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Vermicompost Rate Effects on Soil Fertility and Morpho-Physio-Biochemical Traits of Lettuce

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Abstract: The use of agricultural chemicals has adversely affected soil health and the environment. Organic farming practices, particularly vermicompost (VC), are gaining attention for their potential to improve soil fertility and crop productivity. This study investigated VC rate applications on lettuce growth, yield, soil fertility, nutrient dynamics, enzyme activity, biological parameters, and biochemical aspects under greenhouse conditions in Samsun, Turkey during 2022–2023. Experimentally, VC was applied at rates of V1: 1%, V2: 2%, and V3: 4% *w*/*w*, with a control group without VC application, V0: 0% *w*/*w*. Batavia lettuce, which is sensitive to environmental conditions and nutrient deficiency, was subjected to these treatments in a randomized complete block design, replicated thrice. Results showed consistent improvements in plant dry weight across all VC treatments, with the 2% application rate (V2) yielding the highest increase in lettuce yield (56.43%). Soil pH varied across treatments, with V1 being slightly alkaline and V3 showing high electrical conductivity and increased nitrogen content. Phosphorus content increased in all treatments, while potassium varied, with V3 having the highest values. Soil enzyme activities increased with VC concentrations, with V3 showing the highest urease activity. Pearson correlations confirmed positive associations with growth parameters and soil enzymatic activity. These findings highlight vermicompost as a sustainable solution for lettuce production and soil improvement.

Keywords: sustainable agriculture; recycling organic waste; vermicompost; soil microbial function; enzyme activity; lettuce yield; organic matter; micronutrients

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1. Introduction

The global demand for sustainable agricultural practices has led to the exploration of innovative approaches aimed at enhancing crop yield while minimizing environmental impact. Approximately 50% of the world's population relies on chemical fertilizers for crop production to fulfill their food requirements [1]. Nevertheless, this heavy reliance on chemical fertilizers has resulted in adverse consequences, contributing to environmental pollution and raising health concerns due to the presence of agrochemical residues in food commodities [2]. These residues can have detrimental effects on both the environment and human health [3].

To tackle these issues, scientists have redirected their focus toward discovering "green substitutes" for chemical fertilizers, seeking alternatives with a lower environmental impact [4,5]. Among these alternatives, vermicompost (VC) has emerged as an appealing choice, where earthworms and microbes collaborate to break down organic materials, leading to the production of nutrient-rich and microbiologically active organic amendments [6]. Earthworms play a pivotal role in breaking down organic matter, facilitating partial decomposition as it passes through their digestive systems. Simultaneously, beneficial microorganisms, including bacteria and fungi, contribute to the decomposition process. These microorganisms thrive under specified temperature and aeration conditions, ensuring optimal microbial activity. Consequently, the organic waste undergoes a transformation into VC [6–8]. This microbiologically active vermicompost, naturally enriched with essential nutrients and minerals, becomes a sustainable alternative to synthetic fertilizers [5,9]. When applied to the soil, VC enhances fertility and promotes improved crop growth. It provides readily available nutrients and fosters a conducive environment for beneficial microorganisms [10], ultimately contributing to a healthier and more productive soil-plant system [8]. Furthermore, VC is known for its slow-release properties, which can enhance agricultural production while minimizing nutrient losses [7]. VC contains nitrogen (N), phosphorus (P), potassium (K), and other micronutrients in plant-available forms, enhancing their accessibility through microbial activities [11]. On average, VC typically contains 1.5–2.2% nitrogen (N), 1.8–2.2% phosphorus (P), and 1.0–1.5% potassium (K), along with organic carbon ranging from 9.15% to 17.98%, and includes a range of micronutrients [12]. Therefore, VC can be used as a soil conditioner [5]. Furthermore, mycorrhizal fungi and plant growth-promoting rhizobacteria (PGPR) can be stimulated by VC. These beneficial microorganisms play a crucial role in improving plant nutrient absorption and fortifying plants against various abiotic and biotic stresses [8].

In recent years, several studies have highlighted the potential of plant growth-promoting bacteria (PGPB) and compost in enhancing soil fertility and promoting circular economy principles in agriculture. For example, Stegelmeier et al. [13] demonstrated the role of PGPB in improving nutrient uptake and plant growth in a hydroponic system. Additionally, Ng et al. [14] conducted a comprehensive review of bacterial-based biofertilizers and concluded that the introduction of microbial populations, including plant growth-promoting bacteria (PGPB), as a strategy to enhance soil fertility and stimulate plant growth, aligns with circular economy objectives by closing the life cycles of fertilizer products. The application of PGPB as biofertilizers has shown promising results in enhancing nutrient availability in the rhizosphere, increasing root surface area, inducing beneficial symbioses in plants, and reducing the need for chemical fertilizers [15]. Additionally, the importance of composting practices and their contributions to soil health and carbon sequestration has also been documented by numerous research reports [16–18]. These studies highlight the importance of incorporating microbial-based fertilizers and organic amendments into agricultural practices to reduce reliance on synthetic inputs and promote sustainable nutrient management.

Moreover, aligning with the principles outlined in the "Green Agenda" (European Green Deal), it is imperative to integrate guidelines for sustainable soil management and fertilizer application into agricultural policies and practices. Recommendations such as minimizing chemical fertilizer usage, promoting organic farming practices, and enhancing soil conservation efforts are crucial steps toward achieving environmental sustainability and resilience in agriculture. By adopting these guidelines, farmers can mitigate environmental degradation, improve soil health, and contribute to the circular economy by closing nutrient loops and reducing waste.

Soil quality is an important factor in crop productivity and nutritional value [19]. A well-balanced nutrient supply is essential to achieve high crop yield and nutritional value [20]. VC is a beneficial soil amendment that not only provides essential nutrients but also improves soil physicochemical properties [12]. It enhances water retention, soil structure, organic carbon levels, and nutrient content while reducing bulk density and penetration resistance [21]. Enriched with humic acid and plant growth regulators, VC aids in drainage, nutrient uptake, and root growth [22]. Micronutrient malnutrition is a global health concern that VC can help address by enriching legumes with vital nutrients [23,24]. VC also has antimicrobial properties that protect earthworms during composting [25]. VC contains organic compounds that enhance soil enzyme activity, serving as substrates [23,26]. It also has antimicrobial properties that protect worms from harmful microorganisms [27]. These enzymes, along with plant growth hormones, make VC an effective organic fertilizer for gardening and sustainable farming [5,6,26]. VC application boosts enzyme activity like dehydrogenase, catalase, urease, and protease, enhancing soil biological processes and nutrient dynamics even at low concentrations [28].

Lettuce (*Lactuca sativa* L.) is a popular leafy vegetable grown for its nutritional value and economic value. Due to its rapid growth, lettuce is sensitive to changes in soil fertility and nutrient availability. Investigating the effects of vermicompost (VC) rate applications on lettuce yield can provide valuable information about its potential as a sustainable fertilizer for increasing crop productivity and quality. Furthermore, studying the nutrient composition of lettuce grown with VC can offer insight into the effect of this organic amendment on the nutritional value of the harvested produce [21]. Therefore, in the present study, it was hypothesized that different VC rates significantly influence the composition and activity of microbial communities in the rhizosphere, leading to changes in soil fertility parameters, enzyme activity levels, nutritional content, and agronomic aspects of lettuce. The objectives are threefold: (i) to investigate how vermicompost rates influence the abundance and diversity of microbial communities in the lettuce rhizosphere; (ii) to identify specific alterations in nutrient element content and agronomic characteristics resulting from distinct VC rates; and (iii) to clarify the precise mechanisms through which VC application impacts the microbiological characteristics and enzyme activity within the lettuce rhizosphere. By concentrating on these particular aspects, our study aims to provide targeted and novel insights, advancing the understanding of the relationships between VC rates and the rhizospheric microbial environment of lettuce. This approach distinguishes our work, contributing valuable information to enhance sustainable agricultural practices and broaden the current knowledge in this field.

2. Materials and Methods

2.1. Plant Material

The experimental vegetable material was lettuce *cv.* 'Dragone', a Batavia type of green lettuce supplied by the Vilmorin seed company (Vilmorin, La Ménitré, France). This lettuce variant, characterized as mid-early, requires approximately 55–60 days to reach harvest maturity under favorable temperature conditions when cultivated in soil. 'Dragone' lettuce is recognized for its appealing qualities and resilience against downy mildew and aphids.

2.2. Experimental Site Location

A pot experiment was conducted in a greenhouse situated at Ondokuz Mayıs University in Samsun, Turkey (41°21′49.9″ N, 36°11′19.7″ E) during 2022–2023. The site has an average annual maximum temperature of 27.7 °C, a minimum temperature of 5 °C, a relative humidity of 73%, and an average annual precipitation of 937.26 mm as reported in our previous study [21].

2.3. Experimental Soil Collection and Characterization

A Calcic Chernozem soil according to the World Reference Base (WRB) was collected from an agricultural field located at the Ondokuz Mayıs University, Turkey. Approximately 500 kg of soil was gathered for the current study and was analyzed for physical and chemical properties using standard laboratory methods. Soil acidity (pH) and electrical conductivity (EC) were conducted on 1:5 soil water ratio (*w*/*v*) suspension using a pH meter (Thermo Orion, Waltham, MA USA, Model: 420A+) and an EC meter (Decibel, Model: DB1041A) [29]. The soil organic matter content (SOM) was assessed by employing $K_2Cr_2O_7$ as an oxidizing agent, following the method described by Nelson and Sommers [30]. Analysis of ammonium bicarbonate-diethylenetriaminepentaacetic acid (AB-DTPA) extractable P and K followed the procedure detailed by Soltanpour and Schawab [31]. Briefly, 10 g of soil samples were extracted using a DTPA extracting solution (0.005 mol L^{-1} AB-DTPA and 0.1 mol L^{-1} triethanolamine) adjusted to a pH of 7.6. After shaking for 15 min on a mechanical shaker, the extract was filtered and analyzed for P and K concentrations using a spectrophotometer and a flame photometer, respectively [31]. The total nitrogen content in the soil was determined using the Kjeldahl distillation procedure, following the method described by Bremner [32]. The experimental soil had a clay texture (29.91% sand; 21.68% silt; 48.41% clay) along with a slightly acidic reaction (pH 6.8) and a recorded organic matter content of 2.1%.

2.4. Vermicompost

The vermicompost (VC) used in the experiment was sourced from the Department of Soil Science and Plant Nutrition, Faculty of Agriculture, Ondokuz Mayıs University, Turkey. The chemical analysis of the vermicompost was conducted using standard laboratory methods: pH 1:1, *w*/*v*, waste-water suspension, pH-meter, electrical conductivity 1:1, *w*/*v*, waste-water suspension, EC-meter [29]. Ash content was determined by dry ashing [33], and organic matter content was estimated by dry ashing and calculating the organic carbon using a conversion factor of 1.724 [34]. Total phosphorus in organic wastes was determined by the dry ashing method, and total nitrogen content was determined by the Kjeldahl method [32]. The vermicompost exhibits an alkaline pH of 9.11, elevated soluble salt concentration (EC 5662 μS cm^{-1}), and a substantial organic matter content of 59.25%. It possesses a high total nitrogen content (2.17%), an optimal C/N ratio of 15.84, significant phosphorus content (5361 mg kg^{-1}), 40.75% ash content, and 34.37% organic carbon.

2.5. Experimental Design

A completely randomized design (CRD) was used in the experiment, with one factor: vermicompost rates (V0: 0%, V1: 1%, V2: 2%, and V3: 4% *w/w*). The V0 treatments were given a basic application of NPK fertilizers with the equivalent amount of 100 kg N ha^{-1}, 80 kg P_2O_5 ha^{-1}, and 120 kg K_2O ha^{-1} applied to the pot surface. The standard procedures for calculating fertilizer for a potted experiment were used to determine the amount of fertilizer required for each pot. Each treatment was replicated thrice, including three control pots. The experimental soil was naturally dried in the shade for 15–20 days, crushed with a wooden hammer, and sieved through a 4 mm sieve. This process ensured consistent soil conditions for each treatment. Each pot (diameter 20.3 cm; depth 20 cm) was filled with 3 kg of prepared soil. To ensure consistent and reliable results, the study was conducted in a controlled atmosphere. Temperature was carefully regulated to minimize the impact of fluctuations on the plants. The greenhouse had a relative humidity of 70%. The temperature was kept at 29 °C from 09:00 to 17:00 and 21 °C during the night. The day length followed natural conditions. For irrigation, rainwater was utilized to prevent contamination from other water sources. Soil moisture content was closely monitored and maintained near field capacity, representing the maximum water-holding capacity of the soil. Pots were regularly weighed to assess moisture depletion and replenished as needed to maintain optimal levels for plant growth. After a period of one month and 25 days, each plant was harvested (Figure 1), and soil samples were taken from each harvested pot for further physicochemical and biological analysis.

Figure 1. An overview of Batavia lettuce harvesting.

2.6. Data Collection

2.6.1. Growth Measurements

At the maturity stage (55 days), a measurement tape was used to measure the plant height. On 23 January 2023, Batavia lettuce was harvested and underwent immediate post-harvest handling.

To accurately measure the fresh weight of lettuce (*FW*), a digital scale sensitive to 0.01 g was used after removing any debris. The dry mass (*DW*) was determined by drying the lettuce in an oven at 105 °C for 2 days to obtain constant weight, from which the dry matter content was calculated using a specific formula.

$$\text{Dry matter content(g)} = 100 \times \frac{DW}{FW}$$

2.6.2. Biochemical Analysis

The contents of chlorophyll—chlorophyll a, chlorophyll b, and total chlorophyll—were determined in accordance with Witham et al. [35]. A 0.5 g dried plant sample was weighed and dried at 65 °C with aeration until reaching a constant weight. The sample was then subjected to dry ashing in a furnace at 550 °C for 4 to 8 h. The resulting ash was dissolved in hydrochloric acid (HCl) at a 10% (*v/v*) concentration and heated gently at 65 °C for 30 min, and the iron content was assessed using an atomic absorption spectrophotometer [36].

2.6.3. Post-Harvest Soil Physiochemical Analysis

To assess the effects of vermicompost on soil physiochemical characteristics, all post-harvest soils were analyzed for pH, electrical conductivity (EC), organic matter (OM), total N [32], and AB-DTPA extractable P as described above [31]. Exchangeable base cations, including K^+, Ca^{2+}, Mg^{2+}, and Na^+, were extracted with 1 mol L^{-1} ammonium acetate at pH 7. Calcium and magnesium were determined by atomic absorption spectrophotometry, while sodium and potassium were analyzed by flame emission spectrophotometry [31]. Additionally, an atomic absorption spectrophotometer was used to measure the DTPA extractable contents of Fe, Cu, Mn, and Zn [37]. The solution for extracting these elements was composed of 0.005 mol L^{-1} AB-DTPA and 0.01 mol L^{-1} calcium chloride ($CaCl_2$).

2.6.4. Post-Harvest Soil Biological Analysis

The tests mentioned aim to evaluate different soil biological parameters. These tests collectively provide insights into soil microbial activity, biomass, and functional diversity, essential for understanding soil health, nutrient cycling, and ecosystem functioning, thus informing sustainable soil management practices for optimizing plant productivity and environmental sustainability. The first parameter is microbial carbon biomass, which can be measured using the method developed by Anderson and Domsch [38]. This test measures the amount of carbon present in the microbial cells in the soil. The second parameter is basal soil respiration, which can be evaluated using the method proposed by Anderson [39]. This test measures the rate at which carbon dioxide is released from the soil due to the microbial respiration process. The third parameter is dehydrogenase activity (DHA), which can be assessed by following the procedure of Pepper et al. [40]. This test measures the activity of enzymes that are produced by soil microorganisms and are involved in the process of organic matter decomposition. The fourth parameter is catalase activity, which can be assessed using the method developed by Beck [41]. This test measures the activity of enzymes responsible for breaking down hydrogen peroxide into water and oxygen, indicating the soil microorganisms' ability to defend against oxidative stress. Collectively, these tests offer significant insights into the activity and vitality of soil microorganisms, essential for comprehending soil ecosystems' functionality and their contribution to supporting plant growth and nutrient cycling.

2.6.5. Enzymatic Activity

The β-glucosidase activity was determined according to the procedure outlined by Eivazi and Tabatabai [42]. According to this method, 1 g of soil was incubated with 0.25 mL toluene, 4 mL modified universal buffer (pH 6), and 1 mL PNG solution (25 mmol L^{-1}) for 1 h at 37 °C. Subsequently, 1 mL of 0.5 mol L^{-1} $CaCl_2$ solution and 4 mL of 0.1 mol L^{-1} Tris-hydroxymethyl-aminomethane-HCl (TRIS-HCl) of pH 12 were added [42]. The absorbance was measured at 400 nm using a Rigol Ultra 3660 UV–Vis spectrophotometer (Rigol Technologies, Beijing, China). The β-glucosidase activity was quantified as µg PNG per gram dry weight per hour at 37 °C.

Urease activity was assessed by employing urea as a substrate, following the method outlined by Yao et al. [43]. Specifically, 5 g of moist soil was subjected to incubation with 1 mL of methylbenzene, 10 mL of 10% urea, and 20 mL of citrate buffer (pH 6.7) for 24 h at 37 °C. A citrate buffer with a pH of 6.7 was made by dissolving 24.09 g of sodium citrate (M = 0.0819) and 3.47 g of citric acid (M = 0.0181) in 800 mL of distilled water. The solution was thoroughly mixed for 5 min before additional distilled water was added to reach a volume of 1000 mL, while also adjusting the solution's pH to 6.7. Following the incubation period, 1 mL of the strained soil solution was mixed with 1 mL of a 12.5% w/v sodium phenolate solution and 3 mL of a 12.5% w/v sodium hypochlorite solution. The mixture was then diluted to a final volume of 50 mL for 20 min. The absorbance was then measured at 578 nm using a spectrophotometer (Rigol Technologies, Beijing, China). Urease activity was quantified as NH_3-N per gram per hour at 37 °C.

The analysis of acid phosphatase activity involved the use of p-nitrophenyl phosphate (p-NPP) as a substrate, following the protocol described by Schneider et al. [44]. In this procedure, 1 g portion of wet soil was transferred into a Falcon tube (50 mL) and 4 mL of acetate buffer (pH 5.0, 0.5 mol L^{-1}) and 1 mL of p-NPP substrate (7.5 mmol L^{-1} of 4-Nitrophenyl phosphate disodium salt hexahydrate diluted in buffer) were added. The mixture was incubated at 30 °C for 30 min. After the incubation period, 1 mL of calcium chloride (0.5 mol L^{-1} $CaCl_2$) was added. The reaction was stopped by adding 4 mL of sodium hydroxide (0.2 mol L^{-1} NaOH). The absorbance was then measured at 405 nm using a spectrophotometer (Rigol Technologies, Beijing, China). Acid phosphatase (AP) activity was expressed as µg p-NPP per gram per hour at 30 °C.

2.7. Statistical Analysis

The effect of vermicompost on selected variables was evaluated using a one-way analysis of variance (ANOVA). The Duncan test was used to compare the means of the results at the 0.01 and 0.05 levels, and the F values for significant applications were provided with (**) at the 0.01 level and (*) at the 0.05 level. Pearson's correlation and principal component analysis of the evaluated attributes were conducted by GraphPad Prism V8 software (GraphPad Co., San Diego, CA, USA) and XLSTAT Addinsoft version 2014.5.03 (New York, NY, USA), respectively.

3. Results

3.1. Effect of Vermicompost on Lettuce Growth and Yield

The results demonstrate significant variations in plant-growth parameters with different vermicompost (VC) rates compared to the control (V0). Plant dry weight, a pivotal measure of biomass, consistently improved in all vermicompost treatments. V1, V2, and V3 displayed increments of 81.9%, 85.5%, and 71.7%, respectively, compared to the control (Figure 2). The VC treatments had a significant impact on lettuce yield. Among the various treatments, the plants treated with 2% vermicompost (V2) displayed the highest yield, exhibiting a remarkable increase of 56.43% when compared to the control treatment (V0). Additionally, a notable increase in lettuce yield of 26.15% was recorded with the application of 4% VC (V3) compared to the control treatment. However, it's worth noting that this increase was lower than the yields obtained with 1% and 2% VC applications, which recorded yields of 48.17% and 56.43%, respectively (Figure 2).

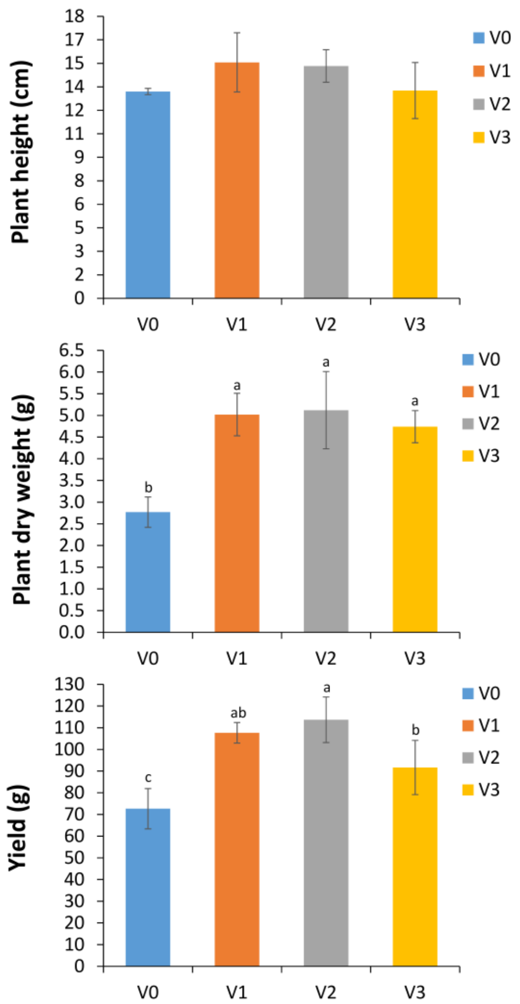

Figure 2. Lettuce growth and yield as influenced by different applications of vermicompost. V0, V1, V2 and V3: 0, 1, 2, 4% of vermicompost. The data are reported as means of 3 replicates. Means followed by the different letters are statistically significant at $p < 0.05$. Error bars represent the standard deviation.

3.2. Impact of Vermicompost Application on Soil Properties and Nutrient Dynamics

The soil pH exhibited significant variations among the VC treatments. Treatment V1 recorded a pH value of 7.21, suggesting a slightly alkaline soil environment. In contrast, V0 and V3 had lower pH values of 7.13 and 7.01, respectively. Treatment V2 showed a pH of 7.02, indicating a moderately alkaline soil condition (Table 1).

Table 1. Effect of vermicompost doses on soil properties and nutrient content of lettuce grown soil.

Vermicompost (%)	pH	EC ($\mu S\ cm^{-1}$)	OM (%)
V0	7.13 ± 0.02 ab	1030.00 ± 51.5 c	2.07 ± 0.18 c
V1	7.21 ± 0.02 a	1216.00 ± 60.8 c	2.24 ± 0.04 bc
V2	7.02 ± 0.12 bc	1669.33 ± 84.97 b	2.53 ± 0.07 b
V3	7.01 ± 0.02 c	2213.33 ± 110.67 a	3.83 ± 0.29 a

Means having different letters showed significant differences between treatments; (V0:0%, V1:1%, V2:2%, and V3:4%). The data are reported as means of 3 replicates ± standard deviations. Means followed by the different letters are statistically significant at $p < 0.05$.

The electrical conductivity (EC) values differed between treatments of lettuce. The highest EC value (2213.33 μS/cm) was recorded for V3, indicating a higher concentration of soluble salts in the soil (214.87%) followed by V2 (62.05%) compared to the control treatment (Table 1). These findings imply that applying vermicompost to the soil may affect its ionic composition and salt levels. Similarly, treatment V3 had the highest OM content of 3.83% followed by organic matter of V2 (2.53%) (Table 1).

The nitrogen (N) content in the VC treatments displayed variability, with V3 exhibiting the highest (0.60%) nitrogen content. This value was significantly (36%) higher than the nitrogen content in the control treatment (Table 2). VC application had a significant impact on the phosphorus (P) content of the soil. There was a substantial increase in P content compared to the control treatment, with V1, V2, and V3 showing increments of 92%, 186%, and 349%, respectively (Table 2).

Examining the potassium (K) content of the VC samples revealed a range from low to high values. V3 exhibited the highest (2.41%) potassium content, followed by V2 (1.4%). The lowest potassium content (0.9%) was recorded in V1, a value statistically similar to that of the control treatment (V0) (0.7%).

The calcium (Ca) content of the VC treatments varied slightly. V0 had the highest (25%) calcium content, which was comparable to the calcium content recorded for V1 and V2 (24% and 24%, respectively). The lowest value of calcium content (20%) was recorded in V3 (Table 2). The magnesium (Mg) content of the VC samples varied significantly from 15 to 29%. The magnesium content was significantly higher (29%) in V3, followed by V2 and V1 (20 and 17%) compared to the control treatment, which had the lowest magnesium content (15%). Sodium (Na) content varied slightly among the VC treatments. V3 had the highest (3%) sodium content, followed by V2 and V1 at 2% and 2%, respectively, compared to the control treatment (V0).

The results revealed significant differences in Fe, Cu, Mn, and Zn levels between VC treatments. Increasing the concentration of VC treatments from 0 to 4% resulted in a significant decrease in Fe concentrations. Treatments V1 and V2 showed a slight decrease of approximately 13.2% and 13.8%, respectively, while V3 exhibited a significant decrease of about 35.8% when compared to the control treatment (V0). Cu concentrations in V1 and V2 decreased by approximately 6.6% and 10.1%, respectively, and V3 showed a substantial decrease of about 34.9% compared to the control treatment (V0). Mn concentrations in V1, V2, and V3 decreased by approximately 11.9%, 7.2%, and 18.3%, respectively, compared to the control treatment (V0). Zinc concentrations saw significant increases across all treatments, with V1 and V2 showing approximately 137.8% and 137.4% increases, respectively, while V3 exhibited a substantial increase of about 288.8% (Table 2).

Table 2. Effect of vermicompost doses on macro- and micronutrient contents of lettuce grown soil.

N (%)	P (mg kg^{-1})	K (cmol(+) kg^{-1})	Ca (cmol(+) kg^{-1})	Mg (cmol(+) kg^{-1})	Na (cmol(+) kg^{-1})	Fe (mg kg^{-1})	Mn (mg kg^{-1})	Cu (mg kg^{-1})	Zn (mg kg^{-1})
0.45 ± 0.01 c	36.49 ± 3.63 d	0.75 ± 0.03 c	25.68 ± 0.36 a	15.58 ± 0.34 d	1.62 ± 0.07 c	49.40 ± 2.83 a	68.8 ± 0.28 a	13.9 ± 2.34 a	9.46 ± 1.27 d
0.4 ± 0.01 d	70.38 ± 4.20 c	0.98 ± 0.04 c	24.64 ± 0.43 a	17.67 ± 0.37 c	2.02 ± 0.10 b	42.89 ± 2.79 b	53.2 ± 0.17 b	12.12 ± 3.12 b	15.32 ± 1.02 c
0.52 ± 0.03 b	104.54 ± 10.46 b	1.44 ± 0.02 b	24.39 ± 1.36 a	20.82 ± 1.29 b	2.06 ± 0.05 b	42.59 ± 1.59 b	51.5 ± 0.84 b	12.27 ± 1.96 b	22.49 ± 1.00 b
0.61 ± 0.02 a	164± 4.9 a	2.41 ± 0.32 a	20.26 ± 0.50 b	29.39 ± 072 a	3.22 ± 0.04 a	31.60 ± 1.84 c	45.2 ± 0.22 c	11.39 ± 1.95 b	36.66 ± 1.96 a

Means having different letters showed significant differences between treatments; (V0:0%, V1:1%, V2:2%, and V3:4%). cmol(+)/kg is the abbreviation for centimoles per kilogram. The data are reported as means of 3 replicates ± standard deviations. Means followed by the different letters are statistically significant at $p < 0.05$.

3.3. Impact of Vermicompost Application on Soil Biological Parameters

The soil's basal respiration rate (BSR) values varied significantly, with V0 having the highest at a 0.0% baseline, while V1, V2, and V3 showed % decreases of 18.8%, 37.5%, and 35.2%, respectively. Microbial biomass carbon (MBC) and dehydrogenase activity (DHA) levels demonstrated substantial increases in V3 compared to V0, with increments of 90.4% and 20.3%, respectively. Soil catalase activity (CA) also varied significantly, with V1 exhibiting the highest increase at 3.8% compared to the control (Figure 3).

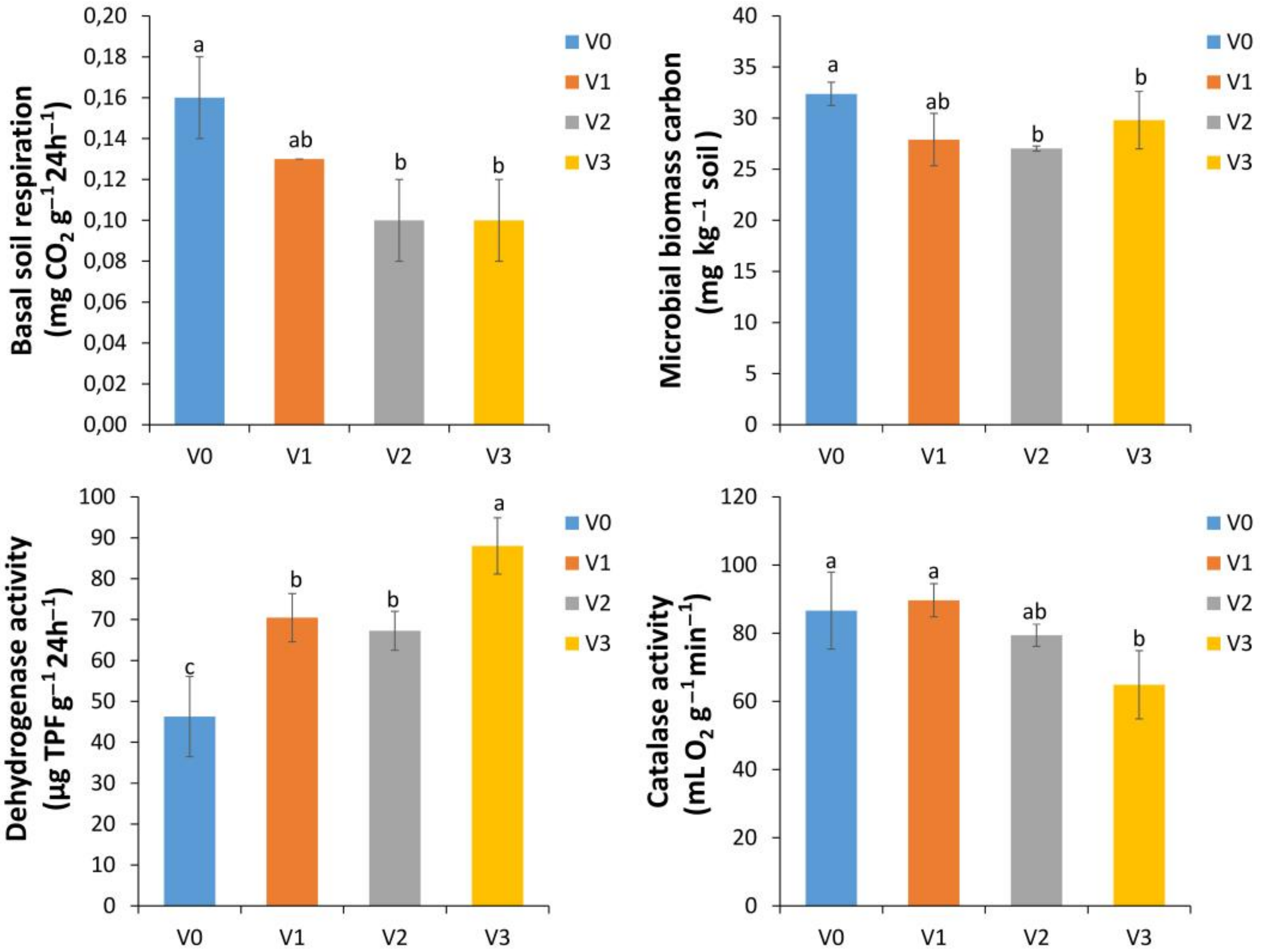

Figure 3. Basal soil respiration, microbial biomass carbon, dehydrogenase activity, and catalase activity as influenced by different applications of vermicompost. V0, V1, V2 and V3: 0, 1, 2, 4% of vermicompost. The data are reported as means of 3 replicates. Means followed by the different letters are statistically significant at $p < 0.05$. Error bars represent the standard deviation.

3.4. Impact of Vermicompost Application on Biochemical Parameters of Lettuce

The examination of lettuce crops with different VC percentages (V0, V1, V2, and V3) revealed significant changes in chlorophyll and carotenoid contents, except for chlorophyll a. Chlorophyll b exhibited fluctuations across treatments. The highest increase in chlorophyll b was observed in V1, with a rise of around 37.93%. Total chlorophyll content demonstrated a similar trend, with the maximum increase of approximately 46% occurring in V1 compared to V0 (Figure 4). Carotenoid content varied among treatments, reaching its peak in V2 with an increase of about 164.28% compared to V0 (Figure 4).

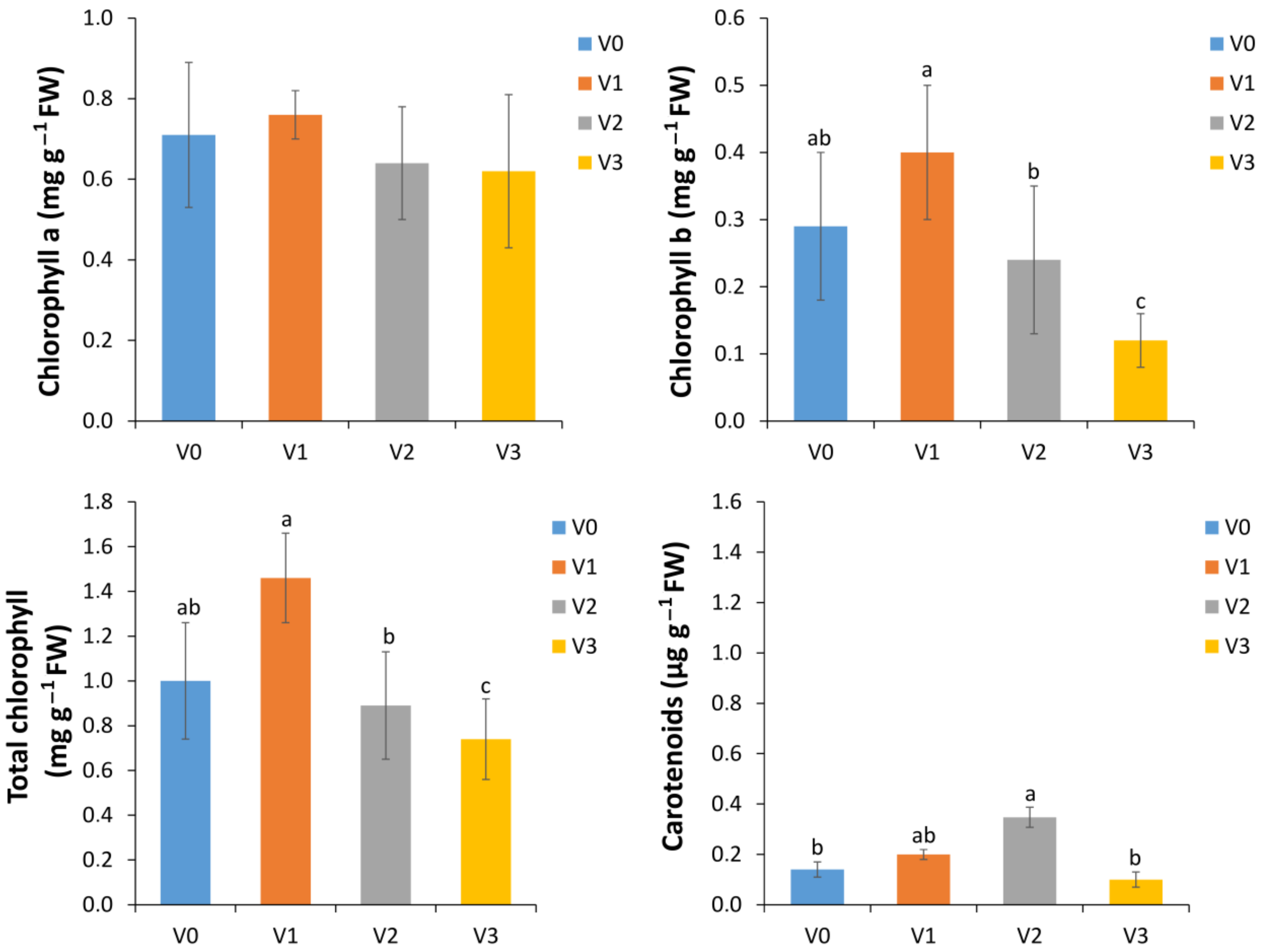

Figure 4. Chlorophyll and carotenoid content in lettuce as influenced by different applications of vermicompost. V0, V1, V2 and V3: 0, 1, 2, 4% of vermicompost. The data are reported as means of 3 replicates. Means followed by the different letters are statistically significant at $p < 0.05$. Error bars represent the standard deviation.

3.5. Effect of Vermicompost on Soil Enzyme Activities

It was noticed that phosphatase activity significantly increased with increasing VC concentrations and varied from 725 to 1570 µg p-nitrophenol g^{-1} soil h^{-1}. Application of VC at V3 resulted in a 53% of increase in phosphatase activity as compared with the control treatment (V0). Similarly, the glucosidase activity was not significantly influenced by VC application. However, increasing the application of VC treatments increased glucosidase activity (335–427 µg p-nitrophenol g^{-1} soil h^{-1}). Similarly, the glucosidase activity increased from V0 to V1 and then decreased from V2 to V3 (Figure 5).

The urease activity ranged from 11.6 to 26.2 µg N g $^{-1}$ soil h^{-1} with the highest value of urease activity noticed in the V2 and V3 treatments and the lowest value was observed in V0 (11.6 µg N g $^{-1}$ soil h^{-1}). There was an increase in urease activity from V0 to V2, followed by a slight decrease from V2 to V3 (Figure 5).

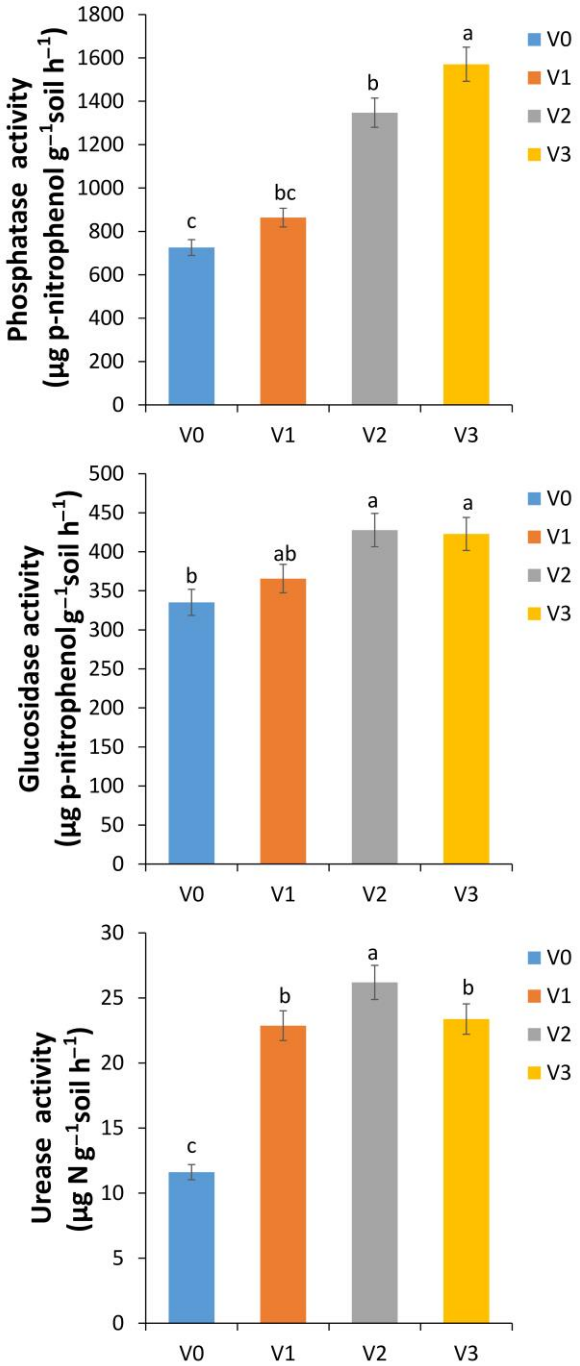

Figure 5. Effects of vermicompost application on soil enzymatic activity of lettuce. V0, V1, V2 and V3: 0, 1, 2, 4% of vermicompost. The data are reported as means of 3 replicates. Means followed by the different letters are statistically significant at $p < 0.05$. Error bars represent the standard deviation.

3.6. Principal Component Analysis and Heat Map Pearson Correlation

The principal component analysis of the studied attributes and their eigenvalue is given in Figure 6. The contributions of different components of PCA are presented on the x-axis (PC1) and y-axis (PC2). PC1 (57.18%) and PC2 (33.9%) exhibited the highest contributions in terms of percentage variance and represented 91% of the total variance in the dataset. Most of the studied attributes showed a positive correlation with each other (Figure 6). Additionally, the relationship between yield, soil enzyme activity, and specific treatments was identified. For instance, yield, growth parameters, and soil enzyme activity were associated with the V2 treatment, while EC and OM were linked with the V3 treatment.

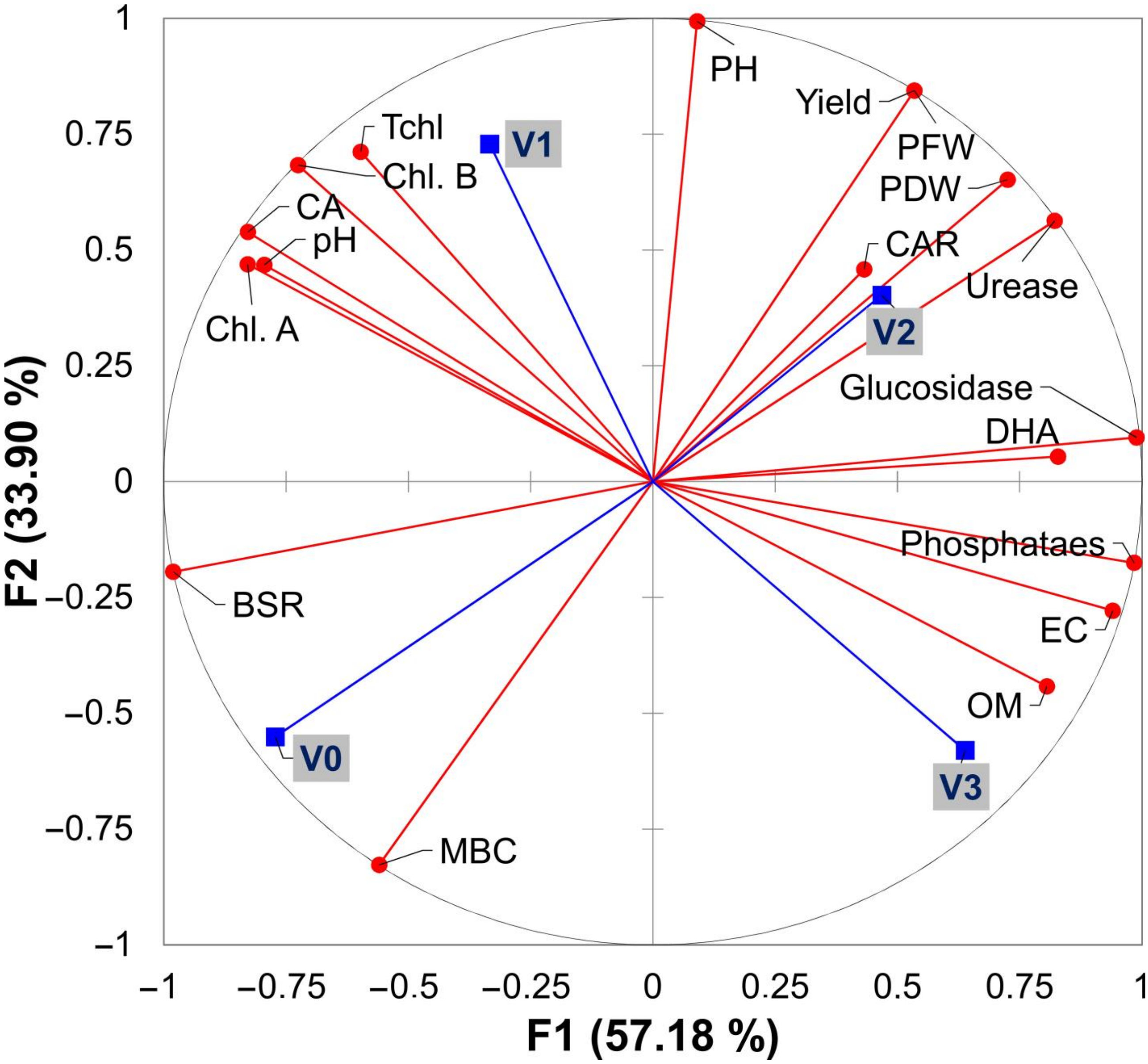

Figure 6. An analysis of the correlations between treatment variables in lettuce plants using principal component analysis (PCA. PH: plant height, PFW: plant fresh weight, PDW: plant dry weight, Chl. B: chlorophyll b, Tchl: total chlorophyll, CAR: carotenoids, BSR: basal soil respiration, MBC: microbial biomass carbon, DHA: dehydrogenase, CAT: catalase. V0, V1, V2 and V3: 0, 1, 2, 4% of vermicompost.

To highlight the key aspects of the interest relationship, we computed the Pearson correlation coefficients between yield and related indicators (Figure 7). The photosynthetic characteristics, soil enzymatic activity, as well as yield and growth metrics of lettuce, all exhibited highly significant and positive correlation coefficients. Conversely, negative, and statistically significant correlation coefficients, were observed between yield and a few biological indicators, such as the rate of basal respiration in the soil and the carbon content of the microbial biomass (Figure 7). The heat map validates the relationship between quantitative statistical parameters based on the average values of various parameters included in this investigation.

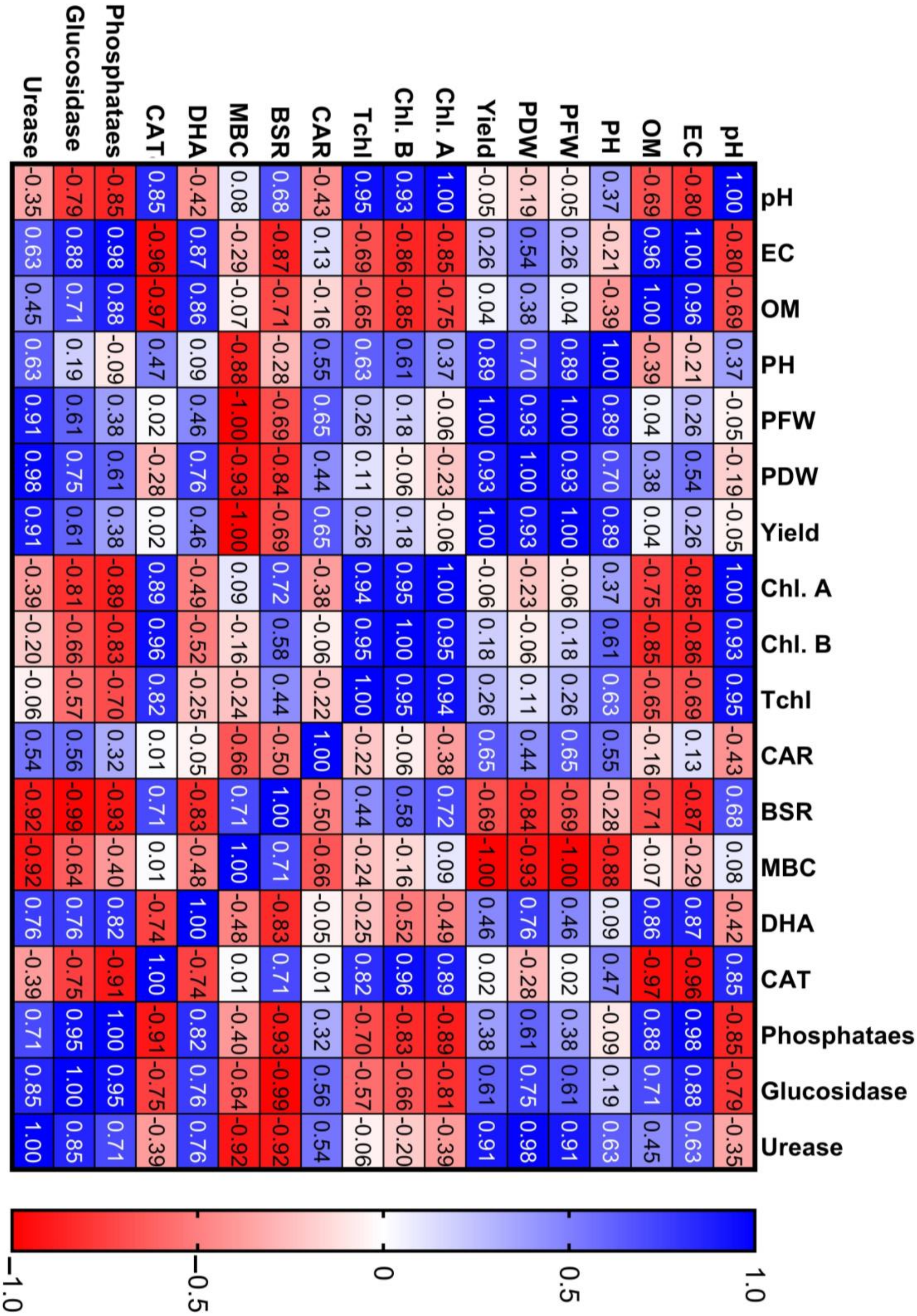

Figure 7. Heat map of Pearson correlation coefficients between various morpho-physio-biochemical attributes of lettuce under vermicompost applications. PH: plant height, PFW: plant fresh weight, PDW: plant dry weight, Chl. B: chlorophyll b, Tchl: total chlorophyll, CAR: carotenoids, BSR: basal soil respiration, MBC: microbial biomass carbon, DHA: dehydrogenase, CAT: catalase.

4. Discussion

The key finding of this study is the significant enhancement in lettuce yield through vermicompost (VC) applications compared to the control group. Notably, the 2% VC treatment (V2) exhibited the most substantial yield increase, reaching an increase of yield of 56.43%. This substantial growth boost demonstrates the value of VC as a natural soil amendment for improving plant growth and crop yields. Our findings are consistent with the results reported by Oyege et al. [6], which emphasized the positive impact of VC on plant growth and crop yields. VC contains readily available nutrients such as nitrogen, potassium, and phosphorus that plants can easily absorb [11]. Furthermore, the presence of humic acids and beneficial microorganisms in VC enhances soil fertility and microbial activity, promoting root growth and overall plant vigor, consistent with the findings of Wu et al. [26]. Additionally, VC possesses diverse properties, such as its capability to elevate soil microbial activity by improving oxygen availability, regulating soil temperature, enhancing porosity, promoting infiltration, and augmenting nutrient content. These characteristics collectively contribute to enhanced plant emergence, vegetative growth, and root development, as supported by numerous studies [5,8,45]. The 1% and 4% VC treatments (V1 and V3) did not yield the same increase in lettuce yield as the 2% treatment (V2). This suggests the presence of an optimal VC concentration for maximizing growth benefits. Our results aligns with the previous study, indicating that excessive VC application may lead to diminishing returns or even toxicity due to elevated soluble salt levels [46]. Our research consistently indicates that the application of VC has positive effects on various plant-growth parameters. Moreover, our results corroborate the existing literature demonstrating the beneficial effects of VC on various plant-growth parameters, including dry weight, height, and leaf area [5,8,47]. Similarly, it has been reported earlier that compared to chemical fertilizers and compost, VC enhances nutrient availability and gradual nutrient release, supporting plant growth [27]. Overall, these findings highlight VC's potential for enhancing plant growth and crop yield, as highlighted by Raza et al. [48]. The variation in soil pH between the different VC treatments, ranging from 7.01 to 7.21, highlights the influence of VC additions on pH levels. However, the observed changes were relatively minor, within a range of only 0.2 pH units. This finding is consistent with the work of Lazcano et al. [49], which reported similar modest shifts in pH of around 0.4 units due to VC application. The minor pH changes signify VC has a weaker effect than inorganic fertilizers, which can dramatically alter pH over time [50]. VC contains organic acids like humic acid that buffer drastic pH swings [21], likely explaining the small variations seen between treatments in our study. Despite the limited influence on soil pH, the organic matter in vermicompost can still positively affect nutrient cycling by stimulating microbial communities [26]. While the observed pH variations may seem insignificant, they should not overshadow the documented benefits of VC for soil health and plant nutrition reported in the scientific literature [12,51]. Our study contributes to a deeper understanding of how VC specifically influences soil pH conditions. However, further controlled trials across diverse crops and soil types are needed to fully elucidate these relationships. Adiloğlu et al. [52] described the beneficial impacts of VC on lettuce, attributing this to enhanced soil properties such as pH, organic matter content, and microbial activity, which are beneficial for the growth of lettuce. The effect of VC on soil pH can vary depending on various factors such as the amount of organic matter added, the initial soil pH, and the decomposition process [53]. VC has been shown to have an alkaline effect on soil pH, which can help raise the pH of acidic soils and create a more balanced environment for plant growth [5]. Some studies have reported an increase in soil pH due to the addition of VC. For example, one study found that the application of VC increased soil pH by 12.38% [54]. However, Atiyeh et al. [55] noted a decrease in soil pH when VC was applied. This discrepancy could be due to the specific conditions of the study, such as the initial soil pH, the amount of VC applied, or the decomposition process. The over 2-fold increase in EC for the V3 treatment versus the control demonstrates that VC can substantially impact soil salinity and ion concentrations. The heightened EC aligns with prior research of Arancon et al. [56], who found a nearly 300% EC increase with VC versus traditional compost. However, the EC increased proportionally more than crop yield, implying the potential for salt accumulation over repeated VC applications that could require monitoring. Though VC salinity levels depend on the initial feedstocks [57], adjustments to application rates and frequencies could optimize nutrient availability while preventing salinization issues long-term. The higher ECs signify the complex effects VC can exert on dissolved ions and conductivity, influencing nutrient retention, mobility, and uptake [58]. The increased EC during the vermicomposting process is probably due to the degradation of organic matter releasing minerals such as exchangeable Mg, K, and P in the available forms, that is, in the form of cations in the VC, as reported by Ceritoğlu et al. [59]. The application of VC can mitigate salinity stress in plants, improving growth and nutrient uptake [60]. The over 50% higher organic matter content in the V3 treatment compared to the control strongly demonstrates vermicompost's capacity to elevate soil organic matter levels. Higher soil organic matter signifies VC may improve other interconnected soil properties like better water-holding capacity, enhanced microbial communities, and increased cation exchange sites for nutrient retention [58]. The heightened organic matter likely stems from vermicompost's high levels of humic acids, carbohydrates, lignin, and other hard-to-degrade compounds [55]. VC, moreover, contains microorganisms that continue synthesizing byproducts when applied to soil [49], helping explain the organic matter accumulation over time here. Though vermicompost shows clear potential for elevating soil organic matter and associated benefits like higher yield here, longer-term carbon dynamics should be clarified. Overall, these results provide strong evidence VC could play a valuable role in supporting soil carbon sequestration and regenerative agriculture goals. In line with our findings, a study evaluating the effectiveness of compost and VC from market organic waste, it was found that a VC application of 10 tons/ha increased the organic C content in the soil by 40.34% [54]. Another study reported that vermicomposting reduced the volume of organic waste, stabilized organic matter, and increased plant biomass production through several mechanisms, such as increasing soil content in organic matter, decreasing soil bulk density, and increasing the availability of water and minerals [61]. In addition to these findings, recent research by Ajijah et al. [62] demonstrated the role of plant growth-promoting bacteria (PGPB) in enhancing soil organic matter content. These bacteria contribute to the decomposition of organic materials, leading to the release of nutrients that are essential for plant growth and soil health [63]. PGPB plays a role in promoting microbial diversity in the soil. By breaking down organic matter, these bacteria create a rich environment that supports the growth of beneficial microorganisms, further enhancing soil health and fertility [64]. Similarly, composting is a valuable practice that enriches soil organic matter content, enhances soil fertility, supports plant growth, improves water retention, aids in bioremediation [16], and contributes to resource conservation in agricultural and gardening systems [18]. The V3 treatment exhibited a 36% higher nitrogen content compared to the control, aligning with previous studies showing elevated total nitrogen levels in VC compared to traditional composts [9,65]. This increase is attributed to accelerated microbial mineralization facilitated by earthworm gut enzymes and excretions [66], indicating vermicompost's potential as a valuable resource for enhancing crop nutrition and yields without additional synthetic fertilizers. We observed a substantial increase in phosphorus content compared to the control treatment, with V3 exhibiting the highest increase. This suggests that higher concentrations of VC may result in more pronounced improvements in soil phosphorus availability. The observed positive impact on phosphorus content can be attributed to several factors associated with VC. Microorganisms present in the gut of earthworms and within the vermicompost actively participate in the decomposition of organic matter, converting organic phosphorus compounds into orthophosphate ions, which are more easily assimilated by plants as reported by Przemieniecki et al. [67]. Similarly, our results are corroborated by the work of Hemati et al. [22], who reported that humic acids, fulvic acids, and other organic components in vermicompost contribute to the chelation and stabilization of phosphorus, preventing its fixation in less available forms. The higher potassium content of VC suggests that it has the potential to be a valuable potassium source for plants. VC, through its derivatives such as leachates, humic substances, and phytohormones, promotes the release and availability of essential nutrients, including potassium, in the soil [8]. Additionally, the published literature revealed that VC enhances potassium absorption by activating microorganisms via the secretion of organic acids, thus stimulating potassium availability and uptake by plants [22]. VC application significantly increases the levels of various essential nutrients in the soil, including calcium, magnesium, iron, sodium, copper, and manganese. VC, a rich source of plant nutrients, contains these elements, contributing to their enhanced availability in the soil [68]. This aligns with previous research findings demonstrating that the application of VC leads to an increase in the content of calcium, magnesium, iron, sodium, copper, and manganese in the soil [67]. This increase in nutrient content is attributed to the role of vermicompost in improving soil quality, increasing nutrient availability, and enhancing crop productivity, as reported by Oyege et al. [5]. In this study, the observed variations in soil basal respiration rate (BSR), microbial biomass carbon (MBC), dehydrogenase activity (DHA), and soil catalase activity (CA) in response to different VC levels highlight the complex interactions between organic amendments and soil microbial dynamics. The significant decrease in BSR in V1, V2, and V3 compared to the baseline (V0) suggests a potential influence of VC on microbial respiration rates. This phenomenon has been noted in the published literature, where the introduction of VC altered microbial community compositions in the soil, favoring populations with lower respiration rates and enhanced efficiency in decomposing organic matter [69]. Additionally, the rich nutrient content in VC initially stimulates microbial activity, but as these nutrients are utilized and stabilized in the soil, microbial activity may decrease, leading to a reduction in BSR [21]. Moreover, VC can affect soil pH and other environmental factors, which in turn can influence microbial activity and BSR [70]. The substantial increase in MBC in the V3 treatment indicates the stimulatory effect of VC on microbial biomass. This aligns with studies demonstrating the positive impact of organic amendments on microbial biomass carbon, emphasizing the role of organic matter in supporting microbial growth and activity [71–73]. Similarly, the rise in DHA levels in the V3 treatment suggests that VC influences soil enzymatic activity, in line with previous research highlighting the enhancement of soil enzyme activities by organic amendments, including VC [74]. Regarding soil catalase activity, the significant increase in the V1 treatment suggests a potential role of VC in promoting soil oxidative processes. Catalase is involved in the decomposition of hydrogen peroxide, and its increased activity may indicate enhanced antioxidant defense mechanisms in the soil, as highlighted by Bhardwaj et al. [75]. We observed a significant positive impact of VC application on various physiological attributes, including increased chlorophyll and carotenoid contents in lettuce leaves. Similar findings were reported in a study involving radishes treated with VC in conjunction with conventional inorganic fertilizers [76]. VC contains a diverse array of plant growth-promoting rhizobacteria (PGPR) that directly enhance plant productivity through processes like biological nitrogen fixation (BNF), nutrient solubilization, and the production of growth hormones. These mechanisms contribute to improved plant growth, development, and physiology, influencing factors such as carotenoid contents and gas exchange attributes like the photosynthetic rate [65,77]. In this study, phosphatase activity was significantly higher in all treatments compared with the control. This might be due to the fact that VC can enhance the physicochemical properties of soil, improve microbial diversity, and increase the activity of various enzymes, including phosphatases [26]. The earthworms in VC create a microenvironment conducive to microbial activity, accelerating the decomposition of organic matter [67]. This increased microbial activity likely results in higher phosphatase enzyme production, facilitating the hydrolysis of organic phosphates and releasing plant-available phosphorus [78]. The application of vermicompost treatments increased glucosidase activity, but the glucosidase activity was not significantly influenced by vermicompost application. The initial increase in glucosidase activity from V0 to V1 may be attributed to the introduction of organic matter and microbial populations associated with vermicompost, providing a conducive environment for enzymatic activities [79,80]. The subsequent decrease in glucosidase activity from V2 to V3 might be indicative of a threshold or saturation effect, where an excess of vermicompost could lead to diminishing returns or even inhibitory effects on the enzyme [24]. Factors such as substrate availability, microbial community composition, and competition for resources may influence the observed trends in glucosidase activity [21]. The introduction of VC into the soil has been found to increase the activity of urease. The highest values in V2 and V3 treatments may be attributed to the increased availability of organic nitrogen from the VC, promoting microbial activity and subsequently enhancing urease production [74]. The initial increase in urease activity from V0 to V2 suggests a stimulatory effect of vermicompost, potentially due to the introduction of earthworms and a diverse microbial community [26]. The subsequent slight decrease from V2 to V3 could be indicative of a saturation point or a balancing effect, where the soil system reaches a threshold of nutrient availability or enzyme production [28].

5. Conclusions

In conclusion, this study highlights the significant impact of vermicompost on lettuce yield, soil characteristics, nutrient dynamics, biochemical and biological parameters, and soil enzymatic activities. Optimal lettuce yields were observed at a 2% vermicompost concentration, underscoring the importance of precise application. The nuanced relationships revealed through correlation and principal component analysis emphasize the complexity of soil-plant interactions influenced by vermicompost, extending beyond yield to encompass soil nutrient dynamics and enzymatic activities. It is essential to recognize the limitations of this study, particularly the lack of open field trials and an evaluation of its effects on lettuce quality. These represent notable constraints that merit attention. Subsequent research endeavors should integrate these aspects to enhance the overall comprehension of vermicompost's influence on agricultural systems. Furthermore, future research should explore mechanistic insights into these interactions, assess long-term effects, investigate microbial community dynamics, and address practical considerations for the sustainable implementation of specific vermicompost concentrations in crop production. This comprehensive approach is essential for optimizing the multifaceted benefits of vermicompost in agricultural systems.

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