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Enhancing the Quality of Tomato Straw Waste Composting: The Role of Earthworm Stocking Density in Composting–Vermicomposting Integrated Systems

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Abstract: Recently, combining composting with vermicomposting has garnered growing interest as an efficient approach for the sustainable processing of agricultural waste. This study utilized composting as a pretreatment method to mitigate the salinity of tomato straw, employing four distinct earthworm stocking densities (0, 40, 80, and 120 worms/kg) to assess the feasibility of a combined composting–vermicomposting approach for stabilizing tomato straw waste. The results indicated that the total nitrogen, total phosphorus, total potassium, humification index, and germination index of the vermicompost products increased by 3.2–10.57%, 3.3–12.69%, 2.65–7.42%, 21.56–30.54%, and 36.57–91.68%, respectively. Relative to the control, the greatest improvement was observed at a density of 80 worms per kilogram of tomato straw compost. Moreover, the earthworm treatment group showed enhanced enzyme activity, bacterial abundance, and diversity relative to the control group. It can, therefore, be concluded that the integrated composting–vermicomposting system contributes to enhancing the quality of tomato straw waste compost. However, to further explore the treatment potential of high-salinity agricultural waste, future research could optimize the integrated composting and vermicomposting system and investigate the synergistic effects between earthworm stocking density and environmental factors.

Keywords: composting; microbial communities; stocking density; tomato straws; vermicomposting; compost quality

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1. Introduction

Tomatoes, a globally popular and economically significant commercial vegetable crop, play a vital role in food processing, fresh consumption, and the production of various agricultural products [1,2]. While their high yield has brought considerable economic benefits, it has also led to the generation of substantial amounts of tomato straw waste [3]. As a common byproduct in agricultural production, if not properly managed, these straws not only increase the burden of agricultural waste management, but also pose a potential risk to the environment [4]. Therefore, the development of effective resource utilization technologies for tomato straw waste is crucial not only for mitigating environmental pollution, but also for enhancing resource efficiency, which plays a key role in the long-term sustainability of

agriculture development. In recent years, the resourceful treatment of agricultural waste has garnered increasing attention, with composting and vermicomposting technologies emerging as widely applied methods in the field [5]. Composting is a well-established technique for treating agricultural waste, harnessing the power of microorganisms to transform such waste into organic fertilizers or soil amendments [6]. Among the leading techniques for managing organic waste, this technology significantly reduces waste volume, lowers electrical conductivity, and facilitates the degradation of harmful substances, effectively preventing environmental pollution [7]. Furthermore, composting can be implemented on an industrial scale, capable of processing large quantities of solid organic waste with high production efficiency [8]. However, the quality of compost products can be inconsistent, not always achieving desirable outcomes in terms of nutrient content, soil amendment effects, and economic costs [9].

Vermicomposting, on the other hand, utilizes the collaborative efforts of earthworms and microorganisms to transform agricultural waste into more beneficial organic fertilizers [10]. Compared to traditional composting, vermicomposting has a superior appearance and higher nutritional content and microbial activity, making it more popular in the market [11]. Despite the obvious advantages of vermicomposting, it has limitations in industrial-scale production and application. Vermicomposting is not entirely adaptable to industrial production; especially when it comes to processing large amounts of waste, it is not as efficient as traditional composting [8]. Moreover, vermicomposting faces a significant challenge when dealing with tomato straw—the raw material often contains high salt levels, making it difficult for earthworms to grow and reproduce [12]. Therefore, the direct use of vermicomposting to handle tomato straw is not ideal.

In recent years, there has been growing interest in combining composting and vermicomposting technologies as a solution to the limitations of using either method alone [13]. Numerous studies have highlighted the use of integrated composting and vermicomposting to stabilize various organic wastes, such as cattle manure [8], human faecal slurry [14], sludge [15], biogas residue [16], and spent mushroom [17]. This integrated technology offers multiple advantages. Firstly, it significantly increases the organic matter content in compost and shortens the composting duration [13]. Secondly, it enhances the nutritional components of the compost, thereby improving the quality of the final compost product [18]. Consequently, this technology transforms the final compost into a more effective soil amendment. However, research on the application of integrated composting and vermicomposting technology in the treatment of tomato straw is still relatively scarce.

The quality of vermicompost is intricately linked to the stocking density of earthworms [19]. The optimal stocking density of earthworms is crucial for enhancing the decomposition efficiency of waste and improving the quality of the compost [20]. Insufficient earthworm density may result in a sluggish composting process, potentially failing to effectively degrade organic matter; conversely, an excessively high density of earthworms could lead to intense competition for resources among them, thereby diminishing their decomposition efficiency [21]. The ideal earthworm stocking density depends on the type of organic waste being processed. Unuofin and Mnkeni [22] found that when processing a mixture of cow dung and waste paper, an inoculation of 12.5 g of earthworms per kilogram yields the highest degree of humification. Mupambwa and Mnkeni [20] recommended an inoculation of 25 g of earthworms per kilogram of substrate in the treatment of fly ash-cow dung-waste paper mixtures to produce highly humified and nutrient-rich vermicompost more rapidly. Yadav and Garg [23] indicated that the optimal stocking density when vermicomposting with a mixture of bakery industry sludge and cow dung is 16 to 32 earthworms per kilogram of waste. Devi et al. [24] showed that in lignocellulosic materials such as kitchen vegetable waste and banana straw, an inoculation of 5 to 7 earthworms

per kilogram is the best condition for producing vermicompost that is rich in nutrients and microbial activity. Chen et al. [25] discovered that in a mixture of corn stover and cow dung, the stocking density of 120 earthworms per kilogram of substrate resulted in the maximum total organic carbon utilization rate and β-glucosidase activity. Consequently, the rate of decomposition during vermicomposting is greatly influenced by the population density of earthworms, with this influence differing based on the type of material used in the process [22].

In the vermicomposting system, waste decomposition is closely linked to changes in the microbial community composition [17]. Furthermore, the symbiotic interaction between earthworms and microorganisms is a key factor in the vermicomposting process, significantly enhancing nutrient release and enzyme activity [21]. Therefore, analyzing microbial community composition and enzyme activity during integrated composting and vermicomposting is crucial for a comprehensive understanding of the waste decomposition mechanisms.

Although previous studies have explored the impact of earthworm stocking density on the degradation of various waste materials, research focusing on high-salinity waste such as tomato straw remains limited. This study addresses this gap by using composting as a pretreatment to reduce the salt content of tomato straw, and by combining compost products with earthworms at different stocking densities to construct an integrated composting and vermicomposting system. The main objectives of this study are as follows: (1) to evaluate the feasibility of using an integrated composting and vermicomposting system to improve the composting quality of tomato straw waste; (2) to investigate the microbial community dynamics and functional succession in the integrated composting and vermicomposting system, and identify the dominant functional microorganisms; and (3) to determine the optimal earthworm stocking density for the integrated composting and vermicomposting system based on tomato straw. This study provides an effective approach for the resource utilization of high-salinity tomato straw waste, offers solid theoretical support for the optimization of integrated composting and vermicomposting system technologies, and provides valuable information for the application of this technology in waste resource recovery and sustainable agricultural development.

2. Materials and Methods

2.1. Method of Compost, Vermicompost, and Sampling

This study was conducted from July to November 2023 at the Horticultural Experimental Garden of Northwestern Agricultural and Forestry University of Science and Technology in Yangling, Shaanxi Province, China (108°00′E, 34°30′N). Tomato straws (TS) were collected from vegetable greenhouses near the test site. After collection, the tomato straws were dried and crushed into pieces of 1–4 cm in size. The moisture content of the crushed straws was adjusted to 60–70%, followed by composting, with the compost pile dimensions approximately 2.0 m × 1.5 m × 1.0 m (length × width × height). Plastic film was applied to cover the pile to minimize moisture loss. Regular manual turning was employed during the composting period to ensure natural aeration. After 65 days of composting, samples were taken from the composted product, designated as TSCP. The physicochemical properties of TS and TSCP are shown in Table 1.

In order to construct an integrated composting and vermicomposting system, vermicomposting was further conducted using TSCP by placing 2 kg (dry weight) of TSCP into pots (size 21 cm high and 31.5 cm in diameter) and then placing earthworms into the pots at different stocking densities, with four stocking densities of 0 (no earthworms), 40, 80, and 120 worms/kg (dry weight) (CK-T3). *Eisenia fetida* was obtained from Tianjin Success Earthworm Farm. All treatments were performed in triplicate. During the experiment, the

temperature was maintained at 25–30 °C, and the substrate was regularly sprayed with sufficient tap water to maintain the moisture content at about 60–70%. The duration of the experiment was 35 days.

Table 1. Physicochemical properties of tomato straw and tomato straw compost products.

Physical and Chemical Properties	TS	TSCP
pH	6.80 ± 0.02	9.39 ± 0.01
EC (mS/cm)	12.05 ± 0.10	4.75 ± 0.10
TOC (g/kg)	332.61 ± 5.03	244.10 ± 2.17
OM (g/kg)	520.17 ± 17.56	396.22 ± 6.61
TN (g/kg)	19.77 ± 1.77	15.64 ± 0.51
TP (g/kg)	2.46 ± 0.03	5.67 ± 0.04
TK (g/kg)	33.82 ± 0.35	42.06 ± 0.58
Cellulose (%)	32.12 ± 1.59	19.86 ± 0.96
Hemicellulose (%)	9.34 ± 0.31	4.17 ± 0.01
Lignin (%)	7.27 ± 1.29	15.54 ± 0.03

Note: TS: tomato straw; TSCP: tomato straw compost product. Values are expressed as mean ± standard deviation (n = 3). (EC: electrical conductivity, TOC: total organic carbon, OM: organic matter, TN: total nitrogen, TP: total phosphorus, TK: total potassium)

Samples were collected on days 0, 10, 20, and 35 of vermicomposting. A portion of each sample was air-dried and ground for the analysis of physicochemical properties, nutrient content, and lignocellulose changes. Another portion of the samples was stored at 4 °C for the determination of enzymatic activities, including dehydrogenase, cellulase, urease, and alkaline phosphatase. Additionally, samples from day 0 and day 35 of vermicomposting were preserved at −80 °C for the MiSeq sequencing analysis of the microbial community.

2.2. Physicochemical Analysis

The pH and EC were assessed using a pH meter and an EC meter (Shanghai, China), with a ratio of air-dried sample to distilled water of 1:10 (w/v, air-dried sample) [26]. Total organic carbon (TOC) and organic matter (OM) were determined following the methods described by Cucina et al. [27] and Zhang and Sun [28], respectively. A 0.1 g sample was digested with 98% (v/v) H_2SO_4 and 30% (v/v) H_2O_2 before being analyzed for nutrient content [29]. Total nitrogen (TN) was quantified using the Kjeldahl method [30]. Total phosphorus (TP) was measured using the anti-Mo-Sb spectrophotometric method [31]. Total potassium (TK) was determined using a flame photometer (Shanghai, China) [26]. Humic acid (HA) and fulvic acid (FA) contents were measured using the oxidative volumetric method with potassium dichromate as outlined by Yu et al. [32]. Cellulose, hemicellulose, and lignin contents were quantified using the method proposed by Chen et al. [33]. Enzyme activities were determined as described previously [34–37], which included the measurement of cellulase, dehydrogenase, alkaline phosphatase, and urease activities.

2.3. Seed Germination Test

A germination test using seeds of pakchoi was conducted to evaluate the maturity and phytotoxicity of the final vermicompost product [34]. The methodology was as follows: The air-dried sample was mixed with distilled water at a ratio of 1:10 (w/v), and the mixture was oscillated at 120 r/min in a thermostat oscillator for 1 h. The water extract was then obtained by filtration. Twenty seeds were evenly arranged on filter paper in 9 cm diameter Petri dishes; the filter paper in each dish was moistened with 5 mL of either a water extract (1:10 w/v) or distilled water (control), with each test conducted in triplicate. The Petri dishes were placed in an incubator at 25 ± 1 °C for three days, after which the germination

rate and root length of the sprouted seeds were recorded to calculate the germination index (GI). The formula for calculating GI is as follows [38]:

$$\mathrm{GI} = \frac{\text{Germination rate of treated seeds (\%)} \times \text{Root length of treated seeds (\%)}}{\text{Germination rate of control seeds (\%)} \times \text{Root length of control seeds (\%)}} \quad (1)$$

2.4. DNA Extraction and Sequencing Analysis

Compost samples were collected on days 0 and 35 of the vermicomposting process to assess the diversity of microbial communities. DNA extraction for each sample was performed in triplicate. Total DNA was extracted from 0.5 g of samples using the E.Z.N.A. Stool DNA Kit (D4015, Omega Corporation, USA) following the manufacturer's instructions. The quality of the extracted DNA was assessed using 1% agarose gel electrophoresis, and its quantity was measured with a NanoDrop™ 2000 spectrophotometer to ensure the required concentration (volume $\geq$ 20 μL, concentration $\geq$ 24 ng/μL) for high-throughput sequencing [39]. The V3-V4 region of the bacterial 16S rRNA gene was amplified using universal bacterial primers 338F (5′-ACTCCTACGGGAGGCAGCA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′). Simultaneously, the fungal ITS rRNA gene was amplified using universal fungal primers ITS1 (5′-CTTGGTCATTTAGAGGAAGTAA-3′) and ITS2 (5′-GCTGCGTTCTTCATCGATGC-3′) [40]. PCR products were reconfirmed using 2% agarose gel electrophoresis, purified with AMPure XT (Beckman Coulter Genomics, Danvers, MA, USA), and quantified with a Qubit fluorometer (Invitrogen, Waltham, MA, USA). The size and quantity of purified PCR products were assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies, USA) and an Illumina library quantification kit (Kapa Biosystems, Woburn, MA, USA). The high-throughput sequencing of PCR products was performed on the NovaSeq PE250 platform (LC-Bio Technology Co., Ltd., Hangzhou, China).

2.5. Statistical Analysis

The data in this research were averaged across three repetitions. Statistical analyses were performed on the obtained data using SPSS 27.0 to calculate the mean and standard deviation for each treatment. All data were analyzed using one-way analysis of variance (ANOVA), and significant differences between group means were assessed using Tukey's test; $p < 0.05$ was considered statistically significant. Data visualization and plotting were conducted using Origin 2022 (USA).

3. Results and Discussion

3.1. Changes in Physicochemical Properties During Composting–Vermicomposting

The vermicomposting of TSCP ended after 35 days, and Table 2 shows some of the parameters used to assess the maturity of the product at the end of the vermicomposting of TSCP. The data showed that at the end of vermicomposting, TN, TP, and TK contents were higher in the vermicomposting treatment group than in the control group. It was also observed that lignocellulose content was reduced more in the vermicomposting treatment group. In conclusion, the addition of earthworms had a better effect on the maturation of tomato straw organic waste compost.

Table 2. Maturity parameters of the products from different treatments at the end of vermicomposting.

Parameters	CK	T1	T2	T3
pH	8.23 ± 0.03 a	8.15 ± 0.02 b	7.80 ± 0.06 c	8.08 ± 0.04 b
EC (mS/cm)	3.54 ± 0.34 a	3.15 ± 0.05 ab	2.75 ± 0.02 b	2.93 ± 0.31 b
TOC (g/kg)	199.03 ± 4.40 a	177.85 ± 2.53 b	150.65 ± 3.42 d	165.87 ± 4.30 c
OM (g/kg)	344.67 ± 4.35 a	339.43 ± 3.12 ab	332.04 ± 3.26 c	334.48 ± 2.62 bc
TN (g/kg)	24.41 ± 0.37 c	25.19 ± 0.59 bc	26.99 ± 0.76 a	26.16 ± 0.12 ab
TP (g/kg)	7.88 ± 0.06 d	8.14 ± 0.03 c	8.88 ± 0.06 a	8.34 ± 0.01 b
TK (g/kg)	53.25 ± 1.54 b	54.66 ± 1.58 ab	57.2 ± 1.68 a	55.93 ± 1.70 ab
HA/FA	1.67 ± 0.02 c	2.03 ± 0.05 b	2.18 ± 0.02 a	2.13 ± 0.00 a
Cellulose (%)	15.18 ± 0.21 a	12.71 ± 0.58 b	11.84 ± 0.29 c	12.39 ± 0.21 bc
Hemicellulose (%)	3.78 ± 0.04 a	3.48 ± 0.03 b	3.16 ± 0.04 c	3.20 ± 0.04 c
Lignin (%)	14.88 ± 0.12 a	14.01 ± 0.08 b	13.03 ± 0.06 c	13.23 ± 0.30 c

Note: CK, T1, T2, and T3 denote inoculating densities of 0, 40, 80, and 120 earthworms per kg substrate. Values are expressed as mean ± standard deviation (n = 3). Within the same row, different letters represent significant differences ($p < 0.05$). (EC: electrical conductivity, TOC: total organic carbon, OM: organic matter, TN: total nitrogen, TP: total phosphorus, TK: total potassium, HA/FA: humic acid/fulvic acid)

3.1.1. Values of pH and Electric Conductivity (EC)

The pH value is considered one of the critical factors influencing the quality of vermicompost products. Over time, the pH values of all treatments exhibited a decreasing trend (Figure 1a). After the vermicomposting process, the other treatments demonstrated a reduction in pH ranging from 0.97% to 5.22% compared to CK (with no earthworms). This indicates that earthworms play a significant role in the degradation process of composting and can effectively lower the pH of the resulting products. The observed decrease in pH during the composting–vermicomposting process aligns with findings from previous studies [8,17,41,42]. The final pH of the product ranged from 7.80 to 8.23, which is consistent with the 7.0–8.5 range recommended for agricultural applications [43]. Among all treatments, T2 (with the addition of 80 earthworms/kg) exhibited the most substantial reduction in pH. Neutral and weakly alkaline pH values are recognized as indicators of the stability of vermicomposting products [44]. Factors contributing to the final reduction in pH may include the mineralization of nitrogen and phosphorus into nitrate and orthophosphate [45]. Furthermore, the generation of humic and fulvic acids through the decomposition of organic matter by microorganisms may also contribute to the observed reduction in pH throughout the vermicomposting process [46].

The EC value is a crucial indicator for gauging the soluble salt content in vermicompost products [47]. At the end of vermicomposting, in comparison with CK, the EC value of all treatments decreased by 11–22%. Among these, T2 (with the addition of 80 earthworms/kg) had the largest reduction in EC value, while T1 (with the addition of 40 earthworms/kg) had the smallest reduction (Figure 1b). These findings indicate that earthworm inoculation effectively reduces the EC of compost products. Ultimately, the EC value of vermicompost products varies from 2.75 mS/cm to 3.54 mS/cm. All of these values are lower than 4.0 mS/cm and conform to the standards for the harmless treatment of compost [48]. The observed reduction in EC is attributed to a decrease in soluble ion concentration, primarily due to ammonia volatilization, which reduces both the quantity and mobility of ions [49].

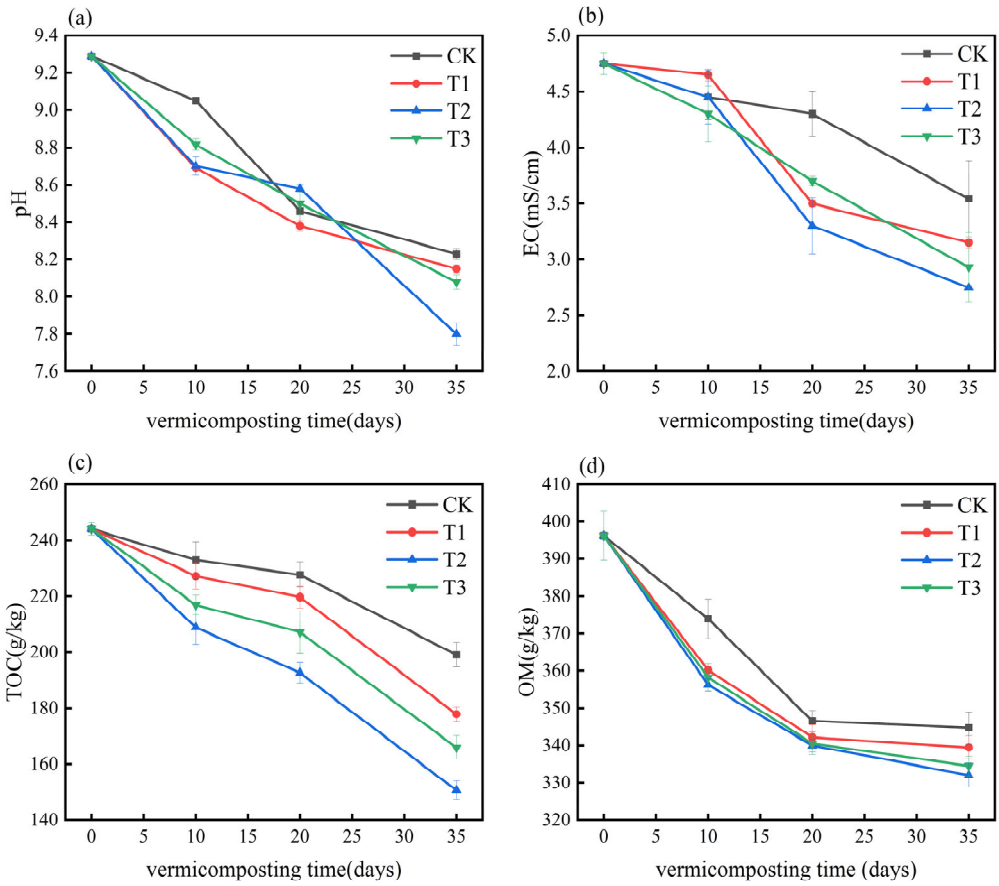


Figure 1. Changes in (**a**) pH, (**b**) EC, (**c**) TOC, and (**d**) OM in different treatments during the vermicomposting process. CK, T1, T2, and T3 denote inoculating densities of 0, 40, 80, and 120 earthworms per kg substrate. Values are expressed as mean ± standard deviation (n = 3).

3.1.2. Total Organic Carbon (TOC) and Organic Matter (OM)

In comparison with TSCP, the content of TOC in the final vermicompost product is decreased (Figure 1c). In different treatments of CK-T3, the reduction percentages of TOC are 18.46%, 27.14%, 37.28%, and 32.05%, respectively. After vermicomposting, in contrast to the control group, the TOC content in the earthworm treatment group is notably reduced, with a reduction range from 10.64% to 24.31%. Among all treatments, T2 (with the addition of 80 earthworms/kg) exhibits the most substantial reduction in TOC content. The utilization of organic carbon by earthworms and microorganisms is one of the primary reasons for the decrease in TOC content [45]. Over time, the degradation and mineralization of organic matter further contribute to TOC loss [48,50,51]. After vermicomposting, the TOC content in the earthworm treatment group is in the following order: T1 (177.85 g/kg) > T3 (165.87 g/kg) > T2 (150.65 g/kg), suggesting that the greatest reduction in TOC content is observed in the medium earthworm stocking density (i.e., T2). The variation in the reduction rate might be attributed to changes in stocking density. Extremely low or high stocking density of earthworms will result in insufficient activity of the microbial community and further restrain the decomposition of organic matter [52]. A lower TOC content indicates a higher humic acid content, suggesting that the final product possesses enhanced stability and has reached maturity [51].

Following the vermicomposting process, the content of OM in all treatments decreased by 13.01%, 14.33%, 16.20%, and 15.58%, respectively (Figure 1d). After vermicomposting, in comparison with the control group, the OM content in the earthworm treatment group decreased by 1.52–3.66%. Among these, T2 (with the addition of 80 earthworms/kg)

exhibited the most significant reduction in OM content. The results indicate that inoculating earthworms notably augments the degradation of organic matter. When earthworms ingest organic matter, it is further decomposed within their intestines and converted into simpler compounds, thus accelerating the degradation of organic matter [53]. When the stocking density is either too high or too low, the increase in earthworm biomass is low, and the decomposition rate of OM decelerates, so the reduction in OM content of T2 is the most significant [54]. The reduction in OM can reflect the extent of degradation and mineralization of the waste and serve as an important indicator of vermicomposting maturity [54].

3.1.3. Contents of Total Nitrogen (TN), Total Phosphorus (TP), and Total Potassium (TK)

Throughout the process of vermicomposting, the TN content of each treatment exhibits an upward tendency (Figure 2a). After vermicomposting, in comparison with TSCP, the TN content of each treatment increased by 56.07–72.52%. Among these, T2 (with the addition of 80 earthworms/kg) witnessed the most substantial increase. In comparison with CK, the TN content of the final products of T1, T2, and T3 increased by 3.2%, 10.57%, and 7.17%, respectively. Similarly, T2 (with the addition of 80 earthworms/kg) exhibited the most significant increase. Earthworms lead to an increase in nitrogen content in the final product by releasing nitrogenous excreta, mucous, body fluids, polysaccharides, hormones, enzymes, and decaying tissues [55–57]. At an optimal stocking density (T2), the earthworm biomass is relatively high, which aids in the digestion of organic waste and the release of more nitrogen-rich excretions [54]. However, when the stocking density is either too low or too high, earthworm activity is reduced, leading to insufficient changes in substrate texture and a decrease in nitrogen mineralization and accumulation [58]. Apart from this, N-fixing bacteria and nitrification also contribute to the increase in the nitrogen content of the final product [59].

Likewise, the TP and TK contents in the earthworm treatment group also exhibit an increasing tendency (Figure 2b,c). In comparison with CK, the TP content of the final products of T1, T2, and T3 increased by 3.3%, 12.69%, and 5.84%, respectively (Figure 2b), and the TK content increased by 2.65%, 7.42%, and 5.03%, respectively (Figure 2c). Among these, T2 (with the addition of 80 earthworms/kg) witnessed the most significant increase in TP and TK contents. Throughout the vermicomposting process, the increase in TP content might be the outcome of the combined effect of various factors such as the decomposition of organic material by earthworms, microbial activities, the role of alkaline bacteria, and the secretion of phosphatase by earthworms [44]. Under the appropriate stocking density (T2), earthworms enhance the activity of microorganisms in the intestine and the secretion of phosphatase, thus resulting in a higher TP content in the final product of T2 [54]. Additionally, the microbial community in the earthworm intestine can transform insoluble phosphorus in organic waste into soluble form, thus increasing the total phosphorus content of the final product [60–62].

Additionally, earthworms also exert an important function in increasing potassium content throughout the composting process, primarily by promoting the decomposition of waste, enhancing microbial activity, generating organic acids, and accelerating the mineralization of potassium [52,63,64]. Earthworms are capable of accelerating the mineralization rate, resulting in changes in the content of exchangeable and non-exchangeable forms of K and ultimately enhancing the total potassium content [65]. The mineralization process of potassium takes place through the action of endogenic and exogenic enzymes [48,51]. In comparison with other treatments, the degree of organic matter degradation in T2 is the most significant. Consequently, its TN, TP, and TK contents are also relatively high.

This indicates that effective organic matter degradation can enhance the nutritional value of products.

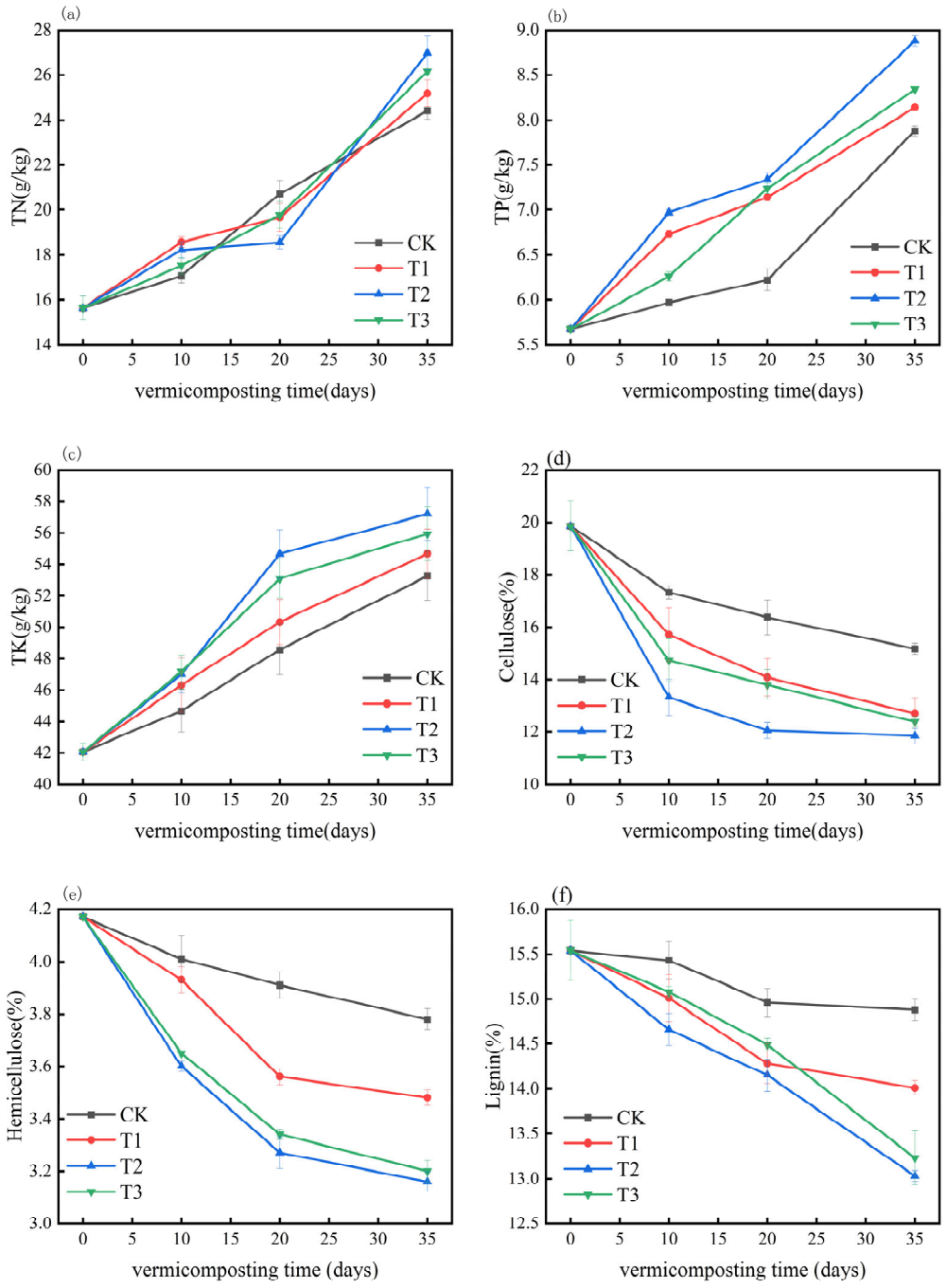


Figure 2. Changes in parameters in different treatments during the vermicomposting process; (**a**–**f**) show the changes in TN, TP, TK, cellulose, hemicellulose, and lignin content, respectively. CK, T1, T2, and T3 denote inoculating densities of 0, 40, 80, and 120 earthworms per kg substrate. Values are expressed as mean ± standard deviation (n = 3).

3.1.4. Lignocellulose Contents

The variations in cellulose, hemicellulose, and lignin contents in each treatment during the vermicomposting process are depicted in Figure 2d–f. During the vermicomposting process, the lignocellulose content exhibits a decreasing tendency in all treatments, but the degrees of reduction vary. In comparison with CK, the lignocellulose degradation rate of other treatments is relatively high, suggesting that the introduction of earthworms accel-

erates the degradation of lignocellulose. The facilitation of lignocellulose decomposition by earthworms stems from its own digestive process as well as from the interaction with microorganisms [66]. Earthworms indirectly facilitate the decomposition of cellulose by microorganisms by further exposing organic matter to microorganisms through mechanical agitation and excretion [33]. After vermicomposting, in comparison with CK, the contents of cellulose, hemicellulose, and lignin in the final products of T1-T3 decreased by 16.27–22%, 7.94–16.40%, and 5.85–12.43%, respectively. Among these, the most significant reduction was observed in T2 (with the addition of 80 earthworms/kg). When the stocking density is either too high or too low, it will impact the physiological activities of earthworms and the growth of microorganisms, further suppressing the activity of cellulase [54]. Consequently, in comparison with other treatments, the decomposition effect of lignocellulose in T2 is superior.

3.1.5. HA/FA Ratio

The ratio of HA/FA is a crucial indicator for assessing the maturity and quality of vermicompost products [67]. The study discovered that the HA/FA ratio of all treatments exhibited a continuous upward tendency over time (Figure 3). After vermicomposting, the HA/FA ratios of CK-T3 were 1.67, 2.03, 2.18, and 2.13, respectively. The HA/FA ratios of the earthworm treatment group were all greater than 1.9, suggesting that the final product has reached a mature state [68]. These results suggest that stocking earthworms can notably enhance the humification degree and maturity of the final product. In comparison with the control group, the HA/FA ratios of the earthworm treatment group increased by 21.56%, 30.54%, and 27.54%, respectively. Among these, the final HA/FA ratio of T2 (with the addition of 80 earthworms/kg) was the highest. Unuofin and Mnkeni [22] assessed the degradation effect of a mixture of cow dung and waste paper by employing four different earthworm stocking densities (0, 12.5, 25, and 37.5 g earthworms/kg). The results indicated that the HA/FA ratio was relatively high when using a medium stocking density.

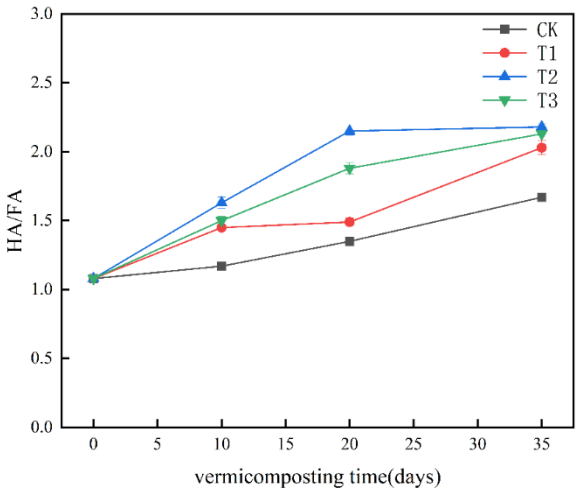


Figure 3. Changes in HA/FA in different treatments during the vermicomposting process. CK, T1, T2, and T3 denote inoculating densities of 0, 40, 80, and 120 earthworms per kg substrate. Values are expressed as mean ± standard deviation (n = 3).

3.2. Enzyme Activity

Enzyme activity is an important indicator to evaluate the characteristics and maturity of vermicompost [69]. In all treatments, urease, cellulase, alkaline phosphatase, and dehydrogenase activities all rapidly increased in the initial stage of vermicomposting and then gradually decreased (Figure 4). This trend is consistent with previous research find-

ings [50,55,58], likely due to the abundance of biodegradable organic matter at the start of vermicomposting, followed by a decrease in enzyme activity as the compost matures and stabilizes [70]. During the vermicomposting process, dehydrogenase activity reflects the biological oxidation process, demonstrating the ability of microorganisms to decompose complex organic materials [51]. Urease activity plays a crucial role in the nitrogen cycle, converting nitrogen in organic waste into a form that can be absorbed by plants [70]. Alkaline phosphatases hydrolyze organic phosphorus compounds, releasing phosphate available for plant use [71]. Cellulase, the key enzyme in the breakdown of cellulose, converts cellulose into simple sugars, thereby promoting the biodegradation of organic matter [72]. Throughout the vermicomposting process, enzyme activities (dehydrogenase, cellulase, urease, and alkaline phosphatase) in treatment T2 were significantly higher than in other treatments, indicating that an appropriate earthworm stocking density provides a better environment and nutritional conditions for microbial growth, promoting the synthesis of microbial enzymes, thereby contributing to the final stabilization of the waste mixture.

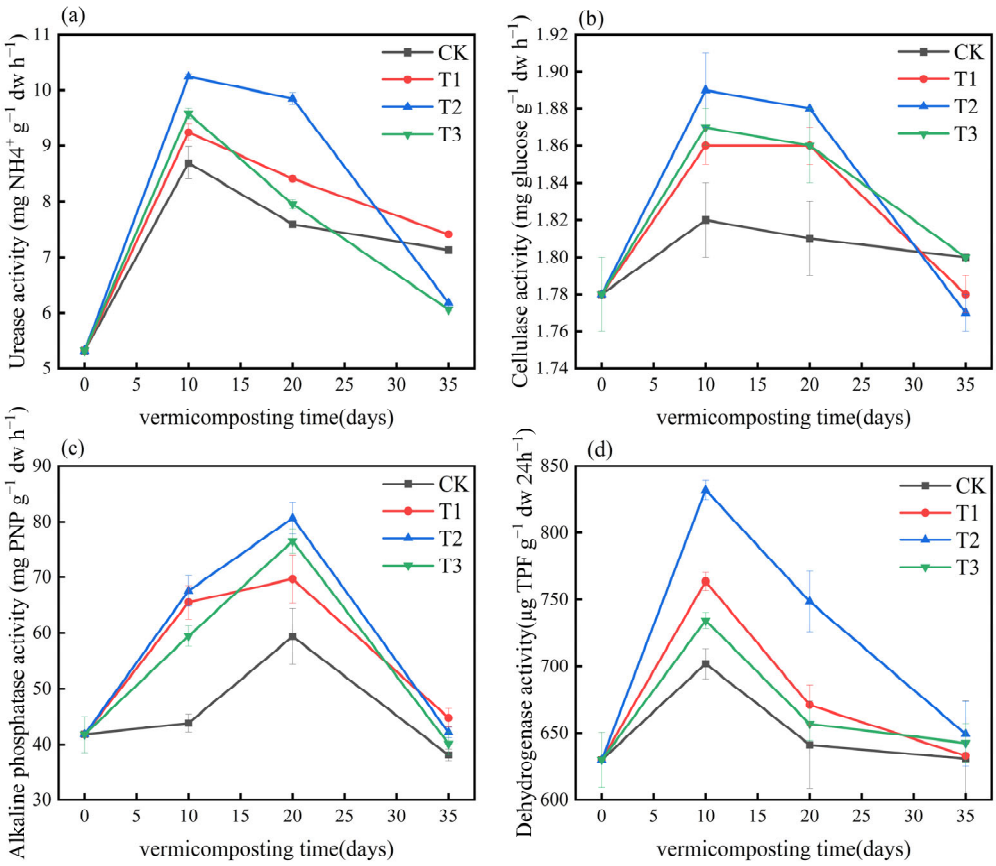


Figure 4. Changes in enzyme activity in different treatments during the vermicomposting process. Panels (**a**–**d**) depict variations in urease activity, cellulase activity, alkaline phosphatase activity, and dehydrogenase activity, respectively. CK, T1, T2, and T3 denote inoculating densities of 0, 40, 80, and 120 earthworms per kg substrate. Values are expressed as mean ± standard deviation (n = 3).

3.3. Germination Assay

The germination index (GI) is a crucial indicator for assessing the maturity and phytotoxicity of vermicompost products [73]. After vermicomposting, the GI values of T1–T3 treatments reached 98.77%, 138.62%, and 106.23%, respectively (Figure 5), conforming to the compost maturity standard, namely, the GI value is higher than 80% [22]. The variations in the maturity of vermicompost products will result in differences in GI values, and these differences in GI values reflect the disparities in the degree of mineralization of organic

matter and the degree of degradation of organic acids in vermicompost [48]. In this study, after vermicomposting, the GI values of T1–T3 were significantly higher than that of CK ($p < 0.05$), suggesting that adding earthworms promotes the maturation of compost and reduces the phytotoxicity of products. Vermicompost products contain plentiful nutrients and growth hormones, such as auxin, gibberellin, and cytokinin, which exert a function in promoting plant growth [50]. T2 (with the addition of 80 earthworms/kg) has the highest GI value and exhibits the highest degree of maturity among all treatments. This might be attributed to the highest nutrient content (TN, TP, and TK) of T2 [54].

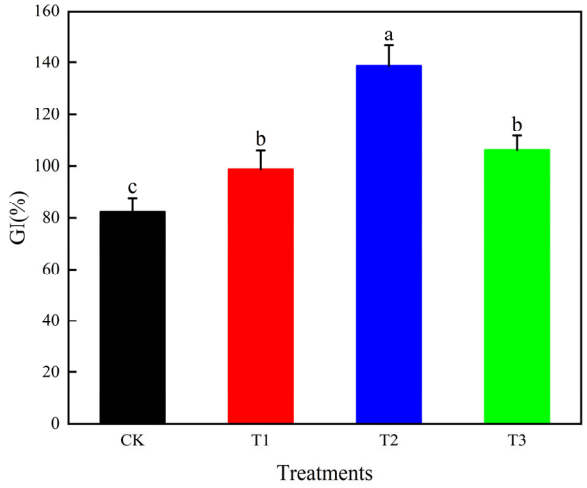


Figure 5. Changes in germination index among different treatments during the vermicomposting process. CK, T1, T2, and T3 denote inoculating densities of 0, 40, 80, and 120 earthworms per kg substrate. Values are expressed as mean ± standard deviation ($n = 3$). Different letters above the bars indicate significant differences between treatments ($p < 0.05$).

3.4. Richness and Diversity of the Microbial Community

OTUs clustering was conducted on non-redundant sequences (excluding singletons) with a 97% similarity threshold. High-quality bacterial and fungal sequences were randomly extracted from samples collected at various stages, with sequence lengths ranging from 64,865 to 74,481 and from 78,011 to 85,206, respectively. The coverage exceeded 100%, indicating that the sequencing results accurately represent the true microbial composition of the samples.

For bacteria, the TSCP contained 1315 bacterial OTUs (Table 3). At the end of the vermicomposting process, bacterial OTUs in the CK, T1, T2, and T3 treatments were 1627, 1991, 2203, and 2153, respectively. This indicates an increase in OTU numbers in the earthworm treatments compared to the control group. Compared to the TSCP, the Chao1 index for bacteria in the earthworm treatments increased by 53%, 70%, and 66%, while the control treatment showed only a 25% increase (Table 3). These results suggest that the earthworm treatments exhibited higher bacterial richness. After the vermicomposting process, the Shannon diversity index for the control group was 8.84, whereas the indices for the earthworm treatments were 9.51, 9.75, and 9.75, respectively (Table 3). The observed increase in bacterial diversity within vermicompost products is consistent with previous research [33,74,75]. The increase in microbial community diversity may be attributed to the stimulation of microbial growth by earthworms, which occurs through the unique microorganisms in their digestive tract, the mucus they secrete, and their biological activities [33,76]. Additionally, earthworms expel millions of microorganisms from their digestive tract through excretion [77].

Table 3. Comparison of bacterial and fungi abundance and diversity indices.

Treatment	OTUs	Shannon	Simpson	Chao1	Goods_Coverage
			Bacterial		
TSCP	1315.15 ± 201.76 c	8.32 ± 0.22 c	0.99 ± 0.00 b	1315.00 ± 106.33 c	1 ± 0.00 a
CK	1627.33 ± 178.53 b	8.84 ± 0.31 b	0.99 ± 0.00 b	1650.30 ± 66.72 b	1 ± 0.01 a
T1	1991.22 ± 88.61 a	9.51 ± 0.06 a	1.00 ± 0.01 a	2022.70 ± 201.36 a	1 ± 0.00 a
T2	2203.18 ± 33.72 a	9.75 ± 0.15 a	1.00 ± 0.00 a	2240.90 ± 52.61 a	1 ± 0.00 a
T3	2153.44 ± 100.52 a	9.75 ± 0.39 a	1.00 ± 0.00 a	2178.40 ± 131.76 a	1 ± 0.00 a
			Fungi		
TSCP	150.00 ± 18.11 b	2.89 ± 0.09 b	0.79 ± 0.01 a	150.00 ± 10.01 b	1 ± 0.00 a
CK	184.11 ± 10.27 a	3.11 ± 0.10 a	0.79 ± 0.00 a	184.00 ± 5.32 a	1 ± 0.00 a
T1	148.26 ± 8.97 b	2.44 ± 0.16 c	0.69 ± 0.00 b	148.00 ± 6.12 b	0.99 ± 0.01 a
T2	92.00 ± 5.36 c	2.29 ± 0.03 c	0.69 ± 0.00 b	92.00 ± 3.19 c	1 ± 0.00 a
T3	140.16 ± 6.25 b	2.41 ± 0.08 c	0.69 ± 0.02 b	140.14 ± 7.36 b	1 ± 0.00 a

Note: TSCP: tomato straw compost product. CK, T1, T2, and T3 denote inoculating densities of 0, 40, 80, and 120 earthworms per kg substrate. Values are expressed as mean ± standard deviation (n = 3). Within the same column, different letters represent significant differences ($p < 0.05$).

For fungi, the TSCP was found to contain 150 OTUs (Table 3). By the conclusion of the vermicomposting process, the control group exhibited 184 OTUs, whereas the earthworm treatments yielded 148, 92, and 140 OTUs, respectively. These results suggest that the number of OTUs in the final products of the earthworm treatments was lower compared to the control group. At the end of the vermicomposting process, the Shannon, Chao1, and Simpson indices for fungi in the earthworm treatments were reduced compared to the TSCP (Table 3). Fernández-Gómez et al. [12] proposed that earthworms acquire nutrients by digesting fungi. Furthermore, earthworms may selectively consume specific types of fungi as part of their diet [78]. At the end of the vermicomposting process, the Shannon, Chao1, and Simpson indices for fungi in the earthworm treatments were consistently lower compared to those in the control group. These findings align with previous research on earthworm composting of corn stover and cow dung [33].

3.5. Composition of the Microbial Community

In the phylum-level relative abundance figure for bacteria, a total of 30 phyla were detected (Figure 6a). Of these, nine phyla exhibited a relative abundance greater than 2%, including *Proteobacteria*, *Actinobacteriota*, *Firmicutes*, *Bacteroidota*, *Verrucomicrobiota*, *Gemmatimonadota*, *Planctomycetota*, and *Deinococcota*. Collectively, these phyla account for 90.26% to 92.96% of the total bacterial count. Among them, *Proteobacteria* is the most dominant phylum, followed by *Actinobacteriota*, *Firmicutes*, and *Bacteroidota*. These four predominant phyla have also been reported in medicinal herbal residues vermicomposting [74], vegetable wastes vermicompost [76], and sludge wastes vermicompost [75,79]. In the TSCP, the relative abundances of *Proteobacteria*, *Actinobacteriota*, *Firmicutes*, and *Bacteroidota* were 24.65%, 15.90%, 15.63%, and 9.87%, respectively.

In the earthworm treatment groups, the relative abundances of *Proteobacteria* and *Actinobacteriota* increased over time, a trend consistent with previous studies [74,79]. Ultimately, the relative abundances of *Proteobacteria* and *Actinobacteriota* in the T1-T3 earthworm treatments increased by 7.32% to 12.89% and 52.03% to 101.14%, respectively, compared to the TSCP. *Proteobacteria* and *Actinobacteriota* effectively degrade lignocellulose and chitin, dominating the vermicomposting process [74,80]. *Actinobacteriota* can serve as an indicator of compost maturity [40]. Among the earthworm treatments, T2 (with the addition of 80 earthworms/kg) exhibited the greatest relative abundance of *Actinobacteriota* (31.99%), which also explains the higher maturity observed in T2. The abundances of *Firmicutes* and

Bacteroidota in the T1-T3 earthworm treatments decreased over time, reducing by 20.95% to 34.99% and 39.45% to 57.44%, respectively. *Firmicutes* can degrade proteins, cellulose, fats, and other organic compounds through the production of cellulases, lipases, proteases, and other extracellular enzymes [74]. *Bacteroidota* can stabilize organic matter during the vermicomposting process [80].

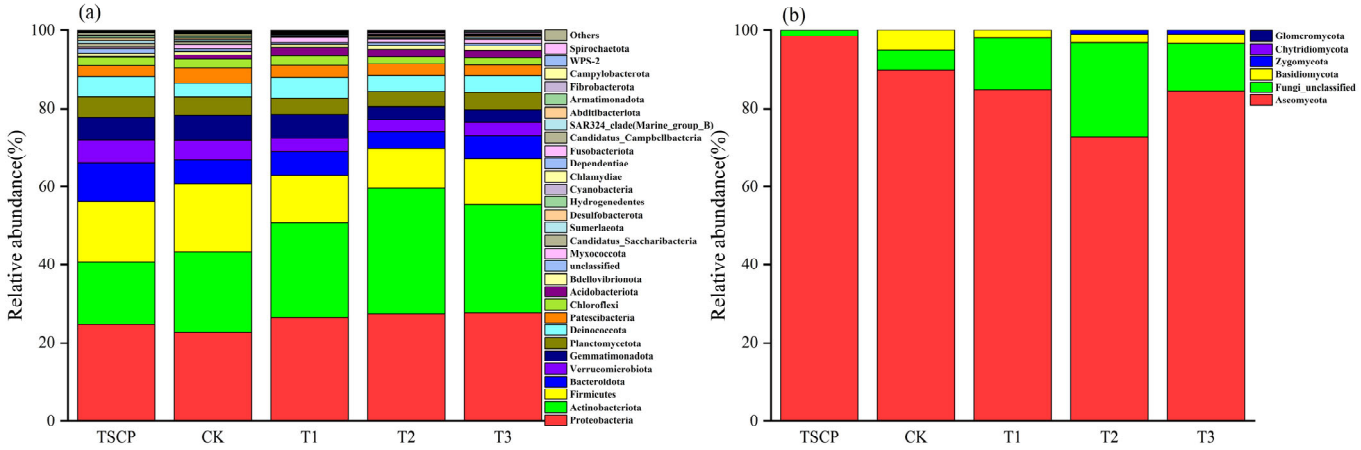


Figure 6. Composition of bacterial (**a**) and fungal (**b**) communities at the phylum level. TSCP: tomato straw compost product. CK, T1, T2, and T3 denote inoculating densities of 0, 40, 80, and 120 earthworms per kg substrate.

For fungi, six phyla were detected in the relative abundance plot at the phylum level (Figure 6b). Among all groups, *Ascomycota* and *Fungi_unclassified* were the dominant microbial phyla. This result is consistent with previous studies [81]. Additionally, *Basidiomycota*, *Zygomycota*, *Chytridiomycota*, and *Glomeromycota* were observed in limited quantities. Specifically, the abundance of *Ascomycota* showed a decreasing trend over time, while the abundance of *Fungi_unclassified* increased. *Ascomycota* and *Basidiomycota* are known to play crucial roles in the degradation of cellulose and lignin [82]. *Ascomycota* can secrete various cellulase and hemicellulase enzymes that effectively degrade lignocellulose [7]. *Basidiomycota* are known to secrete enzymes essential for lignin degradation [83].

4. Conclusions

This study explores the application of integrated composting and vermicomposting in the resource utilization of tomato straw waste. The results show that vermicomposting significantly improved the nutritional content and quality of the compost, particularly in terms of total nitrogen, total phosphorus, total potassium, humification index, and germination index, with increases of 3.2–10.57%, 3.3–12.69%, 2.65–7.42%, 21.56–30.54%, and 36.57–91.68%, respectively, compared to the control group. Among the four earthworm stocking densities, the density of 80 earthworms/kg exhibited the best results. Additionally, the vermicomposting treatment group showed higher enzyme activity, bacterial abundance, and diversity compared to the control group. These findings suggest that the integrated composting and vermicomposting system effectively enhances the quality of compost from tomato straw waste, providing a feasible solution for the resource utilization of agricultural waste. Future research could optimize the integrated composting and vermicomposting system for different high-salinity agricultural wastes and explore the synergistic effects of earthworm stocking density and environmental factors, paving the way for sustainable, intelligent waste treatment systems.

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