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Effect of Biochar on Soil Physiochemical Properties and Bacterial Diversity in Dry Direct-Seeded Rice Paddy Fields

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Abstract: The effects of biochar application on dry direct-seeded rice paddies remains unclear. Therefore, we applied biochar to dry direct-seeded rice paddy fields over 3 consecutive years to assess its effects on soil physicochemical properties and bacterial diversity (conventional fertilization [CK]; biochar + conventional fertilization [BC]). BC increased the content of 0.25–5 mm soil water-stable aggregate particles, but decreased that of <0.25 mm soil water-stable aggregates. At different soil depths, BC significantly reduced sand content and increased silt content. Compared to CK, BC significantly increased the available phosphorus and potassium content of the 0–10 and 10–20 cm soil layers. There were no significant differences in pH, organic matter, total nitrogen, total phosphorus, or total potassium content between the treatments at different soil depths. Compared to CK, BC significantly increased soil neutral phosphatase and catalase activities. Furthermore, BC significantly increased bacterial richness, but had no significant effect on bacterial diversity. According to Qualcomm sequencing analysis, BC increased the relative abundance of Verrucomicrobia, Chloroflexi, Bacteroidetes, Nitrospirae, Verrucomicrobiae, Blastocatellia_Subgroup_4, and Anaerolineae in soil compared to CK. The soil bacterial genera in BC had stronger interrelationships than those in CK. According to redundancy analysis, organic matter was the main environmental factor influencing bacterial community structure. Overall, biochar could promote soil nutrient conversion in dry direct-seeded rice paddies, improve soil effective nutrient content, change the composition of soil bacterial communities, and increase soil bacterial richness. Applying biochar in dry direct-seeded rice cultivation could help realize low-carbon agriculture.

Keywords: biochar; dry direct-seeded rice paddy; soil physiochemical properties; bacterial diversity

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Citation: Lv, Y.; Xu, L.; Guo, X.; Liu, J.; Zou, B.; Guo, Y.; Zhang, Y.; Li, H.; Zheng, G.; Guo, Y.; et al. Effect of Biochar on Soil Physiochemical Properties and Bacterial Diversity in Dry Direct-Seeded Rice Paddy Fields. *Agronomy* **2023**, *13*, 4. https://doi.org/10.3390/agronomy13010004

Academic Editor: Jianjun Yang

Received: 30 November 2022
Revised: 14 December 2022
Accepted: 16 December 2022
Published: 20 December 2022

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1. Introduction

Rice is the world's third most important food crop, and global rice production must increase by 28% to meet the needs of the growing population by 2050 [1]. At present, socioeconomic development and global climate change have caused shortages in labor and water resources, adversely affecting rice cultivation and threatening food security. However, dry direct-seeded rice cultivation is advantageous because of its simple mechanization, and low labor and water requirements [2]. In recent years, dry direct-seeded rice cultivation has been widely utilized throughout China, especially in northeastern China [3]. As the rice yield increases, the amount of straw also increases. In northeastern China, open burning was previously the most common method for farmers to dispose of straw. Open burning of straw can result in a series of problems, such as air pollution which endangers human

health; runaway fires spreading to the surrounding areas; decreased visibility, which threatens traffic safety; and damage to soil structure, thereby decreasing the quality of cultivated land [4]. At present, in addition to returning straw to the field which can solve these problems [5], another effective strategy is to make biochar with straw, as it improves soil quality when returned to the field. Biochar application is conducive to improving soil conditions (aeration and nutrient availability) for crop growth. Furthermore, it directly and practically involves farmers in greenhouse gas reduction policies [6].

Biochar is a solid residue produced by low-temperature pyrolysis of straw wood chips, manure, sewage sludge, and nut shells under anaerobic conditions. It has the potential to not only alter pore size distribution, water retention, seepage mode, and the flow path of soil [7], but also to improve the retention of nitrogen, calcium, phosphorus, and other nutrients. In recent years, studies have highlighted that water-soluble compounds in biochar could alter micronutrients and macronutrients, and soil productivity [8,9]; thus, they were considered effective conditioners for improving soil quality. Moreover, biochar could substantially improve the bioavailability of dissolved organic matter, reduce the humization degree of dissolved organic matter, and improve its activity. Zhang et al. [10] showed that one-time biochar input could considerably reduce the bulk density of soil, thereby improving the content of organic matter, total nitrogen, and available potassium. Biochar application could also increase the pH of acidic soils, and reduce soil nutrient loss by improving soil water retention [11]. Furthermore, some microbial organisms can be protected from predation in the pores of biochar, thereby increasing the soil microbial biomass and diversity [12]. However, the results of the effects of biochar on soil microbial abundance and community composition have been inconsistent [13,14]. This might be because biochar from different sources is often used by different microbial groups owing to structural characteristics and component differences [15]. Thus, the microbial community structure changes would also be different. Biochar is porous, with a large surface area, contains high levels of carbon, and provides an excellent habitat and nutrients for soil microorganisms, which directly affect the growth of microorganisms [16]. Previous research has shown that because the direct use of components of biochar by microorganisms was limited [17], the changes in microbial community structure were mainly observed indirectly. These included the effects on soil pH, nutrient status, enzyme activity, and aggregates [18,19]. In addition, the changes in soil microbial activity and community structure composition from biochar were also affected by the properties of the applied soil itself, different test conditions, and fertility levels [20].

The change in planting mode, to dry direct-seeded rice cultivation, changes the soil environment which changes rice root growth. This may indirectly affect the microbial community structure of the rhizosphere. How biochar affects soil aggregate, pH, organic matter, nutrients, and enzyme activity, which in turn affects soil microbial abundance and community composition, has not been widely reported. The differences in the effects of different types of biochar on soil microbial community structures remain unclear, and the mechanisms affecting microbial populations need to be further evaluated. Therefore, through localized trials for 3 consecutive years, we attempted to understand these specific problems. More specifically, we (1) evaluated the influence of biochar on soil aggregate content, physicochemical properties, and soil enzyme activity in dry direct-seeded rice paddy fields; (2) identified dominant bacteria in dry direct-seeded rice paddy fields after biochar application; and (3) revealed the main environmental factors affecting the bacterial community structure in dry live paddy field soil after biochar application. These results lay a theoretical foundation for the use of biochar for soil improvement, and the use of fertilizer in dry direct-seeded rice paddy fields.

2. Materials and Methods

2.1. Experimental Site and Materials

The fertilization regimes, described in the following section, were applied for three consecutive dry direct-seeded rice campaigns (2018–2020) under field conditions. The plots were located in Youyi farm, Heilongjiang Province (46.45′ N, 131.49′ E). The climate in this region corresponds to the middle temperate continental monsoon, with an annual mean temperature and precipitation of 3.1 °C and 501.2 mm, respectively. The soil type in this area is meadow black soil.

Biochar was provided by Shenyang Longtai Biological Engineering Co., Ltd., Liaoning Province, China. Biochar was made from corn straw via high-temperature pyrolysis at 500 °C for 1 h without oxygen. The basic physicochemical properties of biochar were determined according to Bao's (2000) soil agrochemical analysis protocol [21]. The results were as follows: pore volume, 0.0425 $cm^3\ g^{-1}$; specific surface area, 106.81 $m^2\ g^{-1}$; pH, 9.04; organic carbon, 264.6 g kg^{-1}; total nitrogen, 9.53 g kg^{-1}; total phosphorus, 8.33 g kg^{-1}; total potassium, 30.82 g kg^{-1}.

2.2. Experimental Design

Dry direct-seeded rice was subjected to two experimental treatments under a large-area contrast design, with biochar as the sole variable factor: conventional fertilizer (CK), and biochar with conventional fertilizer (BC). In the CK regime, 204.05 kg N ha^{-1} (urea, 46% N) was applied, distributed as basal fertilizer (88.82 kg N ha^{-1}), tillering fertilizer (69.00 kg N ha^{-1}), and panicle fertilizer (46.23 kg N ha^{-1}). The total amount of phosphate fertilizer (calcium triple superphosphate, 46% P_2O_5) was 80.04 kg ha^{-1}, all as base fertilizer. The total amount of potassium fertilizer (potassium sulfate, 50% K_2O) was 115.00 kg ha^{-1}, divided into basal (63.75 kg K_2O ha^{-1}) and panicle (51.25 kg K_2O ha^{-1}) fertilizers. Biochar was sprinkled (15 t ha^{-1}) on the soil surface before tillage, and fully mixed with the soil through rotary tillage (20 cm deep).

Longjing 31 (11 leaves on the main stem) rice variety was used in this experiment. This genotype has a maturity period of approximately 130 days, and an active accumulated temperature $\geq$ 10 °C of approximately 2350 °C. Before sowing, the seeds were coated with Liangdun seed-coating agent, and the dry seeds were mechanically sown on 30 April 2018; 18 April 2019; and 19 April 2020. In all cases, the seeding rate was 210 kg ha^{-1}, the seeding depth was 2 cm, and row spacing was 20 cm. Each experimental plot covered 300 m^2 (length, 20 m; width, 15 m), and three replicates per treatment were set, yielding a total of six plots. Basal fertilizer was applied 4 cm apart from the seedling belt at 5 cm depth; a soil covering chain was used, and the soil was compacted twice after sowing. Harvesting was performed on 20, 23, and 27 September, in the rice production cycles of 2018, 2019, and 2020, respectively.

2.3. Soil Sampling Collection

Four points in each experimental plot were randomly selected. Eight soil samples were collected from each point: four from the 0–10 cm layer, and four from the 10–20 cm soil layer. A total of 32 samples were obtained. Sampling was undertaken after the rice harvest on 27 September 2020. Subsamples from each layer were collected and mixed (same layer). Soil samples were air-dried, comminuted, and screened with a 1 mm sieve to determine soil chemistry and enzyme activity. Fresh soil samples were delivered to the laboratory in ice bags, and stored at −80 °C to determine soil aggregates and soil bacterial diversity.

2.4. Soil Trait Determination

Soil water-stable aggregates were determined using the wet sieve method [22]; soil particle size distribution was determined using a laser particle analyzer (Mastersizer 3000, Malvern, UK); pH was determined using a pH meter (PHS-3C, Yueping, China); soil organic matter (SOM) content was determined using the potassium dichromate capacity external heating method; soil total nitrogen (TN) was measured using H_2SO_4 accelerator digests and

K360 automated distillation-titration instrument (KjelFlexK-360, Buchi, Switzerland); soil total phosphorus (TP) was determined using the molybdenum-antimony anti-colorimetric method; soil total potassium (TK) was determined using the atomic absorption spectrometry (AAS); soil alkali-hydrolyzed nitrogen (AHN) was determined using the diffusion absorption method; and soil available phosphorus (AP) was determined using sodium bicarbonate extraction-molybdenum-antimony anti-colorimetric method; soil available potassium (AK) was determined using the ammonium acetate leaching- AAS method. SOM, TN, TP, TK, AHN, AP, and AK were determined using the method described by Bao [21].

Soil urease, sucrase, neutral phosphatase, and catalase were determined using the Solarbio Biochemical Technology (Beijing, China) Co., Ltd. kit in three replicates. The sample determination pretreatment and specific operation steps were performed according to the kit instructions. We used the indophenol-blue colorimetric method, 3,5-dinitrosalicylic acid colorimetric method, benzene disodium phosphate hydrolysis colorimetric method, and ultraviolet spectrophotometry method, which have been described in detail by Guo et al. [23].

2.5. Determination of Soil Bacterial Diversity

Total bacterial DNA was extracted from the soil samples using the Soil DNA Isolation Kit (Norgen Biotek, China Regional Agent: Wuhan AmyJet Scientific Co., Ltd., Wuhan, China) in a 96-well plate according to the manufacturer's instructions.

The bacterial 16S rRNA V3 + V4 (338F: 5′-ACTCCTACGGGAGGCAGCA-3′, forward primer), and fungal 16S rRNA ITS1 regions (806R: 5′-GGACTACHVGGGTWTCTAAT-3′, reverse primer) were amplified. The PCR amplification procedure for the target region was as follows: initial denaturation at 95 °C for 5 min, followed by 25 cycles of amplification (30 s at 95 °C, 30 s at 50 °C, and 45 s at 72 °C), and a final extension step at 72 °C for 7 min. The recovered products were subjected to Solexa PCR: after initial denaturation at 98 °C for 30 s, amplification was achieved by 10 cycles for 10 s at 98 °C, 30 s at 65 °C, and 30 s at 72 °C, followed by a final extension step at 72 °C for 5 min. The recovered products were mixed according to the electrophoresis quantification (ImageJ software) results and to a mass ratio of 1:1. The recovered products were purified using the OMEGADNA Purification Kit and recovered using the Monarch DNA Recycling Kit. Sequencing was performed using the Illumina HiSeq platform (Beijing Biomarker Technologies Co., Ltd., Beijing, China).

2.6. Bioinformatics and Statistical Analyses

All statistical analyses were performed using SPSS 21.0 statistical software (SPSS Inc., Chicago, IL, USA). An analysis of variance was performed to test significance between treatments using Duncan's multiple range test at the 5% significance level. Figures were generated using GraphPad Prism 9.4 software (San Diego, CA, USA).

Microbial diversity data were processed using the BMK cloud platform. The reads were spliced using FLASH [24] (version 1.2.11) software. Sequences were analyzed by OTUs clustered using USEARCH [25] (version 10.0) software. Alpha index analysis was performed using Mothur [26] (versionv.1.30) software. A Spearman rank correlation analysis was performed based on Python software, and data with a correlation greater than 0.1 and *p*-value less than 0.05 were filtered to build the correlation network. Linear discriminant analysis (LDA) was used to estimate the effect of the abundance of each component (species), with a significant difference in the logarithmic LDA score of 4.0. For the environmental factor and sample composition correlation analysis, RDA (redundancy analysis) analysis and mapping in the R vegan package were used.

3. Results

3.1. Soil Water-Stable Aggregates

Biochar application for 3 consecutive years significantly changed the ratio of soil water-stable aggregates of different particle sizes (Table 1). Compared with CK, BC treatment increased the 5–0.25 mm water-stable aggregate by 1.60%, 1.22%, 0.98%, 2.78%, and 0.94%; however, the difference between CK and BC was not significant. The <0.25 mm water-stable

aggregate content was reduced by 8.73% by BC. This finding shows that biochar is beneficial for increasing the content of large soil aggregates in dry direct-seeded rice paddies, but is not conducive to the formation of microaggregates.

Table 1. Effects of biochar on soil water-stable aggregates in dry direct-seeded rice field.

Treatment	Percentage Content of Water-Stable Aggregate (%)					
	WSA1 5 mm	**WSA 2 2 mm**	**WSA 3 1 mm**	**WSA 4 0.5 mm**	**WSA 5 0.25 mm**	**WSA 6 <0.25 mm**
CK	27.80 ± 1.01 a	7.73 ± 0.47 a	7.34 ± 0.38 a	9.243 ± 0.92 a	9.44 ± 2.79 a	40.62 ± 1.41 a
BC	29.40 ± 4.54 a	8.95 ± 0.54 a	8.32 ± 2.15 a	11.92 ± 0.61 a	10.38 ± 0.23 a	31.89 ± 1.13 b

CK, conventional fertilizer; BC, biochar with conventional fertilizer; WSA, water-stable aggregate. Different lowercase letters in the same column indicate significant differences among treatments ($p < 0.05$).

3.2. Soil Particle Content

As shown in Figure 1, soil particle content in soil layers of different depths under all treatments were as follows: silt (2–50 μm) > sand (50–200 μm) > clay (0–2 μm). Compared to CK, BC significantly reduced the soil clay content by 3.17% and 3.68% in the 0–10 and 10–20 cm soil layers, respectively. The silt and sand content in the two treatments showed opposite trends at different soil depths. Compared to CK, BC significantly increased the particle content by 3.07% and 3.66%. However, the change in the soil clay content between the treatments did not reach a significant level.

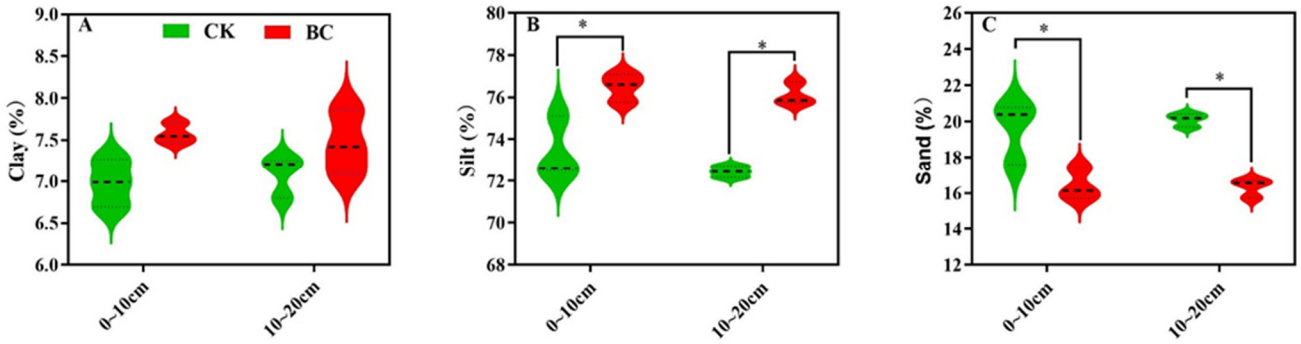


Figure 1. Effect of biochar on the percentage of clay (**A**), silt (**B**) and sand (**C**) in dry direct-seeded rice field. CK, conventional fertilizer; BC, biochar with conventional fertilizer. * Indicates significant difference between CK and BC treatments ($p < 0.05$).

3.3. Soil Nutrients

The changes in soil nutrients at different soil depths are shown in Table 2. From the perspective of the soil profile as a whole, BC significantly increased the content of AP and AK compared to CK. In the 0–10 cm soil layer, the AP and AK content of the BC treatment increased by 22.39% and 18.96% compared with that of CK, and in the 10–20 cm soil layer, the increase was by 22.08% and 10.88% compared with that of CK, respectively. At different soil depths, pH and contents of SOM, TN, TP, TK, and AHN under BC increased compared with those under CK, but there was no significant difference.

3.4. Soil Enzyme Activity

The effects of biochar on soil enzyme activities are presented in Figure 2. Compared to CK, BC significantly increased soil neutral phosphatase and catalase activities by 24.56% and 32.75%, respectively. BC increased the urease and sucrase activities, but there was no significant difference between BC and CK.

Table 2. Effects of biochar on soil nutrients in dry direct-seeded rice field.

Soil Sampling Depth	Treatment	pH	SOM (g kg^{-1})	TN (g kg^{-1})	TP (g kg^{-1})	TK (g kg^{-1})	AHN (mg kg^{-1})	AP (mg kg^{-1})	AK (mg kg^{-1})
0–10 cm	CK	6.53 ± 0.06 a	21.30 ± 0.52 a	1.09 ± 0.06 a	0.92 ± 0.02 a	19.24 ± 0.47 a	110.45 ± 6.74 a	37.74 ± 2.75 b	235.67 ± 16.78 b
	BC	6.55 ± 0.17 a	23.05 ± 0.83 a	1.13 ± 0.04 a	0.93 ± 0.02 a	20.26 ± 0.22 a	112.85 ± 4.78 a	46.19 ± 4.47 a	280.35 ± 17.22 a
10–20 cm	CK	6.55 ± 0.11 a	19.42 ± 0.99 a	1.04 ± 0.05 a	0.83 ± 0.02 a	17.47 ± 0.74 a	100.84 ± 5.11 a	32.29 ± 2.67 b	208.13 ± 13.49 b
	BC	6.60 ± 0.16 a	22.83 ± 2.21 a	1.09 ± 0.07 a	0.89 ± 0.05 a	18.76 ± 0.57 a	109.37 ± 4.65 a	39.42 ± 2.21 a	230.77 ± 18.19 a

CK, conventional fertilizer; BC, biochar with conventional fertilizer. Lowercase letters in the same column indicate significant differences among treatments ($p < 0.05$).

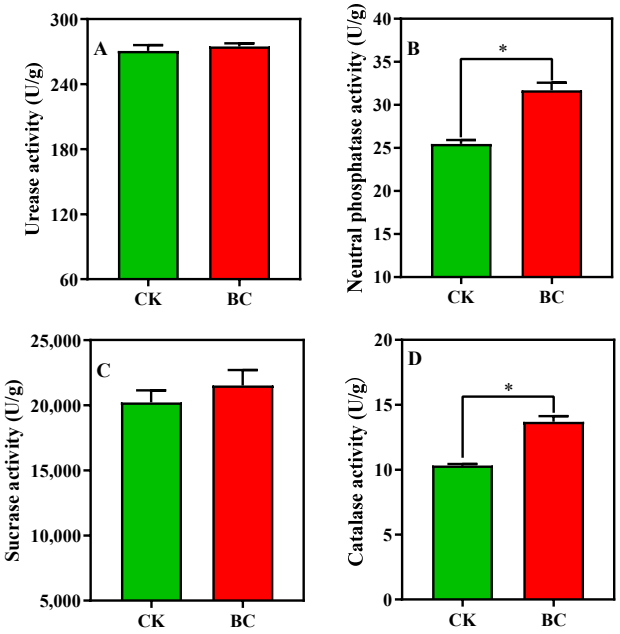


Figure 2. Effects of biochar on soil enzyme activities ((**A**), Urease activity; (**B**), Neutral phosphatase activity; (**C**), Sucrase activity; (**D**), Catalase activity) in dry direct-seeded rice field. CK, conventional fertilizer; BC, biochar with conventional fertilizer. The error line represents the standard error ($n = 6$). * Indicates a significant difference between CK and BC treatments ($p < 0.05$).

3.5. Richness and Diversity of Soil Bacterial Community

As shown in Figure 3, biochar significantly increased soil bacteria richness, with ACE (Abundance-based Coverage Estimator) and Chao indices of BC being 1653 and 1698.52, increasing by 4.38% and 3.66%, respectively, compared with those of CK. There was no significant difference in the bacterial diversity index between the treatments. The Simpson and Shannon indices of CK were 0.9924 and 8.80, respectively, and those of BC were 0.9902 and 8.76, respectively, suggesting that biochar did not increase soil bacterial diversity in dry direct-seeded rice fields but increased bacterial richness.

3.6. Soil Bacterial Community Composition

As shown in Figure 4A, the bacterial composition at the phylum level in the soil was similar according to Qualcomm sequencing analysis. Proteobacteria and Acidobacteria were the dominant bacterial phyla with a relative abundance greater than 1% in the top 10 phyla. The relative abundance of Gemmatimonadetes, Verrucomicrobia, Actinobacteria, and Chloroflexi was low, whereas that of Firmicutes, Bacteroidetes, Nitrospirae, and Cyanobacteria was lower, ranging between 1.11% and 2.44%. Compared to CK, BC reduced the relative abundance of soil Proteobacteria, Acidobacteria, Gemmatimonadetes, Actinobacteria, and Firmicutes. Compared to CK, BC increased the relative abundance of Verrucomicrobia, Chloroflexi, Bacteroidetes, and Nitrospirae by 17.85-, 2.63-, 1.88-, and 1.95-fold, respectively.

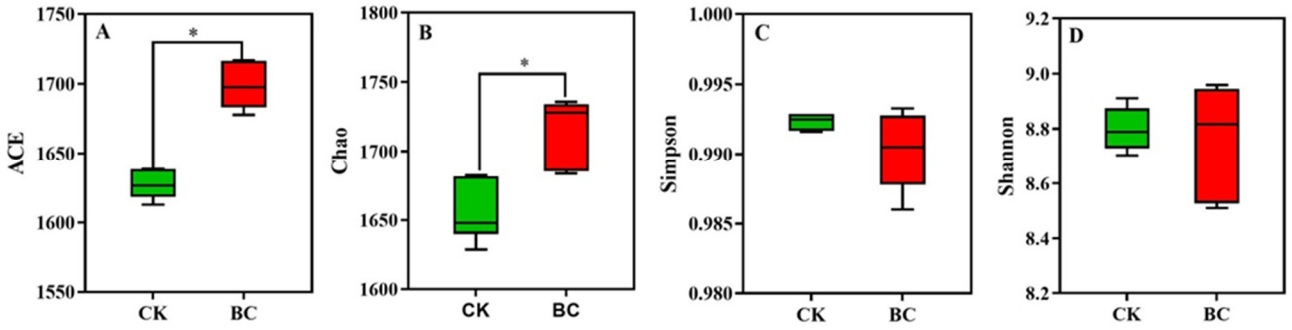


Figure 3. Effect of biochar on richness and diversity index ((**A**), ACE; (**B**), Chao; (**C**), Simpson; and (**D**), Shannon) of soil bacterial communities in a dry direct-seeded rice paddy. CK, conventional fertilizer; BC, biochar with conventional fertilizer. The error line represents the standard error (n = 6). * Indicates a significant difference between CK and BC treatments ($p < 0.05$).

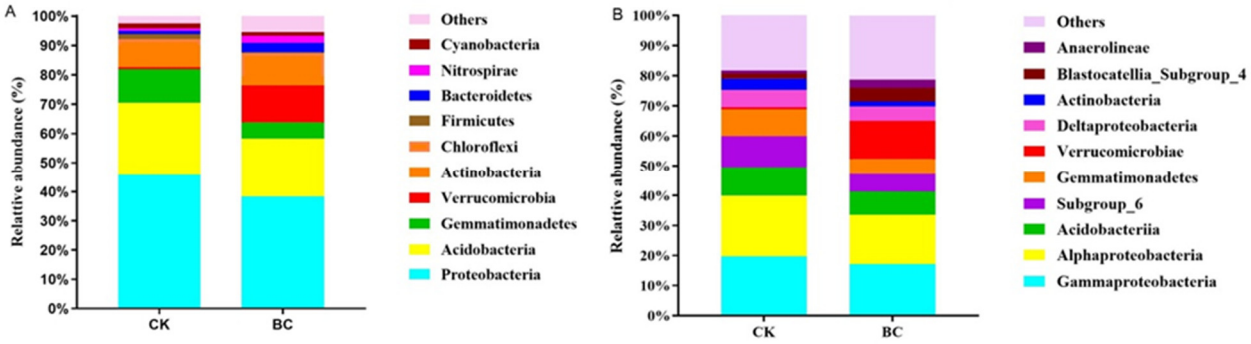


Figure 4. Effect of biochar on relative abundance of bacterial phyla (**A**) and class (**B**). CK, conventional fertilizer; BC, biochar with conventional fertilizer.

At the class level (Figure 4B), the dominant bacteria in CK were members of Alphaproteobacteria, and the dominant bacteria in BC were members of Gammaproteobacteria, indicating that biochar changed the dominant bacterial class. Compared to CK, BC increased the relative abundance of Verrucomicrobiae, Blastocatellia_Subgroup_4, and Anaerolineae by 18.21-, 1.74-, and 1.68-fold, respectively.

3.7. Analysis of Bacterial Taxa

The number of OTUs in each sample was obtained at 97% similarity. Biochar increased the number of bacterial OTUs. There were 1653 bacterial OTUs in BC, a significant increase of 5.62% compared to 1565 OTUs observed in CK.

A Venn diagram was drawn based on the number of OTUs at each depth (Figure 5), which were counted and compared for all samples to determine the common and unique features among the samples. The bacterial Venn diagram obtained from the characteristics of each sample indicated that 1704 bacterial OTUs were shared between the treatments; 18 OTUs were unique to the soil of the CK root region, and 45 OTUs were unique to the soil of the BC root region. These results indicate that biochar could increase the unique bacterial OTUs in the soil of the rice root area, changing the bacterial group composition.

3.8. Species-Correlation Networks

To further explore the changes in the relationship among the soil microbial community species under each treatment, a species correlation network was constructed by conducting a Spearman rank correlation analysis in Python, based on the relative abundance and change in each species in each sample. Data with a correlation value greater than 0.1 and a p-value less than 0.05 were screened.

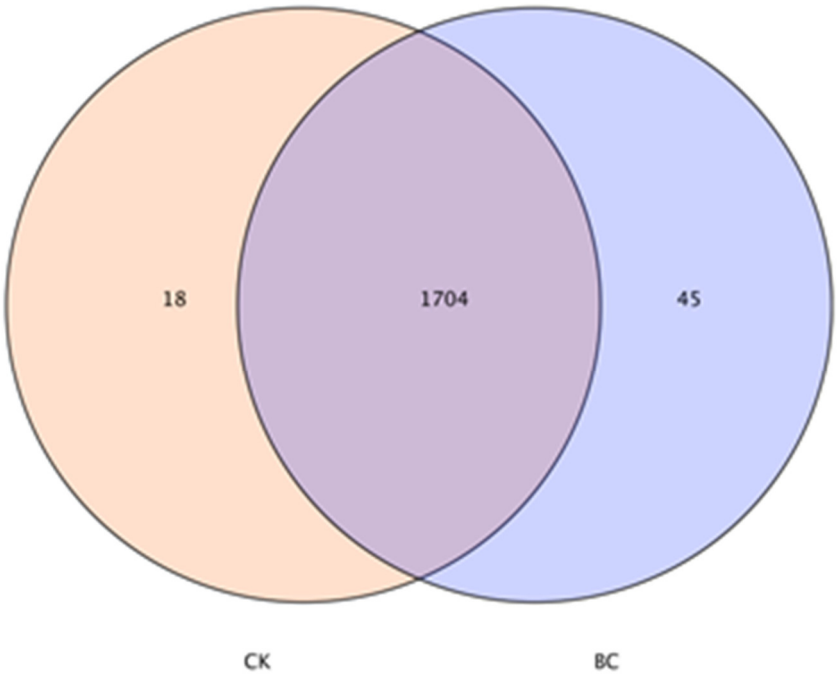


Figure 5. Ven diagram of soil bacteria. CK, conventional fertilizer; BC, biochar with conventional fertilizer.

Changes in species relationships, among the top 50 genera of soil bacteria under each treatment, are shown in Figure 6. There were 84 pairwise related species in CK; there were 35 positively correlated species, and 49 negatively correlated species. The abundance of uncultured_bacterium_c_Subgroup_6, Sphingomonas, Gemmatimonas, and uncultured_bacterium_f_SC-I-84 was high in CK. There were 101 pairwise-related species in BC; there were 47 positively correlated species, and 64 negatively correlated species. The abundance of Candidatus, Udaeobacter, uncultured_bacterium_c_Subgroup_6, and Sphingomonas was high in BC. These findings show that the relationship between the main bacterial genera of biochar-treated soil bacteria was stronger than that of CK, and the number of positive correlations (orange) in BC was significantly higher than in CK.

3.9. Correlation Analysis of Soil Bacterial Community and Soil Nutrients

Based on the RDA linear model, the relationships between soil bacterial communities and soil nutrients under different cultivation methods were explored (Figure 7). The results showed that the contribution rates of principal components 1 and 2 to the bacterial community distribution were 6.69% and 8.59%, respectively, with a total contribution rate of 85.28%. The SOM was the main environmental factor influencing the bacterial community structure.

According to the correlation heat diagram analysis (Figure 8), SOM and TP were significantly and negatively correlated with Proteobacteria, respectively, and significantly and positively correlated with Verrucomicrobia and Nitrospirae, respectively. Acidobacteria members were significantly negatively correlated with TP and AP. SOM, TK, and AK showed extremely significant or significant positive correlations with Chloroflexi, and an extremely significant or significant negative correlation with Actinobacteria.

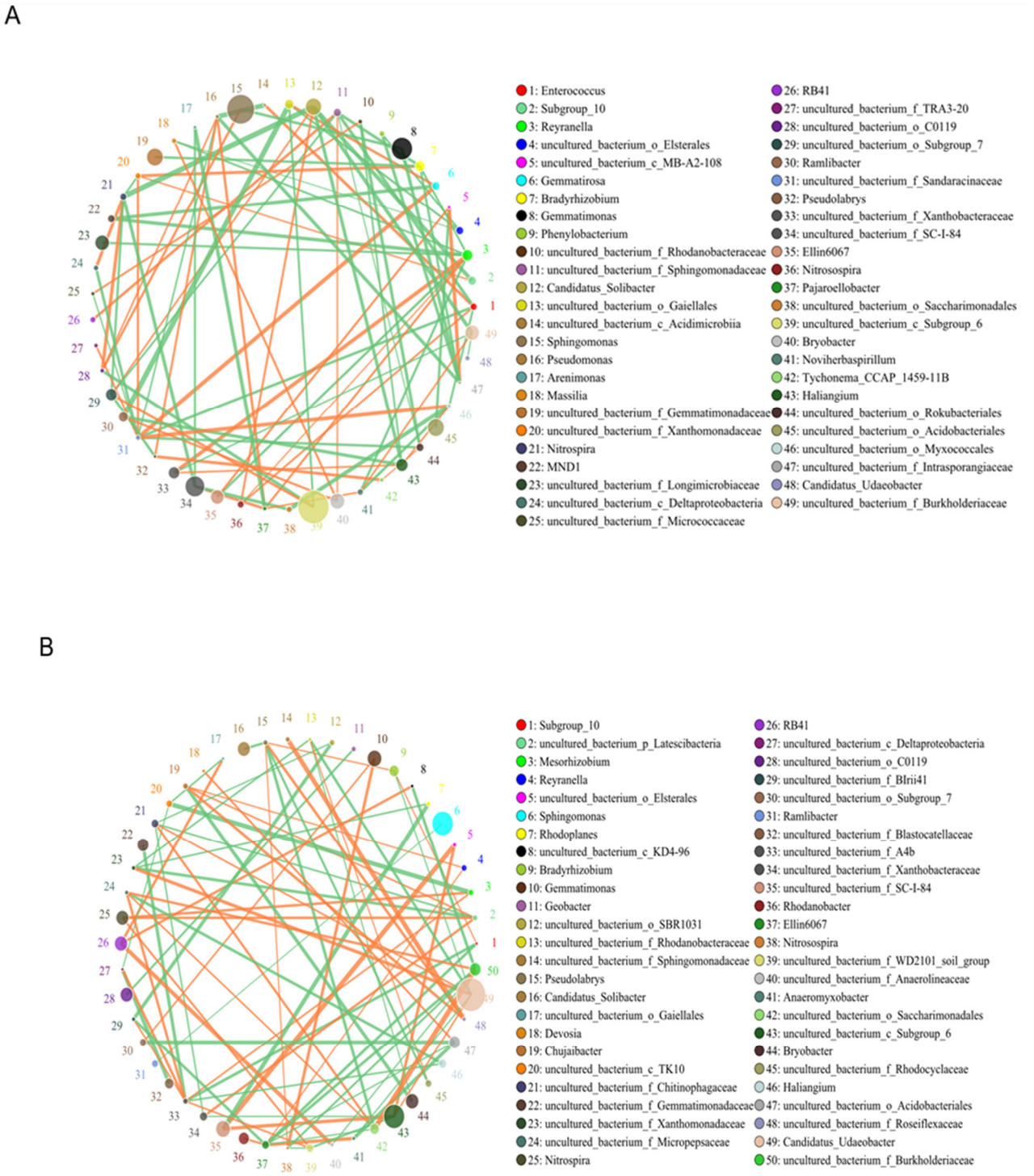


Figure 6. CK (**A**) and BC (**B**) bacterial community species network diagram. CK, conventional fertilizer; BC, biochar with conventional fertilizer. Each node represents each dominant phylum and is identified by a different color. The edge connecting two nodes indicates the correlation between two phyla, with the orange line indicating a positive correlation, and the green line indicating a negative correlation. The node size indicates the degree of association between the g phyla and other phyla of the community. The node size increases with the degree. Different colors represent different degrees of nodes.

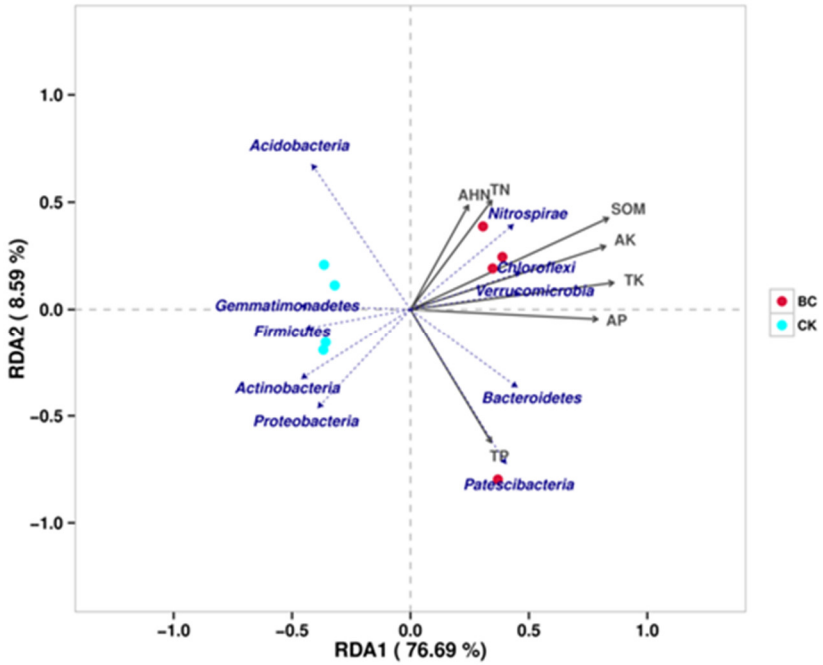


Figure 7. Effects of soil nutrients on the bacterial community composition. CK, conventional fertilizer; BC, biochar with conventional fertilizer. SOM, soil organic matter; TN, total nitrogen; TP, total phosphorus; TK, total potassium; AHN, alkaline hydrolysable-nitrogen; AP, available phosphorus.

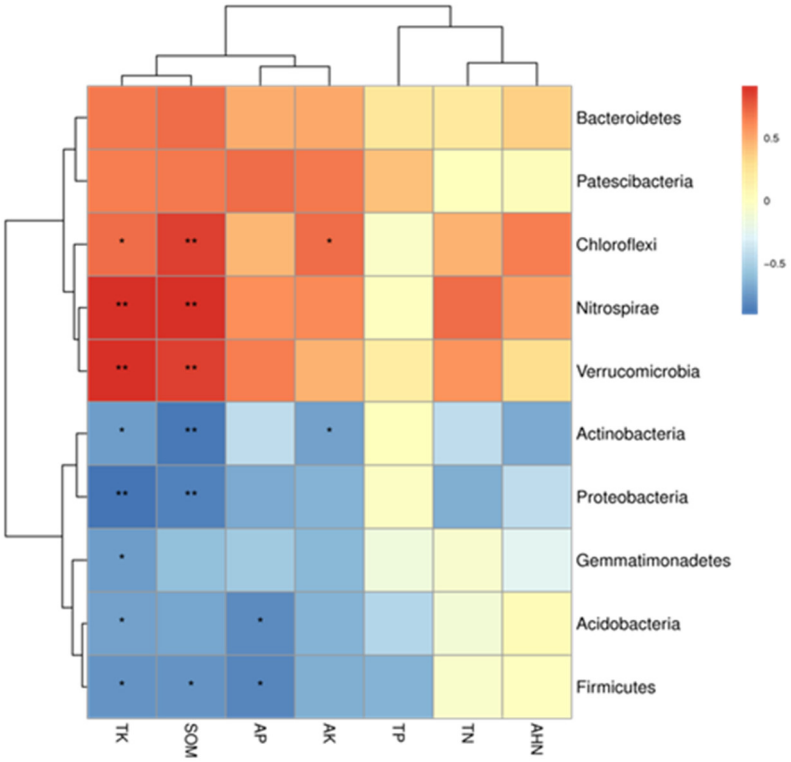


Figure 8. Correlation analysis of soil nutrients and bacterial community structure. SOM, soil organic matter; TN, total nitrogen; TP, total phosphorus; TK, total potassium; AHN, alkaline hydrolyzable-nitrogen; AP, available phosphorus; AK, available potassium. * Significance at $p < 0.05$. ** Significance at $p < 0.01$.

4. Discussion

4.1. Correlation Analysis of Soil Bacterial Community and Soil Nutrients

Soil aggregates are the reservoir of soil nutrients and provide a habitat for various soil microorganisms [27]. The quantitative distribution and stability of aggregates determine the circulation of soil nutrients and microbial activity [28]. The influence of biochar on the distribution and stability of various granular soil aggregates often varies by biochar feedstock, application amount, duration, and soil texture [29]. Previous research has demonstrated that biochar application increases the water-stable aggregate content of silty loam, but has no significant effect on aggregate formation and stability of sandy loam [30]. Biochar applied at 4.5 and 9.0 t ha^{-1} significantly increased the content of the 250–2000 µm aggregates [31]. Situ et al. [32] found that the application of wheat straw biochar at 20 and 40 t ha^{-1} increased the proportion of large soil aggregates in paddy fields after 20 months. Biochar increased the number of >0.25 mm aggregates in red sandstone soil, and the proportion of large water-stable aggregates increased as the application amount increased [33]. Biochar improved the formation of large soil aggregates in saline–alkali rice fields [9]. The results of the present study also demonstrated that corn straw biochar significantly affected the distribution of soil aggregates in a relatively short period (3 years), and BC significantly increased the proportion of 0.25–5 mm water-stable aggregates, with an increase ranging from 0.94% to 2.78%. In particular, >0.5 mm aggregates increased most significantly. The application of biochar significantly promoted the formation of large soil aggregates. Reportedly, biochar application increases the large water-stable aggregate content in the soil. As biochar improves soil biological metabolic activity, more components are secreted to form the cementing substance of the soil aggregates [34]; thus adsorbing mineral particles and cementing the microaggregates to the large aggregates, and ultimately increasing the content of large aggregates in the soil. In addition, the unique highly developed pore structure and high specific surface area of biochar resulted in the adsorption and fixing of inorganic ions and organic compounds in the soil, thus further forming organic–inorganic complexes and large-particle size aggregates [35]. In addition, the present study revealed that biochar significantly reduced the soil sand content and significantly increased the soil silt content, revealing that biochar gradually reduces soil desertification; increases soil bonding; enhances the cementation effect between particles, improving the number and stability of water-stable aggregates, thus having positive implications for improving the soil structure.

4.2. Correlation Analysis of Soil Bacterial Community and Soil Nutrients

Compared to conventional fertilizers, biochar contains organic matter and higher content of phosphorus, potassium, and other nutrient elements. Biochar application to the soil will change the soil structure and texture; increase the porosity and loosen the soil; and reduce the loss of soil alkali hydrolyzable nitrogen, available phosphorus, and available potassium fertilizers. This may be because only biochar application changes the soil structure or has certain adsorption and fixation effects on available nutrients, improving fertilizer utilization rate, and long-term fertilizer supply capacity [36]. The results of the present study showed that biochar could effectively increase soil AP and AK content, which was similar to the results of Yao [37] and Yang et al. [38]. Previous studies have shown that biochar can increase the AP content in the soil, and improve the effectiveness of phosphorus [39]. On the one hand, biochar is rich in phosphate, and most of it is soluble; therefore, biochar can directly increase the AP content after its application to the soil [40]. On the other hand, biochar has adsorption properties that can reduce the contact area between phosphorus and soil particles, as well as the soil fixation effect, improving phosphorus availability [41]. Moreover, it is possible that biochar affects the activity of soil microorganisms, promotes microbial dissolution and mineralization efficiency of phosphorus, and then increases available phosphorus content in the soil [42]. Similarly, the content of AK under biochar treatment was significantly higher than that under treatment without biochar, mainly because biochar is rich in water-soluble potassium

carbonate [43]. Organic carbon can reduce the potassium retention capacity of the soil, thus improving the effectiveness of the potassium in the soil. The application of biochar can also improve the soil temperature, accelerate the release of slow potassium in the soil, and increase the available potassium content [44]. The enzymatic activity of soil is considered a major contributor to soil quality, and is closely related to soil fertility. The application of biochar causes changes in soil enzyme activity to some extent. Related research has demonstrated that the improvement in soil enzyme activity with biochar application is largely related to its adsorption characteristics, which promoted enzymatic reactions by adsorption of the corresponding reaction substrate [45]. Previous research has shown that urease activity was integrally affected by, and depends on, the transformation and decomposition process of organic matter [46]. Soil sucrase is mainly involved in carbohydrate transformation in soil, and its activity was an important indicator of soil fertility. In addition, Forge et al. [47] reported that catalase reflects the strength of the oxidation process of soil microorganisms. The results of the present study showed that BC significantly improved soil neutral phosphatase and catalase activity compared to CK, whereas soil urease and sucrase activities increased; the difference between BC and CK was not significant. He et al. [48] showed that biochar significantly improved soil sucrase and catalase activities, but had no significant effect on urease and phosphatase activity. Gui et al. [49] showed that biochar application increased sucrase and phosphatase activities, but inhibited urease and catalase activities. The results of the present study differed from those of the above studies, possibly due to differences in soil type, crop species, and the amount of biochar applied.

4.3. Effect of Biochar on Soil Bacterial Community Composition

The bacterial reproduction cycle is short and rapid, resulting in rapid decomposition of organic material entering the soil; thus, high bacterial count is one of the best indicators of soil biological traits [50]. Biochar is more conducive to the reproduction of soil bacteria because of its high pore counts and large specific surface area. The effect of biochar on soil improvement and remediation is usually reflected by the number and abundance of soil bacteria [51]. Pietikäinen et al. [52] suggested that bacteria can adsorb to the surface of the biochar, decreasing susceptibility to soil lavage, thus increasing the number of bacteria in the soil. The present study showed that biochar significantly increased bacterial richness. Previous studies indicated that the effect of biochar on soil bacterial diversity is highly time-dependent, and new application of biochar briefly increases bacterial diversity and abundance; however, repeated addition of biochar over time had a lower effect on the bacterial community [53]. The present study also showed that biochar did not significantly affect bacterial diversity, which contrasted with the results of Xu et al. [54]. Changes in the relative abundance of soil bacteria also alter the composition of soil microbial taxa. The present study showed that biochar increased the unique bacterial OTU count in the root soil of dry direct seeded rice, increased the bacterial endemic species, and then changed the bacterial group composition. This was mainly because carbonized corn stalk, similar to other biochar, could affect the physico-chemical properties and biological characteristics of the soil, and could create a specific microenvironment for microorganisms. Yuan et al. [55] found that the main bacteria contained in rice fields were Proteobacteria, Acidobacteria, Chloroflexi, Actinobacteria, Firmicutes, and Verrucomicrobia. The main bacteria found by Song et al. [56] in rice fields were: Proteobacteria, Planctomycetes, Nitrospirae, Firmicutes, Chloroflexi, Actinobacteria, and Acidobacteria. The main bacterial phyla detected in dry direct seeded rice paddy field soil samples in the present study were: Proteobacteria, Acidobacteria, Gemmatimonadetes, Verrucomicrobia, Actinobacteria, and Chloroflexi; among them, Proteobacteria and Acidobacteria were the dominant bacteria in each treatment. Thus, compared with that in conventional rice fields, the composition of soil bacteria in dry direct seeded rice fields changed very little at the phylum level; however, the structure changed significantly. Biochar increased the relative abundance of Verrucomicrobia, Chloroflexi, and Nitrospirae compared with the controls, but decreased the relative abundance

of Proteobacteria, Acidobacteria, Gemmatimonadetes, Actinobacteria, and Firmicutes; this may be due to the utilization of biochar by different bacterial groups and environmental factors being different. Proteobacteria members are ubiquitous, and their high abundance, species richness, and genetic diversity determine their dominance [57]. Acidobacteria members are mostly acidophilic, showing a significant negative correlation with soil pH, and the highest abundance in environments with low soil pH [58]. Therefore, carbonized corn stalks reduced the relative abundance of Acidobacteria by increasing pH in the soil microregion; however, the reduced relative abundance of Acidobacteria is a marker for improved soil quality [59]. The present study showed that Acidobacteria members were significantly negatively correlated with TK and AP. Chloroflexi members can accelerate the decomposition of organic matter in rice fields [60], and the abundance of Chloroflexi members is associated with potassium content [61]. Consistent with previous study results, our RDA analysis showed that the abundance of Chloroflexi was positively correlated with total potassium and available potassium. Gemmatimonadetes members can degrade soil contaminants; the results of the present study indicated that biochar reduced the relative abundance of soil Gemmatimonadetes, indicating that biochar might inhibit soil pollutant degradation. It was worth mentioning that this study revealed that SOM was the main factor influencing the bacterial composition of soil in dry direct-seeded rice fields. SOM was significantly and negatively correlated with Proteobacteria and Actinobacteria, and significantly and positively correlated with Chloroflexi, Verrucomicrobia, and Nitrospirae. These results indicate that soil nutrients promote or inhibit the abundance of soil microorganisms. A previous study has shown that the community composition of bacteria changes after biochar application to the soil; this is because the addition of biochar could promote the growth of some bacteria and also inhibit the growth of other bacteria, resulting in an altered soil bacterial community structure [62]. The findings of the present study revealed that the unique structure of biochar can directly affect the physical and chemical properties and nutrient content of the soil, thus changing the soil environment where the bacteria survive, and thus affecting the bacterial community and diversity.

5. Conclusions

Biochar affected the physicochemical properties of soil in dry direct-seeded rice fields, significantly increasing the content of AP and AK, enhancing the proportion of large water-stable aggregates, and reducing the proportion of micro-water-stable aggregates. Compared to CK, BC significantly increased the available phosphorus and available potassium content of 0–10 cm and 10–20 cm soil layers by 22.39% and 18.96%, and 22.08% and 10.88%, respectively; BC significantly increased soil neutral phosphatase and catalase activities by 24.56% and 32.75%, respectively. The BC treatment significantly increased the ACE and Chao indices of soil bacteria by 4.38% and 3.66% compared to CK, respectively; however, the bacterial diversity index did not significantly differ between the treatments. Proteobacteria and Acidobacteria were the dominant bacterial phyla. SOM was the factor that most significantly influenced bacterial community composition. Dry direct-seeded rice is conducive to realizing the overall goal of low input, high efficiency, and low carbon in the agricultural ecosystem, consistent with the characteristics and requirements of low-carbon agriculture. Biochar application in farmland can enhance the content of soil organic matter, which benefits carbon fixation, and is conducive to the agricultural transformation toward low-carbon, environment-friendly, biodiversity protection.

Author Contributions: Methodology, investigation, data curation, visualization, formal analysis, writing original draft, Y.L.; methodology, investigation, validation, formal analysis, L.X.; investigation, conceptualization, supervision, funding acquisition, X.G.; investigation, J.L.; conceptualization, B.Z.; data curation, Y.G.(Yukun Guo), Y.Z. and H.L.; supervision, funding acquisition, G.Z.; methodology, investigation, conceptualization, writing review and edition, supervision, Y.G.(Yongxia Guo); formal analysis, writing review and edition, funding acquisition, M.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This research was financially supported by the Opening Foundation of Collaborative Innovation Center for Genetic Improvement and High Quality and Efficiency Production of Northeast Japonica Rice (funding number: KF2022-02; funder: X.G.), the Liao Ning Revitalization Talents Program (funding number: XLYC2008025; funder: M.Z.), and the Heilongjiang Bayi Agricultural University Support Program for San Heng San Zong (funding number: TDJH201802; funder: G.Z.).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data available from the author.

Acknowledgments: We thank Guojun Zhang, Qiang Wang, Wenhui Zou, and Wenhao Sun from the Agricultural Technology Extension Center of Heilongjiang Youyi Farm for assisting with the field experiment.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Alexandratos, N.; Bruinsma, J. *World Agriculture towards 2030/2050: The 2012 Revision*; ESA E Working Paper; FAO: Rome, Italy, 2012.
2. Xu, L.Q.; Guo, X.H.; Zhang, J.N.; Zhao, Y.; Li, X.L.; Liu, S.F.; Cui, Z.Y.; An, Y.L.; Lv, Y.D. The effect of organic fertilizer on the quality of dry direct-seeding rice. *ACTA Agric. Boreali Sincia* **2022**, *37*, 137–146. (In Chinese)
3. Tang, Z.Q.; Zhang, L.Y.; He, N.; Ma, Z.B.; Zhao, M.Z.; Wang, C.H.; Zheng, W.J.; Yin, Y.A.; Wang, H. Effects of mechanical direct dry seeding on rice growth, photosynthetic characteristics and yield. *Crops* **2021**, *5*, 87–94. (In Chinese)
4. Cao, G.L.; Zhang, X.Y.; Wang, Y.Q.; Zheng, F.C. Estimation of emissions from field burning of crop straw in China. *Chin. Sci. Bull.* **2008**, *53*, 784–790. [CrossRef]
5. Sun, R.B.; Zhang, X.X.; Guo, X.S.; Wang, D.Z.; Chu, H.Y. Bacterial diversity in soils subjected to long-term chemical fertilization can be more stably maintained with the addition of livestock manure than wheat straw. *Soil Biol. Biochem.* **2015**, *88*, 9–18. [CrossRef]
6. Ghorbani, M.; Amirahmadi, E.; Zamanian, K. In-situ biochar production associated with paddies: Direct involvement of farmers in greenhouse gases reduction policies besides increasing nutrients availability and rice production. *Land Degrad. Dev.* **2021**, *32*, 3893–3904. [CrossRef]
7. Major, J.; Steiner, C.; Downie, A.; Lehmann, J. Biochar effects on nutrient leaching. In *Biochar for Environmental Management: Science and Technology*; Lehmann, J., Joseph, S., Eds.; Earthscan Publishers: London, UK, 2009; pp. 271–287.
8. Ma, N.N.; Zhang, L.L.; Zhang, Y.L.; Yang, L.J.; Yu, C.X.; Yin, G.H.; Doane, T.A.; Wu, Z.J.; Zhu, P.; Ma, X.Z. Biochar improves soil aggregate stability and water availability in a mollisol after three years of field application. *PLoS ONE* **2016**, *11*, e0154091. [CrossRef]
9. Abulaiti, A.; She, D.L.; Liu, Z.P.; Sun, X.Q.; Wang, H.D. Application of biochar and polyacrylamide to revitalize coastal saline soil quality to improve rice growth. *Environ. Sci. Pollut. Res.* **2022**, *11*, 1–17. [CrossRef]
10. Zhang, Y.S.; Tang, G.M.; Long, X.S.; Ge, C.H.; Xu, W.L. Effects of one-time biochar input on soil properties and corn yield in irrigation sandy soil. *Agric. Res. Arid Areas* **2021**, *39*, 137–141. (In Chinese)
11. Joseph, S.D.; Camps-Arbestain, M.; Lin, Y.; Munroe, P.; Chia, C.H.; Hook, J.; Van Zwieten, L.; Kimber, S.; Cowie, A.; Singh, B.P.; et al. An investigation into the reactions of biochar in soil. *Aust. J. Soil Res.* **2010**, *48*, 501–515. [CrossRef]
12. Palansooriya, K.N.; Wong, J.T.; Fung, H.Y.; Huang, L.B.; Rinklebe, J.; Chang, S.X.; Bolan, N.; Wang, H.L.; Ok, Y.S. Response of microbial communities to biochar-amended soils: A critical review. *Biochar* **2019**, *1*, 3–22. [CrossRef]
13. Butnan, S.; Deenik, J.L.; Toomsan, B.; Vityakon, P. Biochar properties affecting carbon stability in soils contrasting in texture and mineralogy. *Agric. Nat. Resour.* **2017**, *51*, 492–498. [CrossRef]
14. Zhang, Q.Z.; Dijkstra, F.A.; Liu, X.R.; Wang, Y.D.; Huang, J.; Lu, N.; Singer, A.C. Effects of biochar on soil microbial biomass after four years of consecutive application in the north China plain. *PLoS ONE* **2014**, *9*, e102062. [CrossRef] [PubMed]
15. Steinbeiss, S.; Gleixner, G.; Antonietti, M. Effect of biochar amendment on soil carbon balance and soil microbial activity. *Soil Biol. Biochem.* **2009**, *41*, 1301–1310. [CrossRef]
16. Azeem, M.; Hale, L.; Montgomery, J.; Crowley, D.; McGiffen, M.E.J. Biochar and compost effects on soil microbial communities and nitrogen induced respiration in turfgrass soils. *PLoS ONE* **2020**, *15*, e0242209. [CrossRef] [PubMed]
17. Lehmann, J.; Rillig, M.C.; Thies, J.; Masiello, C.A.; Hockaday, W.C.; Crowley, D. Biochar effects on soil biota-A review. *Soil Biol. Biochem.* **2011**, *43*, 1812–1836. [CrossRef]
18. Ghorbani, M.; Amirahmadi, E.; Neugschwandtner, R.W.; Konvalina, P.; Kopecký, M.; Moudrý, J.; Perná, K.; Murindangabo, Y.T. The impact of pyrolysis temperature on biochar properties and its effects on soil hydrological properties. *Sustainability* **2022**, *14*, 14722. [CrossRef]
19. Ghorbani, M.; Asadi, H.; Abrishamkesh, S. Effects of rice husk biochar on selected soil properties and nitrate leaching in loamy sand and clay soil. *Int. Soil Water Conserv. Res.* **2019**, *7*, 258–265. [CrossRef]

20. Ameloot, N.; Sleutel, S.; Das, K.C.; Kanagaratnam, J.; De Neve, S. Biochar amendment to soils with contrasting organic matter level: Effects on N mineralization and biological soil properties. *Glob. Chang. Biol. Bioenergy* **2015**, *7*, 135–144. [CrossRef]
21. Bao, S.D. *Soil Agrochemical Analysis*, 3rd ed.; China Agricultural Press: Beijing, China, 2005. (In Chinese)
22. Zheng, B.Z. *Technical Guide for Soil Analysis*; China Agricultural Press: Beijing, China, 2013. (In Chinese)
23. Guo, X.H.; Zou, B.; Xu, L.Q.; Zhang, J.N.; Zheng, G.P.; Wang, H.Z.; Yin, D.W.; Li, H.Y.; Zhang, W.Z.; Lv, Y.D.; et al. Changes in the physical, chemical, and bacterial community characteristics of soil in response to short-term combined organic–inorganic fertilizers in a dry direct-seeded paddy field. *Agronimy* **2022**, *12*, 2808. [CrossRef]
24. Magoč, T.; Salzberg, S.L. FLASH: Fast length adjustment of short reads to improve genome assemblies. *Bioinformatics* **2011**, *27*, 2957–2963. [CrossRef]
25. Edgar, R.C. UPARSE: Highly accurate OTU sequences from microbial amplicon reads. *Nat. Methods* **2013**, *10*, 996–998. [CrossRef] [PubMed]
26. Schloss, P.D.; Westcott, S.L.; Ryabin, T.; Hall, J.R.; Hartmann, M.; Hollister, E.B.; Lesniewski, R.A.; Oakley, B.B.; Parks, D.H.; Robinson, C.J.; et al. Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl. Environ. Microbiol.* **2009**, *75*, 7537–7541. [CrossRef] [PubMed]
27. Six, J.; Bossuyt, B.; Degryze, H.; Denef, K. A history of research on the link between (micro) aggregates, soil biota, and soil organic matter dynamics. *Soil Tillage Res.* **2004**, *79*, 7–31. [CrossRef]
28. Liu, Z.L.; Yu, W.T. Review of researches on soil aggregate and soil organic carbon. *Chin. J. Eco-Agric.* **2011**, *19*, 447–455. (In Chinese) [CrossRef]
29. Han, L.F.; Sun, K.; Yang, Y.; Xia, X.H.; Li, F.B.; Yang, Z.F.; Xing, B.S. Biochar's stability and effect on the content, composition and turnover of soil organic carbon. *Geoderma* **2020**, *364*, 114184. [CrossRef]
30. Liu, X.H.; Han, F.P.; Zhang, X.C. Effect of biochar on soil aggregates in the loess plateau: Results from incubation experiments. *Int. J. Agric. Biol.* **2012**, *6*, 975–979.
31. Du, Z.L.; Zhao, J.K.; Wang, Y.D.; Zhang, Q.Z. Biochar addition drives soil aggregation and carbon sequestration in aggregate fractions from an intensive agricultural system. *J. Soils Sediments* **2017**, *17*, 581–589. [CrossRef]
32. Situ, G.M.; Zhao, Y.L.; Zhang, L.; Yang, X.Q.; Chen, D.; Li, S.H.; Wu, Q.F.; Xu, Q.F.; Chen, J.H.; Qin, H. Linking the chemical nature of soil organic carbon and biological binding agent in aggregates to soil aggregate stability following biochar amendment in a rice paddy. *Sci. Total Environ.* **2022**, *847*, 157460. [CrossRef]
33. Li, J.Z.; Dai, K.; Zhang, L.M.; Ji, S.G.; Qiao, Z.X.; Jiao, Y.G.; Meng, J.; Lan, Y. Effects of biochar application on soil organic carbon distribution and soil aggregate composition of red soils in Yunnan tobacco planting area. *Acta Sci. Circumstantiae* **2016**, *36*, 2114–2120. (In Chinese)
34. Glaser, B.; Lehmann, J.; Zech, W. Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal—A review. *Biol. Fertil. Soils* **2002**, *35*, 219–230. [CrossRef]
35. Zhu, Q.L.; Zeng, D.P.; Wang, C.; Tong, C.; Wang, W.Q. Effects of waste applications on the distribution and stability of soil aggregates in the paddy field of Fuzhou plain. *Acta Sci. Circumstantiae* **2016**, *8*, 3000–3008. (In Chinese)
36. Fang, B.; Li, X.Q.; Zhao, B.; Zhong, L. Influence of biochar on soil physical and chemical properties and crop yields in rainfed field. *Ecol. Environ. Sci.* **2014**, *23*, 1292–1297. (In Chinese)
37. Yao, Q. *Effect of Biochar Addition on Soil Physicochemical Properties and Microbial Diversity in a Black Soil of Northeast China*; Changchun: University of Chinese Academy of Sciences (Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences): Changchun, China, 2017. (In Chinese)
38. Yang, H.H.; Li, M.S.; Wang, L.Y.; Feng, H.L.; Liu, F.T.; Du, J.; Ren, T.B.; Gao, W.K. Effects of biochar on soil nutrients and rhizosphere bacterial community structure of flue-cured tobacco at maturity stage. *J. Huazhong Agric. Univ.* **2021**, *40*, 62–71. (In Chinese)
39. Gao, T.Y.; Li, N.; Peng, J.; Gao, M.H.; Luo, P.Y.; Han, X.R. Effect of consecutive application of biochar on phosphate morphology and availability in brown soil. *J. Plant Nutr. Fertil.* **2019**, *25*, 1451–1460. (In Chinese)
40. Angst, T.E.; Sohi, S.P. Establishing release dynamics for plant nutrients from biochar. *Glob. Change Biol. Bioenergy* **2013**, *5*, 221–226. [CrossRef]
41. Zhan, X.M.; Peng, J.; Wang, Y.; Liu, Y.F.; Chen, K.; Han, X.R.; Wang, H.F.; Lin, W.C.; Li, X.Y. Influences of application of biochar and biochar-based fertilizer on brown soil physiochemical properties and peanut yields. *J. Plant Nutr. Fertil.* **2015**, *21*, 1633–1641. (In Chinese)
42. Wang, N.; Jiao, X.Y.; Wu, A.L.; Wang, J.S.; Dong, E.W.; Guo, J.; Ding, Y.C.; Wang, L.G. Research advances on effects of biochar application on soil phosphorus and potassium. *J. Shanxi Agric. Sci.* **2016**, *44*, 1402–1405, 1420. (In Chinese)
43. Ji, L.D.; Liu, X.T.; Si, H.L.; Sun, Q.; Wang, R. Effects of biomass charcoal on soil physicochemical properties and corn growth. *Agric. Res. Arid Areas* **2021**, *39*, 114–120. (In Chinese)
44. Yao, F.; Ren, T.B.; Xu, M.; Zhang, F.J.; Xu, C.S.; Ma, G.J.; Zhang, Z.Y.; Xie, T.X.; Liu, G.S. Research progress and mechanism of action analysis of biochar improving soil mineral nutrient absorption. *Jiangsu Agric. Sci.* **2020**, *10*, 46–51. (In Chinese)
45. Czimczik, C.I.; Masiello, C.A. Controls on black carbon storage in soils. *Glob. Biogeochem. Cycles* **2007**, *21*, 113. [CrossRef]
46. Feng, H.L.; Xu, C.S.; He, H.H.; Zeng, Q.; Chen, N.; Li, X.L.; Ren, T.B.; Ji, X.M.; Liu, G.S. Effect of biochar on soil enzyme activity and the bacterial community and its mechanism. *Environ. Sci.* **2021**, *42*, 422–432. (In Chinese)

47. Forge, T.; Ehret, D.; Messiga, A.; Dorais, M. Influnences of nitrogen inputs on nematode populations under high bush blueberry. *J. Nematol.* **2020**, *52*, 1–14. [PubMed]
48. He, X.F.; Zhao, F.Y.; Yu, K.; Yang, X.; Wang, J.W.; Yu, S.L. Effect of biochar on nutrient, enzyme activities and microbial diversity of rhizosphere soil of grape seedlings. *Soil Fertil. Sci. China* **2020**, *6*, 19–26. (In Chinese)
49. Gui, Y.Y.; Li, H.B.; Wei, J.J.; Mao, L.Y.; Zhang, R.H.; Qu, H.P.; Zhu, K.; Zhao, P.F.; Zhou, H.; Liu, X.H. Effects of biochar on soil nutrients, enzyme activities and microbial diversity of ratoon sugarcane in dry slope land. *J. South Agric.* **2022**, *53*, 776–784. (In Chinese)
50. Kennedy, A.C. Bacterial diversity in agroecosystems. *Agric. Ecosyst. Environ.* **1999**, *74*, 65–76. [CrossRef]
51. Gu, L.S.; Whalen, J.K.; Thomas, B.W.; Sachdeva, V.; Deng, H.Y. Physico-chemical properties and microbial responses in biochar-amended soils: Mechanisms and future directions. *Agric. Ecosyst. Environ.* **2015**, *206*, 46–59. [CrossRef]
52. Pietikäinen, J.; Kiikkilä, O.; Fritze, H. Charcoal as a habitat for microbes and its effect on the microbial community of the underlying humus. *Oikos* **2000**, *89*, 231–242. [CrossRef]
53. Nguyen, T.T.N.; Wallace, H.M.; Xu, C.Y.; Zwieten, L.V.; Weng, Z.H.; Xu, Z.H.; Che, R.X.; Tahmasbian, I.; Hu, H.W.; Bai, S.H. The effects of short term, long term and reapplication of biochar on soil bacteria. *Sci. Total Environ.* **2018**, *636*, 142–151. [CrossRef]
54. 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]
55. Yuan, H.C.; Wu, H.; Ge, T.D.; Li, K.L.; Wu, J.S.; Wang, J.R. Effects of long-term fertilization on bacterial and archaeal diversity and community structure within subtropical red paddy soils. *Chin. J. Appl. Ecol.* **2015**, *26*, 1807–1813. (In Chinese)
56. Song, Y.N.; Lin, Y.; Chen, Z.Q. Effect of nitrogen fertilizer level on bacterial community and N_2O emission in paddy soil. *Chin. J. Eco-Agric.* **2017**, *25*, 1266–1275. (In Chinese)
57. Chen, X.D.; Jiang, N.; Chen, Z.H.; Tian, J.H.; Sun, N.; Xu, M.G.; Chen, L.J. Response of soil phoD phosphatase gene to long-term combined applications of chemical fertilizers and organic materials. *Appl. Soil Ecol.* **2017**, *119*, 197–204. [CrossRef]
58. Jones, R.T.; Robeson, M.S.; Lauber, C.L.; Hamady, M.; Knight, R.; Fierer, N. A comprehensive survey of soil acidobacterial diversity using pyrosequencing and clone library analyses. *Int. Soc. Microb. Ecol. J.* **2009**, *3*, 442–453. [CrossRef] [PubMed]
59. Ding, X.J.; Huang, Y.L.; Jing, R.Y.; Ma, F.Y.; An, R.; Tian, Q.; Chen, B.J. Bacterial structure and diversity of four plantations in the Yellow River Delta by high-throughput sequencing. *Acta Ecol. Sin.* **2018**, *38*, 5857–5864. (In Chinese)
60. Li, P.; Wu, J.Q.; Sha, C.Y.; Ye, C.M.; Huang, S.F. Effects of manure and organic fertilizer application on soil microbial community diversity in paddy fields. *Environ. Sci.* **2020**, *41*, 4262–4272. (In Chinese)
61. Li, Y.Y. *Microbial Diversity and Enzyme Activity of Soil Reclaimed by Hydraulic Dredge Pump in Coal-Mining Subsidence Areas*; Xuzhou: China University of Mining and Technology: Xuzhou, China, 2015. (In Chinese)
62. Zhang, Y.J.; Wu, T.; Zhao, J.; Liu, S.L.; Yu, Y.G.; Zhang, H. Effect of biochar amendment on bacterial community structure and diversity in straw-amended soils. *Acta Sci. Circumstantiae* **2017**, *37*, 712–720. (In Chinese)

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