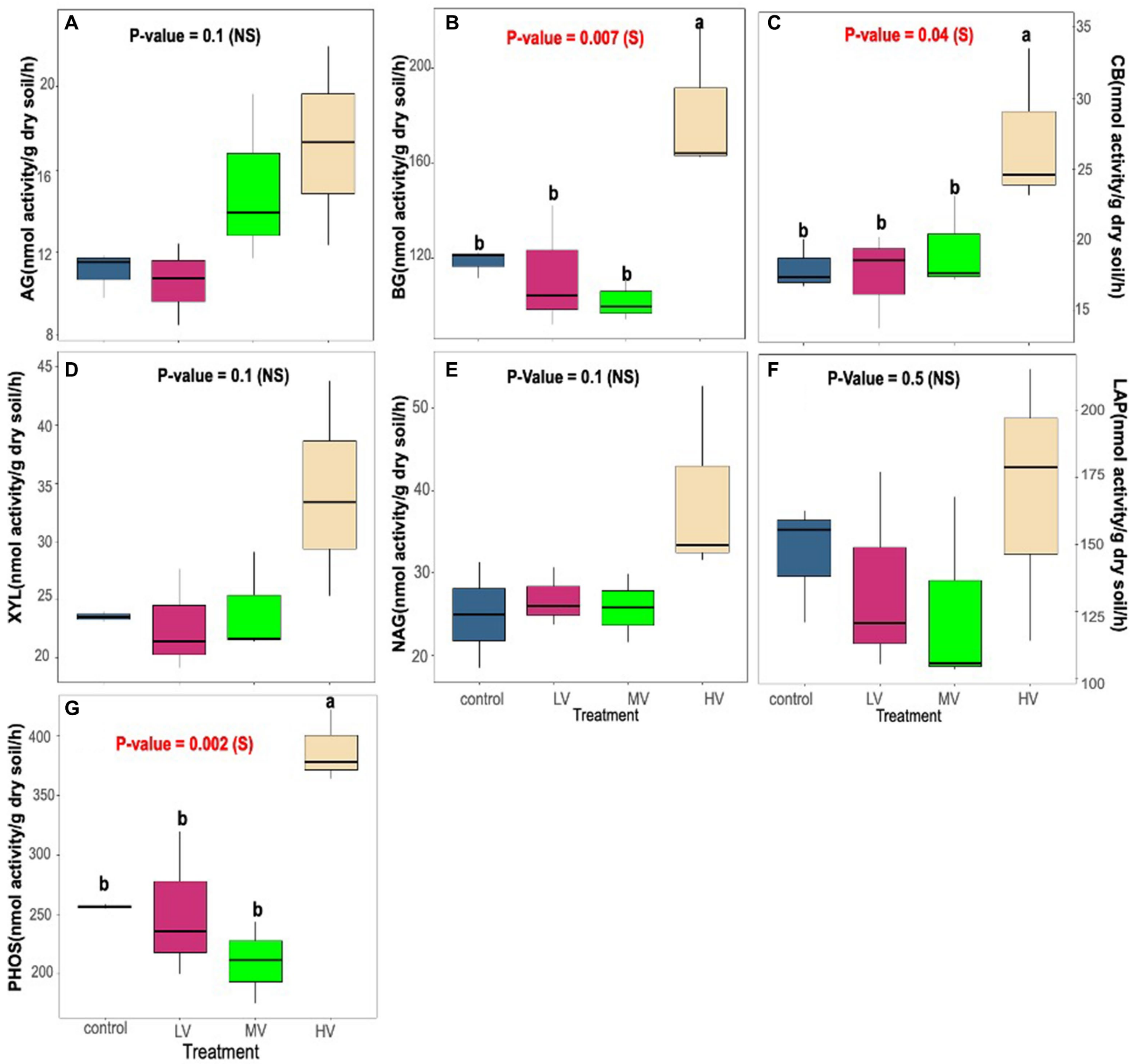

FIGURE 4
Potential enzymatic activities in soil after vermicompost application at different rates. **(A)** Alpha-Glucosidase, **(B)** Beta-Glucosidase, **(C)** Cellulase, **(D)** Xylosidase, **(E)** N-Acetyl-Glucosaminidase, **(F)** Leucine-Amino-Peptidase, **(G)** phosphatase. LV stands for vermicompost applied at a low rate, MV stands for vermicompost at a medium rate, and HV stands for vermicompost at a high rate. NS stands for non-significant when the *p* value is higher than 0.05, and S means a significant effect when the $p < 0.05$. different letters indicate a significant difference between treatments according to the Tukey test results.

4.1 Applying vermicompost improved soil health

Healthy soils play a pivotal role in fostering both environmental resilience and sustainable agriculture (Salomon and Cavagnaro, 2022). Our findings reveal notable improvements in key indicators such as total carbon, total nitrogen, PMN, POC, PON, and enzymatic activity following vermicompost application for two consecutive years. Importantly, our approach of basing application rates on the available nitrogen content of each vermicompost type ensured consistency in nutrient inputs across both years of the study, regardless of the feedstock material used.

Soil organic carbon (SOC) constitutes the largest terrestrial carbon pool and serves as a vital indicator of soil health, and carbon sequestration, (Just et al., 2023; Sun et al., 2023; Tang et al., 2023). The increase in carbon content following the application of vermicompost observed in our study is consistent with previous research on organic amendments and suggests that vermicompost improved soil health (Rahman et al., 2020; Wu et al., 2021; Xu et al., 2022; Bai et al., 2023; Cooper and DeMarco, 2023; Zhang et al., 2023). However not all soil C fractions contributes equality to soil health. To further evaluate the impact of vermicompost use on SOC, we assessed alterations in various SOC fractions, including POC and MAOC.

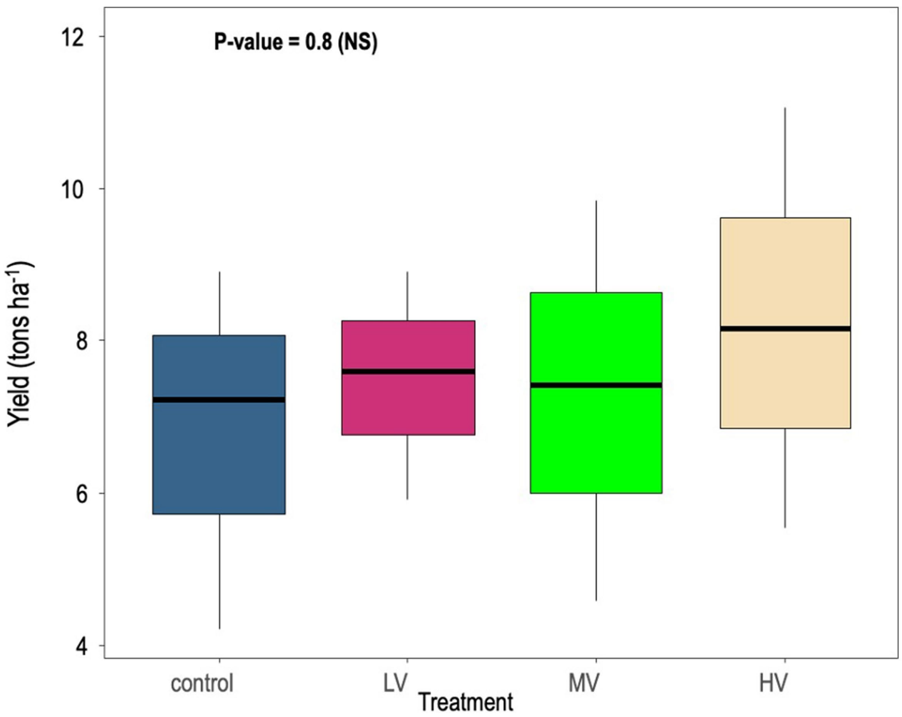

FIGURE 5
Squash yield variation after the application of three rates of vermicompost. LV, low; MV, medium; HV, high. NS stands for non-significant when the *p*-value is higher than 0.05.

TABLE 5 The concentration of squash macro and micronutrients across treatments.

	Control	LV	MV	HV	*P*-value
N (%)	4.04 ± 0.57	3.64 ± 0.53	3.86 ± 0.51	3.96 ± 0.59	0.84
P (%)	0.49 ± 0.05	0.49 ± 0.02	0.47 ± 0.01	0.43 ± 0.02	0.19
K (%)	3.69 ± 0.62	3.62 ± 0.42	3.75 ± 0.19	3.41 ± 0.24	0.77
Ca (%)	0.33 ± 0.08	0.34 ± 0.03	0.35 ± 0.05	0.31 ± 0.03	0.83
Mg (%)	0.41 ± 0.06	0.38 ± 0.06	0.36 ± 0.04	0.39 ± 0.02	0.73
S (ppm)	2,517 ± 482	2,403 ± 431	2,323 ± 225	2,377 ± 238	0.93
B (ppm)	46.9 ± 4.62 b	53.5 ± 6.61 ab	61.9 ± 5.26 a	50 ± 5.90 ab	0.05
Zn (ppm)	59.2 ± 13.3	55.1 ± 9.75	52.5 ± 8.85	56.6 ± 5.24	0.86
Mn (ppm)	14.1 ± 3.70	13.3 ± 1.59	12.8 ± 1.36	12.4 ± 0.69	0.8
Fe (ppm)	122 ± 23.1	119 ± 21.8	113 ± 52.6	108 ± 17.3	0.96
Cu (ppm)	16.7 ± 1.67 ab	22.8 ± 6.54 a	24.7 ± 4.68 a	15.1 ± 0.56 b	0.008

LV stands for vermicompost at a low rate, MV stands for vermicompost at a medium rate, and HV stands for vermicompost at a high rate. Different letters indicate a significant difference between treatments according to the Tukey test results.

Particulate organic carbon is the labile fraction of SOC composed of lightweight fragments of organic matter and characterized by a low turnover time (Sun et al., 2023). The addition of vermicompost in this study introduced labile organic matter into the soil, increasing POC. This is consistent with other studies, such as Plaza et al. (2016) and Giannetta et al. (2024), which also confirm the positive impact of organic amendments on the POC fraction of soil organic matter. Increasing POC can address soil health by improving soil structure, water infiltration, aeration, root growth, and cation exchange capacity (Carter et al., 2003; Fronning et al., 2008). Furthermore, an increase in POC is often associated with a rise in enzymatic activity (Tang et al., 2023). Our experiment observed that the activity of C, N, and P cycling enzymes increased with vermicompost application, in agreement with Li et al. (2021) and Yan et al. (2023). The soil microbiome produces extracellular enzymes that break down the labile fraction of soil organic matter, releasing carbon and nutrients available to microbes and plants (Verrone et al., 2024).

Despite the increase in enzymatic activity following the vermicompost, it was not associated with any changes in microbial biomass carbon and respiration. The rise in soil enzyme activity can be due to the growth of microbes, modifications in the microbial community, or the stimulation of microbial activity through the addition of organic matter (Yan et al., 2023). Therefore, it is crucial to analyze the effects of vermicompost on microbial community's dynamics, composition, and variation in further experiments to gain further insights into the soil's C and N dynamics.

The MAOC fraction of SOC primarily consists of small molecules formed through microbial processing of C inputs. These molecules are subsequently protected through organo-mineral associations or encapsulated within microaggregates. Consequently, MAOC exhibits long turnover time, stability, and greater resistance to microbial degradation (Tang et al., 2023). The MAOC fraction did not change during the study despite the application of vermicompost, probably because the MAOC unlike POC forms slowly over the years. This slow accumulation process makes it difficult to detect significant shifts in MAOC content in short-term studies. Therefore, more time is needed to detect any changes in this fraction resulting from stabilizing the supplemented organic matter (Sokol et al., 2019), or the transformation of POC to MAOC with time (Püspök et al., 2023).

Nitrogen is an essential macronutrient for plant growth, soil health, climate change regulation and carbon sequestration. The application of vermicompost increased soil total nitrogen, particularly in the HV treatment, which is consistent with previous studies demonstrating the positive impact of organic amendments on soil nitrogen levels (Ryals et al., 2014; Wang et al., 2018). The increase in PMN in the vermicompost treatments, combined with no significant difference in inorganic nitrogen (nitrate and ammonium) concentration between the control and the vermicompost-treated groups, suggests that vermicompost addition has affected the organic nitrogen pool without substantially impacting the readily available mineral nitrogen pool. In fact, the use of vermicompost especially the HV treatment also increased the PON fraction.

The organic nitrogen pool is an important indicator of soil health (Lehmann et al., 2020), and considered as a sensitive indicator of changes in agricultural management practices (Hossain et al., 2021). Studies by Hossain et al. (2021) and Yao et al. (2021) indicated that the application of organic amendments increases the PON fraction. This fraction contributes to aggregate formation water holding capacity, nutrient cycling (Hossain et al., 2021) and it may pave the way for gradual nutrient release, which may not be immediately apparent but can benefit soil health in the long run. Similar to the MAOC fraction, the use of vermicompost did not increase the MOAN, needing more time to depict changes in this fraction of soil organic nitrogen.

4.2 The vermicompost application did not enhance the yield but it changed the nutrient status of summer squash

The vermicompost used was rich in nutrients and organic matter, and its application was expected to increase both the yield and nutrient content of the squash. However, there were no differences in yield compared to the control. This may be attributed to many factors; It is possible that the plants were already receiving all the required nutrients through the fertigation system, or that more time was needed to observe any changes in crop performance. According to Ma et al. (2022), repeated application of organic amendment over the years can improve soil conditions and lead to visible effects on crop yield. Additionally, the nature of the irrigation system might explain the limited impact of vermicompost on squash yield. Using a subsurface drip irrigation system could restrict the benefits of applying vermicompost at the soil surface because the moisture is concentrated in the root zone, and the nutrients contained in vermicompost may not move effectively through the soil profile. Liquid fertilizers like vermicompost tea can be utilized to overcome this limitation.

On the other hand, the use of vermicompost, especially at a medium rate, enhanced the nutritional value of squash as it boosted the concentration of B and Cu – two crucial plant micronutrients (Khaliq et al., 2018; Lafuente et al., 2023). Copper, for example, is crucial for brain development and immune system functioning (Chiou and Hsu, 2019). However, excessive amounts of copper can pose a risk to human health (Yang et al., 2002). In this study, the levels of Cu in summer squash samples remained below the established toxicity threshold of 40 ppm as recommended by Codex Alimentarius Commission (1984) and FAO/WHO (1988). As for B, it is an essential micronutrient for forming cell walls and membrane structure and functioning (Läuchli and Grattan, 2014). It is also an essential micronutrient in many physiological activities, including bone growth and nervous system functioning (Nielsen, 2016). The B concentration in squash did not exceed the toxicity threshold of 200 ppm (Vitosh et al., 2006).

The increase in Cu and B concentrations following the application of vermicompost at a medium rate may be attributed to the diverse responses of plants to nutrient uptake, which vary depending on the level of organic amendment applied (Bar-Tal et al., 2004; Chang et al., 2007). Moreover, the same trend is reflected in soil and squash total nutrients, where the medium-rate application of vermicompost resulted in higher concentrations of nutrients. Interestingly, the relationship between nutrient concentration and vermicompost rate does not follow a linear trend. This phenomenon might be elucidated by a synergistic effect between nutrients and organic matter, a phenomenon documented by Bar-Tal et al. (2004). A plausible mechanism could involve the moderate application rate optimizing cation exchange capacity (CEC), thereby enhancing the retention and availability of copper and boron to plants. Importantly, this optimization occurs without the risk of excessive metal fixation that could arise from overly high organic matter application rates (Clemente et al., 2006).

5 Conclusion

The utilization of vermicompost derived from vermifiltration of wastewater as an organic amendment has shown promise as a sustainable means of addressing soil health. The incorporation of vermifiltration-derived vermicompost, especially at a rate of 54 kg N ha^{-1}, improved the nutritional status of the summer squash and improved soil health by increasing the total carbon, nitrogen, particulate organic matter, and microbial activity. These encouraging results, obtained in the context of a short-term investigation, highlighted that vermicompost enabled prompt soil quality recovery and heightened crop nutritional status. However, the long-term evaluation of this practice remains a subject of interest. It is crucial to conduct long-term studies to determine the fate of the added organic carbon and whether it will endure stable sequestration within the soil matrix. Additionally, it is important to investigate whether repeated applications would increase yields and promote overall agricultural sustainability.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

HM: Writing – review & editing, Writing – original draft, Visualization, Investigation, Data curation, Conceptualization. VR: Writing – review & editing, Investigation, Funding acquisition, Data curation, Conceptualization. WH: Writing – review & editing, Resources, Funding acquisition. SD: Writing – review & editing, Conceptualization. PB: Writing – review & editing, Conceptualization. MA: Writing – review & editing, Visualization. HL: Writing – review & editing, Validation, Supervision. CL: Writing – review & editing, Validation, Supervision, Resources.

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Conflict of interest

SD and PB were employed by Biofiltro Inc.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

A'ali, R., Jafarpour, M., Kazemi, E., and Pessarakli, M. (2017). Effects of raw materials on vermicompost qualities. *J. Plant Nutr.* 40, 1635–1643. doi: 10.1080/01904167.2016.1270319

Ayangbenro, A. S., and Babalola, O. O. (2021). Reclamation of arid and semi-arid soils: the role of plant growth-promoting archaea and bacteria. *Curr. Plant Biol.* 25:100173. doi: 10.1016/j.cpb.2020.100173

Bai, X., Tang, J., Wang, W., Ma, J., Shi, J., and Ren, W. (2023). Organic amendment effects on cropland soil organic carbon and its implications: a global synthesis. *Catena* 231:107343. doi: 10.1016/j.catena.2023.107343

Bar-Tal, A., Yermiyahu, U., Beraud, J., Keinan, M., Rosenberg, R., Zohar, D., et al. (2004). Nitrogen, phosphorus, and potassium uptake by wheat and their distribution in soil following successive, annual compost applications. *J. Environ. Qual.* 33, 1855–1865. doi: 10.2134/jeq2004.1855

Bell, C. W., Fricks, B. E., Rocca, J. D., Steinweg, J. M., McMahon, S. K., and Wallenstein, M. D. (2013). High-throughput fluorometric measurement of potential soil extracellular enzyme activities. *J. Vis. Exp.* 81:e50961. doi: 10.3791/50961

Cambardella, C. A., and Elliott, E. T. (1992). Particulate soil organic-matter changes across a grassland cultivation sequence. *Soil Sci. Soc. Am. J.* 56, 777–783. doi: 10.2136/sssaj1992.03615995005600030017x

Carter, M. R., Angers, D. A., Gregorich, E. G., and Bolinder, M. A. (2003). Characterizing organic matter retention for surface soils in eastern Canada using density and particle size fractions. *Can. J. Soil Sci.* 83, 11–23. doi: 10.4141/S01-087

Chang, E.-H., Chung, R.-S., and Tsai, Y.-H. (2007). Effect of different application rates of organic fertilizer on soil enzyme activity and microbial population. *Soil Sci. Plant Nutr.* 53, 132–140. doi: 10.1111/j.1747-0765.2007.00122.x

Chiou, W.-Y., and Hsu, F.-C. (2019). Copper toxicity and prediction models of copper content in leafy vegetables. *Sustain. For.* 11:6215. doi: 10.3390/su11226215

Clemente, R., Escolar, Á., and Bernal, M. P. (2006). Heavy metals fractionation and organic matter mineralisation in contaminated calcareous soil amended with organic materials. *Bioresour. Technol.* 97, 1894–1901. doi: 10.1016/j.biortech.2005.08.018

Codex Alimentarius Commission (1984). *Contaminants, joint FAO/WHO food standards program*, vol. *XVII. 1st* Edn Codex Alimentarius.

Cooper, A., and DeMarco, J. (2023). Composted biosolids amendments for enhanced soil organic carbon and water storage in perennial pastures in Colorado. *Agric. Ecosyst. Environ.* 347:108401. doi: 10.1016/j.agee.2023.108401

Danish Toor, M., Kizilkaya, R., Anwar, A., Koleva, L., and Eldesoky, G. E. (2024). Effects of vermicompost on soil microbiological properties in lettuce rhizosphere: an environmentally friendly approach for sustainable green future. *Environ. Res.* 243:117737. doi: 10.1016/j.envres.2023.117737

De Rooij, M. M. T., Erbrink, H. J., Smit, L. A. M., Wouters, I. M., Hoek, G., and Heederik, D. J. J. (2024). Short-term residential exposure to endotoxin emitted from livestock farms in relation to lung function in non-farming residents. *Environ. Res.* 243:117821. doi: 10.1016/j.envres.2023.117821

Dey Chowdhury, S., Bhunia, P., and Surampalli, R. Y. (2022). Sustainability assessment of vermifiltration technology for treating domestic sewage: a review. *J. Water Proc. Eng.* 50:103266. doi: 10.1016/j.jwpe.2022.103266

Doane, T. A., and Horwath, W. R. (2003). Spectrophotometric determination of nitrate with a single reagent. *Anal. Lett.* 36, 2713–2722. doi: 10.1081/AL-120024647

Dore, S., Deverel, S. J., and Christen, N. (2022). A vermifiltration system for low methane emissions and high nutrient removal at a California dairy. *Bioresour. Technol. Rep.* 18:101044. doi: 10.1016/j.biteb.2022.101044

Enebe, M. C., and Erasmus, M. (2023). Vermicomposting technology—a perspective on vermicompost production technologies, limitations and prospects. *J. Environ. Manag.* 345:118585. doi: 10.1016/j.jenvman.2023.118585

FAO/WHO. (1988). *Report of 33rd meeting, joint FAO/WHO joint expert committee on food additives, toxicological evaluation of certain food additives and contami- nants no. 24.* International Programme on Chemical Safety, WHO, Geneva.

Fernández-Ugalde, O., Virto, I., Barré, P., Gartzia-Bengoetxea, N., Enrique, A., Imaz, M. J., et al. (2011). Effect of carbonates on the hierarchical model of aggregation in calcareous semi-arid Mediterranean soils. *Geoderma* 164, 203–214. doi: 10.1016/j.geoderma.2011.06.008

Fronning, B. E., Thelen, K. D., and Min, D.-H. (2008). Use of manure, compost, and cover crops to supplant crop residue carbon in corn Stover removed cropping systems. *Agron. J.* 100, 1703–1710. doi: 10.2134/agronj2008.0052

Giannetta, B., Plaza, C., Galluzzi, G., Benavente-Ferraces, I., García-Gil, J. C., Panettieri, M., et al. (2024). Distribution of soil organic carbon between particulate and mineral-associated fractions as affected by biochar and its co-application with other amendments. *Agric. Ecosyst. Environ.* 360:108777. doi: 10.1016/j.agee.2023.108777

Horwath, W. R., and Paul, E. A. (1994). "Microbial biomass" In: *Methods of Soil Analysis*. R. W. Weaver, J. S. Angle, P. J. Bottomley, D. F. Bezdicek, M. S. Smith and M. A. Tabatabaiet al. Eds (Hoboken: John Wiley & Sons, Ltd.), 753–774.

Hossain, M. E., Mei, X., Zhang, W., Dong, W., Yan, Z., Liu, X., et al. (2021). Substitution of chemical fertilizer with organic fertilizer affects soil Total nitrogen and its fractions in northern China. *Int. J. Environ. Res. Public Health* 18:12848. doi: 10.3390/ijerph182312848

Just, C., Armbruster, M., Barkusky, D., Baumecker, M., Diepolder, M., Döring, T. F., et al. (2023). Soil organic carbon sequestration in agricultural long-term field experiments as derived from particulate and mineral-associated organic matter. *Geoderma* 434:116472. doi: 10.1016/j.geoderma.2023.116472

Kenneth, M. J., Koner, S., Hsu, G.-J., Chen, J.-S., and Hsu, B.-M. (2023). A review on the effects of discharging conventionally treated livestock waste to the environmental resistome. *Environ. Pollut.* 338:122643. doi: 10.1016/j.envpol.2023.122643

Khaliq, H., Juming, Z., and Ke-Mei, P. (2018). The physiological role of boron on health. *Biol. Trace Elem. Res.* 186, 31–51. doi: 10.1007/s12011-018-1284-3

Köninger, J., Lugato, E., Panagos, P., Kochupillai, M., Orgiazzi, A., and Briones, M. J. I. (2021). Manure management and soil biodiversity: towards more sustainable food systems in the EU. *Agric. Syst.* 194:103251. doi: 10.1016/j.agsy.2021.103251

Kumar, T., Bhargava, R., Prasad, K. S. H., and Pruthi, V. (2015). Evaluation of vermifiltration process using natural ingredients for effective wastewater treatment. *Ecol. Eng.* 75, 370–377. doi: 10.1016/j.ecoleng.2014.11.044

Lafuente, M. T., Sampedro, R., Vélez, D., and Romero, P. (2023). Deficient copper availability on organoleptic and nutritional quality of tomato fruit. *Plant Sci.* 326:111537. doi: 10.1016/j.plantsci.2022.111537

Lahlou, F.-Z., Mackey, H. R., and Al-Ansari, T. (2021). Wastewater reuse for livestock feed irrigation as a sustainable practice: a socio-environmental-economic review. *J. Clean. Prod.* 294:126331. doi: 10.1016/j.jclepro.2021.126331

Läuchli, A., and Grattan, S. R. (2014). Plant abiotic stress: salt. In AlfenN. K. Van (Ed.), *Encyclopedia of agriculture and food systems* (313–329). Oxford: Academic Press.

Lazcano, C., Arnold, J., Tato, A., Zaller, J. G., and Dominguez, J. (2009). Compost and vermicompost as nursery pot components: effects on tomato plant growth and morphology. *Span. J. Agric. Res.* 7, 944–951. doi: 10.5424/sjar/2009074-1107

Lazcano, C., Tsang, A., Doane, T. A., Pettygrove, G. S., Horwath, W. R., and Burger, M. (2016). Soil nitrous oxide emissions in forage systems fertilized with liquid dairy manure and inorganic fertilizers. *Agric. Ecosyst. Environ.* 225, 160–172. doi: 10.1016/j.agee.2016.04.009

Lehmann, J., Bossio, D. A., Kögel-Knabner, I., and Rillig, M. C. (2020). The concept and future prospects of soil health. *Nat. Rev. Earth Environ.* 1, 544–553. doi: 10.1038/s43017-020-0080-8

Li, X., Zhu, W., Xu, F., Du, J., Tian, X., Shi, J., et al. (2021). Organic amendments affect soil organic carbon sequestration and fractions in fields with long-term contrasting nitrogen applications. *Agric. Ecosyst. Environ.* 322:107643. doi: 10.1016/j.agee.2021.107643

Lindsay, W. L., and Norvell, W. A. (1978). Development of a DTPA soil test for Zinc, Iron, Manganese, and Copper. *Soil Sci. Soc. Am. J.* 42, 421–428. doi: 10.2136/sssaj1978.03615995004200030009x

Luo, Y., Chavez-Rico, V. S., Sechi, V., Bezemer, T. M., Buisman, C. J. N., and Ter Heijne, A. (2023). Effect of organic amendments obtained from different pretreatment technologies on soil microbial community. *Environ. Res.* 232:116346. doi: 10.1016/j.envres.2023.116346

Ma, B., Ma, B.-L., McLaughlin, N. B., Li, M., and Liu, J. (2022). Improvement in dryland crop performance and soil properties with multiple annual applications of lignite-derived humic amendment. *Soil Tillage Res.* 218:105306. doi: 10.1016/j.still.2021.105306

Martinez-Burgos, W. J., Bittencourt Sydney, E., Bianchi Pedroni Medeiros, A., Magalhães, A. I., de Carvalho, J. C., Karp, S. G., et al. (2021). Agro-industrial wastewater in a circular economy: characteristics, impacts and applications for bioenergy and biochemicals. *Bioresour. Technol.* 341:125795. doi: 10.1016/j.biortech.2021.125795

Meyer, G. A., and Keliher, P. N. (1992). An overview of analysis by inductively coupled plasma-atomic emission spectrometry. In: *Inductively coupled plasmas in analytical atomic spectrometry*, A. Montaser and D. W. Golightly, Eds (New York, NY: VCH Publishers) 473–516.

Miranda, K. M., Espey, M. G., and Wink, D. A. (2001). A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. *Nitric Oxide Biol. Chem.* 5, 62–71. doi: 10.1006/niox.2000.0319

Naikwade, P. V. (2019). Soil organic carbon sequestration by Long-term application of manures prepared from *Trianthema portulacastrum* Linn. *Commun. Soil Sci. Plant Anal.* 50, 2579–2592. doi: 10.1080/00103624.2019.1671442

Ngo, P.-T., Rumpel, C., Doan, T.-T., and Jouquet, P. (2012). The effect of earthworms on carbon storage and soil organic matter composition in tropical soil amended with compost and vermicompost. *Soil Biol. Biochem.* 50, 214–220. doi: 10.1016/j.soilbio.2012.02.037

Ngo, P.-T., Rumpel, C., Doan Thu, T., Henry-des-Tureaux, T., Dang, D.-K., and Jouquet, P. (2014). Use of organic substrates for increasing soil organic matter quality and carbon sequestration of tropical degraded soil: a 3-year mesocosms experiment. *Carbon Manag.* 5, 155–168. doi: 10.1080/17583004.2014.912868

Nielsen, F. H. (2016). "Boron" in *Encyclopedia of food and health*. eds. B. Caballero, P. M. Finglas and F. Toldrá (Oxford: Academic Press), 451–455.

Nurhidayati, N., Ali, U., and Murwani, I. (2016). Yield and quality of cabbage (*Brassica oleracea* L. var. Capitata) under organic growing media using Vermicompost and earthworm *Pontoscolex corethrurus* inoculation. *Agric. Agric. Sci. Proc.* 11, 5–13. doi: 10.1016/j.aaspro.2016.12.002

Pasha, M. F. K., Yeasmin, D., Zoldoske, D., Kc, B., and Hernandez, J. (2018). Performance of an earthworm-based biological wastewater-treatment Plant for a Dairy Farm: case study. *J. Environ. Eng.* 144:04017086. doi: 10.1061/(ASCE)EE.1943-7870.0001290

Plaza, C., Giannetta, B., Fernández, J. M., López-de-Sá, E. G., Polo, A., Gascó, G., et al. (2016). Response of different soil organic matter pools to biochar and organic fertilizers. *Agric. Ecosyst. Environ.* 225, 150–159. doi: 10.1016/j.agee.2016.04.014

Püspök, J. F., Zhao, S., Calma, A. D., Vourlitis, G. L., Allison, S. D., Aronson, E. L., et al. (2023). Effects of experimental nitrogen deposition on soil organic carbon storage in Southern California drylands. *Glob. Chang. Biol.* 29, 1660–1679. doi: 10.1111/gcb.16563

Rahman, M. L., Rahman, M. M., Rahman, G. K. M. M., Solaiman, A. R. M., Karim, M. A., Bokhtiar, S. M., et al. (2020). Influence of tillage and organic amendments on terrace soil bio-physico-chemical properties and carbon sequestration index. *Commun. Soil Sci. Plant Anal.* 51, 2683–2700. doi: 10.1080/00103624.2020.1845355

Raza, S. T., Wu, J., Ali, Z., Anjum, R., Bazai, N. A., Feyissa, A., et al. (2021). Differential effects of organic amendments on maize biomass and nutrient availability in upland calcareous soil. *Atmos.* 12:1304. doi: 10.3390/atmos12081034

Rocha, D. H. D., Freitas, F. R. S., Sakamoto, I. K., Silva, E. L., and Varesche, M. B. A. (2024). Co-digestion of solid-liquid waste from citrus agroindustrial: effect of hydraulic retention time and organic loading rate on H2 production in a long-term continuous operation leach bed reactor. *Int. J. Hydrog. Energy* 49, 554–571. doi: 10.1016/j.ijhydene.2023.10.289

Ryals, R., Kaiser, M., Torn, M. S., Berhe, A. A., and Silver, W. L. (2014). Impacts of organic matter amendments on carbon and nitrogen dynamics in grassland soils. *Soil Biol. Biochem.* 68, 52–61. doi: 10.1016/j.soilbio.2013.09.011

Rynk, R., Schwarz, M., Richard, T. L., Cotton, M., Halbach, T., and Siebert, S. (2022). "Chapter 4—compost feedstocks" in *The composting handbook*. ed. R. Rynk (Oxford: Academic Press), 103–157.

Sah, R. N., and Miller, R. O. (1992). Spontaneous reaction for acid dissolution of biological tissues in closed vessels. *Anal. Chem.* 64, 230–233. doi: 10.1021/ac00026a026

Saapi, S. S. Y., Andrianisa, H. A., Zorom, M., Mounirou, L. A., Kouassi, H. A. A., and Ahossouhe, M. S. (2024). New developments on vermifiltration as a bio-ecological wastewater treatment technology: mechanism, application, performance, modelling, optimization, and sustainability. *Heliyon* 10:e25795. doi: 10.1016/j.heliyon.2024.e25795

Salomon, M. J., and Cavagnaro, T. R. (2022). Healthy soils: the backbone of productive, safe and sustainable urban agriculture. *J. Clean. Prod.* 341:130808. doi: 10.1016/j.jclepro.2022.130808

Sarkar, B., Chakrabarti, P. P., Vijaykumar, A., and Kale, V. (2006). Wastewater treatment in dairy industries—possibility of reuse. *Desalination* 195, 141–152. doi: 10.1016/j.desal.2005.11.015

Simas, A., Mores, R., Steffens, J., Dallago, R. M., Kunz, A., Michelon, W., et al. (2019). Electrodisinfection of real swine wastewater for water reuse. *Environ. Chem. Lett.* 17, 495–499. doi: 10.1007/s10311-018-0782-z

Singh, R., Samal, K., Dash, R. R., and Bhunia, P. (2019). Vermifiltration as a sustainable natural treatment technology for the treatment and reuse of wastewater: a review. *J. Environ. Manag.* 247, 140–151. doi: 10.1016/j.jenvman.2019.06.075

Sinha, R., Nair, J., Bharambe, G. R., Patil, S. S., and Bapat, P. (2008). *Vermiculture revolution: A low-cost and sustainable technology for management of municipal and industrial organic wastes (solid and liquid) by earthworms with significantly low greenhouse gas emissions*. In: Progress in waste management research (159–227). Nova Science Publishers.

Sokol, N. W., Sanderman, J., and Bradford, M. A. (2019). Pathways of mineral-associated soil organic matter formation: integrating the role of plant carbon source, chemistry, and point of entry. *Glob. Chang. Biol.* 25, 12–24. doi: 10.1111/gcb.14482

Song, X., Li, H., Song, J., Chen, W., and Shi, L. (2022). Biochar/vermicompost promotes hybrid Pennisetum plant growth and soil enzyme activity in saline soils. *Plant Physiol. Biochem.* 183, 96–110. doi: 10.1016/j.plaphy.2022.05.008

Song, S., Wen, Y., Zhang, J., and Wang, H. (2019). Rapid spectrophotometric measurement with a microplate reader for determining phosphorus in NaHCO3 soil extracts. *Microchem. J.* 146, 210–213. doi: 10.1016/j.microc.2019.01.002

Stehouwer, R., Cooperband, L., Rynk, R., Biala, J., Bonhotal, J., Antler, S., et al. (2022). "Chapter 15—compost characteristics and quality" in *The composting handbook*. ed. R. Rynk (Oxford: Academic Press), 737–775.

Sun, T., Mao, X., Han, K., Wang, X., Cheng, Q., Liu, X., et al. (2023). Nitrogen addition increased soil particulate organic carbon via plant carbon input whereas reduced mineral−associated organic carbon through attenuating mineral protection in agroecosystem. *Sci. Total Environ.* 899:165705. doi: 10.1016/j.scitotenv.2023.165705

Tang, B., Rocci, K. S., Lehmann, A., and Rillig, M. C. (2023). Nitrogen increases soil organic carbon accrual and alters its functionality. *Glob. Chang. Biol.* 29, 1971–1983. doi: 10.1111/gcb.16588

Verrone, V., Gupta, A., Laloo, A. E., Dubey, R. K., Hamid, N. A. A., and Swarup, S. (2024). Organic matter stability and lability in terrestrial and aquatic ecosystems: a chemical and microbial perspective. *Sci. Total Environ.* 906:167757. doi: 10.1016/j.scitotenv.2023.167757

Vitosh, M. L., Warncke, D. D., and Lucas, E. R.. (2006). *Secondary and micronutrients for vegetables and field crops*. East Lansing, Michigan: Michigan State University Extension.

Wang, L., Zheng, X., Tian, F., Xin, J., and Nai, H. (2018). Soluble organic nitrogen cycling in soils after application of chemical/organic amendments and groundwater pollution implications. *J. Contam. Hydrol.* 217, 43–51. doi: 10.1016/j.jconhyd.2018.08.003

Weil, R., Stine, M., Gruver, J., and Samson-Liebig, S. (2003). Estimating active carbon for soil quality assessment: a simplified method for laboratory and field use. *Am. J. Altern. Agric.* 18, 3–17. doi: 10.1079/AJAA200228

Western Regional Climate Center. (n.d.). Available at: https://wrcc.dri.edu

Wu, L., Zhang, S., Ma, R., Chen, M., Wei, W., and Ding, X. (2021). Carbon sequestration under different organic amendments in saline-alkaline soils. *Catena* 196:104882. doi: 10.1016/j.catena.2020.104882

Xing, M., Yang, J., and Lu, Z. (2005). *Microorganism-earthworm integrated biological treatment process—A sewage treatment option for rural settlements*. In: ICID 21st European regional conference, Frankfurt (pp. 15–19).

Xu, C., Wang, J., Wu, D., Li, C., Wang, L., Ji, C., et al. (2022). Optimizing organic amendment applications to enhance carbon sequestration and economic benefits in an infertile sandy soil. *J. Environ. Manag.* 303:114129. doi: 10.1016/j.jenvman.2021.114129

Yan, B., Zhang, Y., Wang, Y., Rong, X., Peng, J., Fei, J., et al. (2023). Biochar amendments combined with organic fertilizer improve maize productivity and mitigate nutrient loss by regulating the C-N-P stoichiometry of soil, microbiome, and enzymes. *Chemosphere* 324:138293. doi: 10.1016/j.chemosphere.2023.138293

Yang, X.-E., Long, X.-X., Ni, W.-Z., Ye, Z.-Q., He, Z.-L., Stoffella, P. J., et al. (2002). Assessing copper thresholds for phytotoxicity and potential dietary toxicity in selected vegetable crops. *J. Environ. Sci. Health B* 37, 625–635. doi: 10.1081/PFC-120015443

Yao, Z., Xu, Q., Chen, Y., Liu, N., Li, Y., Zhang, S., et al. (2021). Leguminous green manure enhances the soil organic nitrogen pool of cropland via disproportionate increase of nitrogen in particulate organic matter fractions. *Catena* 207:105574. doi: 10.1016/j.catena.2021.105574

Zhang, Y., Li, F., Lu, Z., Pei, Z., Zhao, H., Shen, Q., et al. (2023). Organic amendments effects on soil aggregation and carbon sequestration in saline-alkaline croplands in China. *Agron. J.* 115, 2083–2095. doi: 10.1002/agj2.21332

Zhang, M., Tashiro, Y., Ishida, N., and Sakai, K. (2022). Application of autothermal thermophilic aerobic digestion as a sustainable recycling process of organic liquid waste: recent advances and prospects. *Sci. Total Environ.* 828:154187. doi: 10.1016/j.scitotenv.2022.154187