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# Combined Use of Biochar and Microbial Agents Can Promote Lignocellulosic Degradation Microbial Community Optimization during Composting of Submerged Plants

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**Abstract:** Aerobic composting is one of the methods for the resource utilization of submerged plant residues. This study investigated the effects of biochar, wetland sediments and microbial agents added individually or combined on the humification process, lignocellulose degradation and microbial communities during *Ceratophyllum demersum* and *Potamogeton wrightii* composting. The results showed that the addition of wetland sediment and biochar was found to significantly elevate the composting temperature and humification of compost products. The average content of lignin in wetland sediment and/or biochar treatments was 12.2–13.5%, which was higher than the control group (10.9–11.45%). Compared with the organic matter (19.4%) and total nitrogen concentration (35.3%) of compost treated with complex microbial agent treatments, the homemade microbial agents significantly increased the values by 22.1% and 41.0%, respectively. By comparing the differences in microbial communities among different treatments, the sediments and homemade agents demonstrated greater increases in activity and diversity of lignocellulose degradation-related microbes, especially for *Truepera* and *Actinomarinale*. Humus component and temperature were the most critical parameters influencing the changes in the bacterial community. Based on these results, a combination of biochar and homemade agents was a promising additive for an effective composting strategy, and sediment was identified as a potential control of bacterial diversity in wetland plant compost.

**Keywords:** submerged plants compost; biochar; lignocellulose degradation; microbial agents; microbial community



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## 1. Introduction

Aquatic plants constitute unique ecological environment elements of wetlands, which become a rich source of biomass raw materials; however, due to untimely harvest and inadequate management, they generate a rich source of water nutrients and cause enormous pressure on the environment. Accumulation and decay of wetland aquatic vegetation residues in water results in increased odor, nitrogen, phosphorus and some organic matter and release of various sulfides (thiol, methyl sulfide, dimethyl disulfide, etc.), causing ecological environment degradation [1]. The concentrations of ammonia nitrogen ($NH_4^+$-N), total phosphorus (TP) and chemical oxygen demand (COD) in the initial stage of lodging and rot decomposition increase sharply, and the pH value and concentration of dissolved oxygen will reduce, which deteriorates the water quality and has a great impact on the

physical and chemical properties of the sediment [2]. Therefore, it is an urgent topic to study the problems caused by the accumulation and decay of submerged plants. Aerobic composting is one of the effective technologies of solid waste recycling treatment and has been widely applied in the reduction and harmless treatment of waste [3,4]. It will be an effective method for the resourceful treatment of aquatic plant residue.

Previous research has indicated that the addition of biochar [5], acid and alkaline substances [6] significantly improved composting efficiency. This is attributed to the increased reaction space and improved ventilation that facilitate the biochemical composting process [7] or the provision of optimal initial conditions for beneficial microorganisms involved in composting. Mineral additives such as phosphogypsum [8], montmorillite [9], illite and limestone [10] serve as conditioning agents that regulate pH, porosity and electrical conductivity during composting while promoting the growth of thermophilic microorganisms. Moreover, the addition of minerals containing specific metal elements enhances the bioactivities of relevant microorganisms; in particular, the inclusion of manganese [11,12] and iron minerals [13,14] substantially diminishes the anaerobic reactions and minimizes the loss of vital nutrients.

Microbial agents, due to their capacity to accelerate decomposition and other specialized functions, could serve as an excellent supplement for optimizing the composting process. For instance, white rot fungi have the capability to accelerate lignocellulose material composting and play a direct role in the initial phase of mineralization. This, in turn, affects colonization by other microorganisms [15], while the actinomycetes are known to prolong the high-temperature period, increasing cellulose degradation and humus formation [16]. In addition, *Pichia* species thrive in composting environments with acidic initial conditions [17], and compost with *B. thuringiensis* could produce potential insecticidal effects [18]. Finally, extensive research is typically conducted to explore the use of various additives for optimizing the composting process using different raw materials [19]. The influence of different additives and microbial agents on the composition of microbial communities has emerged as a hot research topic [7,20–22]. Nevertheless, investigation into the transformation contribution of microorganisms to various submerged plant components remains unclear during composting under different microbial agent amendment strategies. Therefore, we innovatively proposed the effects of various microbial inoculation strategies on the co-composting process of submerged plant lignocellulose residues and animal manure.

Baiyangdian Lake is the largest semi-closed shallow water lake in the North China Plain. The lake area is predominantly occupied by large aquatic plants covering approximately 60% of the waters, and the submerged plant species are abundant. Submerged macrophytes contain a high concentration of nitrogen, phosphorus, potassium and other nutrients. Composting presents an effective method for recycling plant resources and mitigating water pollution. In order to regulate the microenvironment required for composting and optimize the structure of degrading microorganisms, the objectives of this study were to (i) characterize physicochemical properties of compost at different maturity stages, exploring the potential of sediment and biochar as composting additives, (ii) investigate the changes in abundance and diversity of the bacterial community resulting from different microbial agent addition strategies, and (iii) identify the correlation between functional bacterial community composition and changes in plant compost components. This composting incubation process will provide the basic information and technical reference for the utilization of submerged plant resources and optimizing the application of additives in composting production.

## 2. Materials and Methods

### *2.1. Raw Materials*

The submerged plants (*Ceratophyllum demersum* and *Potamogeton wrightii*) and wetland surface sediment for composting experiments were obtained from Baiyangdian Lake in August 2022. Dry chicken manure (purchased from Nanjing Easy Recycling Agricultural

Technology Co., Ltd., Nanjing, China) and urea (Cangzhou Zhengyuan Chemical Fertilizer Co., Ltd., Cangzhou, China) were used to adjust compost carbon–nitrogen ratio (C/N), while the biochar was straw biochar, purchased from Pingdingshan Tannuo Environmental Protection Material Co., Ltd. (Pingdingshan, China). The complex microbial agents (group C) were purchased from Nongkang Storage Trading Co., Ltd. (Xinxiang, China), which contained *lactic acid bacteria* and *yeast*, and the homemade microbial agent (group H) contained *bifidobacteria*, *lactobacillus*, *Bacillus* and other microorganisms.

The experimental procedure for cultivating the homemade microbial agent in this study involved dissolving and evenly mixing 2.5 g effective microorganism (EM) bacteria and 500 g of brown sugar in a plastic bucket containing 9 L of distilled water. The plastic bucket was sealed and placed in a 32 °C incubator for 7 d to obtain the bacterial stock. The original solution was diluted with distilled water at a ratio of 1:10 to achieve the desired bacterial concentration. The properties of composting raw materials are detailed in Table 1.

**Table 1.** Primary properties of raw materials in composts.

| Material | TOC (%) | TN (%) | C/N | Cellulose (%) | Hemicellulose (%) | Lignin (%) |
|---|---|---|---|---|---|---|
| Submerged plant | 84.62 ± 5.26 | 1.33 ± 0.09 | 63.62 | 15.8 ± 0.43 | 29.2 ± 0.76 | 8.90 ± 0.46 |
| Dry chicken dung | 63.11 ± 3.23 | 3.62 ± 0.22 | 17.43 | - | - | - |
| Wetland sediment | 39.64 ± 2.17 | 2.63 ± 0.19 | 15.13 | - | - | - |
| Compost material | 76.36 ± 4.35 | 2.69 ± 0.18 | 28.39 | 7.62 ± 0.26 | 15.9 ± 0.87 | 4.88 ± 0.39 |

Note: Data are reported as mean ± SD with $n$ = 3. TOC, total organic carbon; TN, total nitrogen; -, not detected due to low content. Compost materials consisted of submerged plants mixed with dry chicken manure in a 7:3 ratio; the submerged plants were *Ceratophyllum demersum* and *Potamogeton wrightii* mixed 1:1.

### 2.2. Composting Design and Sampling

Eight treatment groups were established, each incorporating various dosages ($w/w$) of sediment (0, 10%), biochar (0, 2%) and microbial inoculum (complex microbial agent, i.e., group C; homemade microbial agent, i.e., group H) added to the raw materials; the composting treatment groups are shown in Table 2. The composting process was carried out using submerged plants, which were air-dried and crushed into small sections of about 1 cm, then sterilized in a high-temperature sterilization pot at 120 °C and 0.1 M Pa for 20 min. Dry chicken manure and urea were added to act as the bulking agent to adjust the C/N to 28–30, and the addition of wetland sediment and biochar was 10% and 2% of the total dry weight of the material, respectively. The raw materials were placed in a plastic cuboid container (32 cm × 22 cm × 16 cm) and incubated in a thermostat incubator to keep the temperature above 50 °C for 36 days. The moisture content of each container was kept at about 65%. The experimental containers were covered with plastic film with holes to ensure ventilation and reduce water evaporation. At the same time, according to the change in the temperature and humidity, compost in each container was manually turned over every 6 days and supplemented with the bacteria agent. The five-point sampling method was used on days 0, 6, 12, 18, 24, 30 and 36, and 200 g samples were collected at the front, back, left, right and center of each container and evenly mixed. The mixed samples were divided into two parts; one sample was air-dried for determination of physicochemical properties, and the other was stored at room temperature to determine the seed germination index. The fresh samples from days 0, 12 and 36 (8 treatments and each replicated thrice) were stored at −80 °C to determine the genetic diversity of the microbial community; sub-samples were taken from five sampling points and mixed into a composite sample. The samples for microbial analyses were labelled as treatment-d0, -d12 and -d36, representing the prophase, metaphase and anaphase, respectively.

**Table 2.** Standard table of experimental groups.

| Group | Wetland Sediment (%) | Biochar (%) | Bacterial Type |
|---|---|---|---|
| CK-C | 0 | 0 | C |
| S-C | 10 | 0 | C |
| B-C | 0 | 2 | C |
| SB-C | 10 | 2 | C |
| CK-H | 0 | 0 | H |
| S-H | 10 | 0 | H |
| B-H | 0 | 2 | H |
| SB-H | 10 | 2 | H |

Note: CK is the control group, S represents wetland sediment, B represents biochar, SB represents wetland sediment + biochar, C represents complex microbial agent, and H represents homemade microbial.

### *2.3. Measurements of Physical and Chemical Properties*

Daily temperatures were taken above, in the middle and under compost at a fixed time, and the average of these was taken as the temperature of the reactor. Total organic carbon (TOC) was determined by the external heating method [23]. Total nitrogen (TN) was determined by the Kjeldahl method [24]. The pH and electric conductivity (EC) values were determined by a pH/EC instrument (pH/ISE meter, MP522, Shanghai San-Xin Instrumentation, Inc.,Shanghai, China). The determination of hemicellulose, cellulose, and lignin was made using the Van Soest washing method [25]. Fulvic acid (FA) and humic acid (HA) were measured using the method reported by NY/T 1971–2010 [26]. The fresh sample and distilled water were mixed at 1:10 (g:mL) for 2 h, and the purple locust seeds were grown in a 25 °C incubator for 48 h. Then, germination indexes (GI) were measured.

### *2.4. Spectroscopic Metrics Characterization*

The freeze-dried compost sample and potassium bromide (KBr) were mixed at a ratio of 1:100, then ground and mixed in an agate mortar under an infrared lamp, and the sample powder was compressed to form a disc. The Fourier conversion infrared spectroscopy analyzer (FTIR, Perkin Elmer Frontier) was used to determine the main functional groups involved in the compost reaction, with a wave number range ranging from 4000 to 400 $cm^{-1}$. A scanning electron microscope (SEM, Czech TESCAN MIRA LMS) was used for appearance shooting and energy spectrum scanning to observe the composting surface morphology and determine the elemental structure. The samples were previously metallized with a 10 nm layer of gold.

### *2.5. DNA Extraction and High-Throughput Sequencing*

Bacterial 16S rRNA genes were amplified using primers 338F (5′-ACTCCTACGGGA GGCAGCAG-3′) and 806R (5′-GGACTACCAGGGTATCTAAT-3′). PCR was performed in triplicate. The PCR products were extracted from a 2% agarose gel and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, USA) according to the manufacturer's instructions and quantified using a Quantus™ Fluorometer (Promega, Madison, WI, USA). The V4 region of 16S rRNA was sequenced using the Illumina MiSeq PE300 platform (Illumina, San Diego, CA, USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China).

The raw sequence reads were demultiplexed, quality-filtered by fastp (https://github.com/OpenGene/fastp, version 0.20.0) and merged by FLASH (http://www.cbcb.umd.edu/software/flash, version 1.2.7). We used UPARSE (http://drive5.com/uparse/, version 7.1) to cluster operational taxonomic units (OTUs) with 97% similarity and to identify and remove chimeric sequences. The taxonomy of each representative OTU sequence was analyzed by RDP Classifier (http://rdp.cme.msu.edu/, version 2.2) against the 16S rRNA database (v138, https://www.arb-silva.de/) and Unite database (Release 8.0 https://unite.ut.ee/) using a confidence threshold of 70%.

### *2.6. Statistical Analysis*

Experimental results were determined by triplicate replicates. The final results were shown as the average with standard deviations. The arrangement, statistical analysis and chart drawing of the experimental data were performed in Excel 2016 (Microsoft Inc., Redmond, WA, USA), SPSS 26.0 (SPSS Inc., Chicago, IL, USA) and Origin 2021b (Origin Lab Inc., Northampton, MA, USA) software, respectively. Analysis of variance (ANOVA) was used to evaluate the effects of different treatments on compost properties. One-way ANOVA was used for sample data, and the differences were considered statistically significant at $p < 0.05$ using Tukey's test. Redundancy analysis (RDA) was performed using CANOCO 5.0.

## 3. Results and Discussion

### *3.1. Composting Properties*

Temperature can be used to assess compost maturity, which is also an important inducing factor for microbial changes at different composting stages. No significant difference in the temperature change trend was found for all treatments (Figure S1). The temperature rapidly rose to the maximum in the initial stage due to thermophilic microorganisms decomposing organic matter and multiplying rapidly, as well as producing a large amount of heat and gas during this period. The highest temperature of the reactors was 53.1 to 54.8 °C, and subsequently, the temperature fluctuated down and gradually stabilized. EC mainly reflects the concentration of soluble salt present in the stack and can be used as an indicator to determine whether the compost restricts crop growth. The EC value increased in the early stage of composting (Figure 1a) for the reason that some organic matter degraded into soluble small molecular substances, such as ammonium salt, phosphate, small molecule organic acids and other dissolved organic matter. With the progress of the composting reaction, the EC values of different treatments decreased slightly and tended to stabilize as the degradation of organic acid, the formation of humus and $NH_3$, and the emission of $CO_2$ occurred. At the end of the composting, the EC values of different treatments ranged from 2.74 (S-H) to 3.36 (CK-C) mS·cm$^{-1}$, which all met the appropriate EC (<4 mS·cm$^{-1}$) for marking compost maturity and would not inhibit plants [27].

Dynamic profiles of the pH for different treatments were similar (Figure 1b). The stable pH value was 7.5–8.0 in the late stage of composting, which meets the necessary conditions for compost maturity [28]. The initial pH of all treatments showed an increasing trend in the reaction process at days 0–6, mainly because the microorganisms degraded the nitrogenous organic matter and produced a large amount of $NH_4^+$-N under high temperature. In the later stage, the pH value was relatively stable and ultimately between 7.4 and 7.9. The main reason for this is that the porosity of the stack decreased with the decomposition of composting materials, a large amount of organic matter transformed into organic acids of small molecules and gradually accumulated at the same time, and the carbon dioxide released by microbial respiration also led to a decrease in pH [29]. This result was consistent with previous studies of biomass waste compost [15].

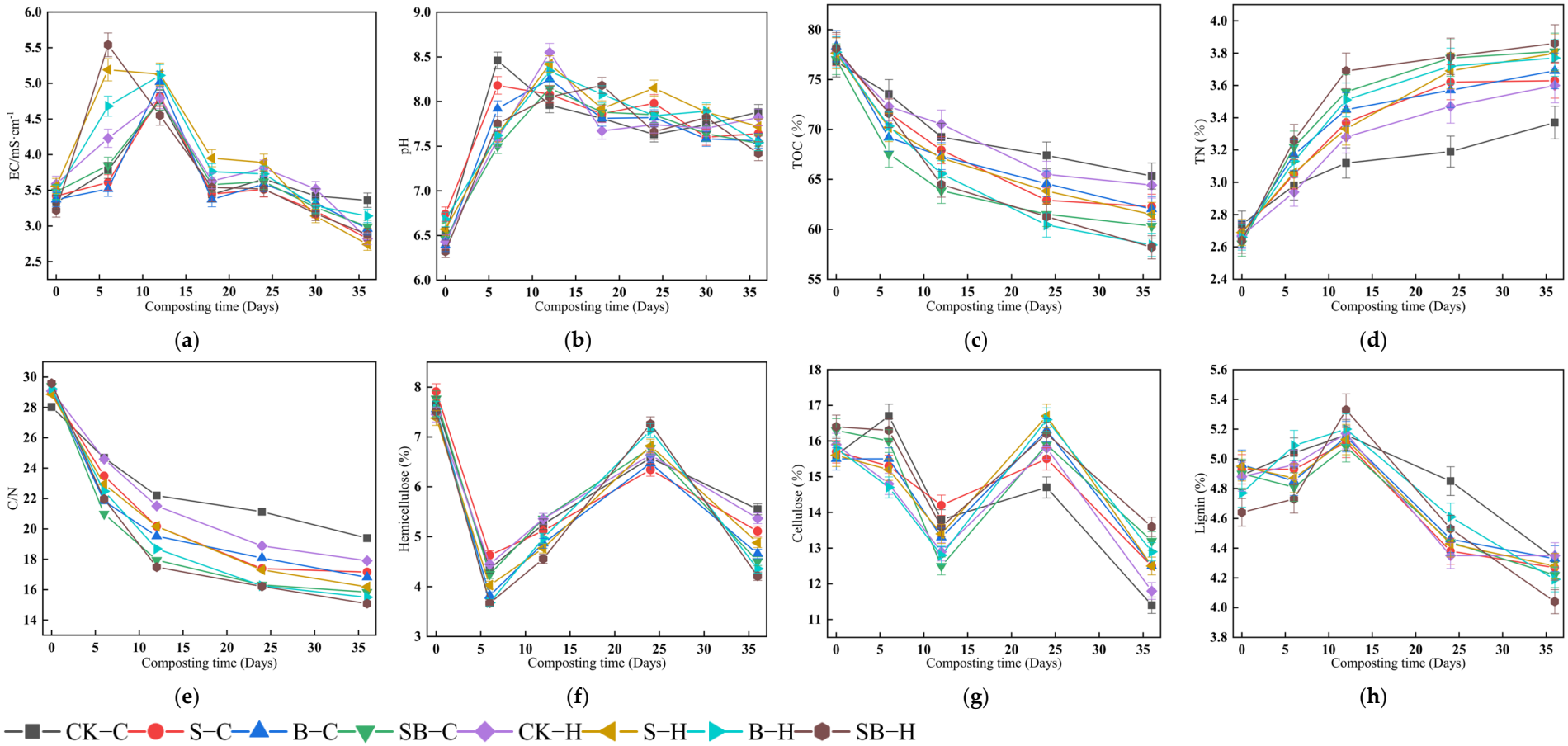



**Figure 1.** Changes in (**a**) EC, (**b**) pH, (**c**) total organic carbon (TOC), (**d**) total nitrogen (TN), (**e**) C/N, (**f**) hemicellulose, (**g**) cellulose, and (**h**) lignin during composting in different treatments. Error bars represent standard deviations (SDs) for each test day ($n$ = 3).

Total organic carbon (TOC) represents the organic carbon content in the compost sample and serves as a source of carbon for microbial metabolism, which exhibited a gradual decreasing trend in the composting process [30]. Generally, solid organic matter forms dissolved organic matter (DOM) that is more easily utilized by microorganisms through metabolic activities, and the small molecule organic matter reorganizes to form humus with a stable structure [31]. During the initial 0–12 days of composting, the TOC in all samples decreased significantly, possibly resulting in the volatilization and rapid loss of carbon in the form of gas due to vigorous microbial activity (Figure 1c). However, the TOC contents showed significant differences due to the different organic matter degradation and humification processes with various conditioners (Table S1). A higher degree of TOC degradation was observed in the SB-H and SB-C treatments, and the average content of TOC in the late composting period was 58.20% and 60.33%, respectively, indicating that the synergy of wetland sediment and biochar additives was more conducive to the decomposition of TOC. Additionally, the loss of TOC content in the biochar treatment was greater than that of the wetland sediment. At the end of composting, the mass fraction of TOC was above 45%, which met the standard of organic fertilizer.

The composting process was also accompanied by nitrogen loss for the decomposition of organic matter, but the nitrogen continued to undergo ammonification, nitrification and denitrification, as well as the condensation of solid matter; the mass fraction of TN in the pile volume generally showed an increasing trend. When the composting completed, the TN content in each treatment ranged from 3.37 to 3.86% (Figure 1d), and the combination of sediment and biochar significantly increased the nitrogen content due to the abundant nitrogen source provided by sediments and the reduction of nitrogen loss facilitated by biochar. The carbon to nitrogen (C/N) ratio is another important indicator for judging compost maturity. When the C/N < 20, the compost can be considered to be decomposed [32]. At the end of composting for each treatment, C/N was between 15.08 and 19.39 for reaching the maturity standard (Figure 1e).

As shown in Figure 1f and Table S1, the hemicellulose content in composts also had a fluctuating decreasing trend. In the early 6d of composting, the average hemicellulose concentration decreased from 7.38–7.91% to 4.21–5.55% because hemicellulose can be the preferred carbon source, as it is easily degradable organic matter. Hemicellulose in aquatic plants is the most easily degradable part due to its relatively simple structural characteristics, and its degradation directly affects the composting process and humification degree of submerged plants [33]. At days 24–36, microbial agent supplementation accelerated the degradation of hemicellulose, and the hemicellulose content decreased slightly in the late compost period. At the end of composting, the hemicellulose in both wetland sediment and biochar addition treatments showed a lower concentration of hemicellulose, and the change in hemicellulose in the biochar treatment groups was significantly more obvious than that in the sludge treatment group ($p < 0.05$). The variation of cellulose content in different compost treatments showed a similar trend as hemicellulose. After 36 d of composting, the average content of cellulose in each treatment was basically stable between 11.4% and 13.2%. The results showed that the average content of cellulose for the control group was lower than that in the other treatments, which was in contrast to hemicellulose treatment, likely due to the synergistic effect of the microbial agent with wetland sediment or biochar not being able to promote the decomposition of cellulose. In addition, compared with hemicellulose and cellulose, lignin has a more complex structure, and the molecular structure of lignin is more difficult for microorganisms to degrade. The microbial degradation process of lignin mainly occurs in the high-temperature and cooling decay stage. At the end of composting, the average content of lignin in each treatment ranged from 4.04 to 4.35%. Biochar and wetland sediment played an important role in lignin degradation, as the lignin in the SB-H treatment (4.04%) was significantly lower than that in the control treatments (CK-C 4.33% and CK-H 4.35%). This could be attributed to the structural properties of biochar being beneficial to the survival of microorganisms, as well

as a richer and higher-quantity microbial community in the wetland sediment, producing more enzymes conducive to lignin decomposition [16,19].

For the other important indexes for evaluating the quality of compost, the HA contents showed an overall increasing trend, which was basically stable at 4.82% to 5.86% in each treatment (Figure 2a). Among them, the HA in the SB-C treatment group ranged from 3.16% at the beginning to 5.73% at the end of composting, which was significantly higher than that in the S-C treatment group (47.00%). Due to the relatively small molecular weight and simple structural characteristics, FA in the raw material was greatly decomposed by microorganisms. The decomposition of FA under the microbial action and the rate of disintegration or polymerization into HA affected the dynamic change in FA content [10]. At the end of the composting, the FA content of each treatment was 2.41% to 2.66% (Figure 2b). It is generally believed that when HA/FA is greater than 1.9, the conversion of HA and FA tends to be stable. The HA/FA of each treatment showed an increasing trend, and the HA/FA value ranged from 1.9 to 2.4 at the end of this compost experiment (Figure 2c). The treatment groups with the highest humification were SB-H and SB-C, indicating that the addition of wetland sediment, biochar and microbial agents could promote humification and polymerization of submerged plant composting, while the microporous biochar structure provided more space for microbial growth and reproduction [15]. Wetland sediment could significantly increase the diversity and abundance of microorganisms, and the homemade microbial agent promoted the degradation of refractory compounds. Therefore, a synergistic effect between additives effectively promoted the formation of HA and FA degradation, improving the degree of heap humification [3].

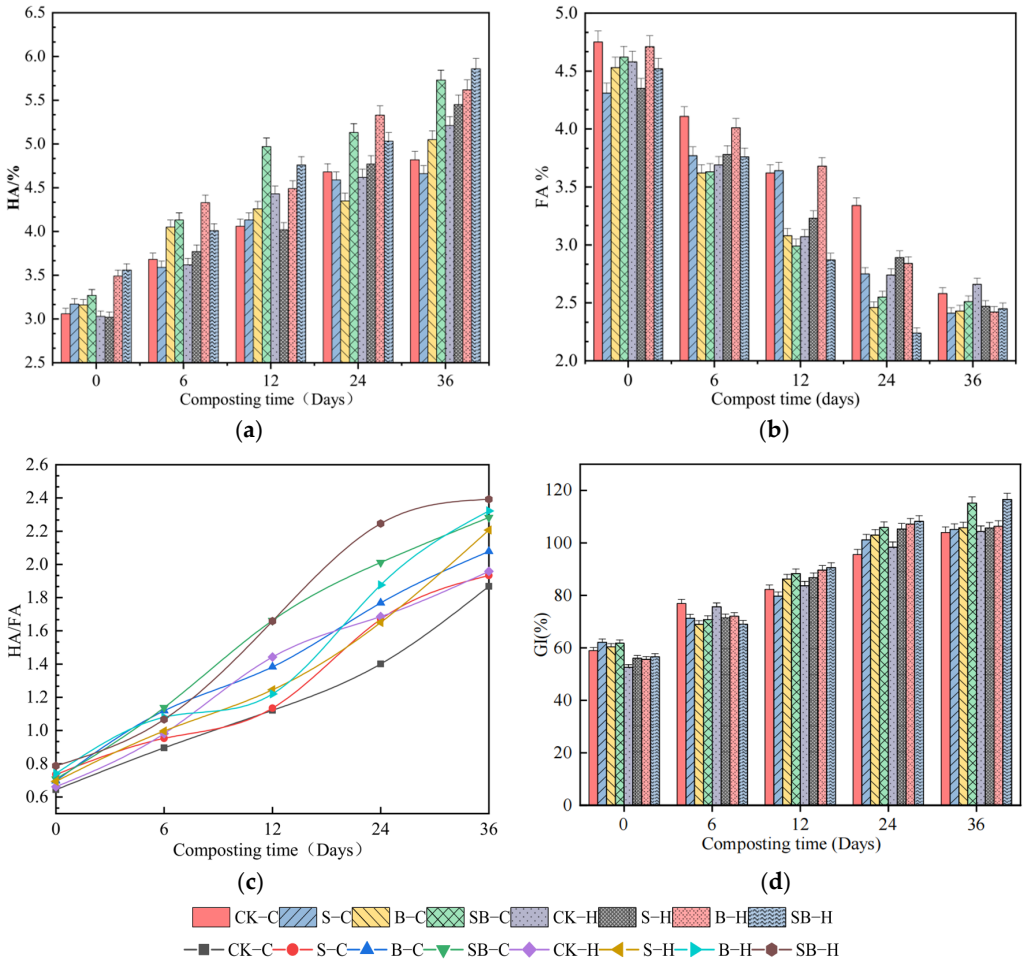



**Figure 2.** Changes in HA, FA, HA/FA and seed germination index (**a**–**d**) during composting in different treatments. Error bars represent standard deviations (SDs) for each test day ($n$ = 3).

The germination index (GI) is an important biological indicator for the evaluation of the maturity and biotoxicity of compost. A GI > 80% can be suggested as an indicator of the disappearance of phytotoxicity, and a GI > 100% is considered to have a positive effect on plant seed development [8]. After 36 days of composting, the seed germination index increased significantly to 104.00–117.00% (Figure 2d). Similarly, the combination of wetland sediment and biochar had the greatest promoting effect on seed germination.

### *3.2. Infrared and SEM Characterization*

As seen in the FTIR analysis of submerged plant composts presented in Figure 3, the humus contained in different raw material compost products may have similar structural composition and functional groups [34]. The main absorption zones of various composts were identical, indicating that the functional groups were not significantly different, and the disparity in peak absorption intensity reflected the difference in the characteristic functional groups of organic matter in the process of adding different regulating agents. The characteristic absorption peaks specific to the submerged plant composts were predominantly concentrated at 3400, 2930, 1650, 1430, 1030, 870 and 460 $cm^{-1}$, with their intensity closely related to the functional group content. At 3400 $cm^{-1}$, the O-H absorption peaks increased for the S-C, B-C, SB-C, CK-H and SB-H groups, which indicated the breakdown of lignocellulose and an increase in small molecules within the treated stack. Conversely, for CK-C, S-H, B-H and B-H groups, there was an indication of a decrease in the content of small polysaccharides [35]. The stretching band at 2930 $cm^{-1}$ decreased, except for SB-C and SB-H, indicating a reduction in the aliphatic compound content. Absorption bands at 1650 $cm^{-1}$ were characteristic of carboxylate lipid compounds or ketones, which formed during submerged plant composting, probably because of the decomposition of organic matter at high temperatures. Moreover, the functional groups present in the biochar have complexed with other substances in the pile [36]. The change in spectral peak at 1430 $cm^{-1}$ corresponded to the shift in aromatic substances during the composting process, which mainly arose from fluctuations in the relative abundance of complex aromatic functional groups in humic acid and the increased degree of humification and polymerization of organic matter by microorganisms [37]. However, the adsorption bands observed here were also associated with the decomposition of cellulose, sugar and other organic matter, resulting in the formation of carbonate root and subsequent carbonate–metal ion complexation. In addition, the stretching band at 1030 $cm^{-1}$ increased for CK-C, S-C, CK-H, S-H and B-H groups, signifying an augmented presence of polysaccharide substances in compost products. The alteration in the peak at 870 $cm^{-1}$ also suggested the generation of $CO_3^{2-}$ during the compost fermentation process.

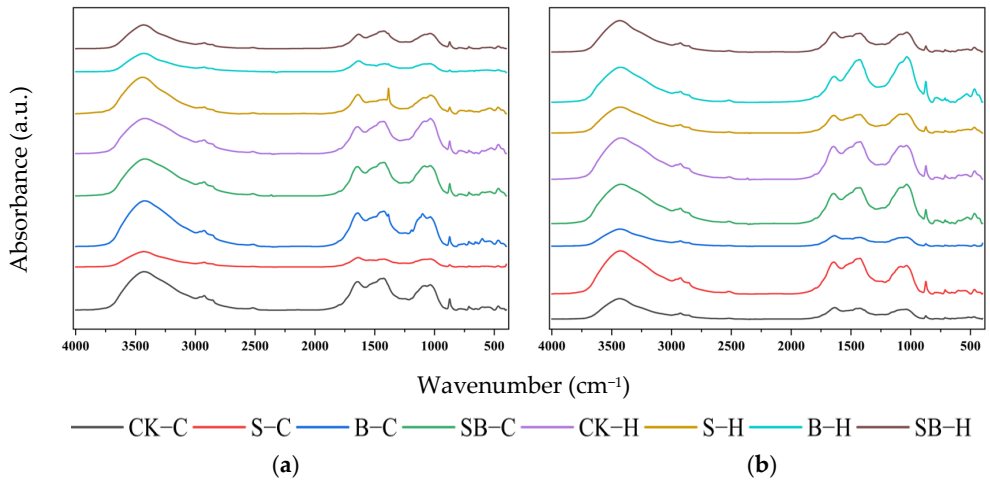



**Figure 3.** FTIR spectra of composting in different treatments. (**a**) Represents 0 d, (**b**) represents 36 d. Note: CK-C, S-C, B-C, SB-C, CK-H, S-H, B-H and SB-H are compost samples.

The SEM images (Figure 4a–h) reveal the irregular surface of composts amplified 6000 times. The particles of final compost were larger in the CK-C and CK-H treatment groups, particularly for the SB-C treatment. On the contrary, the SB-H treatment groups exhibited the smallest particles. Compost obtained from wetland sediment as the conditioner was relatively smooth and regular, while the compost with biochar as an additive displayed irregularities and a more diverse surface morphology. Studies have indicated that biochar can be enriched by absorbing leachate on its surface and pores, and co-composting with biochar has been found to help reduce the loss of plant-available nutrients that occurs during composting [35]. In contrast, the microscopic surface pore structure of the SB-C and SB-H groups was observed to be highly varied and intricate, contributing to a significant increase in specific surface area, which is likely to have a considerable positive impact on the adsorption and fixation of nutrients.

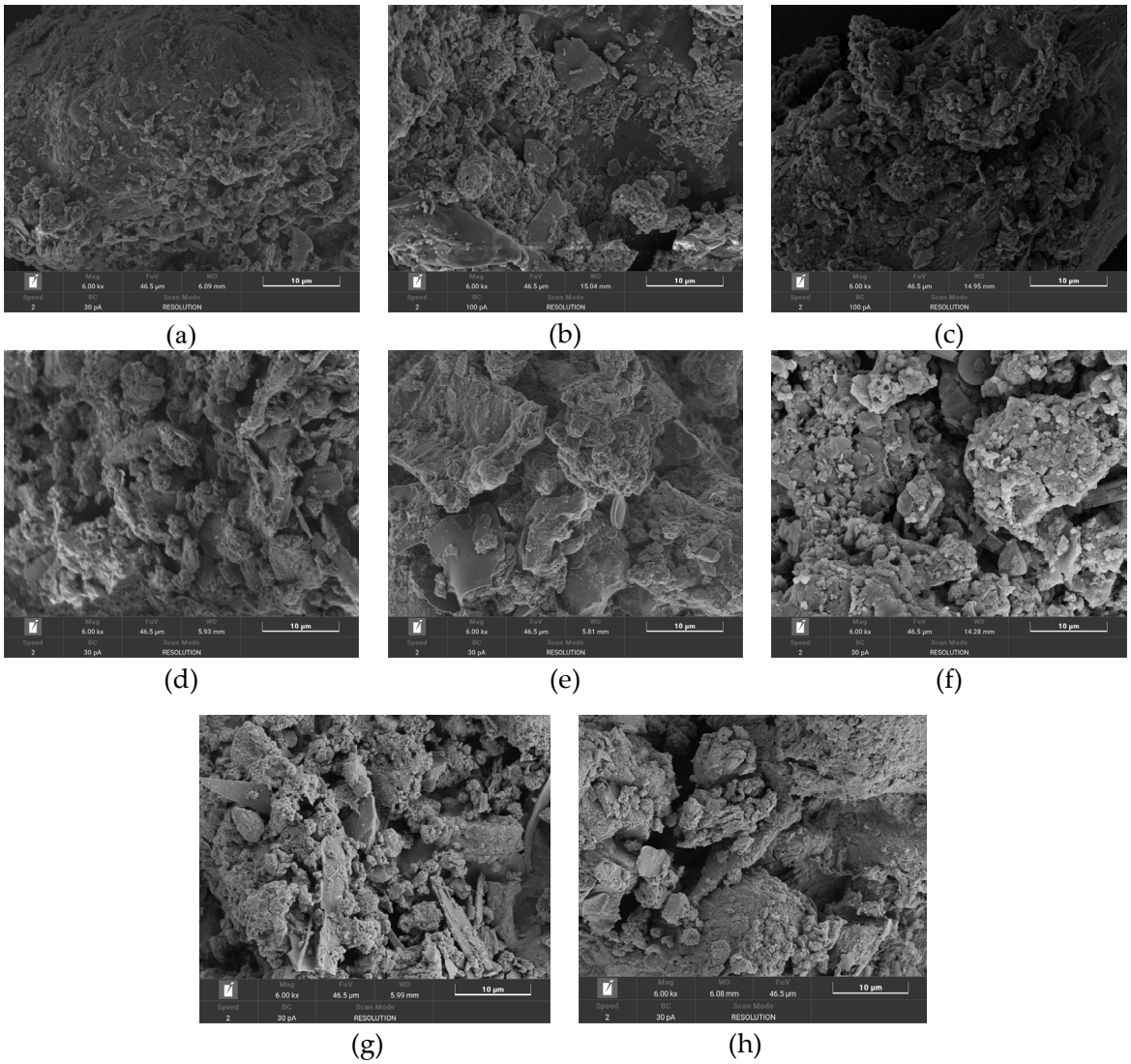



**Figure 4.** Scanning electron micrographs of composting in different treatments. Note: Shows the SEM view at the end of the different treatment compost at a 10 μm scale. (**a**–**h**) CK-C, S-C, B-C, SB-C, CK-H, S-H, B-H and SB-H are compost samples.

### *3.3. Changes in Composition and Abundance of Microbial Community during Composting*

#### 3.3.1. Microbial Community Richness and Diversity

A total number of 1,077,933 high-quality 16S rRNA sequences were obtained from compost samples of all treatments, leading to the identification of 1382 OTUs in any

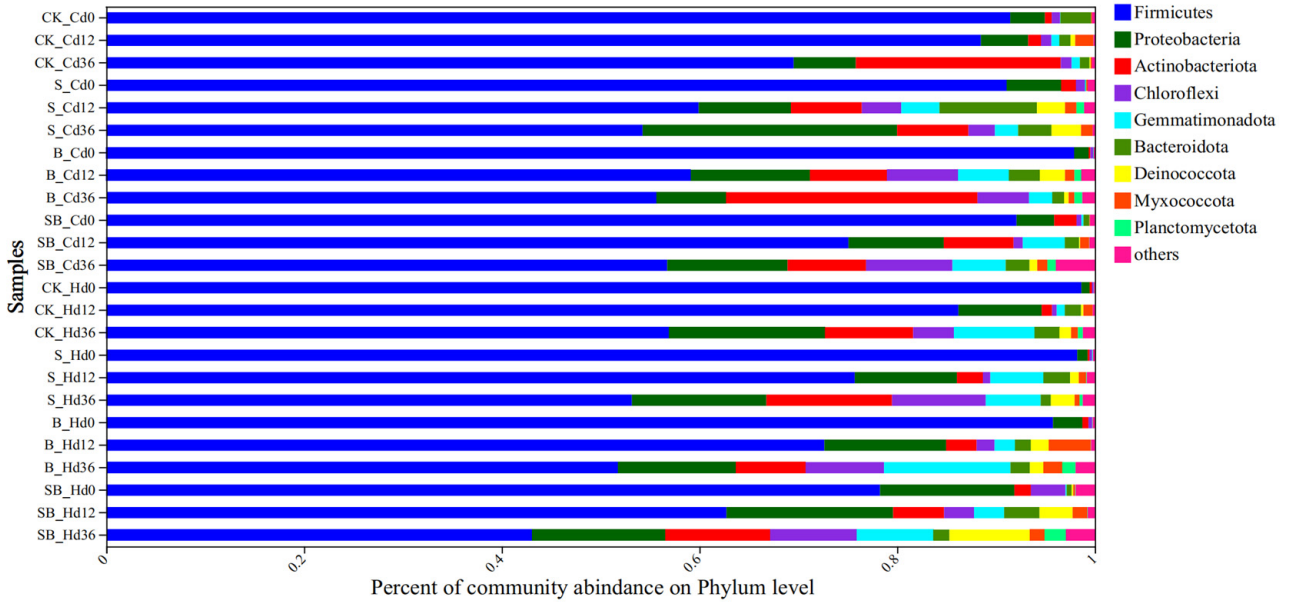
given library. The rarefaction curves were flat (Figure S2), indicating that the sequencing sample provided ample data for subsequent research. The Chao and Shannon indexes were employed as alpha diversity indices to further compare and analyze the richness and diversity of the bacterial community across different treatments [38], and the indexes ranged from 483.1 to 924.6 and from 2.60 to 4.48, respectively. The diversity index was used to characterize the dynamics of the bacteria community (Table S2, Figure S3). Group CK-C and CK-H showed a decrease in both the Chao index and the Shannon index during the thermophilic period; this decline might be attributed to the high temperature, which could lead to the inactivation of certain microorganisms and play a role in microbial population screening [39]. These findings suggest that elevated temperatures are not conducive to enhancing community diversity [40], but the problem can be mitigated by incorporating biochar and sediment [41].

The Chao and Shannon indexes of the complex microbial agent groups (group C) were significantly higher than those of the control CK-C, while the indexes of group H were higher in the thermophilic period compared to those of the CK-H group. This indicates that the incorporation of biochar and sediment increased the whole-process community diversity [42,43]. In contrast to the two microbial agent groups, the richness of the CK-C group decreased in the later stage compared with the early stage, whereas the CK-H group showed a significantly increasing trend, albeit followed by a decrease in the metaphase. This result indicates that the addition of homemade microbial agent favored the recovery of the community in the later compost period [44], which suggests that it may be used in conjunction with the compost products. Similarly, the same conclusion could be reached by comparing the S-C (B-C) group with the S-H (B-H) group. In addition, the Chao index of the biochar addition groups was lower than that of the sediment addition groups, indicating that the biochar screening microorganisms were more effective, while the sediment addition retained a greater number of low-abundance microorganisms [43]. The results reveal that different bacterial agents showed a significant impact on the alterations in microbial community diversity, which was in agreement with the conclusion of a previous study on sediment composting [45]; meanwhile, sediments were also part of the source of microorganisms [41]. In this study, the introduction of a complex microbial agent led to an increase in the population of effective microorganisms in the early composting phase, but the population slightly decreased in the later stage of composting, potentially hindering the degradation of lignin and other refractory organics [46]. However, the addition of homemade microbial agent resulted in a consistent rise in the abundance of effective microorganisms throughout the composting period, potentially fostering a diverse community structure and subsequently enhancing the efficient degradation of various organic materials [47]. Previous research has demonstrated that the inclusion of biochar can promote compost heating and ventilation, creating a conducive environment for their biological activities [48]. In comparison to the biochar compost groups, the homemade microbial agents also provided more stable microbial diversity during the composting process, suggesting that appropriate bacterial agents are more favorable to the composting process [49].

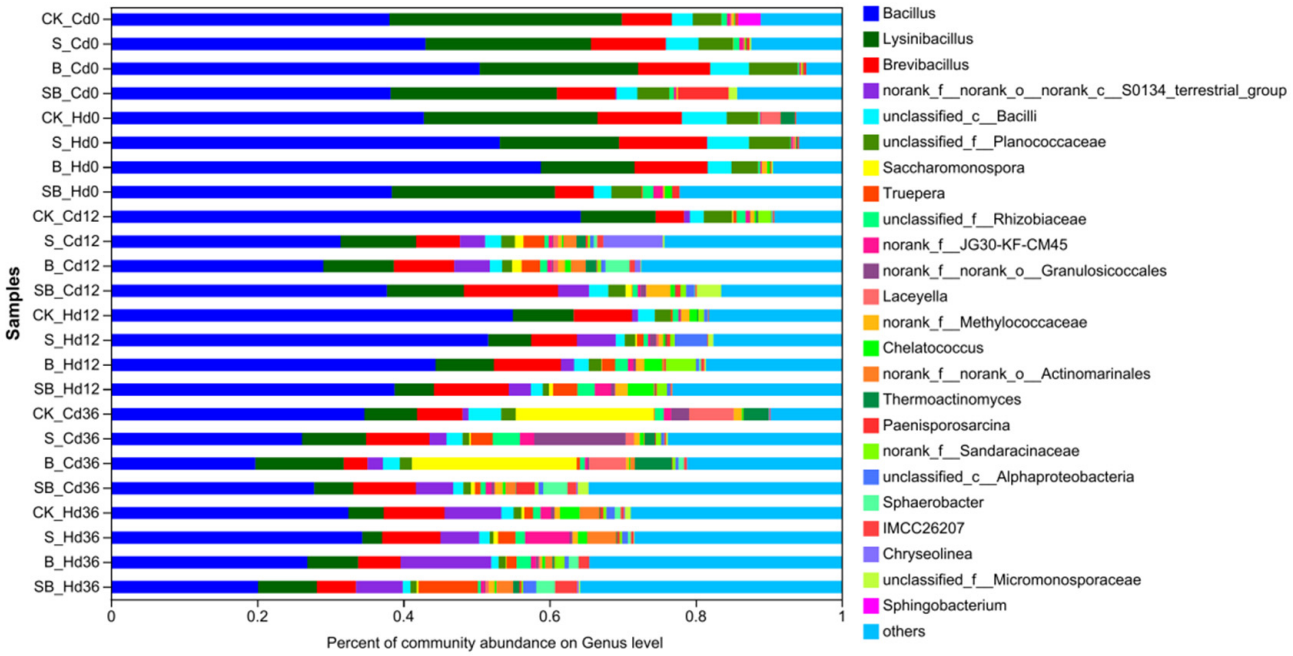
#### 3.3.2. Analysis of the Microbial Community Composition

Different composting materials influence the composition of the bacterial community [50,51]. As illustrated in Figure 5 and Figure S4, the predominant phyla in the compost samples included *Firmicutes* (43.1–98.6%), *Proteobacteria* (0.8–25.7%), *Actinobacteriota* (0.2–25.4%), *Chloroflexi* (0.2–9.5%), *Gemmatimonadota* (0.04–12.8%), *Bacteroidota* (0.03–9.9%), etc., collectively accounting for a relative abundance exceeding 96%. This bacterial community distribution was consistent with most studies of composting [52]. It is noticeable that the elevated levels of *Firmicutes*, *Proteobacteria*, *Actinobacteria* and *Bacteroidetes* in composts were indicative of their predominant role in the metabolism and conversion of organic matter [52]. The relative abundance of *Firmicutes* decreased significantly in all treatments, with

the addition of biochar and sediment promoting the reduction in *Firmicutes*; meanwhile, this increased the levels of other phyla.

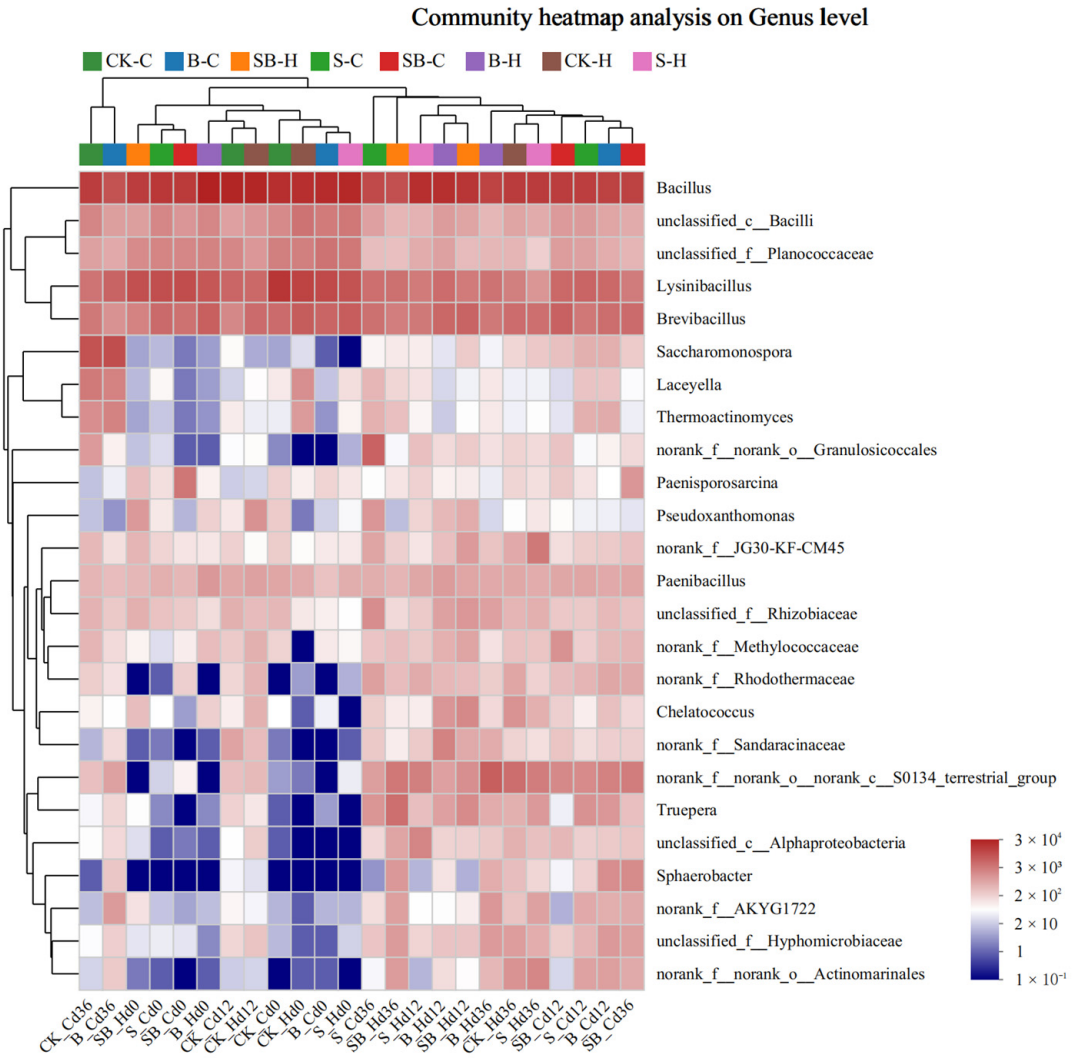



(**a**)




(**b**)

**Figure 5.** *Cont*.




(**c**)

**Figure 5.** Bacterial community at phylum and genus level of composting in different treatments. Bar graph of phyla level (**a**) and genus level (**b**) for different treatments. (**c**) The community heatmap of genera in the top 25 genera in abundance.

In the initial stage, *Firmicutes* were the most abundant bacteria phyla (91–98.64%), followed by *Actinobacteria* and *Chlorophytis*, which was consistent with the previous results identifying these phyla as abundant during lignocellulosic waste composting [53] and other organic waste composting processes [54]. The wetland sediment contained a relatively diverse microbial community, and the treatments with sediment as an additive exhibited a more favorable microbial community structure in the early composting stage [45]. The bacteria abundance increased during the metaphase for all treatments while decreasing through the anaphase. On day 12, the average abundance of *Firmicutes* decreased to 72.49%, lower than that in the early stage; however, *Firmicutes* remained the first dominant bacteria—known for the ability to degrade polysaccharides such as cellulose—to play a crucial role in lignocellulose degradation [55]. The relative abundance of *Proteobacteria* ranged from 4.79% to 16.86%, showing a rapid increase compared to the initial stage. *Proteobacteria* are key players in the degradation of glucose, propionate, butyrate and other small molecules [56]. Compared with the control, the relative abundance of *Proteobacteria* significantly increased in the biochar and sediment treatments, which could be attributed to the high organic matter content in sediment and the simulating impact of biochar on

pile aeration, thereby providing *Proteobacteria* with suitable environmental conditions and nutrients [48]. The addition of homemade microbial agent concurrently increased the abundance of *Proteobacteria*. The relative abundance of *Bacteroidetes* reduced in the late stage due to their significant role in the decomposition of lignin and cellulose, with their changes mirroring the variation in lignin content. In the anaphase of composting, the relative abundance of *Proteobacteria* was increased; the difference within the complex microbial agent treatments (6.28–25.74%) was greater than that of homemade microbial treatments (11.90–15.78%). *Proteobacteria* and *Actinobacteria* showed a gradual increase in relative abundance during the composting process, which facilitated the degradation of the cellulose-rich agricultural residues [57] and played a crucial role in the maturation stage [58]. This antagonistic effect inhibited the activities of *Bacteroidetes* and other bacteria [52].

At the genus level, *Bacillus*, *Lysinibacillus*, *Brevibacillus* and *Saccharomonospora* were the dominant genera in compost samples obtained from the initial composting stage (Figure 5c). This suggested that these genera have the capacity to produce enzymes involved in polysaccharides or lignocellulose degradation [56]. In composting raw materials, *Bacillus*, *Lysinebacillus* and *Brevibacillus* were the dominant bacteria, constituting over 50% of the total bacteria (Table S3). *Bacillus*, *Lysinebacillus* and *Brevibacillus* belong to *Firmicutes*, which are capable of producing spores to withstand high temperatures [59] and are important thermophilic decomposition agents in lignocellulosic composting [60]. Throughout the composting period, the abundance of the *Bacillus* remained relatively stable during mesophilic and thermophilic periods, when it slightly changed, but decreased significantly during the cooling period. Additionally, the abundance of *Lysinibacillus* decreased continuously. Meanwhile, the abundance of *Brevibacillus* exhibited a continuous slight decrease during the thermophilic period and showed a mild increase in the cooling period, in contrast to the initial stage. Despite a rapid decline in the abundance of these three genera, they still persisted as the most abundant in the bacterial community, which indicated their capability to degrade cellulose [61].

In the various treatment groups, *Bacillus* exhibited the highest relative abundance in the biochar and sediment treatments in the early stage, reaching 50.46% and 58.84%, respectively. In contrast, the average abundance of *Bacillus* in group C was 24.72%; however, the abundance in group H was 18.79%, which was the highest in the control group. This suggests that the microbial diversity in the control group was lower compared to the treatments with biochar and sediment, but some dominant genera accounted for a higher proportion of the microbial community. According to the relationship between treatments and species, the *Brevibacillus* emerged as the dominant genus in the group treated with homemade microbial agent and sediment–biochar synergistic addition. *Bacillus*, *Lysinebacillus* and *Brevibacillus* remained the relatively dominant bacteria at the metaphase. In particular, the highest relative abundance of *Bacillus* in the CK-C treatment rose to 62.79%, surpassing that in the B-C treatment. Compared with the prophase of composting, the average abundance of *Lysinebacillus* declined to 9.64%; their average abundance in group C was 10.18%, and it was higher than that in group H by 6.89%. Regarding the addition of biochar and sediment (Figures 6 and S5), the biochar group showed 48 nodes and 321 edges, whereas there were 48 nodes and 209 edges in the sediment group within the top 50 genera, indicating that biochar addition has a stronger impact on microbial connectivity.

In addition, *Granulosicoccales*, *Chelatococcus* and *Alphaproteobacteria* were the genera with lower relative abundance and are classified under *Proteobacteria*; the majority of these were thermotolerant and involved in carbon and nitrogen mineralization during composting [32]. *Truepera*, *Actinomarinale* and *Sphaerobacter* showed a notable increase in composting and displayed a significant negative correlation with lignocellulosic content, suggesting their primary function in contributing to lignocellulose degradation [62]. This finding is in line with the outcome of a previous study on microbial functional structure [63]. Furthermore, the abundance of other bacterial genera (whose abundance was not among the top 25 genera) increased significantly in the anaphase. The SB-H treatment showed the highest relative abundance at 35.76%, followed by the SB-C treatment at 34.62%, indicating an

increased microbial diversity during the composting with biochar and sediment. This was consistent with the Shannon index change and the number of co-occurrence network nodes and edges (Table S4). This aligns with the conclusions of an earlier study, conjecturing that the genera with initially low abundance make important contributions to the composting process [64].

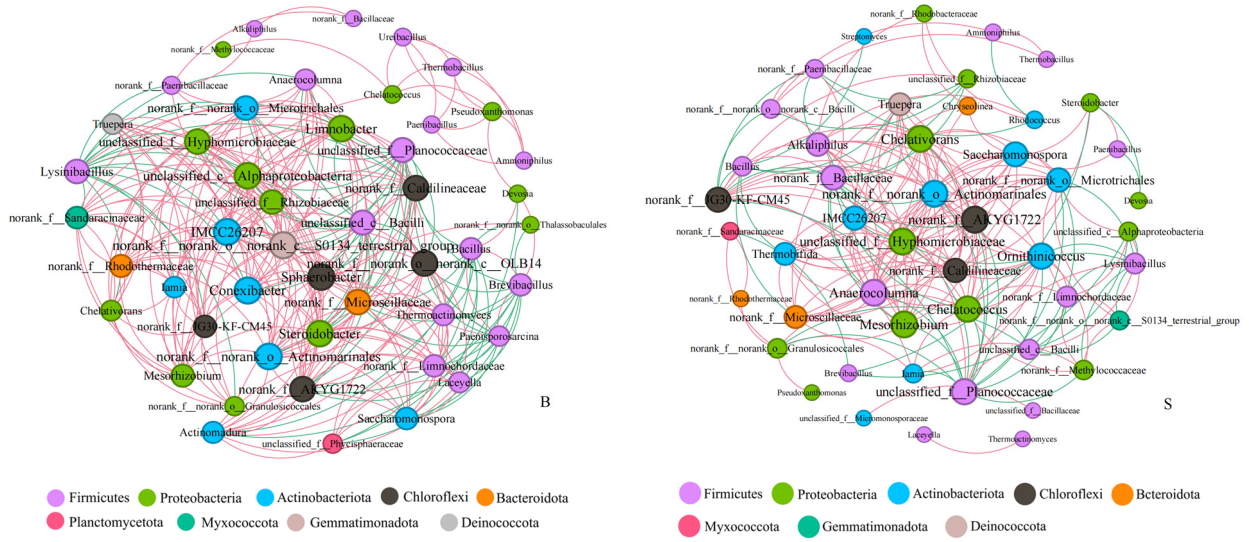


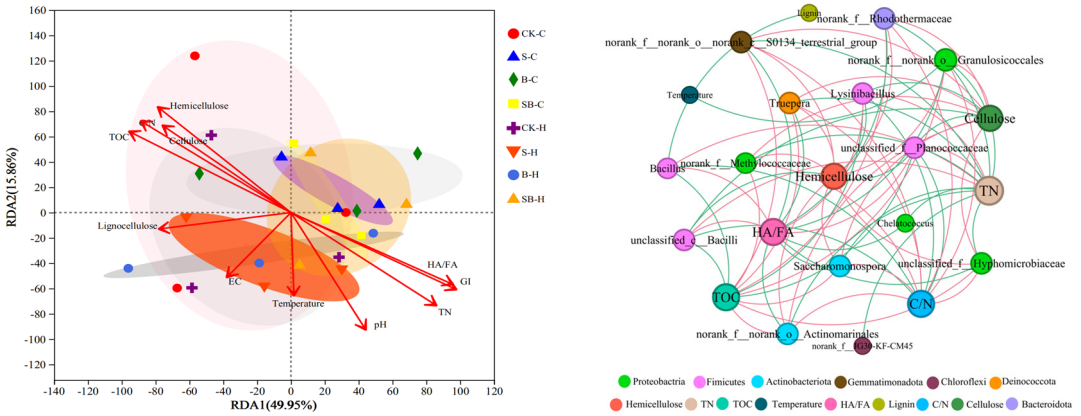

**Figure 6.** Co-occurrence network diagram of top 50 bacterial genera in biochar groups (B-H, B-C) and sediment groups (S−H, S-C). (Spearman's $r > 0.5$, FDR-adjusted $p < 0.05$) (red represents positive effect; green represents negative effect).

### 3.3.3. Relationship between the Bacteria Community and Environmental Factors

To further evaluate the relationship between lignocellulose degradation and bacteria community composition and environmental factors of compost, RDA analysis was conducted using physicochemical parameters and bacterial abundance (Figure 7a). Environmental factors, including pH, TN, HA/FA and GI, accounted for 49.59% and 15.86% of the variations in the bacterial community in compost. The influence of environmental factors revealed that HA/FA and GI exerted a significantly greater impact on the microbial community structure than other factors. Previous studies have also considered the affiliate index as a guiding factor in determining microbial community structure of compost [38]. HA and FA are rich in phenolic hydroxyl groups, alcoholic hydroxyl groups and carboxyl groups, with HA additionally containing abundant benzene ring structures [65]. It is generally believed that the gradual rise in HA/FA is positively correlated with the degree of humification, subsequently impacting microbial abundance and community composition. The impact of nitrogen concentration, crucial for microbial proliferation, on the microbial community was significant, but microorganisms may suffer under the conditions of ammonium production [66]. GI represents the toxicity of compost products to plants, which can indirectly reflect biological hazards such as ammonia nitrogen and high redox potential during composting. It is also noteworthy that the compost material was rich in organic carbon components, comprising water-soluble organic matter, cellulose, hemicellulose and lignin. Water-soluble organic material served as the direct material and energy source for microbial activities [67] while necessitating the degradation of high molecular weight organic matter such as lignocellulose. Consequently, the total organic matter content was negatively correlated with the development trajectory of the microbial community, implying that microorganisms were involved in the degradation of lignocellulose.

Figure 7a illustrates that HA/FA and TN showed a strong positive correlation with GI; meanwhile, they displayed a strong negative correlation with hemicellulose, C/N and cellulose. Significant indicative factors in compost maturation were higher HA/FA, TN and GI, leading the sample to slope towards the lower right corner as composting

progressed, signifying the degradation of lignocellulose. The treatments involving sediment and biochar addition were primarily associated with lignocellulose degradation, confirming that the sediment and biochar addition had the most significant impact on lignocellulose degradation during composting. In comparison to the CK-C and CK-H groups, the SB-C and SB-H groups showed a more favorable degradation of lignocellulose with the homemade microbial agent treatment, and there was no significant difference in lignocellulose degradation observed among the S-C, S-H, B-C and B-H groups. Therefore, the combination of biochar and sediment, along with the addition of effective bacterial agents, will promote the decomposition of lignocellulose [68,69].




(**a**) (**b**)

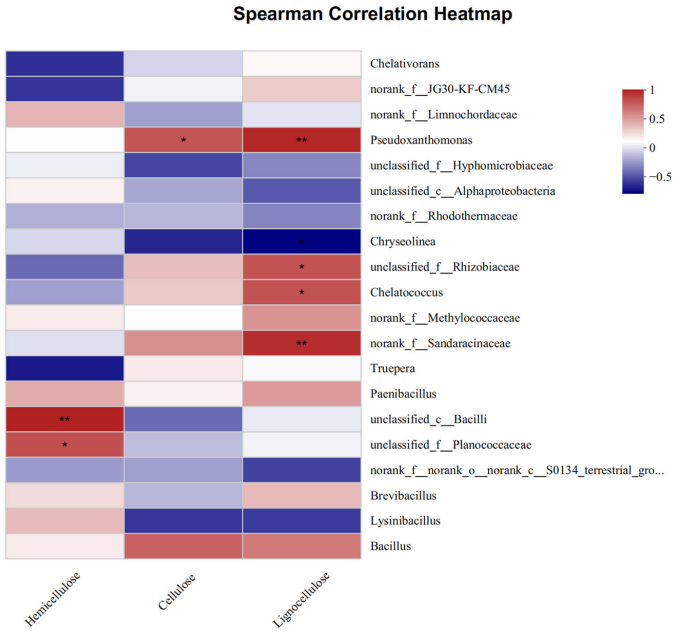
**Figure 7.** Relationship between the bacteria community and environmental factors. (**a**) RDA analysis of sample distribution and environmental factors in composting in different treatments. (**b**) Cooccurrence network between environmental factors and major genera in the top 20 in abundance during (Spearman's $r > 0.5$, FDR-adjusted $p < 0.05$) (red represents positive effect; green represents negative effect).

### 3.3.4. Analysis of the Factors That Affected Microbial Communities and Lignocellulose Degradation

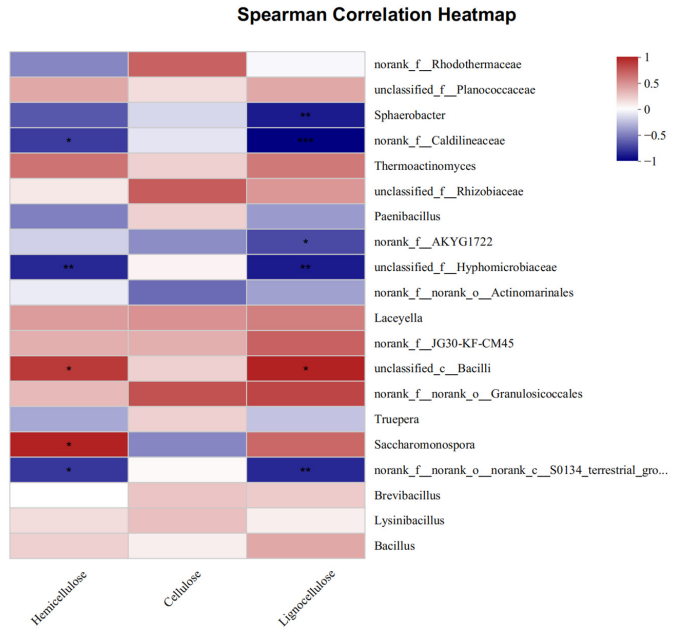
The physicochemical properties of composting systems can be directly or indirectly related to the microbial community composition [70]. Network analysis was performed based on the top 20 bacterial genera and the selected physicochemical factors (temperature, TC, TN and HA/FA) in the composting system (Figure 7b). We observed a significant positive correlation between temperature and *Granulosicoccales*, suggesting their greater resistance to high temperature compared to other genera. Previous studies have highlighted temperature as a crucial factor in affecting bacterial community diversity [71,72]. *Firmicutes* showed a negative correlation with TN and a positive correlation with TOC, as mentioned by a relevant study, indicating that the *Firmicutes* do not favor nitrogen retention but have a greater affinity for organic carbon [73]. According to the result that the lignin, cellulose and hemicellulose contents were positively correlated with *Firmicutes*, we propose that *Firmicutes* may utilize lignocellulose degradation products for their essential activities, particularly in the carbon cycle [74].

The function of bacteria in composting generally relates to carbohydrate metabolism, amino acid metabolism, energy metabolism, etc. However, functional changes coincide with the dynamics of lignin, cellulose and hemicellulose during composting. Specifically, cellulose degradation occurs rapidly during the thermophilic phase and lignin in the anaphase [75]. The Spearman Correlation Heatmap in Figure 8a shows the investigation of the impact of major bacteria on lignocellulose degradation, indicating a positive correlation between *g__Pseudoxanthomonas*, *g__unclassified_f__Planococcaceae* and *g_unclassified_c_Bacilli* and cellulose and hemicellulose during the thermophilic stage; this finding was consistent with previous study results regarding the microbial characteristics [62]. Furthermore,

Figure 8b illustrates the gradual degradation of hemicellulose and lignin in the anaphase of composting, and the *g_unclassified_c_Bacilli* and *g_Saccharomonospora* exhibited a stimulative effect on the degradation process. Indeed, the degradation necessitates the interaction of multiple microorganisms and the formation of symbiotic relationships to collectively facilitate the degradation of organic matter [76]. It is possible that some species could adapt to the changes in material composition characteristics and environmental factors in composting, resulting in the succession of functional microbial composition.




(**a**)




(**b**)

**Figure 8.** The relationship between major genera and lignocellulose composition during composting. Pearson correlations between lignocellulosic content and the major 20 genera in abundance during the (**a**) thermophilic (12 d) and (**b**) cooling stages (36 d). ("*", Spearman's $r > 0.5$, FDR-adjusted $p < 0.05$; "**", Spearman's $r > 0.5$, FDR-adjusted $p < 0.01$; "***", Spearman's $r > 0.5$, FDR-adjusted $p < 0.001$).

## 4. Conclusions

The results of this study show that biochar and sediment as compost conditioners can improve the humus, TN content and GI at the end of composting. The promotion effect of compost maturation in the biochar treatment group was more obvious than in the sediment treatment group, whereas sediment treatments exhibited greater microbial diversity. *Bacillus*, *Lysinebacillus* and *Brevibacillus* were the dominant bacteria for lignocellulose degradation; meanwhile, *Truepera*, *Actinomarinale* and *Sphaerobacter* had important roles in the degradation of lignocellulose. In the co-occurrence network, the key groups that significantly promote lignocellulose degradation are *Firmicutes* and *Actinobacteria.* The structure and function of the microbial co-occurrence network are dominated by low-abundance species and environmental factors affecting lignocellulose degradation. Based on the relationship between exogenous additives with humic substances and lignocellulose, an optimized strategy of biochar–homemade microbial agent co-inoculation was proposed to improve compost maturity and adjust the microbial community according to the functional needs during the composting process. This study provides new ideas for the resource utilization and harmless treatment of submerged plants.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fermentation10010070/s1, Figure S1: Variation in temperature during co-composting; Figure S2: Sobs (**a**) and Shannon (**b**) rarefaction curves; Figure S3: The αdiversity of different treatments or additives (a: Chao index; b: Shannon index); Figure S4: Community heatmap analysis on phyla level; Figure S5: Co-occurrence network between lignocellulosic content and major genus in the top 20 in abundance during the thermophilic (12 d) (**a**) and cooling stages (36 d) (**b**) (Spearman's $r > 0.5$, FDR-adjusted $p < 0.05$) (red represents positive effects; green represents negative effects). LIG, lignocellulose; CELL, cellulose; HEMI, hemicellulose; Table S1: Changes in physical-chemcal parameters for 8 treatments during composting process; Table S2: Richness and diversity index of composting in different treatments at 97% level; Table S3: The twenty most abundant OTUs identified to genus and sorted by total bacteria sequences; Table S4: The network nodes and edges number in co-occurrence.

**Author Contributions:** Conceptualization, X.G., H.W. and L.L.; methodology, Z.S. and S.R.; software, Z.S.; validation, P.Z. and S.R.; formal analysis, H.L. and L.L.; investigation, Z.S. and S.R.; resources, S.R.; data curation, Z.S. and S.R.; writing—original draft preparation, H.W. and Z.S.; writing—review and editing, X.G., P.Z. and L.L.; visualization, S.R.; supervision, X.G. and L.L.; project administration, S.R. and L.L.; funding acquisition, H.L., H.W. and L.L. All authors have read and agreed to the published version of the manuscript.

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