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 vermicfiltration 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.

\textbf{KEYWORDS} agricultural wastewater, vermifiltration, vermicompost, soil organic matter, microbial activity, micro and macro nutrients

\textbf{1 Introduction}

Agricultural and agro-industrial activities have grown considerably over the last few decades, leading to substantial environmental pollution (Martínez-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 vermicomposting 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, vermicomposting is an eco-friendly and cost-effective biological aerobic process that can be used for secondary wastewater treatment using epigeic 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 vermicomposting treatment in agriculture could increase the attractiveness and efficacy of vermicomposting 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 vermicomposting 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 vermicomposting 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 (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 vermicomposting 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 vermicomposting 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 vermicompost 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 ‘Ananashy’ variety planted in 2022.

2.2 Characterization of the organic amendment

We employed vermicompost generated as a by-product of the vermicomposting system. The vermicomposting system is operated by Biofiltro Inc. The system consisted of a rectangular concrete enclosure (49 m x 11 m x 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 vermicompost surface every 30 min. The applied influent percolated through the vermicompost to the underlying drainage space and drained under gravity in about 4 h. The vermicompost 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 vermicomposting 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
Briefly, we mixed 2.5 g of air-dried soil samples with 2.02 g of moist soil was mixed to remove plant matter and organic debris. The 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. 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.

### 2.3 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.

Nitrates (NO₃⁻N) and ammonium (NH₄⁺-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 K₂SO₄ (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 N₂ gas and incubated for 7 days at 37°C. A soil extract was subsequently prepared using 0.67 M of K₂SO₄, and NH₄⁺-N was determined colorimetrically in the soil extracts. The difference between NH₄⁺-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 K₂SO₄. 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).

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

<table>
<thead>
<tr>
<th>Vermicompost (pepper plant)</th>
<th>Vermicompost (dairy manure)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-NO₃⁻ (mg g⁻¹ dw)</td>
<td>0.378</td>
</tr>
<tr>
<td>N-NH₄⁺ (mg g⁻¹ dw)</td>
<td>0.170</td>
</tr>
<tr>
<td>Inorganic N (mg g⁻¹ dw)</td>
<td>0.548</td>
</tr>
<tr>
<td>Total N (%) dw</td>
<td>2.02</td>
</tr>
<tr>
<td>Organic N (mg g⁻¹ dw)</td>
<td>19.65</td>
</tr>
<tr>
<td>C (%)</td>
<td>42</td>
</tr>
<tr>
<td>C/N</td>
<td>20.79</td>
</tr>
<tr>
<td>BD' Wet (g cm⁻³)</td>
<td>0.48</td>
</tr>
<tr>
<td>BD' Dry (g cm⁻³ dw)</td>
<td>0.17</td>
</tr>
</tbody>
</table>

BD, bulk density.

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

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 CO₂. The soil respiration was estimated through the CO₂ evolved from the samples measured with a continuous flow LICOR 850 IRGA CO₂/H₂O 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).

#### Table 2

<table>
<thead>
<tr>
<th>Vermincoompost (dairy manure)</th>
<th>Nitrate (NO₃⁻N)</th>
<th>Ammonium (NH₄⁺-N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 g</td>
<td>0.285</td>
<td>0.143</td>
</tr>
<tr>
<td>3 g</td>
<td>0.423</td>
<td>0.212</td>
</tr>
<tr>
<td>5 g</td>
<td>0.561</td>
<td>0.281</td>
</tr>
</tbody>
</table>

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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...

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

<table>
<thead>
<tr>
<th>Year</th>
<th>Low rate (LV)</th>
<th>Target available N (kg ha⁻¹)</th>
<th>Vermicompost applied (dry ton ha⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2021</td>
<td>Low rate (LV)</td>
<td>18</td>
<td>2.8</td>
</tr>
<tr>
<td></td>
<td>Medium rate (MV)</td>
<td>28.8</td>
<td>4.48</td>
</tr>
<tr>
<td></td>
<td>High rate (HV)</td>
<td>54.0</td>
<td>8.39</td>
</tr>
<tr>
<td>2022</td>
<td>Low rate (LV)</td>
<td>18</td>
<td>4.22</td>
</tr>
<tr>
<td></td>
<td>Medium rate (MV)</td>
<td>28.8</td>
<td>6.56</td>
</tr>
<tr>
<td></td>
<td>High rate (HV)</td>
<td>54.0</td>
<td>12.20</td>
</tr>
</tbody>
</table>
TABLE 3: Variation in soil physicochemical properties after vermicompost application.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>LV</th>
<th>MV</th>
<th>HV</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.29 ± 0.16</td>
<td>7.42 ± 0.21</td>
<td>7.43 ± 0.26</td>
<td>7.54 ± 0.15</td>
<td>0.5</td>
</tr>
<tr>
<td>EC (μS cm⁻¹)</td>
<td>345 ± 115</td>
<td>259 ± 62.5</td>
<td>280 ± 60.4</td>
<td>276 ± 83.3</td>
<td>0.6</td>
</tr>
<tr>
<td>Bulk density (g cm⁻³)</td>
<td>1.07 ± 0.05</td>
<td>1.04 ± 0.07</td>
<td>1.14 ± 0.14</td>
<td>1.09 ± 0.08</td>
<td>0.6</td>
</tr>
<tr>
<td>TIN (kg ha⁻¹)</td>
<td>47.1 ± 29.2</td>
<td>38.1 ± 18</td>
<td>38.1 ± 18</td>
<td>27.3 ± 8.39</td>
<td>0.4</td>
</tr>
<tr>
<td>PMN (kg ha⁻¹)</td>
<td>18.1 ± 4.37 b</td>
<td>20.1 ± 7.68 b</td>
<td>27.8 ± 12.8 ab</td>
<td>51.6 ± 17.5 a</td>
<td>0.02</td>
</tr>
</tbody>
</table>

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.

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.
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 \(\beta\)-glucosidase (Figure 4B) and cellulase (Figure 4C), where the HV treatment had the highest enzymatic activity among the four treatments. The activity of \(\alpha\)-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 vermicompost, 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 vermicompost 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.
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 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 value is less than 0.05. 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

![Figure 5](image-url)  
**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.
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.

### 5 Conclusion

The utilization of vermicompost derived from vermicomposting as an organic amendment has shown promise as a sustainable means of addressing soil health. The incorporation of vermicompost as an organic amendment, especially at a rate of 54kg N/ha⁻¹, improved the nutritional status of the squash. This improvement was achieved 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 enhanced 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 addition of vermicompost is effective in improving soil health.
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. KL: 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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