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Metagenomics Analysis of the Impact of Protein-Degrading Functional Microbial Agents on Composting of Chicken Manure from Cereal Hulls

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Abstract: In this study, four highly efficient protein-degrading bacteria (*Siccibactercolletis*, *Bacillus thuringiensis*, *Bacillus cereus*, and *Bacillus* sp. (in: Firmicutes)) were screened from soil and fermentation beds and prepared into a mixed microbial agent in a ratio of 1:1:1:1. The effects of inoculation with protein-degrading functional bacteria on nitrogen transformation rate, microbial community, and functional genes during chicken manure–rice husk composting were studied. With the addition of functional agents, the nitrogen loss in chicken manure composting was reduced to 17.05%, and ammonia emissions were also reduced. Firmicutes, Proteobacteria, Bacteroidetes, Cocci, and Actinobacteria became the dominant bacterial communities, accounting for 85.41%~98.52% of the overall bacterial community in the compost; it promoted the growth of microorganisms such as *Pseudogracilibacillus* and *Lachnospiraceae* in the compost. Metagenomic analysis revealed that the addition of functional bacterial agents enhanced the expression of nitrogen fixation genes (*nif*K, *nif*H, and *gln*A) during the high-temperature phase, increased the diversity of bacteria associated with the nitrogen cycle in the compost, and improved the absorption and fixation of nitrogen source elements by microorganisms. Additionally, it strengthened the correlation between microbial communities, the composting environment, and functional genes. This study provides a theoretical basis for the efficient application of microbial agents and the reduction of pollution in chicken manure hull composting.

Keywords: composting; nitrogen conversion; functional microbial agent; microbial communities; metagenomica

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1. Introduction

With the continuous expansion of animal husbandry, large-scale and intensive breeding has become the mainstream mode of animal husbandry, producing a large amount of livestock and poultry manure. Currently, China generates approximately 3.8 billion tons of livestock and poultry manure annually, but the overall utilization rate is less than 60% [1,2]. The large-scale generation of livestock and poultry manure, coupled with inappropriate disposal methods, poses a series of problems for soil, water, and atmospheric environments. Excessive use of untreated manure can lead to the accumulation of heavy metals and particulate matter, and increased greenhouse gas emissions [3]. Therefore, composting plays a crucial role in promoting the safe and effective reuse of livestock and poultry manure, contributing to environmental improvement.

Previously, landfill, anaerobic digestion, and composting were prominent methods of treating animal and poultry waste. Among them, composting is one of the environmentally

friendly methods for recovering organic waste, which is widely accepted for reducing secondary pollution and having economic benefits [4]. Composting is a series of complex biological and chemical interactions. Under the influence of microorganisms, the organic matter in the pile creates a relatively stable humus, which is advantageous for lowering environmental dangers [5]. However, because standard composting takes a long time, adding additives to the compost can increase composting efficiency. Studies have shown that add can reduce the emission of greenhouse gases during composting and increase the degradation of organic matter. Adding organic carbon in the process can reduce carbon and nitrogen losses [6], but it is accompanied by the production of greenhouse gases and other gases, including methane (CH_4) and carbon dioxide (CO_2) [7]. In the composting process, microbial agents and crop waste (such as rice husk, sawdust, straw, etc.) are added to increase the content of humic acid [8] and adjust the C/N ratio, pH, water, and nutrient content [9]. Meanwhile, microbial agent can promote microbial succession in the process of rotting, accelerating the transformation of livestock and poultry manure into safe rotting fertilizer, so as to realize the reduction, harmlessness, and resource utilization of livestock and poultry manure [2]. Li et al. [5] improve the mineralization and humification of organic matter and the stability of phosphorus in the composting process through added microbial agents composed of *Trichoderma*, *Bacillus*, actinomycetes, lactic acid bacteria, *Pseudomonas* and magnesium ammonium phosphate combined with pig manure–corn straw composting. Chi et al. [10] found that Streptomyces straw JSD-1 remodeled the microbial community of pig manure and rice straw co-composting. Zhang et al. [11] injected mink dung composting with actinomycetes to improve the quantity of bioavailable organic nitrogen and the number of beneficial microorganisms, consequently enhancing composting quality. Liu et al. [12] found that the activation of key resident microorganisms in the early stage of inoculation of microbial agents had a great contribution to the formation of humic acid and improved composting efficiency. Li et al. [13] inoculated the compost with bioinoculants consisting of *Bacillus subtilis*, *Enterobacter hallii*, and *Trichoderma reesei*, and composted it with a mixture of chicken manure and mushroom waste, which effectively elevated the levels of humus, humic acid, and available phosphorus in the compost, while bolstering the stability of the predominant microbial communities within the compost. Ji et al. [14] introduced biochar and lignocellulose-degrading bacteria during the composting process of pig manure, which effectively inhibited denitrification and significantly improved the nitrogen retention rate. Figure 1 shows the possible mechanism of action of microbial agents in the composting process. In addition, researchers have found that bacterial communities play a crucial role in the decomposition and mineralization of organic matter under high-temperature conditions, particularly during the thermophilic phase of composting [15,16].

More than half of the world's population relies on rice as their staple food. It is projected that from 2022 to 2023, global rice production will reach 515 million tons [17]. Rice husk is an unused byproduct in the rice industry, accounting for about 20% [18]. Its density is 80–125 kg/m^3, which is composed of volatile substances, ash, and so on [19]. It is commonly used as an additive for many materials, including compost, animal feed, insulation materials, flame-retardant materials, rubber composites, etc. [18,20,21]. Liu et al. [9] added 50% rice husk to chicken manure, which significantly improved the composting efficiency and final germination index. Meanwhile, the relative abundance of *Bacillus*, cellulose, and β-glucosidase activity was increased in the middle temperature period and high-temperature period. Therefore, it promotes the degradation and humification of cellulose in compost. Takaku et al. [22] found that the microbial community can be changed dramatically during the composting process with rice husk as an additive, and the main bacterial group changed from nitrogen-fixing bacteria to Bacteroidetes. Yu et al. [23] added rice husk and minerals to chicken manure compost to promote the formation of humic acid driven by improved core microorganisms. Kim et al. [24] used rice husk as padding for cow dung compost, which could lower nitrogen loss than wood chips. Composting is a self-heating process driven by microorganisms and environmental factors. The addition of

microbial agents in this composting process can improve bacterial communities to adapt to adverse different environmental conditions.

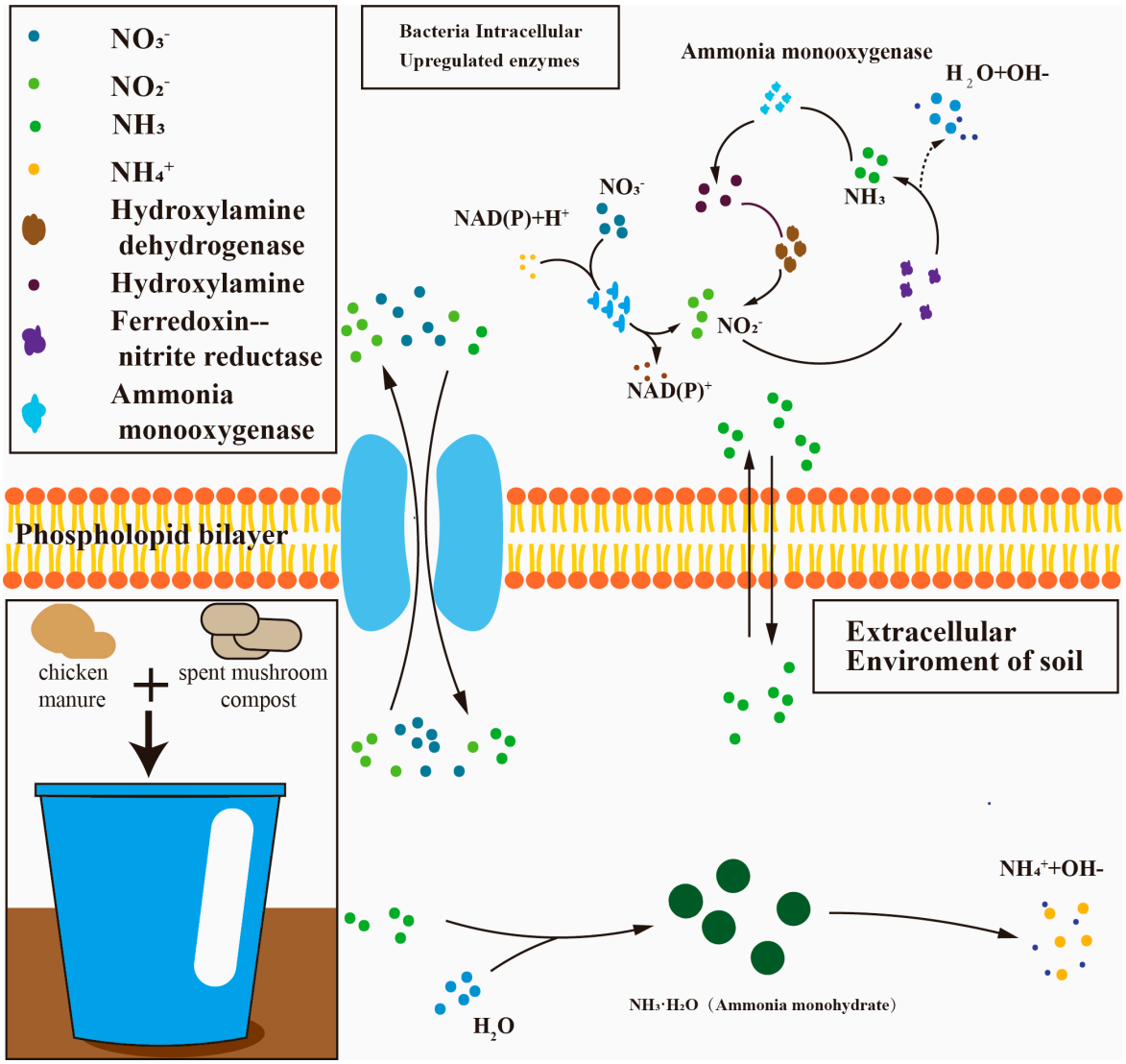

Figure 1. Effects of microbial preparations on microbial community succession, nitrogen, and physicochemical properties in piles.

In this study, cereal husk–chicken manure co-composting was utilized to investigate the changes in physical and chemical qualities, as well as the bacteria community in composing process, which is expected to provide valuable reference for promoting the composting technology of livestock and poultry manure.

2. Materials and Methods

2.1. Composting Materials and Experimental Design

Fresh chicken manure was obtained from Linxi Farm, China (112°53′4.39″ E, 28°31′9.05″ N, Changsha, China), and rice husks (Changsha, China) were collected by farmers. Their physicochemical properties were shown in Table 1. Four strains of highly efficient protein-degrading bacteria from soil and fermentation beds (*Siccibacter colletis*, *Bacillus thuringiensis*, *Bacillus cereus*, and *Bacillus* sp. (in: Firmicutes)) were screened to make functional bacterial agents in a 1:1:1:1 ratio. Composting was carried out with 2.5% functional bacterial agent additions.

Table 1. Physicochemical properties of composting materials.

	TN (g/kg)	TOC (g/kg)	Organic Matter (%)	Water Content (%)
Chaff	11.64 ± 0.40	38.32 ± 0.36	66.08 ± 0.63	—
Chicken manure	14.96 ± 1.04	13.26 ± 0.78	22.86 ± 1.35	71.14 ± 0.12

Note: values indicate the mean of six replicates ± standard deviation.

Mix fresh chicken manure with rice husk was mixed in a ratio of 4:1. The C/N ratio was adjusted to around 20. The compost pile should be 20 kg [25]. We used ultrapure water to achieve a moisture content of 60%. The experimental design treatment groups were the CK group (CM + chaff) and T1 group (microbial agent + CM + chaff). Functional bacterial agents were added during the mixing of the pile to ensure that they were uniformly present in the pile. The compost bin size was L × W × H = 460 mm × 420 mm × 570 mm. The entire composting process lasted 20 days. Throughout the composting process, water was replenished as appropriate to ensure proper composting. The temperature was measured at 9 a.m. every day using a thermometer at the top, middle, and bottom of the compost bin. A five-point sampling method was used, with 100 g of compost sampled from the top, middle, and bottom of the pile every three days, and the samples were then mixed thoroughly. During composting, the pile was turned every two days. The samples were divided into two parts and stored in a −20 °C refrigerator for the determination of physical and chemical parameters, and in a −80 °C refrigerator for the determination of microorganisms and the metagenomic analysis.

2.2. Physicochemical Analyses

The ammonia concentration in the drums was measured every day at 9:00 a.m. The ammonia was measured using a gas analyzer. We measured the temperature of five different points in the middle layer of the pile with a thermometer every day, and took the average as the temperature of the pile on the day. The compost samples were obtained by adding ultrapure water (1:10) and shaking for 30 min with a pH meter (PHS-25, Lai-Chern, Shanghai, China). Ammonia and nitrate nitrogen were determined by a flow analyzer (Auto Analyzer 3-AA3, Brown Rupee, Hamburg, Germany). Total nitrogen (TN) was determined by the Kjeldahl method [26]. The above measurements were repeated three times. We used a portable ammonia detector (HG-MDK-NH3, Haiku, Changsha, China) to measure the daily ammonia emissions from the compost pile (measurements were taken three times per compost barrel each day at 9:30 a.m.).

2.3. 16S rRNA Analysis

We took 0.25 g of compost samples stored at −80 °C and sent them to Biomarker Technologies Co., Ltd. (Beijing, China). Using the PowerSoil DNA Extraction Kit for DNA extraction, the V3–V4 sequences of the 16S rRNA gene were amplified from the genomic DNA extracted from each sample with the primers 27F-AGGTTTGATYNTGGCTCAG/1492R-TASGGHTACCTTGTTASGACTT. The amplified products were purified using Agencourt AMPure XP Beads (Beckman Coulter, Indianapolis, IN, USA), and quantitatively analyzed with the Qubit dsDNA HS Assay Kit and Qubit 4.0 Fluorometer (Invitrogen, Thermo Fisher Scientific, Hillsboro, OR, USA).

2.4. Metagenomics Analysis

Using the TGuide S96 Magnetic Soil/Stool DNA Kit (Tiangen Biotech (Beijing) Co., Ltd., Beijing, China), DNA was extracted from the compost samples. The DNA concentration of the samples was measured with the Qubit dsDNA HS Assay Kit and Qubit 4.0 Fluorometer (Invitrogen, Thermo Fisher Scientific, OR, USA). Subsequently, each genomic DNA sample was randomly fragmented into approximately 300 bp segments using an ultrasonic instrument, the Covaris M220 (Covaris, Woburn, MA, USA). The DNA library was constructed using the NEBNext® Ultra™ DNA Library Prep Kit for Illumina® (NEB, Ipswich, MA, USA), purified with Agencourt AMPure XP (Beckman, Brea, CA, USA), and

quantified and analyzed using the GenNextTM NGS Library Quantification Kit (Toyobo, Japan). Sequencing was performed on the Illumina NovaSeq platform (No-va-Seq6000), and the sequencing results were assigned to the sample reads through the index to obtain the original sequence of each sample. The original sequences were identified and trimmed (Trimmatic, 0.33; QIIME2, 2020.6; Cutadapt, 1.9.1), connections and chimeras were removed (USEARCH, 10.0; UCHIME, 8.1), and the remaining sequences were clustered using USEARCH (10.0) (OTU; similarity 97%, threshold 0.005%) to improve the quality and reliability of subsequent analysis. Metagenomic assembly was performed using MEGAHIT (version 1.2.9), and the results were evaluated using QUAST software (version 5.2.0), filtering for contigs smaller than 100 bp. The assembled sequences were processed using MetaGeneMark (https://exon.gatech.edu/meta_gmhmmp.cgi, version 3.26, accessed on 29 December 2022) to identify coding sequences and remove redundancy (https://www.bioinformatics.org/cd-hit/, version 4.6.6, accessed on 7 January 2023, 95% similarity, 90% coverage). Salmon (Version 1.6.0) was used to determine the gene abundance in each sample, and the results were compared with the NCBI database to perform species annotation at the taxonomic level. QIIME2 (https://qiime2.org/, accessed on 2 February 2023) was used to analyze the microbial diversity and similarity in different composting materials, and a heat map was created based on standardized OTU data for data visualization. At the same time, functional annotation was performed with the help of the KEGG database to establish an interaction network between functional genes and microorganisms. The metagenomic dataset was submitted to the National Center for Biotechnology Information database with accession number SRP484657.

2.5. Data Analysis

The data were analyzed using SPSS (version 26.0, IBM, Armonk, NY, USA) for single-factor analysis (analysis of variance). GraphPad software (version 8.0.2, GraphPad Software, San Diego, CA, USA) was used to draw charts.

Microbial diversity analysis, clustering analysis, and the creation of heat maps were conducted following the methods consistent with Wang et al. [27].

3. Results and Discussion

3.1. Changes of Physicochemical Parameters

Temperature is an important indicator of microbial changes in the composting process as well as compost maturity [28]. The temperature variations for the two treatments are shown in Figure 2a, composting process through four stages, which are the mesophilic stage, the thermophilic stage, the cooling stage, and the maturation stage [29]. The maximum temperatures of the two treatment groups were 50 °C (CK) and 52 °C (T1), while the thermophilic period (temperature greater than 50 °C) of the T1 treatment group with the addition of functional bacterial agents was 2, 3, 4, and 5 d. The reason may be that turning the heap puts the heap in an aerobic fermentation state, which provides a better aerobic environment for the microorganisms in T1 and increases the bioheat in T1. In the later stages of composting, there was little difference in temperature between the two groups of treatments; all were 2–3 °C above room temperature.

The pH trends in different treatment groups are shown in Figure 2b. Microbial decomposition of organic matter results in the accumulation of organic acids in the compost pile, a decrease in pH for both treatment groups during the first 3 days. As composting progresses, the pH of the pile increases and then decreases, with the overall conversion from a weakly acidic to a weakly alkaline state [30]. With the decomposition of organic acids by microbial activities, pH increases, and then stabilizes. During the high-temperature period, the lowest pH value in T1 is lower than in CK (7.36 > 7.06), suggesting that T1 during the high-temperature phase may have produced more organic acids. The higher microbial activity in T1 during the high-temperature phase is consistent with the findings of Kiyohiko Nakasaki et al. [28]. The pH of the T1 group was more stable in the maturity period of composting relative to CK, and the pH was lower than in CK, probably because

the added bacterial agent regulated the microbial community in the compost, and the lower pH was also able to reduce the nitrogen loss due to the volatilization of ammonia.

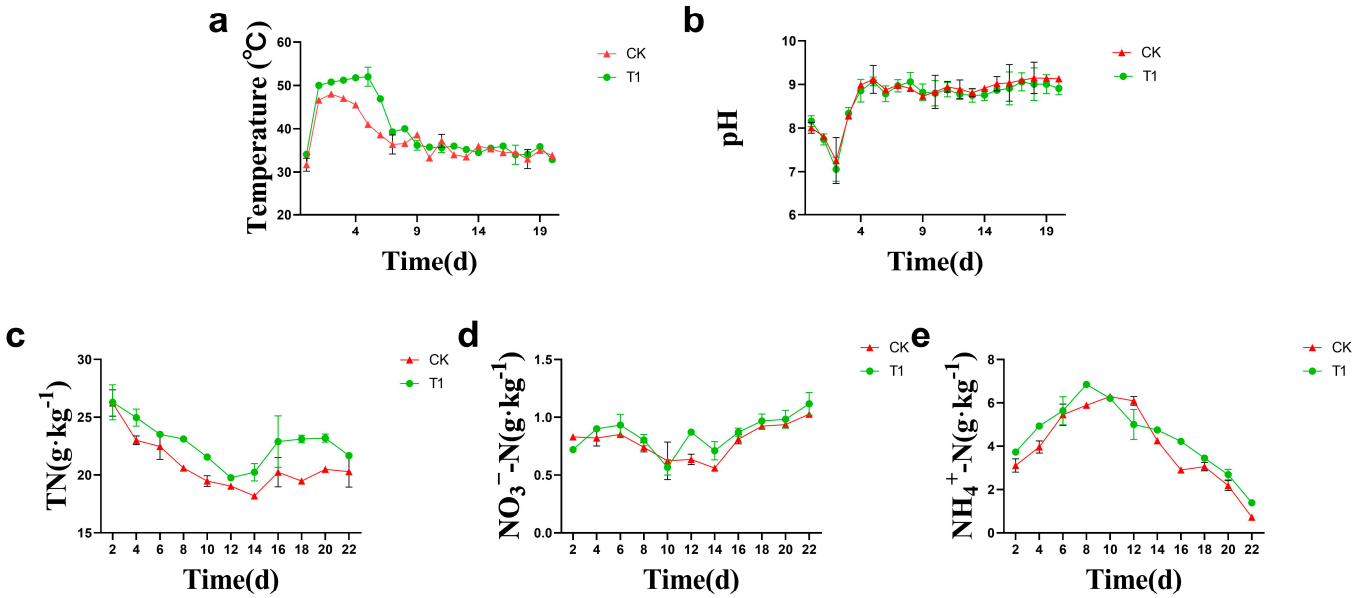

Figure 2. Changes in temperature (**a**), pH (**b**), TN (**c**), NO_3^--N (**d**), and NH_4^+-N (**e**) under different treatments during the composting process of chicken manure (n = 6). The error bar represents the standard deviation of three repeated measurements.

3.2. Nitrogen Conversion

During the composting process, organic nitrogen is utilized by the bacteria community to produce ammonium (NH_4^+-N) and nitrate nitrogen (NO_3^--N). In the early stages of composting, the NH_4^+-N produced is lost as a gas as temperatures rise [31]. For the first 2–8 days, ammonium nitrogen (NH_4^+-N) showed an increasing trend (Figure 2c) and CK decreased faster than T1; the reason for this may be that the temperature and duration of the high-temperature period were greater in T1 than in CK. The trend of NH_4^+-N in the two treatments were similar to the study of Ma et al. [32]. When the temperature stabilized, NH_4^+-N was produced as the organic nitrogen was continuously utilized by microorganisms and the NH_4^+-N salts were reelevated with the turning of the pile. This phenomenon is more pronounced in T1, indicating that the addition of microbial agents accelerates the decomposition of organic matter and the conversion of organic nitrogen into inorganic nitrogen. During the mature phase, the trend of NH_4^+-N stabilizes, indicating that microbial decomposition activity is becoming stable. This could be attributed to the influence of a stable microbial community.

The NO_3^--N content during composting varied, as seen in Figure 2d. The NO_3^--N in the two treatment groups showed an increasing trend at the mesophilic stage of composting, and reached the peak value on the 6th day, which was 0.93 g/kg and 0.85 g/kg in CK and T1, respectively. This may be due to the increase in ammonium nitrogen being utilized by the bacteria community to form nitrate nitrogen [33]. Meanwhile, the high nitrate nitrogen content in T1 is due to the addition of functional bacterial agents, which promote the growth of indigenous microorganisms that are able to convert more ammonium nitrogen into nitrate nitrogen. With the temperature, nitrifying bacteria were affected by high temperature increase, microbial activity decreased, and the conversion rate of nitrate nitrogen decreased. At the same time, part of the nitrate nitrogen was utilized by microorganisms to form organic nitrogen, so the nitrate nitrogen content of the two treatments showed a decreasing trend [34]. In the maturity period of the compost, the suitable temperature allowed for the nitrifying bacteria to increase their activity, which increased the content of nitrate nitrogen in the compost. At the end of composting, the content of nitrate nitrogen

in T1 was 1.12 g/kg, which was higher than that of CK (1.03 g/kg), indicating that the addition of bacterial agents could fix part of the nitrogen by increasing nitrification in the compost, and reduce the nitrogen loss in the compost.

TN in compost showed a decreasing trend as ammonia was emitted (Figure 2e). The TN contents of composting process T1 were all higher than CK, which may be caused by the role of microbial in nitrogen fixation. The content of urea in chicken manure is relatively high compared to pig manure; the hydrolysis of urea produces ammonia, and frequent turning of the pile during the high-temperature period also contributed to the decrease of TN in the pre-compost pile [35]. At the end of composting, the TN content of T1 was 2.17% and that of CK was 2.03%. Nitrogen loss of T1 was 17.05%, which was lower than that of CK (22.64%). The incorporation of the microbial agent effectively reduced the nitrogen loss, which indicated that the microbial agent reduced the nitrogen loss from chicken manure composting through the reduction in ammonia emission and the immobilization of inorganic nitrogen into organic nitrogen in the composting process.

One of the main reasons for nitrogen loss during the composting process is the substantial emission of ammonia [31]. Ammonia emissions from all treatment groups increased rapidly at first and then declined (Figure 3). The high temperatures in the first period are responsible for the increase in ammonia emissions. Ammonia emissions rose again on day 6 for both treatment groups, presumably due to the increased oxygen content in the compost from turning the pile on day 6, which accelerated the fermentation of the compost and led to increased ammonia emissions. Towards the end of the composting period (18–20 days), the ammonia emissions stabilized; the reasons for this were stable pH and temperature and a stable microbial community. The highest daily ammonia emissions were significantly lower ($p < 0.05$) in T1 than in CK during the composting process. Therefore, the incorporation of functional bacterial agents can reduce ammonia emission during the high-temperature period; improve the pH of compost, thereby reducing the conversion of NH_4^+-N to ammonia emission; and thus reduce nitrogen loss from compost. In addition, the addition of the functional bacterial agent may have promoted the early microbial activity in the compost and increased the utilization of NH_4^+-N by microorganisms in the compost. For the high-temperature period, the increase in temperature was important in leading to ammonia emission. Ammonia is insoluble in water when the temperature is too high, and since this experiment was conducted under laboratory conditions, again, some of the N losses were reduced.

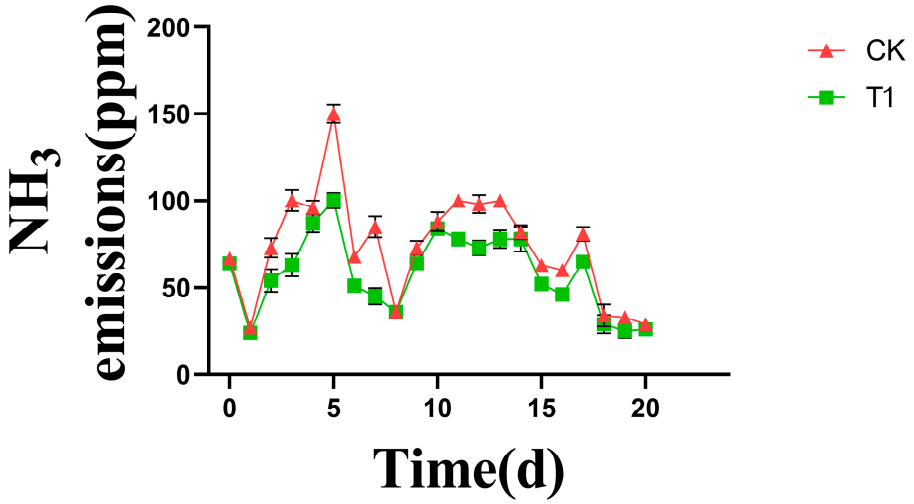

Figure 3. The trend of daily ammonia emissions during composting (n = 6).

3.3. Composition and Change of Microbial Community

The effect of functional bacterial agents on bacteria composed in compost was revealed using 16S rRNA gene sequencing. At the gate level, the top 5 bacteria in terms of abundance in both treatment groups were Firmicutes, Proteobacteria, Bacteroidota, Deinococcota, and

Actinobacteriota, which accounted for 85.41–98.52% of the overall bacterial community in the compost (Figure 4a); this is similar to the study by Zhang et al. [36]. In the early stages of composting, Firmicutes, a major constituent of the animal gut flora, make up a large portion of the microbial community of the compost [37]. With the prolonged composting time and a decrease in temperature, there is a decline in the relative abundance of Actinobacteria, while the relative abundance (RA) of Firmicutes increases. On the second day, the relative abundance of Bacteroidota in CK is higher than in T1. This could be attributed to an increase in Actinobacteria in the early stages of composting, leading to elevated temperatures in the pile, which may have inhibited the growth in Bacteroidota. Bacteroidota growth is inhibited under high temperature conditions [38]. During the mature phase, the abundance of Firmicutes in T1 is higher than in CK, which is conducive to the degradation and utilization of complex organic matter in the later stages of composting. Also, the presence of bacteria screened from the gut in the functional bacteria explains the higher relative abundance of Firmicutes in the maturation of compost in the T1 treatment group than in CK. Deinococcota appeared during the cooling and maturation phases, and the addition of the agent increased the relative abundance of Deinococcota during these two periods. The medium temperature was more suitable for the growth of Proteobacteria, so Proteobacteria showed a tendency of decreasing and then increasing, probably because the addition of the functional bacterial agent accelerated the rate of the heap into high temperature.

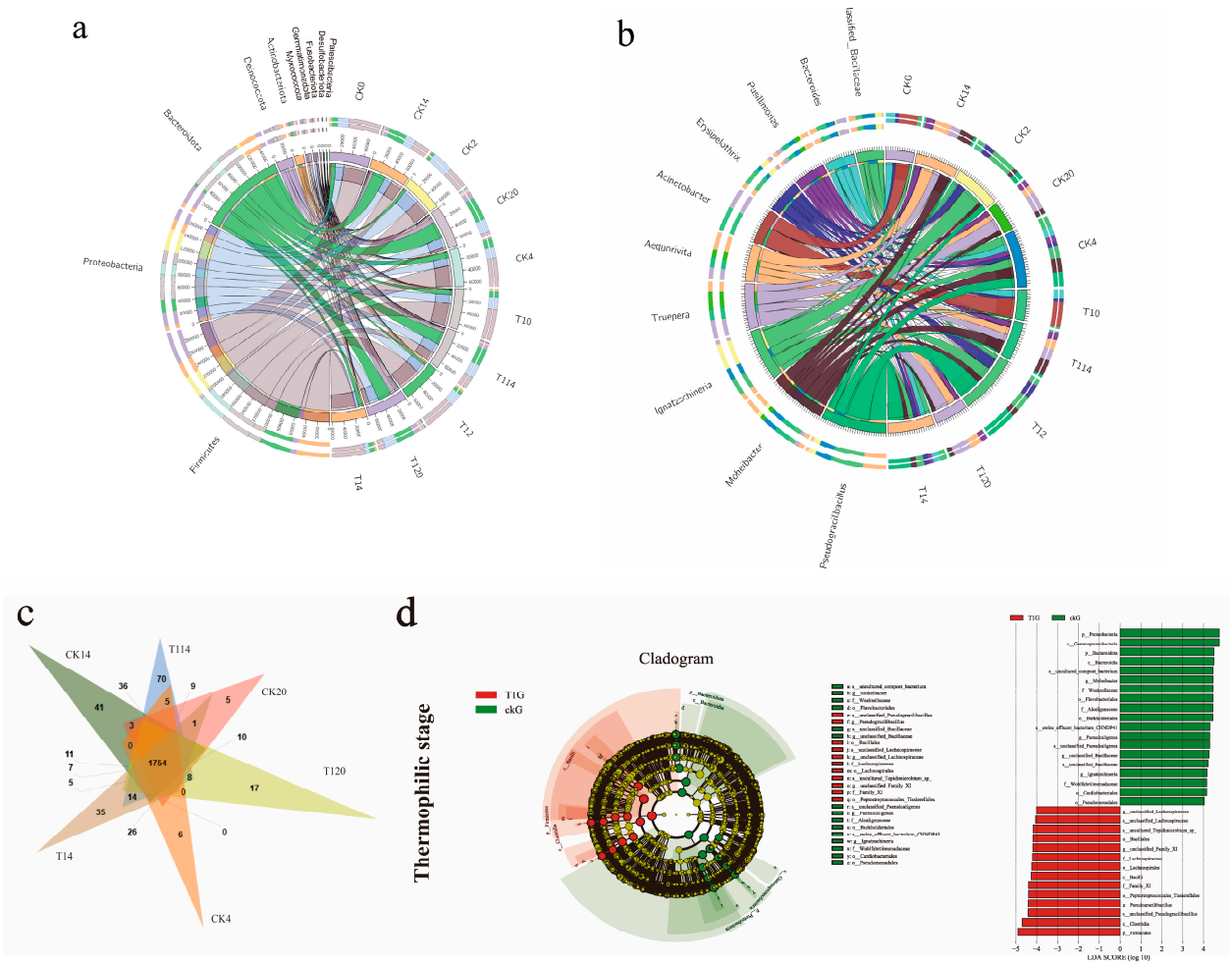

Figure 4. Microorganisms at the phylum (**a**) and genus (**b**) levels with relative abundance in the top 15 during the composting process. The composition of bacterial genera related to nitrogen cycling annotated in the Nr database (**c**). Linear discriminant analysis effect size (LEfSe): the evolutionary branching diagram during the high-temperature period, and the distribution histogram (**d**). (n = 6).

The microbial communities of the two treatment groups also differed at the genus level between the hot and cool periods of composting (Figure 4b). *Acinetobacter* was mainly found in the pre-composting stage and decreased as composting progressed. The microbial communities of the two treatment groups also differed considerably at the genus level between the hot and cool phases of composting. *Pseudogracilibacillus*, a moderately halophilic bacterial genus, was more common during the thermophilic phase of chicken manure composting [39], where it helped to promote cellulose decomposition and humic acid production, to improve the quality of the compost product. Bacteria of the genus Bacillus were included in the compost, while the addition of *Pseudogracilibacillus* increased the relative abundance of *Pseudogracilibacillus*. *Ignatzschineria* is a denitrifying bacterium [40]; during the high-temperature phase, the relative abundance of *Ignatzschineria* in T1 is lower than in CK. This is also the reason why the nitrate nitrogen content in CK is lower than in T1 during the pre-composting stage. Adding of functional bacterial agents suppressed the growth of *Ignatzschineria* in the compost and reduced denitrification in the compost. *Moheibacter* was able to promote the stable transformation of DOM. During the high-temperature phase, the relative abundance of *Moheibacter* in T1 is higher than in CK. This indicates that the addition of functional microbial agents promotes the growth of *Moheibacter*, accelerating the decomposition of organic matter in the compost. This is also a possible reason for T1 having a higher maximum temperature and a longer duration of the high-temperature phase compared to CK.

The number of microorganisms associated with the nitrogen cycle in each period of composting in the two treatment groups was annotated through the NR database (Figure 4c). The total number of bacteria related to nitrogen cycling is 3318, and at each stage, the quantity of bacteria related to nitrogen cycling in the T1 group is higher than in CK. The addition of functional bacterial agents can increase the diversity of microorganisms in the composting process [41]. Especially in the high-temperature period and the cooling period, the microbial diversity of microorganisms associated with the nitrogen cycle was significantly more than that in the T1 than that in the CK, which indicates that the addition of functional bacterial agent not only promotes the nitrogen conversion during the composting process, but also promotes the growth of the indigenous functional microorganisms.

LEfSe analysis identified differential microorganisms between the two treatment groups during the high-temperature period (Figure 4d). At the genus level, *Pseudogracilibacillus*, *Lachnospiraceae* were the two bacterial counts that were significantly higher in T1 than in CK. *Pseudogracilibacillus* contributes to lignocellulose decomposition and utilization, increasing beneficial nutrients and thus promoting microbial community diversity. The addition of the bacterial agent increased the *Pseudogracilibacillus* in T1 during the high-temperature period, which produced by-products capable of promoting the growth of indigenous microorganisms, and the growth of microorganisms cumulated the bioheat of the heap, resulting in a higher temperature in the high-temperature period of T1 than that of CK. *Lachnospiraceae* can participate in a wide range of carbohydrate metabolism production of acetic and butyric acids, which may contribute to the lower pH of T1 relative to CK during periods of high temperatures.

3.4. Bacterial Community Network Analysis

The network analysis is able to show the trend of microbial de-community structure changes in compost [42]. Microbial network analyses demonstrated microbial interactions between the two treatment groups at various times (Figure 5). The microbial network properties are shown in Table 2. The network structure of both treatment groups ranged from complex to simple to complex, which was attributed to the fact that some heat-intolerant bacterial genera activity inhibited in the composting environment as the composting temperature increased, and bacterial activity was reelevated after entering the cooling period.

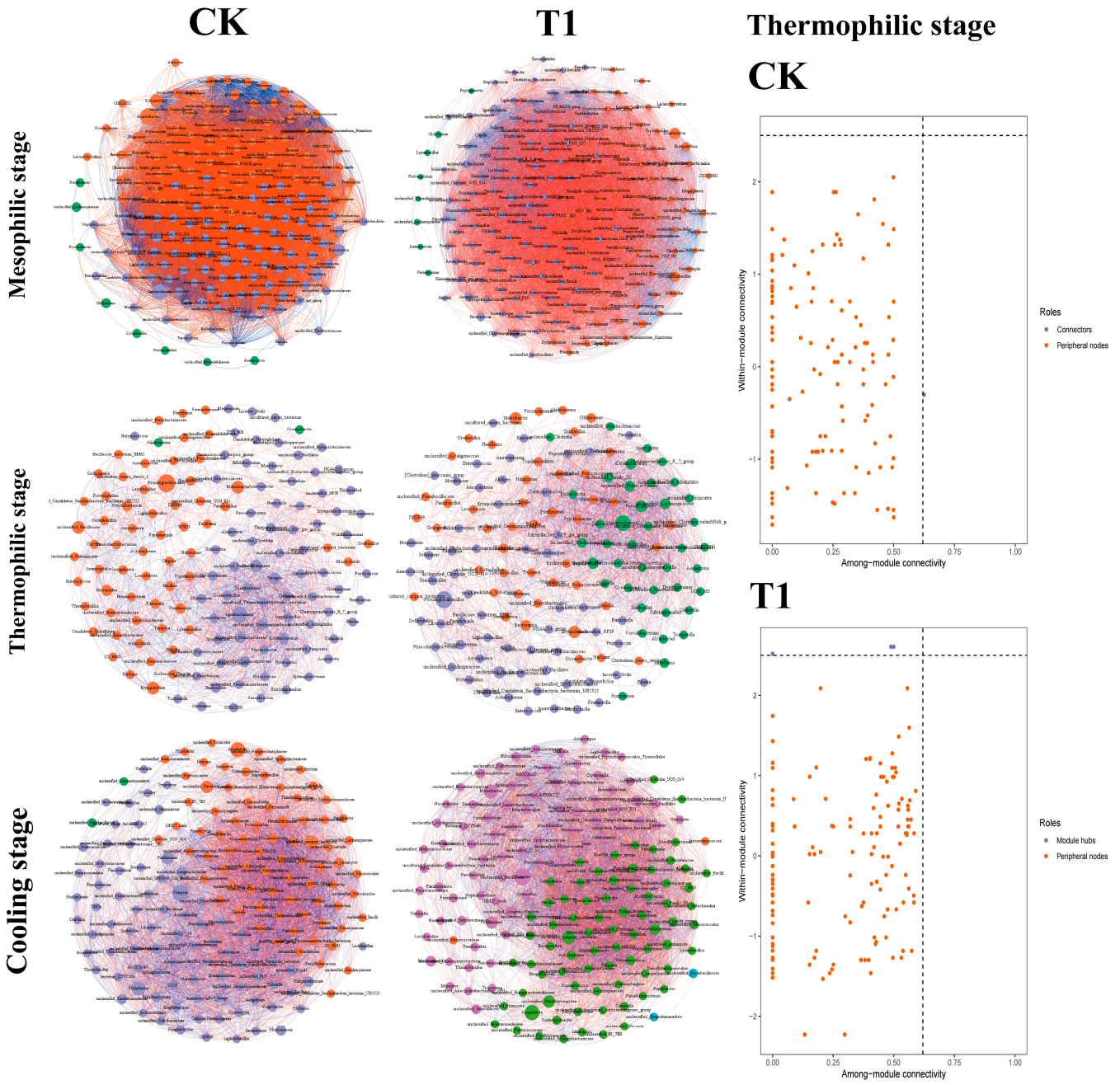

Figure 5. Network structure diagram and ZIPi diagram of microbial community genus level at different stages of two treatment groups (n = 6).

Table 2. Properties of microbial networks across time.

	Mesothermal Stage			High-Temperature Stage			Maturation Stage		
	Nodes	**Edges**	**Modularity**	**Nodes**	**Edges**	**Modularity**	**Nodes**	**Edges**	**Modularity**
CK	188	12,112	0.063	139	1766	0.384	162	5236	0.205
T1	187	12,098	0.060	145	1988	0.352	161	5218	0.200

Note: values indicate the mean of six replicates ± standard deviation.

In the early stages, the points and edges of the networks of the two treatment groups were similar. In the thermophilic stage, the edges of T1 were higher than those of CK, although the points of the two treatment groups were similar. This suggests that the

incorporation of functional bacterial agents enhanced the interrelationships among microorganisms in the compost at the thermophilic stage as the lower degree of modularity of T1. During the high-temperature phase, the positive correlations among microorganisms in T1 are greater than in CK, indicating that the addition of functional microbial agents increases bacterial competition and accelerates the stability of the microbial community structure in the later stages of composting. However, the lower modularity in T1 compared to CK suggests that the microbial community in T1 is more resistant and stable during this period, indicating that the addition of functional microbial agents also promotes microbial diversity during the high-temperature phase. In the mature phase, the modularity in T1 is similar to CK, indicating that the microbial community structures in both treatment groups tend to stabilize, and the compost reaches an equilibrium state.

The microbial network diagram for the high-temperature period was selected to make a ZiPi diagram, which is proposed to be based on module connections (sub) and inter-module connections (Pi): peripherals (Zi $\leq$ 2.5 and Pi $\leq$ 0.62), modular hubs (Zi > 2.5 and Pi < 0.62), and network hubs (sub > 2.5 and Pi > 0.62). T1 contains several modular hubs, which suggests that the microbial network structure of T1 is more stable than that of CK. Three key OTUs (bacterial genera) were identified based on Zi and Pi values. In T1, *Thiopseudomonas* (Proteobacteria 10.6%), *Caldicoprobacter* (Firmicutes 73.8%), and *Rikenellaceae_RC9_gut_group* (Bacteroidetes 11.1%) served as the key microbial inT1. The microbial network was classified into three modules based on the degree of modularity of the microbial structure, where *Rikenellaceae_RC9_gut_group* and *Caldicoprobacter* belong to the M1 module, *Thiopseudomonas* belongs to the M2 module, and the *Rikenellaceae_RC9_gut_group* and *Caldicoprobacter* were able to break down organic matter and degrade cellulosic polysaccharide production to provide beneficial metabolites for other microorganisms [43], while *Thiopseudomonas* were able to utilize nitrogen source elements in the compost and promote nitrogen fixation in the compost [44]. The addition of functional microbial agents promotes the growth of indigenous microorganisms, making them core members of the microbial community within the compost pile.

3.5. Bacterial Metagenomics and Nitrogen Metabolism—Related Genes Analysis

Functional genes in the composting process of two composting treatment groups were investigated using KEGG pathway composting (Figure 6a,b). Cellular processes in the primary pathway of T1 were higher in the high-temperature period compared to CK, which was the same as the previous speculation. In the secondary pathway, xenobiotics biodegradation and metabolism were significantly higher in T1 than in CK, which was mainly related to the utilization by microorganisms of some chemicals present in the external environment that may be in contact with the organisms and may enter the organisms in one way or another, suggesting that the addition of the bacterial agent promoted the microbial cellular activities during the high-temperature period, and enhanced the microbial degradation in the compost. This suggests that the addition of the functional bacterial agents enhanced the cellular activity of the microorganisms during the high-temperature period and enhanced the ability of the microorganisms in the compost to degrade the organic matter and utilize nitrogen.

Figure 6c mainly demonstrates the diversity of pathway II expression between the two treatment groups at various times during the composting process. As shown in Figure 5c, the diversity of the pathways in the T1 was higher than that of CK in the cooling period and maturation periods. Meanwhile, there was a significant difference in the expression of the pathways between the two treatment groups in the maturation period of the compost ($p < 0.05$), which indicated that the addition of functional bacterial agents made an important impact on the KEGG gene dynamics in the composting process. The same finding was reported by Wu et al. [45]. The addition of functional bacterial agents had a significant effect on the structure of the microbial community, which accelerated the composting process and improved the quality of the compost.

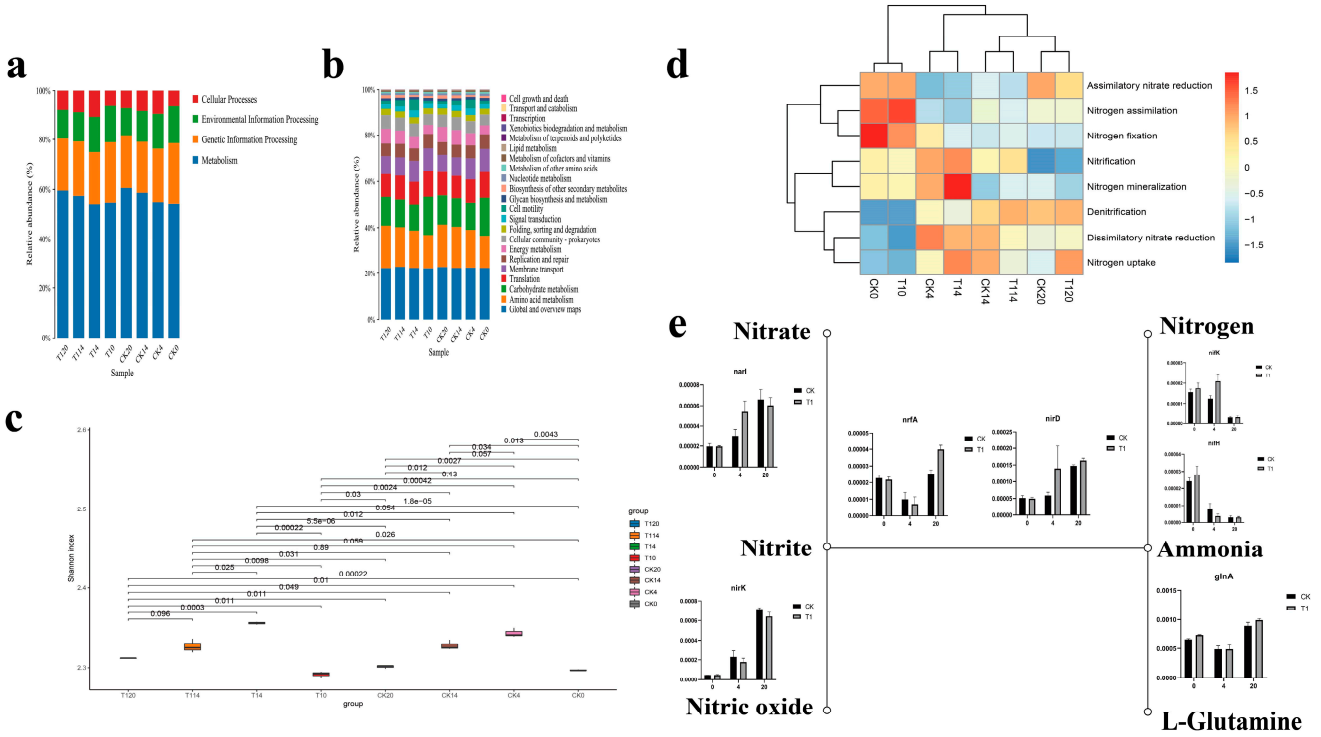

Figure 6. The two treatment groups annotated by KEGG, showing primary pathway (**a**), secondary pathway (**b**), secondary pathway diversity (**c**), nitrogen cycling-related gene expression heatmap (**d**), and nitrogen cycling gene expression level (**e**) at different stages. (n = 6).

NCycDB (https://github.com/qichao1984/NCyc, accessed on 25 May 2023) is an artificially corrected database of nitrogen cycling-related genes that can be used to annotate nitrogen cycling functional genes [46]. The expression of nitrogen cycling genes in the two treatment groups was annotated by the Ncyc database (Figure 6d,e). The heatmap corresponded to the abundance of genes related to nitrogen cycling in the two treatment groups in each period (Figure 6d). Assimilatory nitrate reduction and nitrogen assimilation were higher in the T1 treatment group than in the CK during the compost warming period and the high-temperature period, which was in accordance with the previous physico-chemical performances. Nitrogen mineralization is the process of converting nitrogenous compounds into inorganic nitrogen under the action of microorganisms, and nitrogen mineralization in T1 was significantly higher than that in CK, suggesting that the addition of mycorrhizal agents enhanced the microbial activity in the compost and enhanced the degradation of proteins and other substances in the compost. Interestingly, the genes concerning nitrogen uptake were higher in T1 than in CK when the compost entered the maturity stage, indicating that the uptake capacity of nitrogen in the composting environment rose, which may also be the reason for the smaller nitrogen loss at the end of composting in the T1 group. The genes with significant differences were mainly *nar*I, *nir*K, *nrf* A, *nir*D, *nif* K, *nif* H, and *gln*A. *nif* K and *nif* H can fix ammonia in the environment to form ammonia nitrogen fixed in the compost, and the addition of microbial agents enhanced the expression of these nitrogen fixation gene expressions in compost. Notably, as the compost entered the high-temperature period, the expression of *nif* H began to decrease, while the expression of *nif* K began to increase. This indicates that the addition of functional bacterial agents can promote the expression of nitrogen-fixing genes. Simultaneously, it enables bacteria with nitrogen-fixing genes to grow during the high-temperature phase. In the mature phase of composting, the expression levels of both nitrogen-fixing genes show a decreasing trend. This also explains why T1 has higher total nitrogen and ammonium nitrogen content than CK in the early to middle stages of composting, which is consistent with the increase in nitrogen mineralization during the high-temperature phase as shown

in Figure 6d. During the high-temperature phase, the expression level of *nar*I in T1 is higher than in CK, which might be caused by the higher ammonium nitrogen content in the compost. When ammonia nitrogen accumulates in the compost, the expression product of *gln*A (glutamine synthetase) promotes the conversion of ammonia nitrogen to organic nitrogen. The relative abundance of *gln*A accounted for a relatively high percentage in T1; this also results in a higher total nitrogen content in T1 during the high-temperature and mature phases, and the nitrogen was immobilized in the form of organic nitrogen in the compost, which improved the quality of the compost. *nir*K expressed the nitrite reductase (NO-forming), which degrades nitrite to NO. *nir*K expression in T1 was less than that in CK during the composting process, which reduced the nitrogen loss caused by the emanation of some of the nitrogen when it was emitted as a gas. The incorporation of functional bacterial agents reduced NO emission and lowered nitrogen loss during the high temperature and maturation periods of the compost.

3.6. Relevance Analysis

The relationship between microbial communities, composting environments, and functional genes can be demonstrated by Mantel test analyses [47] (Figure 7a,b). The temperature was significantly and positively correlated with microbial communities and functional genes ($p < 0.05$); increasing temperature can increase microbial activity in the compost. The temperature as well as the duration of high temperatures were higher in T1 than in CK, which promoted the growth of microorganisms in T1. The positive correlations between pH, ammonia, nitrate nitrogen, ammonia, and functional genes were increased when microbial agent was added to the heap, which suggests that the addition of microbial agent indicates an improvement in the conversion rate of nitrogen. In both treatment groups, TN and pH were significantly negatively correlated ($p < 0.0.5$), while ammonia nitrogen and ammonia, and ammonia and temperature were significantly positively correlated ($p < 0.05$). The increase in temperature promoted the conversion of ammonium nitrogen to ammonia, and the emission of ammonia also led to nitrogen loss. It is worth noting that the addition of functional bacterial agents changed the relationship between nitrate nitrogen, pH, and TN in the heap, which could be due to the increased microbial activity leading to more expression of enzymes related to the nitrogen cycle and increased interconversion of various forms of nitrogen.

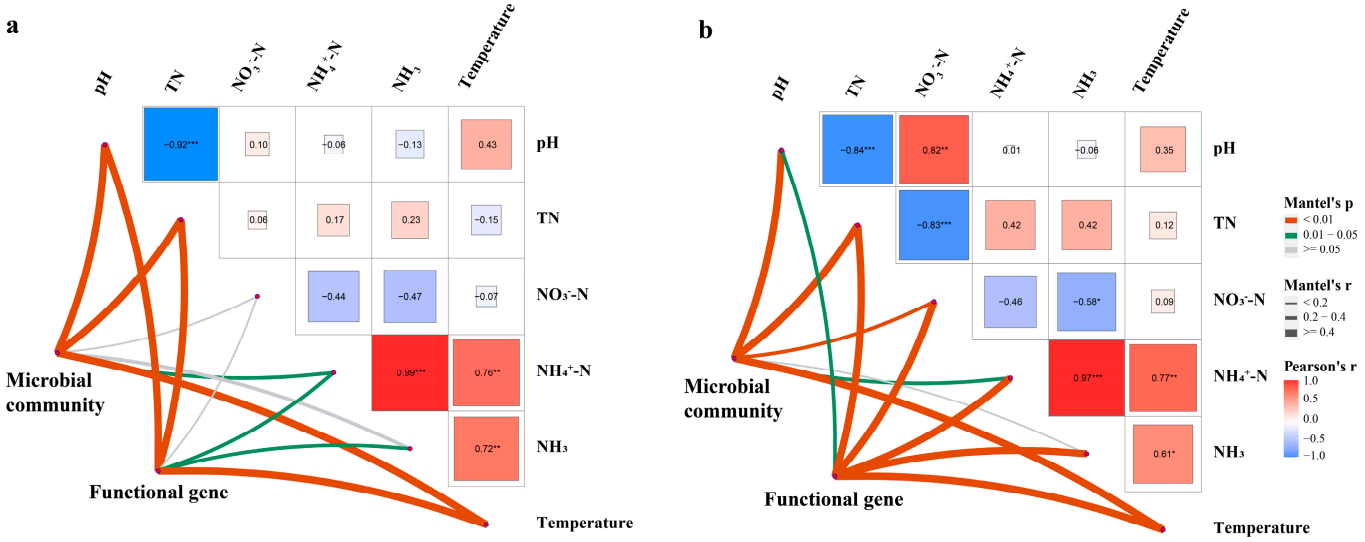

Figure 7. Correlation analysis of bacterial communities, nitrogen cycling functional genes, and physicochemical properties of compost: CK (**a**); T1 (**b**). (n = 6). "*" indicates $p < 0.05$, "**" indicates $p < 0.01$, "***" indicates $p < 0.001$.

4. Conclusions

This study showed that protein-degrading bacterial agents had a significant effect on nitrogen cycling genes in grain compost. The incorporation of the bacterial agent reduced the emission of ammonia in the grain husk compost. The incorporation of the microbial agent promoted the growth of the original microorganisms in the compost and stabilized the microbial community structure. Meanwhile, it improved alien organism biodegradation and metabolism in the compost, increased the expression of nitrogen fixation genes (*nif*K, *nif*H, and *gln*A), and enhanced the correlation between microbial community, compost environment, and functional genes. In conclusion, this study provides a theoretical basis for enhancing the types of microbial agent selected and improves the understanding of the response of functional genes to microbial agent in the composting process. It provides a theoretical basis for the efficient production of microbial agents and pollution reduction in chicken manure–husk composting and promotes the recycling of agricultural waste resources.

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References

1. Liu, Z.; Wei, Y.; Li, J.; Ding, G.-C. Integrating 16S RRNA Amplicon Metagenomics and Selective Culture for Developing Thermophilic Bacterial Inoculants to Enhance Manure Composting. *Waste Manag.* **2022**, *144*, 357–365. [CrossRef]
2. Wang, Z.; Xu, Y.; Yang, T.; Liu, Y.; Zheng, T.; Zheng, C. Effects of Biochar Carried Microbial Agent on Compost Quality, Greenhouse Gas Emission and Bacterial Community during Sheep Manure Composting. *Biochar* **2023**, *5*, 3. [CrossRef]
3. Ravindran, B.; Nguyen, D.D.; Chaudhary, D.K.; Chang, S.W.; Kim, J.; Lee, S.R.; Shin, J.; Jeon, B.-H.; Chung, S.; Lee, J. Influence of Biochar on Physico-Chemical and Microbial Community during Swine Manure Composting Process. *J. Environ. Manag.* **2019**, *232*, 592–599. [CrossRef]
4. Wang, Q.; Awasthi, M.K.; Ren, X.; Zhao, J.; Li, R.; Wang, Z.; Wang, M.; Chen, H.; Zhang, Z. Combining Biochar, Zeolite and Wood Vinegar for Composting of Pig Manure: The Effect on Greenhouse Gas Emission and Nitrogen Conservation. *Waste Manag.* **2018**, *74*, 221–230. [CrossRef]
5. Li, H.; Zhang, T.; Shaheen, S.M.; Abdelrahman, H.; Ali, E.F.; Bolan, N.S.; Li, G.; Rinklebe, J. Microbial Inoculants and Struvite Improved Organic Matter Humification and Stabilized Phosphorus during Swine Manure Composting: Multivariate and Multiscale Investigations. *Bioresour. Technol.* **2022**, *351*, 126976. [CrossRef]
6. Barrington, S. Effect of Carbon Source on Compost Nitrogen and Carbon Losses. *Bioresour. Technol.* **2002**, *83*, 189–194. [CrossRef]
7. Chan, M.T.; Selvam, A.; Wong, J.W.C. Reducing Nitrogen Loss and Salinity during 'Struvite' Food Waste Composting by Zeolite Amendment. *Bioresour. Technol.* **2016**, *200*, 838–844. [CrossRef]
8. Wei, Z.; Ahmed Mohamed, T.; Zhao, L.; Zhu, Z.; Zhao, Y.; Wu, J. Microhabitat Drive Microbial Anabolism to Promote Carbon Sequestration during Composting. *Bioresour. Technol.* **2022**, *346*, 126577. [CrossRef]
9. Liu, H.; Huang, Y.; Wang, H.; Shen, Z.; Qiao, C.; Li, R.; Shen, Q. Enzymatic Activities Triggered by the Succession of Microbiota Steered Fiber Degradation and Humification during Co-Composting of Chicken Manure and Rice Husk. *J. Environ. Manag.* **2020**, *258*, 110014. [CrossRef]
10. Chi, C.P.; Chu, S.; Wang, B.; Zhang, D.; Zhi, Y.; Yang, X.; Zhou, P. Dynamic Bacterial Assembly Driven by Streptomyces Griseorubens JSD-1 Inoculants Correspond to Composting Performance in Swine Manure and Rice Straw Co-Composting. *Bioresour. Technol.* **2020**, *313*, 123692. [CrossRef]
11. Zhang, Y.; Wei, Z.; Guo, J.; Zhang, S.; Zhao, L.; Pan, C.; Wang, L.; Zhang, R.; Chen, Y. Resource Utilization of Mink Manure: Functional Microbial Inoculation to Elevate the Bioavailability of Organic Nitrogen during Composting. *Bioresour. Technol.* **2022**, *353*, 127149. [CrossRef]

12. Liu, H.; Huang, Y.; Duan, W.; Qiao, C.; Shen, Q.; Li, R. Microbial Community Composition Turnover and Function in the Mesophilic Phase Predetermine Chicken Manure Composting Efficiency. *Bioresour. Technol.* **2020**, *313*, 123658. [CrossRef] [PubMed]
13. Li, H.-J.; Yang, Z.-H.; Zhang, C.-Y.; Shang, W.-W.; Zhang, T.-L.; Chang, X.-J.; Wu, Z.-S.; He, Y.-H. Effect of microbial inoculum on composting efficiency in the composting process of spent mushroom substrate and chicken manure. *J. Environ. Manag.* **2024**, *353*, 120145. [CrossRef] [PubMed]
14. Ji, Y.-H.; Cao, Y.-Z.; Wang, Y.; Wang, C.; Qin, Z.-H.; Cai, W.-R.; Yang, Y.; Yan, S.-D.; Guo, X.-H. Effects of adding lignocellulose-degrading microbial agents and biochar on nitrogen metabolism and microbial community succession during pig manure composting. *Environ. Res.* **2023**, *239 Pt 1*, 117400. [CrossRef] [PubMed]
15. Kong, W.; Sun, B.; Zhang, J.; Zhang, Y.; Gu, L.; Bao, L.; Liu, S. Metagenomic Analysis Revealed the Succession of Microbiota and Metabolic Function in Corncob Composting for Preparation of Cultivation Medium for *Pleurotus ostreatus*. *Bioresour. Technol.* **2020**, *306*, 123156. [CrossRef] [PubMed]
16. Qiao, C.; Ryan Penton, C.; Liu, C.; Shen, Z.; Ou, Y.; Liu, Z.; Xu, X.; Li, R.; Shen, Q. Key Extracellular Enzymes Triggered High-Efficiency Composting Associated with Bacterial Community Succession. *Bioresour. Technol.* **2019**, *288*, 121576. [CrossRef]
17. Yılmaz Tuncel, N. Stabilization of Rice Bran: A Review. *Foods* **2023**, *12*, 1924. [CrossRef] [PubMed]
18. McCaffrey, Z.; Cal, A.; Torres, L.; Chiou, B.-S.; Wood, D.; Williams, T.; Orts, W. Polyhydroxybutyrate Rice Hull and Torrefied Rice Hull Biocomposites. *Polymers* **2022**, *14*, 3882. [CrossRef] [PubMed]
19. Coronado, M.; Blanco, T.; Quijorna, N.; Alonso-Santurde, R.; Andrés, A. Types of Waste, Properties and Durability of Toxic Waste-Based Fired Masonry Bricks. In *Eco-Efficient Masonry Bricks and Blocks*; Elsevier: Amsterdam, The Netherlands, 2015; pp. 129–188.
20. Torres, L.F.; McCaffrey, Z.; Washington, W.; Williams, T.G.; Wood, D.F.; Orts, W.J.; McMahan, C.M. Torrefied Agro-industrial Residue as Filler in Natural Rubber Compounds. *J. Appl. Polym. Sci.* **2021**, *138*, 50684. [CrossRef]
21. Attia, N.F.; Afifi, H.A.; Hassan, M.A. Synergistic Study of Carbon Nanotubes, Rice Husk Ash and Flame Retardant Materials on the Flammability of Polystyrene Nanocomposites. *Mater. Today Proc.* **2015**, *2*, 3998–4005. [CrossRef]
22. Takaku, H.; Kodaira, S.; Kimoto, A.; Nashimoto, M.; Takagi, M. Microbial Communities in the Garbage Composting with Rice Hull as an Amendment Revealed by Culture-Dependent and -Independent Approaches. *J. Biosci. Bioeng.* **2006**, *101*, 42–50. [CrossRef] [PubMed]
23. Yu, H.; Zhao, Y.; Zhang, C.; Wei, D.; Wu, J.; Zhao, X.; Hao, J.; Wei, Z. Driving Effects of Minerals on Humic Acid Formation during Chicken Manure Composting: Emphasis on the Carrier Role of Bacterial Community. *Bioresour. Technol.* **2019**, *294*, 122239. [CrossRef] [PubMed]
24. Kim, Y.-J.; Choi, W.-J.; Lim, S.-S.; Kwak, J.-H.; Chang, S.X.; Kim, H.-Y.; Yoon, K.-S.; Ro, H.-M. Changes in Nitrogen Isotopic Compositions during Composting of Cattle Feedlot Manure: Effects of Bedding Material Type. *Bioresour. Technol.* **2008**, *99*, 5452–5458. [CrossRef] [PubMed]
25. Xie, Y.; Zhou, L.; Dai, J.; Chen, J.; Yang, X.; Wang, X.; Wang, Z.; Feng, L. Effects of the C/N Ratio on the Microbial Community and Lignocellulose Degradation, during Branch Waste Composting. *Bioprocess Biosyst. Eng.* **2022**, *45*, 1163–1174. [CrossRef] [PubMed]
26. Vázquez, M.A.; Soto, M. The Efficiency of Home Composting Programmes and Compost Quality. *Waste Manag.* **2017**, *64*, 39–50. [CrossRef] [PubMed]
27. Wang, X.-Y.; Liu, N.-Y.; Zeng, R.; Liu, G.; Yao, H.; Fang, J. Change of core microorganisms and nitrogen conversion pathways in chicken manure composts by different substrates to reduce nitrogen losses. *Environ. Sci. Pollut. Res. Int.* **2024**, *31*, 14959–14970. [CrossRef] [PubMed]
28. Nakasaki, K.; Hirai, H. Temperature Control Strategy to Enhance the Activity of Yeast Inoculated into Compost Raw Material for Accelerated Composting. *Waste Manag.* **2017**, *65*, 29–36. [CrossRef]
29. Chen, H.; Awasthi, S.K.; Liu, T.; Duan, Y.; Ren, X.; Zhang, Z.; Pandey, A.; Awasthi, M.K. Effects of Microbial Culture and Chicken Manure Biochar on Compost Maturity and Greenhouse Gas Emissions during Chicken Manure Composting. *J. Hazard. Mater.* **2020**, *389*, 121908. [CrossRef] [PubMed]
30. Nakasaki, K.; Yaguchi, H.; Sasaki, Y.; Kubota, H. Effects of PH Control On Composting of Garbage. *Waste Manag. Res. J. Sustain. Circ. Econ.* **1993**, *11*, 117–125. [CrossRef]
31. Agyarko-Mintah, E.; Cowie, A.; Van Zwieten, L.; Singh, B.P.; Smillie, R.; Harden, S.; Fornasier, F. Biochar Lowers Ammonia Emission and Improves Nitrogen Retention in Poultry Litter Composting. *Waste Manag.* **2017**, *61*, 129–137. [CrossRef]
32. Ma, Q.; Li, Y.; Xue, J.; Cheng, D.; Li, Z. Effects of Turning Frequency on Ammonia Emission during the Composting of Chicken Manure and Soybean Straw. *Molecules* **2022**, *27*, 472. [CrossRef] [PubMed]
33. Kong, Z.; Wang, X.; Liu, Q.; Li, T.; Chen, X.; Chai, L.; Liu, D.; Shen, Q. Evolution of Various Fractions during the Windrow Composting of Chicken Manure with Rice Chaff. *J. Environ. Manag.* **2018**, *207*, 366–377. [CrossRef] [PubMed]
34. Lai, T.V.; Ryder, M.H.; Rathjen, J.R.; Bolan, N.S.; Croxford, A.E.; Denton, M.D. Dissimilatory Nitrate Reduction to Ammonium Increased with Rising Temperature. *Biol. Fertil. Soils* **2021**, *57*, 363–372. [CrossRef]
35. Ksheem, A.M.; Bennett, J.M.; Antille, D.L.; Raine, S.R. Towards a Method for Optimized Extraction of Soluble Nutrients from Fresh and Composted Chicken Manures. *Waste Manag.* **2015**, *45*, 76–90. [CrossRef] [PubMed]
36. Zhang, L.; Li, L.; Pan, X.; Shi, Z.; Feng, X.; Gong, B.; Li, J.; Wang, L. Enhanced Growth and Activities of the Dominant Functional Microbiota of Chicken Manure Composts in the Presence of Maize Straw. *Front. Microbiol.* **2018**, *9*, 1131. [CrossRef] [PubMed]

37. Sun, Y.; Zhang, S.; Nie, Q.; He, H.; Tan, H.; Geng, F.; Ji, H.; Hu, J.; Nie, S. Gut Firmicutes: Relationship with Dietary Fiber and Role in Host Homeostasis. *Crit. Rev. Food Sci. Nutr.* **2023**, *63*, 12073–12088. [CrossRef] [PubMed]
38. Cui, H.; Ou, Y.; Wang, L.; Yan, B.; Bao, M. Tetracycline Hydrochloride-Stressed Succession in Microbial Communities during Aerobic Composting: Insights into Bacterial and Fungal Structures. *Chemosphere* **2022**, *289*, 133159. [CrossRef] [PubMed]
39. Qiu, X.; Zhou, G.; Chen, L.; Wang, H. Additive Quality Influences the Reservoir of Antibiotic Resistance Genes during Chicken Manure Composting. *Ecotoxicol. Environ. Saf.* **2021**, *220*, 112413. [CrossRef] [PubMed]
40. Zhang, W.; Yu, C.; Wang, X.; Hai, L. Increased Abundance of Nitrogen Transforming Bacteria by Higher C/N Ratio Reduces the Total Losses of N and C in Chicken Manure and Corn Stover Mix Composting. *Bioresour. Technol.* **2020**, *297*, 122410. [CrossRef]
41. Chen, X.; Du, G.; Wu, C.; Li, Q.; Zhou, P.; Shi, J.; Zhao, Z. Effect of Thermophilic Microbial Agents on Nitrogen Transformation, Nitrogen Functional Genes, and Bacterial Communities during Bean Dregs Composting. *Environ. Sci. Pollut. Res.* **2022**, *29*, 31846–31860. [CrossRef]
42. Duan, Y.; Awasthi, M.K.; Yang, J.; Tian, Y.; Li, H.; Cao, S.; Syed, A.; Verma, M.; Ravindran, B. Bacterial Community Dynamics and Co-Occurrence Network Patterns during Different Stages of Biochar-Driven Composting. *Bioresour. Technol.* **2023**, *384*, 129358. [CrossRef] [PubMed]
43. Che, J.; Bai, Y.; Li, X.; Ye, J.; Liao, H.; Cui, P.; Yu, Z.; Zhou, S. Linking Microbial Community Structure with Molecular Composition of Dissolved Organic Matter during an Industrial-Scale Composting. *J. Hazard. Mater.* **2021**, *405*, 124281. [CrossRef] [PubMed]
44. Tan, W.; Huang, C.; Chen, C.; Liang, B.; Wang, A. Bioaugmentation of Activated Sludge with Elemental Sulfur Producing Strain *Thiopseudomonas denitrificans* X2 against Nitrate Shock Load. *Bioresour. Technol.* **2016**, *220*, 647–650. [CrossRef] [PubMed]
45. Wu, D.; Wei, Z.; Gao, X.; Wu, J.; Chen, X.; Zhao, Y.; Jia, L.; Wen, D. Reconstruction of Core Microbes Based on Producing Lignocellulolytic Enzymes Causing by Bacterial Inoculation during Rice Straw Composting. *Bioresour. Technol.* **2020**, *315*, 123849. [CrossRef] [PubMed]
46. Tu, Q.; Lin, L.; Cheng, L.; Deng, Y.; He, Z. NCycDB: A Curated Integrative Database for Fast and Accurate Metagenomic Profiling of Nitrogen Cycling Genes. *Bioinformatics* **2019**, *35*, 1040–1048. [CrossRef]
47. Shi, M.; Song, C.; Xie, L.; Zhang, G.; Wei, Z. Role in Aromatic Metabolites Biodegradation and Adverse Implication of Denitrifying Microbiota in Kitchen Waste Composting. *Environ. Microbiome* **2023**, *18*, 44. [CrossRef]