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A Study of the Effect of Biochar Additive on the Manure–Compost–Soil Process and Its Bacterial Succession

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Abstract: The manure–compost–soil process is the main avenue for using agricultural livestock waste. Biochar not only impacts the composting process but also enhances the soil's organic matter and nitrogen content when applied with compost. This paper explores the profound impact of biochar as an additive on the manure–compost–soil process, uncovering novel mechanisms driving enhanced soil health and bacterial succession. The basic physicochemical properties (organic matter, total nitrogen, etc.) and microbial structure of the composting and soil samples were analyzed. Biochar additive increased the C/N ratio and the total carbon content of the compost. Biochar, compost, and biochar-based compost improved the total carbon and organic matter of the soil. After high-temperature composting, *Saccharomonospora* (from 2.68% to 0.80%), *Atopostipes* (from 5.71% to 0.13%), and *Lactobacillus* (from 5.27% to 0.04%) were almost eliminated. *Lysobacter*, *Glutamicibacter*, and *Streptomyces* were the dominant genera in the soil samples, promoting plant growth. *Nocardiopsis*, *Saccharomonospora*, *Bacillus*, and *Oceanobacillus* dominated the genera in the whole manure–compost–soil process. Thus, composting could eliminate the toxic or negative bacteria directly deposited into soil by manure. Those genera arising from compost or biochar-based compost in the soil could contribute to organic matter's cycle.

Keywords: aerobic composting; black soil; biochar; microbial migration; pig manure; 16S rRNA

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1. Introduction

According to statistics from the Ministry of Agriculture and Rural Affairs, the total amount of livestock and poultry manure produced in China is about 3.8 billion tons per year, of which the annual output of pig manure is about 1.8 billion tons, accounting for 47% of the total. If not handled properly, manure can cause environmental pollution, for example, hygiene hazards and odor pollution [1]. Aerobic composting offers a green technology for treating agricultural waste [2]. With microbial activity and ample oxygen, aerobic composting produces high-quality compost suitable for soil enhancement [3]. These composts, regarded as organic fertilizers, can enrich soil fertility, enhancing agronomy and crop productivity [4,5]. Compost application boosts soil carbon and nitrogen content, stabilizes soil organic matter, improves soil structure for root growth, and introduces microorganisms into the soil [6]. In summary, the manure–compost–soil pathway is crucial for managing agricultural livestock and poultry waste.

The manure–compost–soil process plays a pivotal role in microbial transfer and succession. Microbial diversity undergoes significant changes during aerobic composting, and the composition of different composts varies due to factors like raw materials [7–9]. The soil microenvironment was rich in microbiological composition, making it a key focus in soil ecosystem management [10]. Soil health refers to the overall quality and functioning of the soil as an ecosystem, which is also related to the bacterial structure within the soil [11]. Fierer et al. [11] proposed that microbial information could be used to yield relevant and actionable assessments of soil health. Zhou et al. [5] indicated that organic fertilizer enhanced

bacterial network complexity, metabolic function, and the convergence of the community structure of native soils' nearby roots. Maron et al. [12] prolonged that when nutrient inputs in soil, its carbon cycling may be more vulnerable to microbial diversity changes. Compost, which has a certain amount of carbon- and nitrogen-containing organic matter, might affect the microbes in soil; for example, soil microbes convert organic nitrogen into ammonia or nitrate that were available for plants to use [11]. Therefore, investigating microbial diversity and structure in the manure–compost–soil process is crucial for understanding the effects of manure utilization.

Material transformation occurs throughout the manure–compost–soil process. Applying compost and manure to soil can improve fertility, but organic matter rapidly mineralizes, with only a small fraction remaining stable in the soil in the long term [13]. Recent studies have shown that biochar and compost application can effectively increase organic matter retention, enhancing soil quality and farm productivity [14–16]. Biochar is a highly aromatic, insoluble solid produced by biomass thermal cracking and carbonization in anoxia conditions. It has physicochemical properties such as a high stability and developed pore structure, promoting composted organic matter degradation and well enhancing microbial richness [17,18]. Applying biochar to soil can improve physical structure aspects, such as the pore structure and soil water-holding capacity, facilitating the conversion of organic nutrients [16]. Agegnehu et al. [19] also noted that biochar and compost can improve soil quality and crop yields. We hypothesized that biochar-based compost made by aerobic composting combines the functions of compost and biochar, promoting organic matter storage and microbial activity in the soil. Mawof et al. [15] found that biochar, compost, and biochar–compost mixtures significantly improved soil's physicochemical properties and crop yield under wastewater irrigation ($p < 0.05$). Applying biochar and biochar-based compost to soil can improve soil fertility, water-holding capacity, crop yield, and carbon sequestration, which is vital in agriculture's green and sustainable development [20–24]. Recent studies have focused only on the effect of compost or biochar on the structure of soil microbes. For instance, Yin et al. [25] found that biochar accelerated the humification composting process, improving compost quality. Xu et al. [26] explored the effects of corn stover biochar in soil on nitrogen leaching and bacterial community structure, and found that biochar addition helped reduce soil nitrogen leaching and increased bacterial diversity in the soil. However, the mechanism of microbial transfer or succession during the production and application of biochar-based compost remains unclear. We assume that manure carries some nutrients and microbes. When manure becomes fertilizer or compost via aerobic composting through high-temperature fermentation and microbial activity, the microbial structure changes. When compost is applied, compost microbes flow into the soil, which also has its microbial structure. Thus, a key question is whether compost and biochar application can alter microbial succession.

This paper delves into the multifaceted effects of incorporating biochar as an additive in the manure–compost–soil process, with a particular emphasis on its unprecedented impact on bacterial succession. By examining the complex interactions between biochar, compost, and soil microbial communities, we conducted an aerobic composting experiment using pig manure with biochar as an additive. Subsequently, we utilized the compost products in a potting experiment. Throughout this process, we collected representative samples from the raw composting materials, final compost, and soil samples near the cucumber root system in the experimental group. We measured and analyzed the basic physicochemical properties of the soil samples and utilized 16S rRNA high-throughput data to assess microbial structure. Our research pioneers a deeper comprehension of how biochar not only modifies the physical and chemical properties of compost and soil but also fundamentally transforms the microbial landscape, fostering a more diverse and beneficial bacterial succession.

2. Materials and Methods

2.1. Aerobic Composting and Cucumber Potting Experiment

This study encompassed two primary sets of experiments: aerobic composting and cucumber potting. The composting experiment was divided into control (A and C) and experimental (B and D) groups, while the potting experiment evaluated different combinations of compost and biochar.

The primary raw materials for composting were fresh pig manure and wheat straw cut to 1~3 cm. These were sourced from the pig farm of the Chinese Academy of Agricultural Sciences (Beijing, China) and the Shangzhuang experimental base of China Agricultural University in Beijing, China. The control groups (A and C) consisted of a mixture of swine manure and wheat straw at an initial moisture content of 60% and a C/N ratio of 15, with a weight ratio of 5:1 (Table 1) [4]. The experimental groups (B and D) incorporated wheat straw biochar, prepared via high-temperature pyrolysis at 500 °C for 12 h, into the mix at a swine manure–wheat straw–biochar ratio of 10:2:1 according to Liu et al. [27]. The mixtures were placed into four 90 L aerobic composting reactors and composted for 28 days under intermittent oxygen supply (30 min on/30 min off) with a ventilation rate of 0.2 $L \cdot min^{-1} \cdot VS^{-1} \cdot kg^{-1}$ [28]. Samples were collected from the top, middle, and bottom of the reactors on days 0, 3, 7, 14, 21, and 28 for physicochemical and microbiological analysis.

Table 1. Ingredients and group name of aerobic composting and potting experiments.

Experiment	Group Name	Ingredients [a]
Aerobic Composting	Control groups (A and C)	Swine manure and wheat straw (Ratio of 5:1)
	Experimental groups (B and D)	Swine manure, wheat straw, and wheat straw biochar (Ratio of 10:2:1)
Potting [b]	BLANK	0.25 kg nutrient soil
	BIOCHAR	0.225 kg nutrient soil + 0.025 kg biochar
	AA	0.225 kg nutrient soil + 0.025 kg compost from the Group A composting
	BB	0.225 kg nutrient soil + 0.025 kg biochar-based compost from Group B
	CC	0.225 kg nutrient soil + 0.025 kg compost from Group C
	DD	0.225 kg nutrient soil + 0.025 kg biochar-based compost from Group D

Notes: Nutrient soil–black soil and vermiculite in the ratio of 5:1. [a]: Based on wet basis. [b]: The weight of ingredients in the potting experiment was for one pot (six pots in each group).

The cultivation substrate for the potting trials consisted of a 5:1 mix of black soil and vermiculite. Six groups were set up, including a blank control (no additives), a biochar-only treatment, and four treatments with composts from the composting experiment (Table 1). The additive ratio of compost and biochar was based on a fertilizer application rate of 250 kg N/hm^2 [26]. The details of the group name and ingredients are shown in Table 1. Figure S1 shows the cucumber potting experiment set up with six groups (one group for each six parallel pots). The square pots used for the potting experiment were 9.6 cm in height and 10 cm in inner diameter. Each pot contained ~0.25 kg of substrate and was seeded with five cucumber seeds (Zhongnong 6 variety). The plants were grown in an artificial climate chamber (MGC-350HP-2, China) at 25–30 °C with a 16:8 h light–dark cycle for two months. The moisture content of the material in the pots was adjusted to 60–70%. Soil samples around the root were collected on days 0, 7, 21, 42, and 64 for physicochemical and microbiological analysis. The basic physicochemical properties of the raw materials for aerobic composting and the pot experiments are shown in Table 2.

Table 2. Basic physicochemical properties of raw materials for aerobic composting and pot experiments.

Name	MC [a]	pH [a]	OM [b]	TC [b]	TN [b]
Pig manure	66.00 ± 0.72	/	74.70 ± 0.01	37.41 ± 0.14	3.19 ± 0.01
Wheat straw	6.07 ± 0.14	/	89.81 ± 0.03	43.43 ± 0.09	0.99 ± 0.06
Biochar	3.21 ± 0.15	10.21 ± 0.07	80.99 ± 0.28	70.60 ± 0.16	1.08 ± 0.01
Black soil	2.71 ± 0.15	6.87 ± 0.01	4.51 ± 0.06	12.60 ± 0.24	1.12 ± 0.02
Vermiculite	5.62 ± 0.03	7.25 ± 0.01	25.05 ±0.68	0.16 ± 0.01	0.20 ± 0.01
Compost A	13.32 ± 0.06	7.96 ± 0.01	65.57 ± 0.07	33.36 ± 0.59	3.97 ± 0.06
Compost C	13.38 ±0.15	8.07 ± 0.03	65.30 ± 0.22	32.51 ± 0.02	3.86 ± 0.01
Compost B	10.80 ± 0.36	8.54 ± 0.04	67.98 ± 0.09	41.41 ± 0.15	3.34 ± 0.01
Compost D	10.31 ± 0.10	8.15 ± 0.03	71.47 ± 0.28	43.20 ± 0.06	2.67 ± 0.02

Notes: MC: moisture content, %; OM: organic matter, %; TC: total carbon, %; TN: total nitrogen, %, [a]: Based on wet basis, [b]: Based on dry basis. Composts A and C: without biochar additive during aerobic composting. Composts B and D: with biochar additive during aerobic composting.

2.2. Basic Physical and Chemical Indicators

The samples were dried at 105 °C for 24 h until a constant weight to measure the moisture content (MC). Total carbon (TC) and total nitrogen (TN) content were determined using an elemental analyzer (Vario EL CHNOS; Elemental Analysensystems GmbH, Langenselbold, Germany). These values were used to calculate the C/N ratio [29]. Organic matter (OM) and germination index (GI) were determined and calculated according to the methods provided by He et al. [28]. NH_4^+-N were extracted from the compost samples by leaching with 2 mol/L KCL and the content was determined by a continuous flow analyzer (SEAL Analytical AutoAnalyzer 3, Hamburg, Germany) [30]. The supernatant was extracted and filtered at a solid–liquid ratio of 1:10 (*w*/*v*), and the pH values were determined using a pH meter (SC8231, Yokogawa Electric Corporation, Tōkyō, Japan) [29]. The chemical functional groups of the samples were determined using a Fourier transform infrared spectrometer (FT-IR, PerkinElmer Spotlight 400, Waltham, MA, USA), according to Fang et al. [31].

2.3. Microbial Diversity Determination and Analysis

2.3.1. DNA Extraction and Purification

Total DNA from the compost samples was extracted using a soil genomic DNA extraction kit (DP336, Tianhe Biotech (Beijing, China). Extracted DNA was purified using a Puc-T TA cloning kit (CWBIO, Beijing, China), and then purified DNA was analyzed by 1% (*w*/*v*) agarose gel electrophoresis.

2.3.2. High-Throughput Sequencing

A fluorescence spectrophotometer (NANODROP 2000, Thermo Scientific, Waltham, MA, USA) was used to detect the concentration of DNA, and the quality of DNA was checked via 1% agarose gel electrophoresis. The concentration of the DNA solution was adjusted, and the DNA working solution was stored at −4 °C. The V3–V4 region of the 16S rRNA gene was selected for bacterial-specific fragmentation with primers 338F (5′-ACTCCTACGGGGAGGCAGCAG-3′) and 806R (3′-GGACTACHVGGGTWTCTAAT-5′) [32]. The PCR amplification program consisted of pre-denaturation at 95 °C for 5 min, denaturation at 95 °C for 45 s, annealing at 55 °C for 50 s, and extension at 72 °C for 45 s for a total of 28 cycles; and a final extension at 72 °C for 10 min. The PCR amplification products were sequenced using the Illumina MiSeq PE300 sequencing platform.

The raw data generated from high-throughput sequencing runs were processed and analyzed following the flow of the QIIME platform (quantitative insights into microbial ecology; v1.2.1). Sequence reads were trimmed so that the average quality of each read was higher than 20 and then assembled using Flash software (version 1.2.11). Chimeric sequences were identified and removed using UCHIME. Taxonomic analysis of OTU representative sequences with a 97% similarity level was performed using uclust classification or an RDP classifier Bayesian algorithm. Sequence clustering was performed by uclust

(QIIME) with a similarity cut-off value of 97%, after which the samples were clustered into operational taxonomic units. QIIME software (version 1.2.1) was used to calculate the Chao1 index and Shannon index of the samples, and the R language toolkit plotted the remaining graphs. A typical correlation analysis (CCA) was performed using Canoco for Windows (v.5.0) to determine the correlation between biochar and microorganisms in compost and microorganisms in soil. Firstly, DCA analysis was performed on the sample data, and then the maximum Pearson correlation coefficient of the environmental factors with the differences in the distribution of the sample communities and the subset of the environmental factors were determined by the bioenv function. The distribution tables of the sample species with respect to the environmental factors or subsets of environmental factors were then analyzed separately, and finally their significance was obtained by using ANOVA software (IBM Corporation, Armonk, NY, USA) [33].

2.4. Statistics and Data Analysis

Statistical analyses of the basic physical and chemical indicators were performed using Excel 2019 (Microsoft Corporation, Redmond, WA, USA). The data were expressed as the mean ± standard deviation values of repeated measurements. Dynamic changes in the physicochemical properties were plotted using OriginPro 2022b software (OriginLab, Northampton, MA, USA). Significant difference analysis was calculated by ANOVA analysis using SPSS statistics v19.0 (IBM, Armonk, NY, USA) ($p < 0.05$).

3. Results and Discussion

3.1. Physicochemical Changes in the Manure–Compost–Soil Process

3.1.1. Manure–Compost during Aerobic Composting

The key findings from Figure 1 highlight the beneficial effects of incorporating biochar into composting. Notably, there were no significant differences in temperature or oxygen concentration among the four groups (control A/C and experimental B/D) ($p > 0.05$). Figure 1a shows the four groups treated at temperatures >50 °C for more than three days; the compost met the non-hazardous criteria [4]. In addition, throughout the composting process, the experimental groups (B and D) exhibited a longer duration of high temperatures (>50 °C) and higher oxygen concentrations during the initial days when temperatures exceeded 50 °C. This statistically significant improvement ($p < 0.05$) in oxygen penetration indicates that biochar enhances the internal aeration of the compost heap [33,34].

Regarding the physicochemical properties, although the pH, germination index (GI), organic matter (OM), ammonia (NH_4^+), total nitrogen (TN), and total carbon (TC) did not differ significantly between the control and experimental groups within their respective categories (A vs. C and B vs. D) ($p > 0.05$), some interesting trends emerged. As shown in Figure 1b, the pH initially rose and then declined in all groups, likely due to the production and subsequent dissipation of ammonium ions [33]. Notably, the experimental groups (B and D) maintained lower pH values, suggesting ongoing microbial activity and organic acid production [35]. In Figure 1b, the germination index (GI) values of the four groups increased gradually due to the degradation of toxic substances such as low-molecular-weight fatty acids, ammonia, and toxic nitrogen compounds [36]. The GI of the compost products of all four groups was greater than 80%, which indicated that the final compost maturity was up to the required level [37,38]. The GI of group D was significantly higher than that of the other three groups ($p < 0.05$), indicating that the addition of biochar may reduce harmful substances in compost to an extent [36]. In Figure 1c, organic matter was continuously consumed during the composting process and the degradation rate of the experimental groups (B and D) was higher than that of the control groups (A and C). The organic matter's degradation rates in the four groups were 3.92%, 10.45%, 4.73%, and 7.41%, respectively. This may be because biochar promotes organic matter degradation and water evaporation during aerobic composting [39]. The NH_4^+ content of the four groups showed a decreasing trend (Figure 1c). Eventually, the NH_4^+ content of the control groups (A and C) was slightly higher than that of the experimental groups (B and D), which was due to

the retention of NH_4^+ by the biochar slowing down the activity of denitrifying bacteria [40]. In Figure 1d, the total nitrogen content in the experimental groups (B and D) was slightly higher than that in the blank groups (A and C). This is due to the ability of biochar to absorb ammonia and other nitrogenous substances, which explains the higher total nitrogen levels in treatments using biochar as an additive [41]. The total carbon content of the experimental groups (B and D) with biochar addition was significantly higher than in the blank groups (A and C) ($p < 0.05$). Biochar has a better absorption ability to conserve carbon [41]. Therefore, the addition of biochar increased the carbon/nitrogen ratio in the composting experiment.

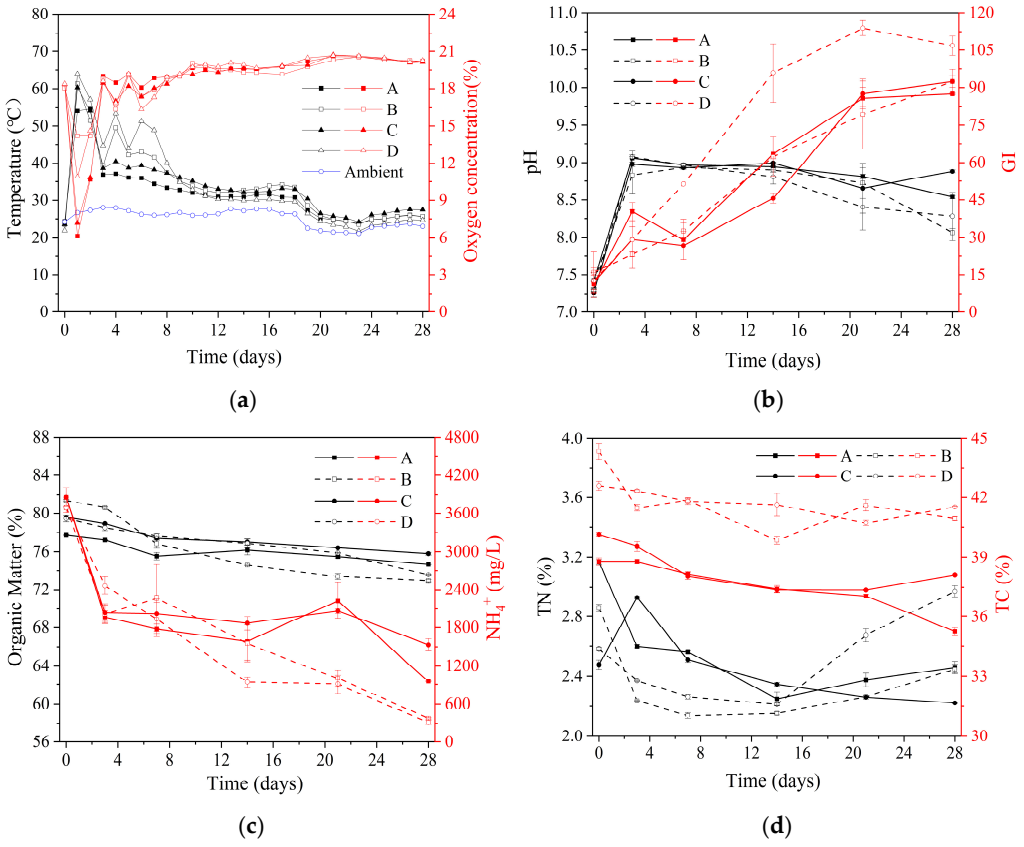


Figure 1. Dynamical changes in (**a**) temperature and oxygen concentration, (**b**) pH and germination index (GI), (**c**) organic matter and ammonia (NH_4^+), (**d**) total nitrogen (TN) and total carbon (TC) in the physicochemical properties of compost samples.

3.1.2. Compost–Soil in the Potting Experiment

The cucumbers in the pot trial germinated and grew well, sprouting around day 7 (Figure S2). Based on the statistical analysis, there was no significant difference in the pH, OM, and TC and TN contents between the AA and CC groups ($p > 0.05$), as well as between the BB and DD groups. As shown in Figure 2a, the pH changes in the soil samples in the BLANK group fluctuated less. The pH of the soil samples in other groups showed a tendency of decreasing and then increasing, with the range mostly between 6.7 and 7.8. On days 0, 10, and 60, the pH of the BIOCHAR group was higher than that of the BLANK group. The pH of the AA, BB, CC, and DD groups was significantly higher than that of the BLANK group ($p < 0.05$). Adding biochar and compost increased the soil pH, as also noted by Naeem et al. [42]. This is mainly because biochar and compost are mostly weakly alkaline [43]. As shown in Figure 2b, the organic matter and total carbon contents of the BIOCHAR group were significantly higher than in the control group (BLANK) ($p < 0.05$). This indicated that compost can be used as a source of organic matter, providing much-needed nutrients to the soil [44]. As shown in Figure 2c,d, the total nitrogen contents of the AA and CC groups were significantly higher than that of the control group (BLANK)

($p < 0.05$). This is because biochar can directly improve the nitrogen cycle in the soil by dissolving organic nitrogen and fixing nitrogen [44]. Conclusively, the application of compost and biochar increased the organic matter, total carbon, and total nitrogen contents in the soil samples.

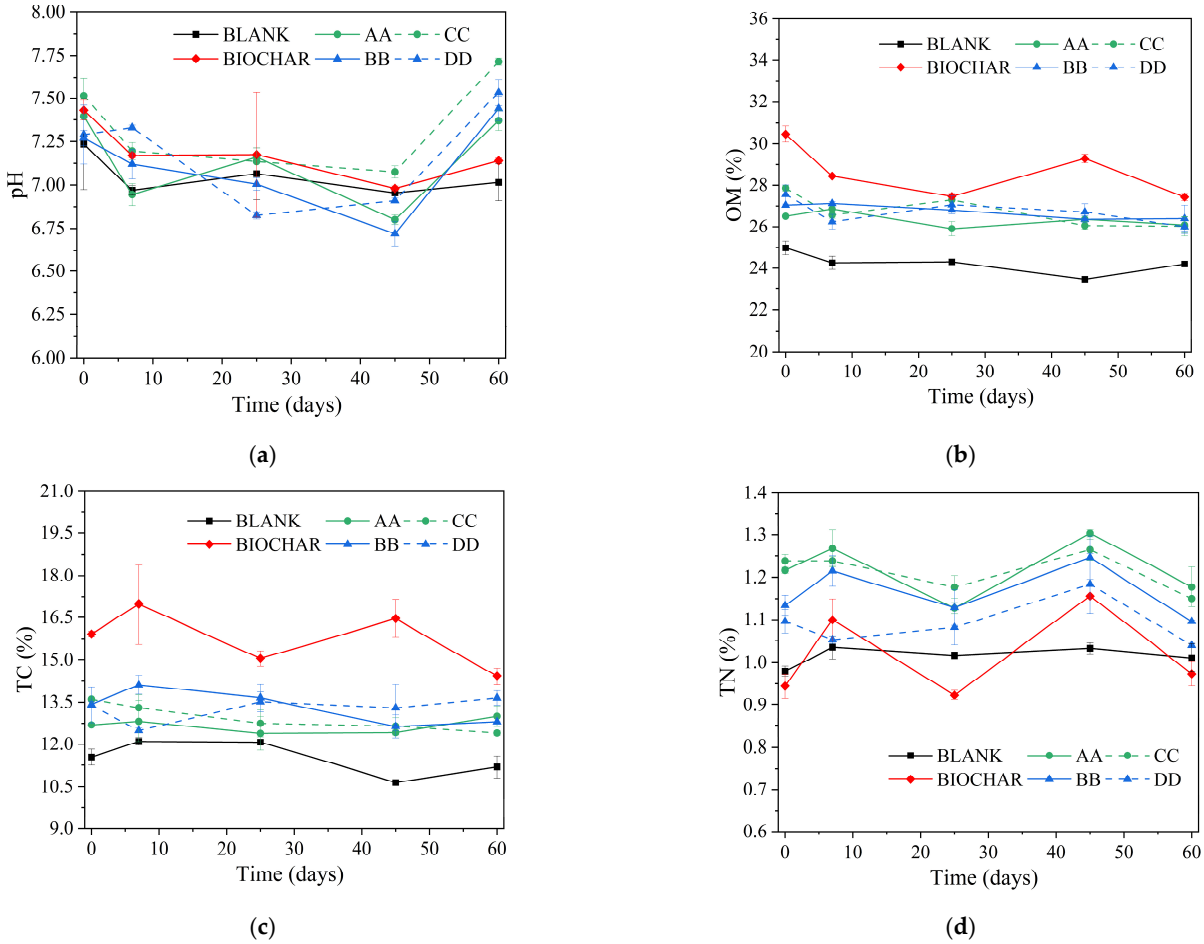


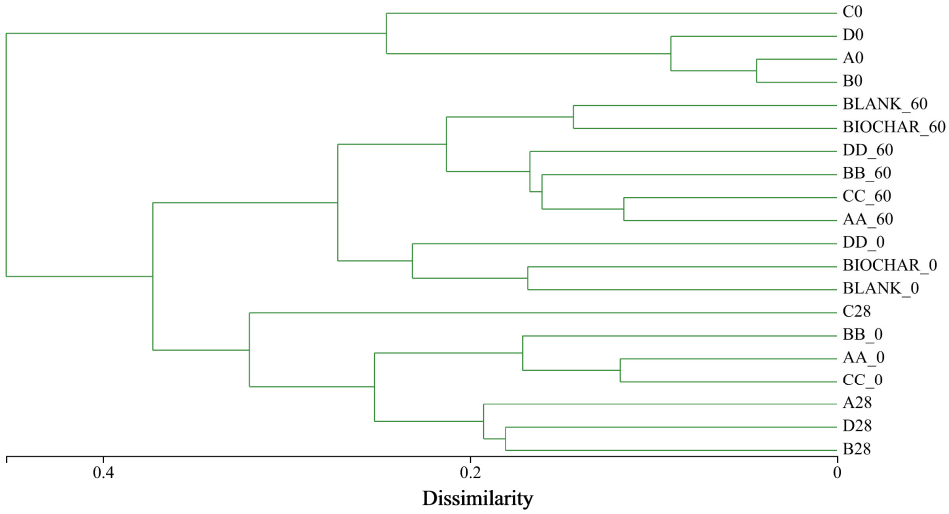
Figure 2. Dynamic changes in soil samples' physicochemical properties: (**a**) pH, (**b**) organic matter, (**c**) total carbon (TC) and (**d**) total nitrogen (TN).

In Figure S3, the wave peaks at 1077 cm^{-1} for different substances were Si-O-Si vibrational peaks [45]. Vermiculite and black soil had higher absorption values compared to the other substances, probably due to the greater abundance of elemental Si. The peak distributions of the added biochar (BIOCHAR) were similar to those of the control (BLANK) groups. The compost samples from A, B, C, and D showed C-H vibrational peaks at 2928 cm^{-1} and N-H vibrational peaks at 3313 cm^{-1}, which represent the alkyl group and amide, respectively. This indicated a higher content of carbon-based material and protein content flow into the soil samples of the AA, BB, CC, and DD groups. This is because compost is rich in nitrogen- and carbon-containing nutrients. Agegnehu et al. [19] proposed that the application of biochar to acidic soils not only improves the pH of the soil but also provides the soil with influential nitrogen. Compost is also rich in NH_4^+, which can be utilized to improve the physicochemical properties of soil and thus provide nutrients for cucumber seedlings.

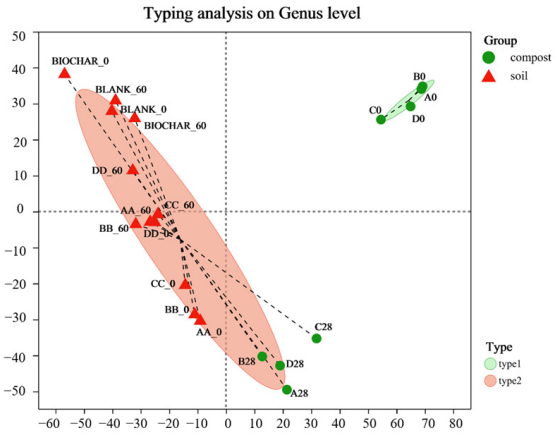
3.2. Analysis of the Bacterial Diversity in the Manure–Compost–Soil Process

In Figure 3a, the microbial composition of the initial compost sample was significantly different ($p < 0.05$) from that of the final compost sample and soil sample. However, there was a high similarity in microbial composition between the final compost samples (A28,

B28, C28, and D28) and the potting soil samples (AA0, BB0, CC0, and DD0) to which the compost was applied. This indicates that as the compost was applied to the soil, the microorganisms that survived in the compost samples flowed into the soil. As shown in Table 3, the microbial abundance of compost decreased significantly during the heating period of the aerobic composting process because bacterial activity was inhibited by the high temperature [8,46,47]. The microbial diversity index of the potted final soil samples (AA60, BB60, CC60, DD60, and BLANK60) increased compared to their respective initial soil samples. The microbial diversity index was lower in soil samples with added compost and biochar-based compost (AA0, BB0, CC0, and DD0) than in the control soil group (BLANK0), because of more bacteria from compost (Figure 3b). The microbial diversity index was higher in the soil sample with biochar application (BIOCAHR0) compared to the control (BLANK0) due to the increased nutrient retention capacity of the soil [48]. Xu et al. [26] found that the microbial diversity in the soil increased as the amount of biochar applied increased. Overall, the microbial composition of the soil was richer than that of the raw compost material (agricultural solid waste), making the study of microbial function in the soil microstructure complex.



(**a**)



(**b**)

Figure 3. Hierarchical clustering of microbes (**a**) and typing analysis (**b**) on the genus level.

Table 3. Microbial diversity by Alpha index of compost and soil samples.

Sample Name	Chao	Shannon	Simpson	Shannoneven	Simpsoneven
A0	252	3.314	0.111	0.599	0.036
C0	243	3.131	0.155	0.570	0.026
B0	294	3.542	0.098	0.623	0.035
D0	260	3.273	0.128	0.589	0.030
A28	199	3.840	0.060	0.725	0.084
B28	136	3.231	0.123	0.658	0.060
C28	268	4.092	0.058	0.732	0.064
D28	145	3.430	0.068	0.689	0.101
BLANK_0	635	5.449	0.014	0.844	0.114
AA_0	351	4.466	0.045	0.762	0.063
BB_0	278	3.372	0.199	0.599	0.018
CC_0	330	4.515	0.031	0.778	0.096
DD_0	436	4.967	0.019	0.817	0.121
BIOCHAR_0	1035	5.739	0.014	0.827	0.070
BLANK_60	896	6.259	0.004	0.921	0.298
AA_60	761	5.830	0.008	0.879	0.171
BB_60	856	5.771	0.014	0.855	0.083
CC_60	661	5.739	0.008	0.884	0.179
DD_60	987	6.288	0.004	0.912	0.243
BIOCHAR_60	758	6.145	0.004	0.927	0.332

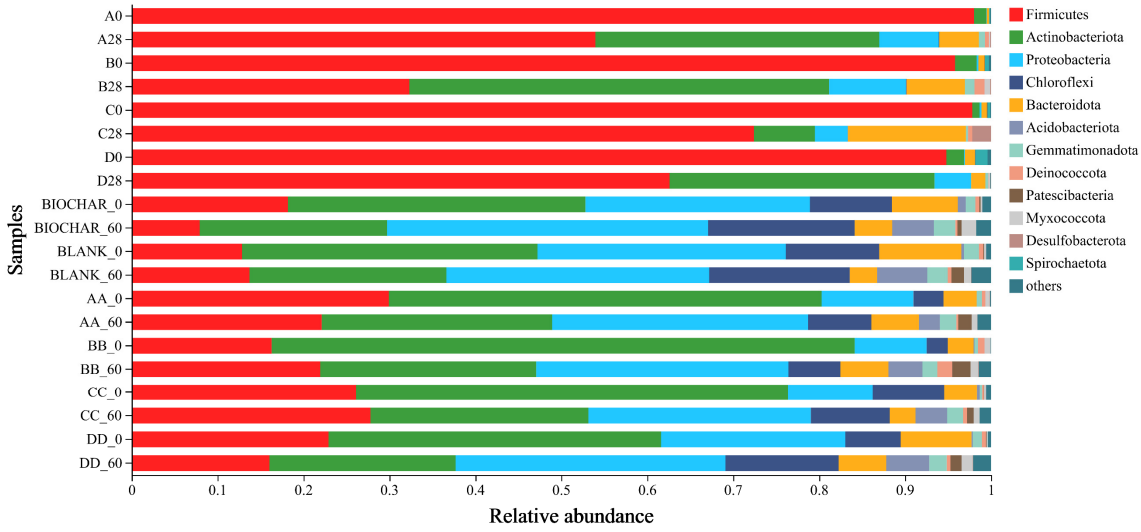
Notes: Alpha index is mainly used to study diversity of communities in certain habitats (or samples), reflecting information such as species, richness, and diversity of samples. Chao reflects community richness; Shannon and Simpson reflect community diversity; and Simpsoneven and Shannoneven reflect community evenness.

3.3. Bacterial Community Composition in the Manure–Compost–Soil Process

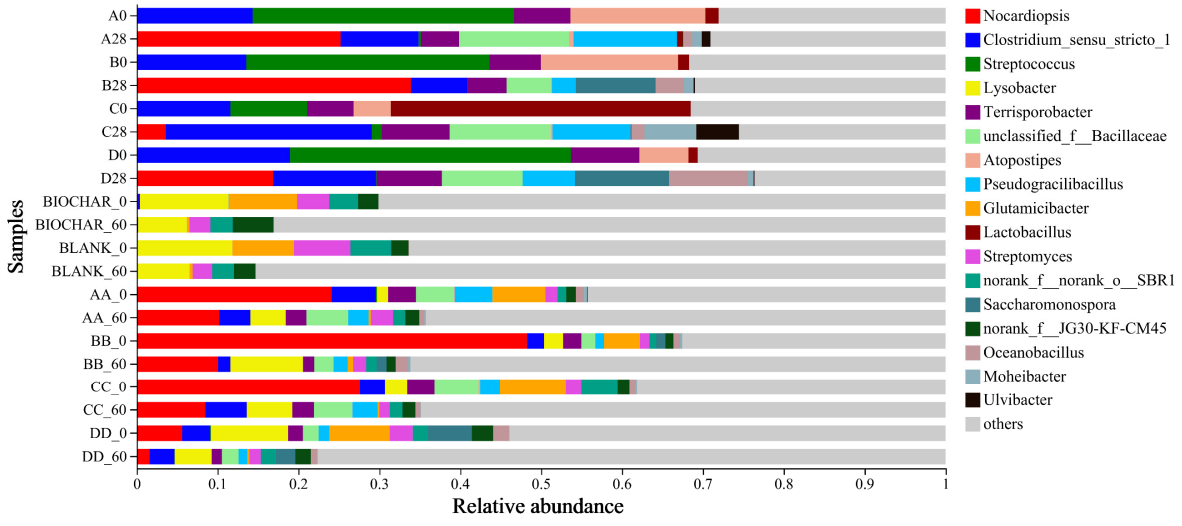
As seen in Figure 4a, the dominant phyla in the composting stage of the composting samples were *Firmicutes*, *Actinobacteriota*, and *Proteobacteria*, whose relative abundance (RA) accounted for more than 90% of the RA of the total bacterial flora. Mao et al. [47] found that the dominant bacterial phyla in the high-temperature period samples of pig manure compost with added bamboo biochar and bacterial agents were *Firmicutes* and *Proteobacteria*, and that the activity of the bacteria in the high-temperature period was affected by the soluble organic carbon content and temperature. The RA of *Firmicutes* exceeded 90% in the initial compost samples. *Actinobacteriota* gradually developed into the dominant bacterial phylum during the composting reaction, and biochar increased its RA in the compost samples. *Chloroflexi*, *Bacterioidota*, and *Acidobacteriota* accounted for more than 90% of RA in the soil samples. Previous studies found that *Proteobacteria*, *Acidobacteria*, *Chloroflexi*, and *Bacteroidetes* were also more abundant, which related to nitrogen transfer in the soil [26,47]. Biochar-containing soil samples (BIOCHAR group) had lower RAs of *Acidobacteria*, *Chloroflexi*, and *Gemmatimonadetes* and higher RAs of *Proteobacteria*, *Bacteroidetes*, and *Actinobacteria*, which indicated that biochar helped reduce soil nitrogen leaching and increased bacterial diversity in the soil. Thus, *Firmicutes* was the most abundant bacterial phylum in the compost samples. Among the soil samples, the largest number of bacterial species belonged to the *Actinobacteriota*. The composition of the bacterial phylum did not change much from the compost samples to the soil samples, but there were some differences in the subordinate bacterial species.

As known in Figure 4b, *Nocardiopsis* was more abundant in the soil samples of the compost (AA, CC) or biochar-based compost (BB, DD) application groups than of the control (BLANK) and biochar additive (BIOCHAR) groups. *Nocardiopsis* belongs to the *Actinobacteriota* and has been detected in compost and soil, producing a number of antimicrobial compounds, including thiopeptides [49,50]. It contributes to the recycling of organic compounds. *Streptococcus* was mostly present in the initial samples of compost, originating from manure, and was almost absent after aerobic high-temperature fermentation. It serves as an indicator of harmful microorganisms for the environmentally sound treatment of agricultural wastes [51]. *Atopostipes* (from around 0.2% to zero) and *Lactobacillus* (from

around 1.6% to zero) were only present in the initial samples of the compost in the A and C groups and had low tolerance in the B and D groups, which were decreased by biochar and the high-temperature fermentation of the compost [52], and biochar additive. *Clostridium_sensu_stricto_1* and *Terrisporobacter* were present in the later stages of composting and belong to the *Firmicutes*, the main genera carrying resistance genes [53]. *Lysimachia* belongs to the genus *Ganoderma* in the family *Xanthomonadaceae* and is a member of an ecologically important microbial community associated with soils and plants. It has been shown to induce systemic resistance in certain plant species, thereby protecting plants from pathogen infection [54,55]. *Glutamicibacter* is derived from soil samples that show a variety of potential plant growth-promoting properties and can tolerate high NaCl concentrations and a wide pH range [56]. *Streptomyces* is derived from soil samples and produces volatile organic compounds that have the potential to inhibit soil diseases [57]. Thus, aerobic composting can eliminate toxic or negative bacteria directly deposited in the soil by manure. Compost or biochar-based compost applied to soil as a fertilizer can introduce some genera into the soil, such as *Nocardiopsis* (RA over 10%) and *Clostridium_sensu_stricto_1* (RA over 5%), which have a positive influence on the microbial diversity of the soil.



(**a**)



(**b**)

Figure 4. Microbial composition histogram at phylum level (**a**) and genus level (**b**).

3.4. Difference Analysis of Bacteria from Pig Manure–Compost to Compost–Soil

Figure 5 presents a detailed analysis of bacterial diversity at the genus level in both compost and soil samples. While certain genera like *Nocardiopsis*, *Saccharomonospora*, *Bacillus*, and *Oceanobacillus* exhibited differences in abundance between compost and soil, these differences were not statistically significant. However, at a 95% confidence interval, notable variations emerged for other genera, including *Clostridium_sensu_stricto_1*, *Streptococcus*, *Terrisporobacter*, *Lysobacter*, *Atopostipes*, *Lactobacillus*, *Glutamicibacter*, *Romboutsia*, and *Streptomyces*. Notably, *Lysobacter*, *Glutamicibacter*, and *Streptomyces* were considerably more abundant in soil samples compared to compost samples, highlighting their dominance in the soil environment.

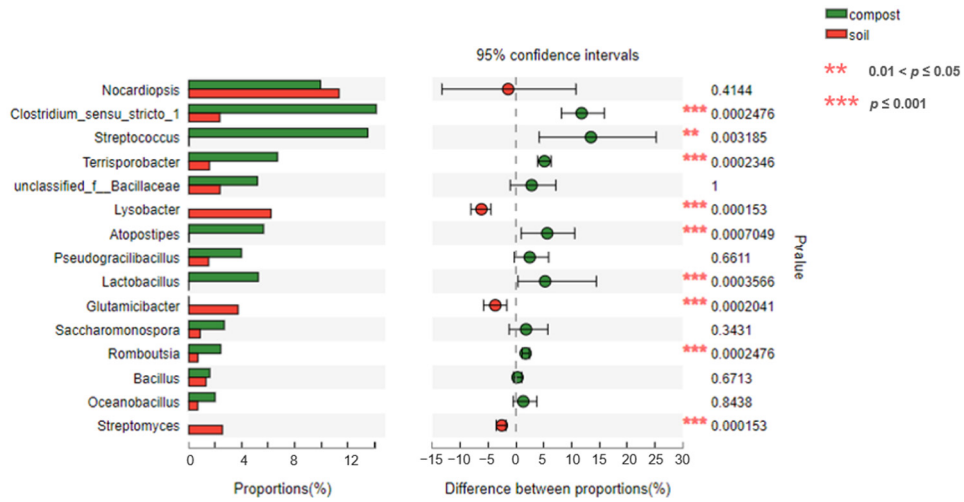


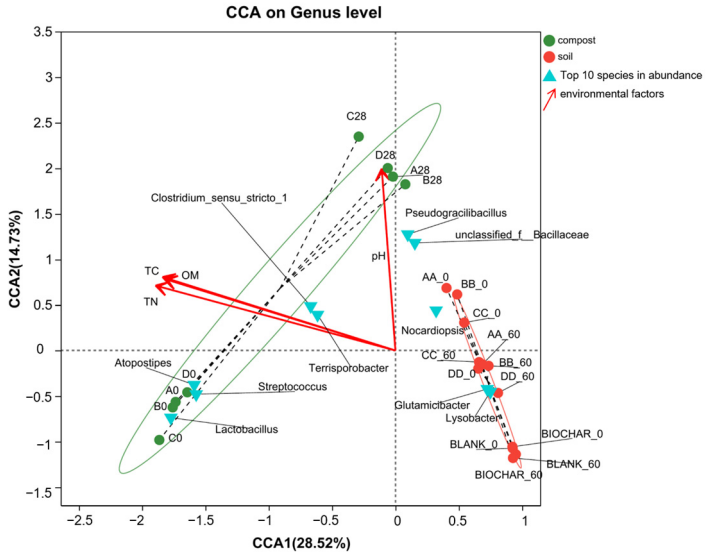
Figure 5. Difference analysis of microbes at the genus level in compost and soil samples.

Intriguingly, the manure initially contained potentially contaminating and toxic genera such as *Saccharomonospora* (from 2.68% to 0.80%), *Atopostipes* (from 5.71% to 0.13%), and *Lactobacillus* (from 5.27% to 0.04%), which were effectively eliminated through the high-temperature aerobic composting process. This finding underscores the sanitizing effect of composting on manure-derived microbial contaminants.

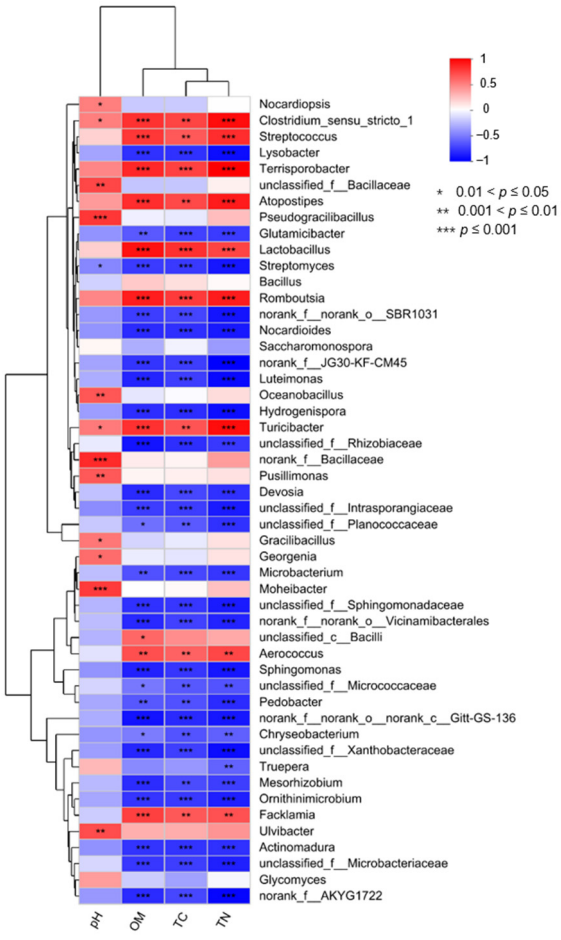
In contrast, soil samples from the potting experiment revealed *Lysobacter*, *Glutamicibacter*, and *Streptomyces* as the dominant genera, contributing positively to the microbial diversity of the soil. Furthermore, the application of compost or biochar-amended compost as fertilizer introduced additional beneficial genera like *Nocardiopsis* and *Clostridium_sensu_stricto_1* into the soil, further enriching its microbial diversity. These results demonstrate the potential of biochar-based compost to promote a healthy and diverse microbial ecosystem in agricultural soils.

3.5. Correlation Analysis of Bacteria and Physicochemical Properties in the Manure–Compost–Soil Process

In Figure 6a, the structure of bacterial communities in the four soil experimental groups (AA, BB, CC, and DD) underwent a notable transformation over the 60-day period. Initially dispersed across different regions, the groups converged into a single region by the end of the experiment, indicating that both compost and biochar-amended compost exert comparable influences on bacterial genetics in soil. In contrast, during the composting phase, the four compost groups remained clustered together, whereas the biochar-supplemented group in the soil experiment exhibited a distinct spatial pattern. This suggests that while biochar did not directly alter the genetic makeup of bacteria during composting, it began to exert its effects once introduced into the soil system. Regarding the nutrient dynamics, the synchronous variations in total carbon and nitrogen concentrations reflect their mutual dependence on organic matter degradation [33].



(**a**)



(**b**)

Figure 6. Canonical correlation analysis (CCA) (**a**) and Spearman correlation heatmap (**b**) between physicochemical properties and microbes at the genus level.

In Figure 6b, notable associations were observed between specific genera and physicochemical properties. For instance, *Pseudohyphobacterium* and *Marsupialia* displayed strong correlations with the pH, while *Aerococcus* and *Facklamia* were positively linked to mois-

ture content Moreover, bacterial genera such as *Romboutsia*, *Turicibacter*, and *Lactobacillus* exhibited positive correlations with organic matter, total carbon, and total nitrogen levels. In contrast, *Lysobacter*, *Streptomyces*, and *Ornithinimicrobium* showed negative correlations with these parameters [57]. Notably, *Lactobacillus*, known for its strict fermentation and anaerobic nature, was among the genera positively associated with soil fertility indicators.

4. Conclusions

This paper's novelty stems from its analysis of the complex interactions between biochar, compost, and soil microbial communities, which led to new insights into how biochar-based compost can enhance soil health and microbial succession. The results demonstrate that biochar addition during aerobic composting increased the C/N ratio and total carbon content of the compost. When applied to soil, composts containing biochar increased the soil's pH, organic matter, total carbon, and total nitrogen contents compared to composts without biochar. Microbial diversity decreased during the high-temperature phase of aerobic composting but increased significantly in the final compost products. Soil samples amended with composts showed higher microbial diversity than unamended soil. The microbial communities in the composts and soil samples were distinct, but some bacterial genera (e.g., *Nocardiopsis*, *Clostridium_sensu_stricto_1*) persisted and contributed positively to the microbial diversity of the soil. Correlation analysis revealed significant relationships between bacterial genera and soil physicochemical properties, providing insights into the microbial mechanisms underlying the observed effects of biochar on soil quality.

Future studies could further analyze the microbial structure during the manure–compost–soil process by taking plant samples in conjunction with assessing plant growth. The use of different types of soils could also be considered to conduct in-depth and systematic research related to improving the physicochemical properties or microbial succession induced by compost, biochar, or biochar-based compost.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su16187910/s1, Figure S1. Soil raw materials and mixed actual substrate of pot experiments in different groups. Figure S2. Progress in cucumber potting trials. Figure S3. Wave number diagrams for different substances.

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List of Abbreviations

Properties	abbreviations
moisture content, %	MC
organic matter, %	OM
total carbon, %	TC
total nitrogen, %	TN
Initial aerobic composting samples on Day 0 of A	A0
Initial aerobic composting samples on Day 0 of B	B0
Initial aerobic composting samples on Day 0 of C	C0
Initial aerobic composting samples on Day 0 of D	D0
Final aerobic composting samples on Day 28 of A	A28
Final aerobic composting samples on Day 28 of B	B28
Final aerobic composting samples on Day 28 of C	C28
Final aerobic composting samples on Day 28 of D	D28
Initial soil samples of potting experiment on Day 0 of BLANK	BLANK_0
Initial soil samples of potting experiment on Day 0 of BIOCHAR	BIOCHAR_0
Initial soil samples of potting experiment on Day 0 of AA	AA_0
Initial soil samples of potting experiment on Day 0 of BB	BB_0
Initial soil samples of potting experiment on Day 0 of CC	CC_0
Initial soil samples of potting experiment on Day 0 of DD	DD_0
Final soil samples of potting experiment on Day 60 of BLANK	BLANK_60
Final soil samples of potting experiment on Day 60 of BIOCHAR	BIOCHAR_60
Final soil samples of potting experiment on Day 60 of AA	AA_60
Final soil samples of potting experiment on Day 60 of BB	BB_60
Final soil samples of potting experiment on Day 60 of CC	CC_60
Final soil samples of potting experiment on Day 60 of DD	DD_60

References

1. Ma, S.; Sun, X.; Fang, C.; He, X.; Han, L.; Huang, G. Exploring the mechanisms of decreased methane during pig manure and wheat straw aerobic composting covered with a semi-permeable membrane. *Waste Manag.* **2018**, *78*, 393–400. [CrossRef] [PubMed]
2. Ravindran, B.; Nguyen, D.D.; Chaudhary, D.K.; Chang, S.W.; Kim, J.; Lee, S.R.; Shin, J.; Jeon, B.; 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] [PubMed]
3. Haug, R. *The Practical Handbook of Compost Engineering*; CRC Press: Boca Raton, FL, USA, 1993.
4. Zhou, S.; Chang, T.; Zhang, Y.; Shaghaleh, H.; Zhang, J.; Yang, X.; Qin, H.; Talpur, M.M.A.; Alhaj Hamoud, Y. Organic fertilizer compost alters the microbial composition and network structure in strongly acidic soil. *Appl. Soil. Ecol.* **2024**, *195*, 105263. [CrossRef]
5. Morra, L.; Bilotto, M.; Baldantoni, D.; Alfani, A.; Baiano, S. A seven-year experiment in a vegetable crops sequence: Effects of replacing mineral fertilizers with Biowaste compost on crop productivity, soil organic carbon and nitrates concentrations. *Sci. Hortic.* **2021**, *290*, 110534. [CrossRef]
6. Scotti, R.; Pane, C.; Spaccini, R.; Palese, A.M.; Piccolo, A.; Celano, G.; Zaccardelli, M. On-farm compost: A useful tool to improve soil quality under intensive farming systems. *Appl. Soil. Ecol.* **2016**, *107*, 13–23. [CrossRef]
7. Awasthi, M.K.; Zhang, Z.; Wang, Q.; Shen, F.; Li, R.; Li, D.; Ren, X.; Wang, M.; Chen, H.; Zhao, J. New insight with the effects of biochar amendment on bacterial diversity as indicators of biomarkers support the thermophilic phase during sewage sludge composting. *Bioresour. Technol.* **2017**, *238*, 589–601. [CrossRef]
8. He, X.; Yin, H.; Han, L.; Cui, R.; Fang, C.; Huang, G. Effects of biochar size and type on gaseous emissions during pig manure/wheat straw aerobic composting: Insights into multivariate-microscale characterization and microbial mechanism. *Bioresour. Technol.* **2019**, *271*, 375–382. [CrossRef]
9. Ma, S.; Fang, C.; Sun, X.; Han, L.; He, X.; Huang, G. Bacterial community succession during pig manure and wheat straw aerobic composting covered with a semi-permeable membrane under slight positive pressure. *Bioresour. Technol.* **2018**, *259*, 221–227. [CrossRef]
10. Babin, D.; Leoni, C.; Neal, A.L.; Sessitsch, A.; Smalla, K. Editorial to the Thematic Topic "Towards a more sustainable agriculture through managing soil microbiomes". *FEMS Microbiol. Ecol.* **2021**, *97*, fiab094. [CrossRef]
11. Fierer, N.; Wood, S.A.; Bueno De Mesquita, C.P. How microbes can, and cannot, be used to assess soil health. *Soil. Biol. Biochem.* **2021**, *153*, 108111. [CrossRef]

12. Maron, P.; Sarr, A.; Kaisermann, A.; Lévêque, J.; Mathieu, O.; Guigue, J.; Karimi, B.; Bernard, L.; Dequiedt, S.; Terrat, S.; et al. High Microbial Diversity Promotes Soil Ecosystem Functioning. *Appl. Environ. Microbiol.* **2018**, *84*, e02738-17. [CrossRef] [PubMed]
13. Bol, R.; Amelung, W.; Friedrich, C.; Ostle, N. Tracing dung-derived carbon in temperate grassland using 13C natural abundance measurements. *Soil. Biol. Biochem.* **2000**, *32*, 1337–1343. [CrossRef]
14. Duan, M.; Li, H.; Gu, J.; Tuo, X.; Sun, W.; Qian, X.; Wang, X. Effects of biochar on reducing the abundance of oxytetracycline, antibiotic resistance genes, and human pathogenic bacteria in soil and lettuce. *Environ. Pollut.* **2017**, *224*, 787–795. [CrossRef]
15. Mawof, A.; Prasher, S.; Bayen, S.; Nzediegwu, C. Effects of Biochar and Biochar-Compost Mix as Soil Amendments on Soil Quality and Yield of Potatoes Irrigated with Wastewater. *J. Soil. Sci. Plant Nutr.* **2021**, *21*, 2600–2612. [CrossRef]
16. Purakayastha, T.J.; Bera, T.; Bhaduri, D.; Sarkar, B.; Mandal, S.; Wade, P.; Kumari, S.; Biswas, S.; Menon, M.; Pathak, H.; et al. A review on biochar modulated soil condition improvements and nutrient dynamics concerning crop yields: Pathways to climate change mitigation and global food security. *Chemosphere* **2019**, *227*, 345–365. [CrossRef] [PubMed]
17. Nguyen, M.K.; Lin, C.; Hoang, H.G.; Sanderson, P.; Dang, B.T.; Bui, X.T.; Nguyen, N.S.H.; Vo, D.N.; Tran, H.T. Evaluate the role of biochar during the organic waste composting process: A critical review. *Chemosphere* **2022**, *299*, 134488. [CrossRef]
18. Sanchez-Monedero, M.A.; Cayuela, M.L.; Roig, A.; Jindo, K.; Mondini, C.; Bolan, N. Role of biochar as an additive in organic waste composting. *Bioresour. Technol.* **2018**, *247*, 1155–1164. [CrossRef] [PubMed]
19. Agegnehu, G.; Srivastava, A.K.; Bird, M.I. The role of biochar and biochar-compost in improving soil quality and crop performance: A review. *Appl. Soil. Ecol.* **2017**, *119*, 156–170. [CrossRef]
20. Fu, T.; Shangguan, H.; Wu, J.; Tang, J.; Yuan, H.; Zhou, S. Insight into the synergistic effects of conductive biochar for accelerating maturation during electric field-assisted aerobic composting. *Bioresour. Technol.* **2021**, *337*, 125359. [CrossRef]
21. Guo, X.; Liu, H.; Zhang, J. The role of biochar in organic waste composting and soil improvement: A review. *Waste Manag.* **2020**, *102*, 884–899. [CrossRef]
22. Kung, C.; Mu, J.E. Prospect of China's renewable energy development from pyrolysis and biochar applications under climate change. *Renew. Sustain. Energy Rev.* **2019**, *114*, 109343. [CrossRef]
23. Sun, X.; Ma, S.; Han, L.; Li, R.; Schlick, U.; Chen, P.; Huang, G. The effect of a semi-permeable membrane-covered composting system on greenhouse gas and ammonia emissions in the Tibetan Plateau. *J. Clean. Prod.* **2018**, *204*, 778–787. [CrossRef]
24. Xiao, L.; Feng, L.R.; Yuan, G.D.; Wei, J. Low-cost field production of biochars and their properties. *Environ. Geochem. Health* **2020**, *42*, 1569–1578. [CrossRef] [PubMed]
25. Yin, Y.; Tao, X.; Du, Y.; Li, M.; Yang, S.; Zhang, W.; Yang, C.; Li, H.; Wang, X.; Chen, R. Biochar improves the humification process during pig manure composting: Insights into roles of the bacterial community and metabolic functions. *J. Environ. Manag.* **2024**, *355*, 120463. [CrossRef]
26. Xu, N.; Tan, G.C.; Wang, H.Y.; Gai, X.P. Effect of biochar additions to soil on nitrogen leaching, microbial biomass and bacterial community structure. *Eur. J. Soil. Biol.* **2016**, *74*, 1–8. [CrossRef]
27. Liu, N.; Zhou, J.; Han, L.; Ma, S.; Sun, X.; Huang, G. Role and multi-scale characterization of bamboo biochar during poultry manure aerobic composting. *Bioresour. Technol.* **2017**, *241*, 190–199. [CrossRef] [PubMed]
28. He, X.; Yin, H.; Fang, C.; Xiong, J.; Han, L.; Yang, Z.; Huang, G. Metagenomic and q-PCR analysis reveals the effect of powder bamboo biochar on nitrous oxide and ammonia emissions during aerobic composting. *Bioresour. Technol.* **2021**, *323*, 124567. [CrossRef]
29. He, X.; Chen, L.; Han, L.; Liu, N.; Cui, R.; Yin, H.; Huang, G. Evaluation of biochar powder on oxygen supply efficiency and global warming potential during mainstream large-scale aerobic composting. *Bioresour. Technol.* **2017**, *245*, 309–317. [CrossRef]
30. Xiong, J.; Ma, S.; He, X.; Han, L.; Huang, G. Nitrogen transformation and dynamic changes in related functional genes during functional-membrane covered aerobic composting. *Bioresour. Technol.* **2021**, *332*, 125087. [CrossRef]
31. Fang, C.; Yuan, X.; Liao, K.; Qu, H.; Han, L.; He, X.; Huang, G. Micro-aerobic conditions based on membrane-covered improves the quality of compost products: Insights into fungal community evolution and dissolved organic matter characteristics. *Bioresour. Technol.* **2022**, *362*, 127849. [CrossRef]
32. Mori, H.; Maruyama, F.; Kato, H.; Toyoda, A.; Dozono, A.; Ohtsubo, Y.; Nagata, Y.; Fujiyama, A.; Tsuda, M.; Kurokawa, K. Design and Experimental Application of a Novel Non-Degenerate Universal Primer Set that Amplifies Prokaryotic 16S rRNA Genes with a Low Possibility to Amplify Eukaryotic rRNA Genes. *DNA Res.* **2014**, *21*, 217–227. [CrossRef] [PubMed]
33. López-Cano, I.; Roig, A.; Cayuela, M.L.; Alburquerque, J.A.; Sánchez-Monedero, M.A. Biochar improves N cycling during composting of olive mill wastes and sheep manure. *Waste Manag.* **2016**, *49*, 553–559. [CrossRef] [PubMed]
34. Sánchez-García, M.; Alburquerque, J.A.; Sánchez-Monedero, M.A.; Roig, A.; Cayuela, M.L. Biochar accelerates organic matter degradation and enhances N mineralisation during composting of poultry manure without a relevant impact on gas emissions. *Bioresour. Technol.* **2015**, *192*, 272–279. [CrossRef]
35. Wei, Y.; Zhao, Y.; Shi, M.; Cao, Z.; Lu, Q.; Yang, T.; Fan, Y.; Wei, Z. Effect of organic acids production and bacterial community on the possible mechanism of phosphorus solubilization during composting with enriched phosphate-solubilizing bacteria inoculation. *Bioresour. Technol.* **2018**, *247*, 190–199. [CrossRef]
36. Xie, T.; Zhang, Z.; Zhang, D.; Wei, C.; Lin, Y.; Feng, R.; Nan, J.; Feng, Y. Effect of hydrothermal pretreatment and compound microbial agents on compost maturity and gaseous emissions during aerobic composting of kitchen waste. *Sci. Total Environ.* **2023**, *854*, 158712. [CrossRef]

37. Tiquia, S.M.; Tam, N.F.Y. Elimination of phytotoxicity during co-composting of spent pig-manure sawdust litter and pig sludge. *Bioresour. Technol.* **1998**, *65*, 43–49. [CrossRef]
38. Kong, Y.; Zhang, J.; Yang, Y.; Liu, Y.; Zhang, L.; Wang, G.; Liu, G.; Dang, R.; Li, G.; Yuan, J. Determining the extraction conditions and phytotoxicity threshold for compost maturity evaluation using the seed germination index method. *Waste Manag.* **2023**, *171*, 502–511. [CrossRef]
39. Zhang, L.; Sun, X. Changes in physical, chemical, and microbiological properties during the two-stage co-composting of green waste with spent mushroom compost and biochar. *Bioresour. Technol.* **2014**, *171*, 274–284. [CrossRef] [PubMed]
40. Ottani, F.; Parenti, M.; Santunione, G.; Moscatelli, G.; Kahn, R.; Pedrazzi, S.; Allesina, G. Effects of different gasification biochar grain size on greenhouse gases and ammonia emissions in municipal aerated composting processes. *J. Environ. Manag.* **2023**, *331*, 117257. [CrossRef]
41. Yu, H.; Xie, B.; Khan, R.; Shen, G. The changes in carbon, nitrogen components and humic substances during organic-inorganic aerobic co-composting. *Bioresour. Technol.* **2019**, *271*, 228–235. [CrossRef]
42. Naeem, M.A.; Khalid, M.; Aon, M.; Abbas, G.; Amjad, M.; Murtaza, B.; Khan, W.; Ahmad, N. Combined application of biochar with compost and fertilizer improves soil properties and grain yield of maize. *J. Plant Nutr.* **2018**, *41*, 112–122. [CrossRef]
43. Liu, H.; Awasthi, M.K.; Zhang, Z.; Syed, A.; Bahkali, A.H. Evaluation of gases emission and enzyme dynamics in sheep manure compost occupying with peach shell biochar. *Environ. Pollut.* **2024**, *351*, 124065. [CrossRef] [PubMed]
44. Aina Najwa Mohd Nor Azman, N.; Inderakusumowati Md Khalid, P.; Aishah Saidina Amin, N.; Yamani Zakaria, Z.; Mohammad Zainol, M.; Ilham, Z.; Phaiboonsilpa, N.; Asmadi, M. Effects of biochar, compost, and composted biochar soil amendments on okra plant growth. *Mater. Today Proc.* 2023; *in press*. [CrossRef]
45. Neville, F.; Seyfaee, A. Real-Time Monitoring of in Situ Polyethyleneimine-Silica Particle Formation. *Langmuir* **2013**, *29*, 14681–14690. [CrossRef]
46. He, X.; Han, L.; Fu, B.; Du, S.; Liu, Y.; Huang, G. Effect and microbial reaction mechanism of rice straw biochar on pore methane production during mainstream large-scale aerobic composting in China. *J. Clean. Prod.* **2019**, *215*, 1223–1232. [CrossRef]
47. Mao, H.; Lv, Z.; Sun, H.; Li, R.; Zhai, B.; Wang, Z.; Awasthi, M.K.; Wang, Q.; Zhou, L. Improvement of biochar and bacterial powder addition on gaseous emission and bacterial community in pig manure compost. *Bioresour. Technol.* **2018**, *258*, 195–202. [CrossRef]
48. Wang, P.; Wang, S.; Chen, F.; Zhang, T.; Kong, W. Preparation of two types plant biochars and application in soil quality improvement. *Sci. Total Environ.* **2024**, *906*, 167334. [CrossRef]
49. Bennur, T.; Kumar, A.R.; Zinjarde, S.; Javdekar, V. Nocardiopsis species: Incidence, ecological roles and adaptations. *Microbiol. Res.* **2015**, *174*, 33–47. [CrossRef]
50. Ibrahim, A.H.; Desoukey, S.Y.; Fouad, M.A.; Kamel, M.S.; Gulder, T.A.M.; Abdelmohsen, U.R. Natural Product Potential of the Genus *Nocardiopsis*. *Mar. Drugs* **2018**, *16*, 147. [CrossRef]
51. Jepsen, S.E.; Krause, M.; Gruttner, H. Reduction of Fecal Streptococcus and Salmonella by selected treatment methods for sludge and organic waste. *Water Sci. Technol.* **1997**, *36*, 203–210. [CrossRef]
52. Li, X.; Shi, X.; Lu, M.; Zhao, Y.; Li, X.; Peng, H.; Guo, R. Succession of the bacterial community and functional characteristics during continuous thermophilic composting of dairy manure amended with recycled ceramsite. *Bioresour. Technol.* **2019**, *294*, 122044. [CrossRef] [PubMed]
53. Wang, J.; Gu, J.; Wang, X.; Song, Z.; Dai, X.; Guo, H.; Yu, J.; Zhao, W.; Lei, L. Enhanced removal of antibiotic resistance genes and mobile genetic elements during swine manure composting inoculated with mature compost. *J. Hazard Mater.* **2021**, *411*, 125135. [CrossRef] [PubMed]
54. Exposito, R.G.; Postma, J.; Raaijmakers, J.M.; De Bruijn, I. Diversity and Activity of *Lysobacter* Species from Disease Suppressive Soils. *Front. Microbiol.* **2015**, *6*, 1243.
55. Puopolo, G.; Tomada, S.; Pertot, I. The impact of the omics era on the knowledge and use of *Lysobacter* species to control phytopathogenic micro-organisms. *J. Appl. Microbiol.* **2018**, *124*, 15–27. [CrossRef]
56. Qin, S.; Feng, W.; Zhang, Y.; Wang, T.; Xiong, Y.; Xing, K. Diversity of Bacterial Microbiota of Coastal Halophyte *Limonium sinense* and Amelioration of Salinity Stress Damage by Symbiotic Plant Growth-Promoting Actinobacterium *Glutamicibacter halophytocola* KLBMP 5180. *Appl. Environ. Microb.* **2018**, *84*, e01533-18. [CrossRef]
57. Cordovez, V.; Carrion, V.J.; Etalo, D.W.; Mumm, R.; Zhu, H.; van Wezel, G.P.; Raaijmakers, J.M. Diversity and functions of volatile organic compounds produced by *Streptomyces* from a disease-suppressive soil. *Front. Microbiol.* **2015**, *6*, 1081. [CrossRef]