EFFECTS OF STRAW RETURNING AND BIOCHAR ON SOIL BACTERIAL COMMUNITY DIVERSITY AND CO-OCCURRENCE NETWORK IN PADDY FIELD

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Abstract. Applying biochar to soil has been proposed as a strategy to enhance soil quality and crop productivity. Four treatments were undertaken, including no amendment of biochar and straw addition (CK), original straw return only (SR), biochar return only (BR), and straw return with biochar addition (SB). The results showed that compared with the CK treatment, SR, BR and SB significantly increased soil bacterial community diversity, and the contents of soil organic matter (SOM), soil total nitrogen (TN), soil total phosphorus (TP), soil available phosphorus (AP), soil available potassium (AK), soil total potassium (TK) and soil cation exchange capacity (CEC) could be significantly increased by applying straw returning and biochar. Based on NMDS analysis at the OTU level for the soil bacterial community compositions, SB clearly separated from CK, SR and BR. The results of RDA for bacteria showed that TP, TN, TK, SOM, CEC and AK were the main factors affecting the composition of bacterial communities, and simple plot of BR and CK had highly significant effects with TN. Compared with CK and SR co-occurrence networks, BR and SB form a more complex co-occurrence network structure.
Keywords: *biochar, straw returning, soil microbial ecology, co-occurrence network, miseq sequencing*

Introduction

Over 1,348.8 million hectares of crops are produced annually in China, with an annual yield of 819 million tons, or close to one-third of global production (Yin et al., 2018). The majority of plant waste is normally removed from the field after harvesting and used for biomass, animal feed, and biofuel (Guo et al., 2019). Usually, the straw is burned or discarded, depleting resources and harming the environment. However, straw resources must be utilized sensibly if agricultural output is to be sustainable. Even if it has become the favored method, returning the straws to the soil in large paddy fields in the cold region is rather challenging. The creation of biochar by carbonizing straw has become a cutting-edge technique in the past few years (Meng et al., 2019).

Application of biochar and reusing straw are crucial steps to maintain soil fertility and soil microbial activity (Wang et al., 2019). Biochar has a high carbon content, a sizable specific surface area, a low bulk density, rich porosity, and outstanding adsorption capability, making it a widely utilized soil improvement material (Chen et al., 2017). It gives soil bacteria and other microorganisms a place to live and is hospitable to bacterial growth and reproduction (Zhang et al., 2017). Application of biochar can alter soil nutrient content to some extent while also reducing soil bulk density, effectively increasing soil available potassium, available phosphorus, and organic carbon content (Kuang et al., 2021; Luo et al., 2013). China is rich in straw resources. Crop straw contains a variety of nutrient elements, which can better provide

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nutrients for the soil (Wu et al., 2015). Straw returning to the field increases the content of soil organic carbon, which is conducive to organic cementation, promoting the formation of soil structure, reducing soil bulk density, and thus creating a good living environment for soil bacteria (Hao et al., 2019). There are also studies that show that straw returning can effectively alleviate a series of problems caused by the abuse of nitrogen fertilizer on soil (Alfadil et al., 2021). Both biochar and straw contain organic carbon. As the main factor to improve the soil environment, organic carbon can stimulate the soil bacterial community, thus promoting the soil nutrient cycle and improving the activity of soil bacterial community. Several research suggest that the biochar produced by agricultural waste and straw under oxygen-limited conditions is crucial for promoting soil nutrients, lowering carbon dioxide emissions, and increasing organic carbon storage (Chen et al., 2019). The effects of adding biochar or straw directly to the soil on crop yields, soil organic carbon, and greenhouse gas emissions have been well documented (Hu et al., 2021; Edwards et al., 2006; Blanchet et al., 2020). Agricultural waste, animal manure, and industrial wood by-products were used to create biochar, a solid substance rich in carbon with a high cation exchange capacity (CEC), wide porosity, and high surface area (Yuan et al., 2011; Poole et al., 2016). Biochar's aromatic structure has traits that make it highly resistant to biological and chemical deterioration as well as stable in soil. When biochar is added to the soil, it has the potential to increase the pH levels due to its high pH value (Benjamini et al., 2006). Additionally, it can enhance the overall health of the soil by increasing carbon stocks, retaining essential nutrients, improving fertility, and ultimately increasing crop yields (Ma et al., 2016). This can be achieved by optimizing fertilizer usage efficiency, the use of biochar amendments resulted in a substantial increase in rice output, ranging from 15.3% to 44.9% compared to chemical fertilizer (Montoya et al., 2006). Additionally, over a four-year period, the application of biochar led to an increase in the soil's total carbon and nitrogen by 27.6% and 75.6%, respectively (Zhou et al., 2011). Furthermore, the yield of peanuts also saw a significant improvement of 50.6% (Sui et al., 2016).

The main objectives of this study were thus to identify (1) their differential changes in the composition and diversity of soil bacterial communities in cold region; and (2) to evaluate the network complexity and the abundance of keystone taxa vary between conventional farmland, straw return and biochar treatments; (3) the potential key soil properties that could affect the structure and diversity of bacterial communities.

Materials and methods

Site description

The field experiment was located in Harbin, Heilongjiang Province, China. This study was conducted in the field trial area of the Modern Agricultural Demonstration Park at Heilongjiang Academy of Agricultural Sciences in Harbin (126° 50' E, 45° 50' N), Heilongjiang Province in 2022. The study site is classified as a typical temperate and monsoonal climate with a maximum potential rainfall of 550 mm and the annual average temperature in the reserve is -0.7°C, and the average relative humidity is 71.1% (Ding, 2023).

Experimental design

In this study, the paddy soil in cold area was taken as the research object, and the rice cultivar of Longdao 21 (*Oryza sativa* L. subsp. Japonica cv.), the main rice variety in

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Heilongjiang Province, was selected as the planting material, which is continuously planted once a year. Rice seedlings were cultivated in greenhouse on March 25, and then transplanted into the field on May 10. The transplanting density was 30 cm × 15 cm for each hole, with three seedlings in each hole. Fertilizers were applied to the soil at rates of 90 kg N ha^{-1}, 100 kg P_2O_5 ha^{-1} and 100 kg K_2O ha^{-1} per year, after which the fertilizers were mixed into the 0−20 cm soil layer. The crop is harvested at the end of September each year, and the land is idle from the end of October to the middle of next April in 2022.

The experiment was design with four treatments: no amendment of biochar and straw addition (CK), amendment of original straw return only (SR), amendment of biochar return only (BR), and amendment of straw return with biochar addition (SB). Each treatment had six replicates with a complete randomized design. Each plot was 4 m^2 (2 m × 2 m) and equipped with artificial penetration filters to avoid a possible surface runoff. All plots had similar soil and climatic conditions and were subjected to the same fertilization and field management during the experimental period.

In brief, rice straw was grinded to 3 mm and pyrolysed at 500°C according to the method of Abujabhah et al. (2016). The biochar material was spread evenly on the soil surface and harrowed thoroughly into the topsoil to 20 cm. All biochar was sieved through a 2-mm sieve and contained 62.45 (%) total C, 1.1 (%) total N, 56.78C/N and pH 8.56. The straw amount used for the biochar preparation was the same with that directly applied into the soil. Thus, to keep the same amount of straw return, the application rate of biochar and straw return was set as 1500 kg ha^{-1} a^{-1} and 3500 kg ha^{-1} a^{-1}, respectively.

Soil sampling

Soil samples were collected on 7 October 2022 from four plots, which had all been planted to rice. Samples were collected after the rice harvest. Soil was sampled from soil 0–20 cm depth in each replicated plot using a soil auger. Five samples were collected following an “S” shape, and then thoroughly mixed to obtain one composite sample for each replication, resulting in a total of 24 plot-level samples. After removing materials, such as roots and debris, each composite field soil sample was homogenized, placed in sterile plastic bags, and immediately shipped to the laboratory. The soil was then passed through a 2 mm sieve and visible debris was removed. Every soil sample was separated into two parts. One part was stored at -80°C for DNA extraction and the other part was air dried at room temperature to test soil properties.

The soil in this area was classified as an Alfisol (USDA Taxonomy), and one of the main cultivated soil types in Northeast China. In the soil of the tillage layer (0-20 cm), the soil organic matter content was 16.2 g kg^{-1}; the total N, P, and K contents were 0.90, 0.62, and 18.1 g kg^{-1}, respectively; the available N, P, and K were 86.5, 11.6, and 115.0 mg kg^{-1}, respectively; and the pH was 6.05. In this experimental field, the continuous rice cropping system has been practiced since 2012.

Soil chemical analysis

Soil pH was determined in a 1:2.5 soil/water suspension using a pH meter. The methods of concentrated H_2SO_4 digestion and Kjeldahl were used to determine the total nitrogen content (TN) of the soil samples (Abujabhah et al., 2016). Total phosphorus content (TP) of the soil samples was determined by $HClO_4$ and H_2SO_4 digestion molybdenum antimony anti-colorimetry (Pan et al., 2016). The soil’s available nitrogen

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(AN) was measured using the Alkali-diffusion method (Deng et al., 2016). Determination of the available phosphorus (AP) in soil was measured by using $NaHCO_3$ extraction- Mo-Sb Anti-colorimetry (Mehlich, 1984). The Walkley-Black titration method was carried out to determine the soil's organic matter (SOM) content (Faina et al., 2012). CEC was determined by the $BaCl_2$ compulsive exchange method (Gillman and Sumpter, 1986). The soil available phosphorus (AP) and potassium (AK) were described by previous studies (Chen et al., 2020).

Soil DNA extraction and quantitative PCR (qPCR)

Genomic DNA of the soil microorganisms was extracted with an Omega E.Z.N.A DNA Kit (Omega Bio-tek, Norcross, GA, USA). The extracted genomic DNA was detected by 1% agarose gel electrophoresis. The PCR was performed on a Geneamp 9700 PCR system (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA). The universal primers 515f (5'-gtgccagcmgcgg-3') and 907r (5'-ccgtcaattcmttragtt-3') were used to amplify the V3-V4 region of the bacterial 16S rRNA gene. The PCR products were quantified using a QuantiFluor® – ST fluorometer (Promega, Madison, WI, USA), and the samples were adjusted as needed for sequencing. Finally, they were sent to Shanghai Meiji Biotechnology Co., Ltd. (Shanghai, China) for high-throughput sequencing using an Illumina HiSeq 2500 PE250 platform (San Diego, CA, USA). Real-time quantitative PCR (RT-qPCR) analysis was conducted on 0.25 g of fresh soil. The DNA was extracted using the Mo Bio's PowerSoil® DNA Extraction Kit (Qiagen, Germany). The quality and concentration of extracted DNA were measured using NanoDrop Spectrophotometer (NanoDrop Technologies, Wilmington, DE) (Horwath, 2017). By using an ABI7500 fluorescence quantitative PCR instrument (Applied Biosystems, USA) and SYBR® Premium Ex Taq Kit (Takara, Japan).

Construction and analysis of microbial co-occurrence network

Because the microbial co-occurrence network model is affected by some ecological processes such as diffusion restrictions and environmental filtering (Blanchet et al., 2008), this study builds an integrated network of soil microbial communities at each sampling point, which can minimize the impact of the above ecological processes on the microbial network. Before constructing the network, remove OTUs with an abundance of less than 0.015% and a single sample site species occurrence frequency of less than 5 (Yuan et al., 2011). By calculating the Spearman correlation coefficient and Jaccard distance, the threshold for constructing a microbial co-occurrence network is determined based on the Random Matrix Theory (RMT) method. Through Permutation and Bootstrap, iterate 1000 times to obtain the P values of Spearman correlation coefficient and Jaccard distance (Poole et al., 2016). Then use the Brown's method to merge the above P-values. According to Benjamin et al. (2006) method of controlling False Discovery Rate (FDR) to correct P-values, a comprehensive network was constructed using FDR corrected relationships with statistical significance ($p < 0.05$). Visualize the network using the R language igraph package and Gephi. Subsequently, we use the subgraph function of the R language igraph package to extract sub networks for each sample from the comprehensive network constructed above. Each sub network is composed of the OTUs contained in each sample and the relationships between OTUs (Ma et al., 2016a). Due to the significant correlation between nodes, links, connectivity, and edge density that characterize the complexity of the network. The nodes are OTUs,

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and the edges are the connections between OTUs. Connectivity represents the proportion of the sum of the actual number of interactions between nodes in the network (the sum of edges) to the total potential number of interactions (the number of edges). The edge density is obtained by dividing the number of edges by the number of nodes (Montoya et al., 2006).

Statistical analysis

The R programming language (R Core Team, 2013) was used to visually analyze and describe the soil physicochemical parameters and bacterial community structure. The Chao1, Shannon, Simpson, and Ace indices were calculated using QIIME V1.9.1. One-way ANOVA and LSD were used to analyze the significance of differences between treatments ($p < 0.05$). Based on the Operational Taxonomic Units (OTUs) data of bacteria obtained by Illumina sequencing, the microbial ecological network was constructed using the CoNet plug-in in the Cytoscape (3.5.0) software. Analysis procedures and network parameter selection were conducted per the operation methods provided by Zhou et al. (2017). Network topology parameters, such as the characteristic path length, number of connections, number of nodes, clustering coefficient, network density, and average connectivity, were obtained using the Network Analyzer tool. The bacterial co-occurrence network diagram was constructed using the CoNet plug-in in the Cytoscape 3.7.0 software.

Results

Validation of the sequencing accuracy and community diversity

The microbial raw sequences were quality-controlled and filtered to obtain 168,604.69 valid sequences for cluster analysis of bacterial OTUs (*Table 1*). In the soil bacterial community, the Chao1 index was significantly higher in BR and SB than in CK and SR ($p > 0.05$). The Shannon index and ACE index of the alpha diversity increased in SB treatment, and the differences were significant among treatments ($p > 0.05$).

Microbial community composition

Venn diagram was used to distinguish the difference of soil bacterial community based on unique and shared OTUs across four treatments (*Fig. 1A*). A total of 15,890 OTUs were observed all treatments, CK and SR treatments, there was 402 shared OTUs (0.06% of the total). The number of shared OTUs was 89 in the group of SR and BR (0.01% of the total), while the number of shared OTUs was 1,035 in the group of BR and SB (0.22% of the total). From the perspective of the overall bacterial community structure, all OTUs belong to 60 bacterial phyla, 178 classes, 388 orders, 612 families, 1,125 genera, and 2,501 species were obtained. At the taxonomic level of bacterial community in the soil samples tested, the top five dominant phyla were Actinobacteriota, Proteobacteria, Acidobacteriota, Chloroflexi and Firmicutes (*Fig. 1B*). Among them, the relative abundance of Actinobacteriota decreased in the BR and SB treatments, whereas it increased in CK and SR. The relative abundance of Proteobacteria increased in the SB treatment, and the relative abundance of Acidobacteriota increased in BR and CK treatments. In addition, SR resulted in the largest relative abundance of Chloroflexi, respectively, in all treatments. Meanwhile, CK and SR reduced the relative abundance of Firmicutes, when compared with BR and SB treatments.

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Table 1. *Sequencing data summary and soil bacterial community diversity*

Items	CK	SR	BR	SB
Sequences	33992.33 ± 3092.08 a	33925.66 ± 8575.49 a	36201.54 ± 3489.41 a	30560.16 ± 1598.91 a
OTUs	2941	3564	4106	5279
Chao 1	2159.10 ± 247.27 b	2271.03 ± 317.61 b	3128.41 ± 230.52 a	3949.11 ± 312.97 a
Shannon	5.84 ± 0.21 b	5.87 ± 0.21 b	6.13 ± 0.07 b	6.81 ± 0.12 a
Ace	2166.23 ± 252.71 b	2289.34 ± 344.92 b	3119.48 ± 214.93 b	3993.57 ± 290.85 a

Data represents as mean ± standard deviation (S.D.) Statistical analyses were performed with Mann-Whitney U test between the two groups. The number of OTUs, richness estimator Chao, Ace and diversity estimator Shannon were calculated at 3% distance. n = 6, in each group. Values labeled with the same lowercase letter were not significantly different ($p > 0.05$). OTU, operational taxonomic unit. CK, no amendment of biochar and straw addition. SR, amendment of original straw return only. BR, amendment of biochar return only. SB, amendment of straw return with biochar addition. The same below

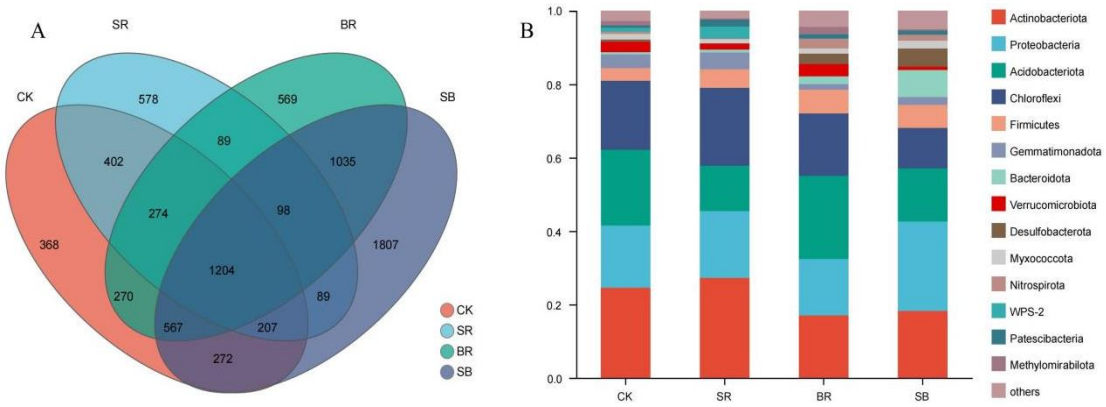

Figure 1. *Bacterial community structure and distribution of the soil samples at phylum level. (A) Venn diagram of OTU numbers for different treatments. Every circle indicates a treatment; the number of OTUs shared between different treatments was interpreted with the number in the overlapping circles, while the number in the non-overlapping area represented the number of unique OTUs for the specific treatment. (B) Bar plot of relative abundance of bacterial communities based on phylum*

The beta diversity for all treatments based on analyses of dimensionality reduction demonstrated the variation of bacterial communities between different treatments (*Fig. 2*). The PCoA plot ($R = 0.9448$, $P = 0.0010$) showed that the bacterial communities of different treatments separated, whereas the clusters for the CK and SR treatments did not show obvious dissimilarity between each other (*Fig. 2A*). Based on NMDS analysis at the OTU level for the soil bacterial community compositions (stress: 0.053, $R = 0.9448$, $P = 0.0010$), SB clearly separated from CK, SR and BR (*Fig. 2B*).

Effect of soil physical and chemical properties on microbial communities

As shown in *Table 2*, the pH of all soils was the differences were not significant among treatments. The SOM, TN, TP, TK and CEC contents of SB were significantly higher than that of CK, SR and BR treatments ($p < 0.05$). Soil AN in SR treatment were 18.28%, 61.11% and 50.14% fold higher than in CK, BR and SB, respectively. Soil AP in SB treatment were 39.56%, 9.41% and 37.72% were higher than in CK, SR and BR, respectively.

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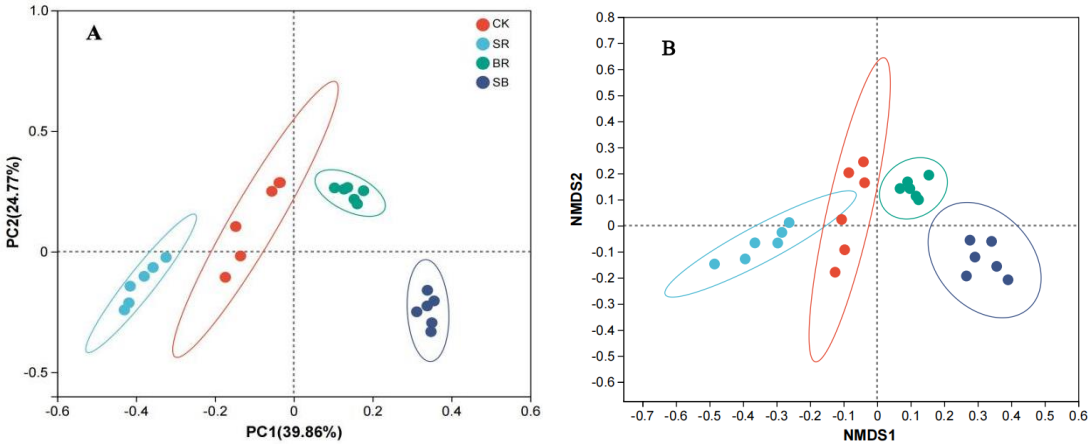

Figure 2. *Beta diversity analysis of different treatments. (A) Principal co-ordinates analysis (PCoA) plot; (B) Non-metric multidimensional scaling (NMDS) plot*

Table 2. *Soil physical and chemical properties in different treatments*

Index	Sample plot							
	CK		SR		BR		SB	
pH	6.24 ± 0.07	a	6.30 ± 0.11	a	6.41 ± 0.08	a	6.40 ± 0.30	a
SOM (g·kg^{-1})	25.51 ± 2.40	d	32.07 ± 1.75	c	35.31 ± 2.81	b	39.38 ± 1.63	a
TN (g·kg^{-1})	2.53 ± 0.85	c	2.86 ± 0.33	c	3.45 ± 0.45	b	4.87 ± 1.42	a
AN (mg·kg^{-1})	245.47 ± 37.65	b	300.41 ± 66.32	a	116.80 ± 14.27	c	149.78 ± 27.57	c
TP (g·kg^{-1})	0.03 ± 0.00	d	0.11 ± 0.01	c	0.22 ± 0.02	b	0.48 ± 0.13	a
AP (mg·kg^{-1})	42.38 ± 12.73	c	63.52 ± 5.10	ab	43.67 ± 7.80	c	70.12 ± 28.23	a
TK (g·kg^{-1})	1.94 ± 0.09	c	2.44 ± 0.62	b	2.08 ± 0.47	bc	3.98 ± 0.31	a
AK (mg·kg^{-1})	150.35 ± 3.39	b	153.61 ± 8.93	b	182.34 ± 3.81	a	179.08 ± 7.73	a
CEC	4.96 ± 0.86	b	5.53 ± 2.30	b	7.53 ± 2.47	ab	9.88 ± 3.30	a

Data represent the mean ± standard deviations, Analysis of variance (Duncan's multiple comparison test) was used to test the significance of differences. The different lowercase letters represent significant differences ($p < 0.05$). pH, the soil pH value. TN, the total nitrogen content of the soil samples. TP, the total phosphorus content of the soil samples. AN, the available nitrogen of the soil samples. AP, the available phosphorus content of the soil samples. SOM, the soil's organic matter content. CEC, the cation exchange capacity in soils. The soil available phosphorus (AP) and TK, the total potassium content of the soil samples. AK, the available potassium content of the soil samples

The results of RDA for bacteria are shown in *Figure 3A*. The first axis of bacteria explained 27.37% and the second axis explained 12.25% of all information. TP, TN, TK, SOM, CEC and AK are the main factors affecting the composition of bacterial communities. As shown in *Figure 3B*, TN, SOM, TP and AK had highly significant effects on the bacterial community, while pH had insignificant effects. Simple plot of BR and CK had highly significant effects with TN.

Significantly different taxa between treatments

To further explore the structural differences of the bacterial communities among different treatments, investigations corresponding to differential genera were performed (*Fig. 4*). The cladogram showed that when the LDA value was 2.0, there were 132 bacterial taxa that were significantly different among the treatments, including 5 phyla,

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14 classes, 28 orders, 38 families and 47 genera. Among the 47 bacterial genera, 8 genera in CK, 10 genera in SR, 12 genera in BR, and 17 genera in SB had significantly higher relative abundance, for example, Gaiellales (LDA = 4.669), JG30-KF-AS9 (LDA = 4.415), Subgroup_7 (LDA = 4.362), and Ktedonobacteraceae (LDA = 4.346).

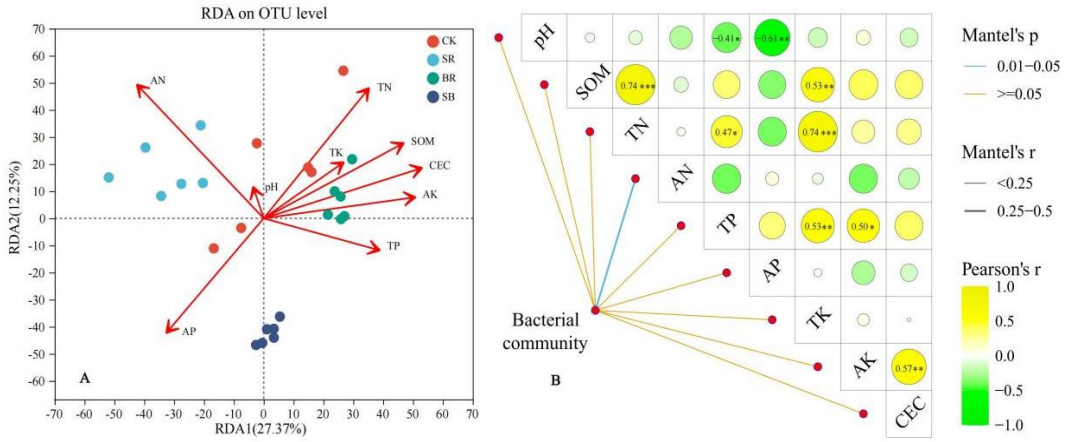

Figure 3. *RDA analysis of soil bacterial community structure and soil physical and chemical properties (A). Mantel test analysis based on the relative abundance matrix of the genera with LDA value > 2.0 (B). Arrows indicate the direction and magnitude of the environmental parameters associated with bacterial community structures, respectively. pH, soil pH vales; SOM, soil organic matter; TN, total N; AN, alkali-hydrolyzed N; TP, total P; AP, available P; TK, total K; AK, available K; CEC, soil cation exchange capacity*

Soil bacterial co-occurrence network

It can be seen from *Figure 5* that the soil bacterial communities showed different patterns of co-occurrence networks. Compared with CK and SR co-occurrence networks, BR and SB form a more complex co-occurrence network structure. Based on this observation, it was proposed that bacterial microorganisms, such as Acidobacteria, Actinobacteria, Bacteroidetes, Chloroflexota, Firmicutes and Myxococcota may be key species in all soil treatment samples. The number of network nodes, edges, and average degree can reflect the scale and complexity of the network. Through analysis, it was found that the soil bacterial network nodes of CK, SR, BR, and SB were 620, 475, 658, and 895, with edge numbers of 2969, 1564, 3691, and 2254, and average degrees of 9.577, 6.585, 11.218, and 5.036, respectively. The overall trend showed that BR > SB > SR > CK. The detailed information of these keystone taxa was shown in *Table 3*.

Discussion

In this study, we investigated the response of bacterial communities to four treatments in agricultural field experiment. The results clearly showed that original rice straw return on biochar products addition influenced soil bacterial community composition. It is well known that straw addition has a strong influence on bacterial communities (Zhang et al., 2014; Chen et al., 2017). However, little was known about the response of bacterial community composition to straw return and biochar products, especially at the phyla and genus level.

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Table 3. *The topological properties of the functional gene co-occurrence networks on four dilution levels and their respective identically sized random networks*

Topological properties	CK	SR	BR	SB
No. of original OTUs	713	588	709	1055
Nodes	620	475	658	895
Edges	2969	1564	3691	2254
Average degree	9.577419	6.585263	11.21884	5.036872
No. of clusters	44	80	51	83
clustering coefficient	0.7335603	0.8006119	0.8090007	0.5991478
Average path distance	7.537327	6.898469	5.845242	14.41858
Connectedness	0.01547241	0.01389296	0.01707587	0.005634084
Positive links	2490	1300	3691	1809
Negative links	192	264	479	445

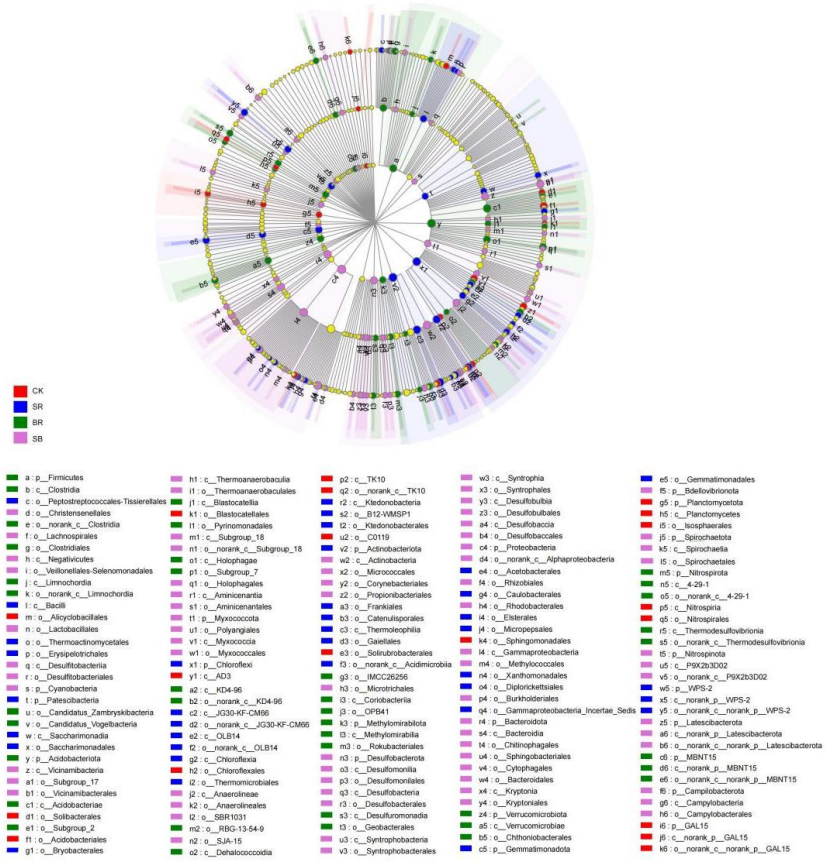

Figure 4. *LEfSe analysis (LDA > 2) of the soil bacterial community. The levels of phylum, class, order, family and genus are arranged from the inside to the outside, and the genera are shown in the figure. The yellow circle represents the taxa with no significant difference among the treatments*

Previous studies have demonstrated that the composition and diversity of crop microbial communities differ based on the specific plant residues used in a given field (Zhao et al., 2018). However, these studies have focused on upland crops or were

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conducted under greenhouse conditions. The study aimed to analyze the microbial communities in paddy soil associated with rice plants. The Chao1 Shannon and Ace diversity indices were assessed for these communities following different treatments, including SR (3500 kg ha^{-1}), BR (1500 kg ha^{-1}), SB (4500 kg ha^{-1}), or CK treatment. The results showed significant variations in soil microbiome diversity. (*Table 1*). The study found that the OTUs in the samples did not show significant differences across all treatment conditions. However, the relative abundance of associated microbes at the phyla level was altered by SR and BR amendment. The study also determined that the addition of RS and BC can increase available soil organic C, providing a niche for soil microbial growth. Original straw return only and biochar treatments led to a minor increase in α diversity of bacterial community compared to CK. In addition, the bacterial communities of straw return and control soils grouped closely. These results were inconsistent with other studies (Li et al., 2020; Palansooriya et al., 2019; Kong et al., 2011). These minor influences of straw return were likely to involve minor changes in pH, TN, AK, and CEC.

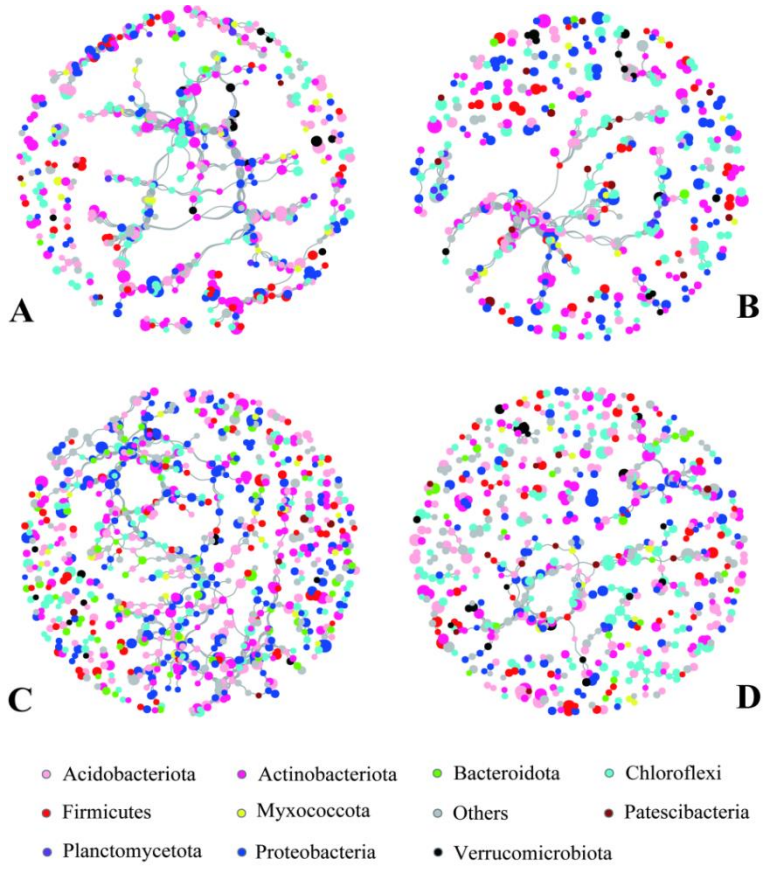

Figure 5. *Properties of soil microbial correlation-based network under different treatments. Network analysis showing the intra-associations inter-associations among different bacterial taxa. Networks were constructed at the operational taxonomic unit (OTU) level. The size of each node is proportional to the number of connections (i.e., degree). Edges between nodes indicate significant correlations among nodes (Spearman's r > 0.08, P-value < 0.01). Red and green edges represent T positive and negative associations between taxa. A, CK, no amendment of biochar and straw addition; B, SR, amendment of original straw return only; C, BR, amendment of biochar return only; D, SB, amendment of straw return with biochar addition*

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Actinobacteriota, Proteobacteria, Acidobacteriota, Chloroflexi and Firmicutes were the dominant phyla (*Fig. 2*). These bacteria are found in various environments, such as soil, water, and the human body (Sun et al., 2015). They play important roles in various biological processes, including organic matter decomposition, nutrient cycling, and symbiotic relationships with plants (Jeffery et al., 2015). This is consistent with reports that Proteobacteria are typically the dominant microbial phylum found in soil samples. Proteobacteria can readily grow in nutrient-rich soil, and SR decomposition can facilitate significant nutrient release into the soil ecosystem (Spain et al., 2009). One of the most prevalent types of bacteria in soil is Proteobacteria. They aid in the decomposition of organic materials in the soil and serve as translators and fixers of important elements including nitrogen, carbon, and phosphorus (Liao et al., 2016). Proteobacteria have the ability to regulate the structure and function of soil microbial communities through interactions with other microorganisms (Shin et al., 2015). One such example is the ability of certain Proteobacteria to coexist with plant roots to produce rhizobia, which provide plants with supplies of nitrogen and other nutrients (Hart et al., 1994). A vital biocontrol bacterium in soil, Proteobacteria can stop the development and reproduction of harmful germs like plant pathogens (Gupta and Germida, 2015). Proteobacteria have an important ecological role in soil and have a significant influence on the efficiency and stability of soil ecosystems (Mukhopadhya et al., 2012). Proteobacteria have an important ecological role in soil and have a significant influence on the efficiency and stability of soil ecosystems. As carbon sources, biochar and straw have different characteristics (Baiamonte et al., 2019). Straw mostly releases carbon sources through microbial decomposition, whereas biochar does so through microbial metabolism (Wang et al., 2019). Therefore, the carbon source characteristics of straw and biochar may have an effect on the composition and diversity of soil microbial communities. Straw and biochar might affect the composition of the microbial community, which is necessary for the growth and reproduction of microorganisms, and increase the amount of organic matter and nutrients in the soil (Hartley et al., 2016; Midwood et al., 2020). But because straw and biochar have different chemical and nutritional properties, they may have different effects on the soil microbial community, affecting the abundance of Actinobacteriota (He et al., 2020). In general, the characteristics of the carbon source, the quantity of nutrients available, and the composition of the microbial community may all have an impact on the impacts of straw and biochar on Actinobacteriota abundance (Bai et al., 2020). Microbes that can use a wider variety of carbon sources are more likely to proliferate in environments with high bacterial diversity and richness (Huang et al., 2019). Related research has demonstrated that using biochar can lessen the amount of Chloroflexi in paddy soil. This may be because biochar, which is compatible with the study's findings, can increase the diversity of soil microbes and compete with them for the nutrients necessary for Chloroflexi's development and reproduction (Hug et al., 2013). According to the findings, SB treatment decreased the abundance of Actinobacteria, Acidobacteriota, and Chloroflexi, respectively (*Fig. 1B*). These could be some of the causes: (1) with dietary restrictions, using just biochar and straw returned to the field can increase the soil's organic matter content and nutrient levels, allowing these microbial species greater sources of carbon and nitrogen for growth and reproduction. However, when biochar and previously-used straw are joined, they could fight for nutrients and impede the development and reproduction of microorganisms (Fan and Wu, 2020). (2) Antibiotic effect: if only biochar is used to cover a field after straw has

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been eliminated from it, it may release specific beneficial bacterial chemicals that promote microbial regrowth and growth (Sollins et al., 1996). Combining biochar and straw waste could interfere with one another or have antibiotic-like properties, which would stop microbial growth and reproduction (Zhang et al., 2020).

To improve the soil's chemical and biological environment, soil physical quality is essential. Biochar has been used as a soil amendment to repair eroded or deteriorated soils (Ma et al., 2016b). Biochar's high porosity, high inner surface area, and numerous micropores have the potential to enhance the physical characteristics of the soil and produce an environment that is more favorable for plant root development and nutrient uptake (Qiao et al., 2018). The ability of organic molecules to adsorb onto straw or the blockage of coarse straw components both control how much SOM is sequestered by straw (Zhang et al., 2017). Returning straw can enhance the proportions of labile organic matter, demonstrating that short-term wheat/rice straw return considerably increases SOM (Guo et al., 2015). The fact that straw contains a lot of alkaline materials may be the cause of the rise in soil pH. However, due to various feedstock types, biochar and straw production temperatures, soil characteristics, and other environmental factors, variable responses of biochar and straw applications to chemical properties have also been observed in earlier investigations (Zhang et al., 2016). Straw had a less noticeable impact on SOM and the C/N ratio than biochar (Jeffery et al., 2011). This demonstrated that biochar is more advantageous for enhancing the chemical characteristics of soil. This might be explained by the fact that biochar is more stable than easily decomposable straw due to its porous structure, high CEC, and surface area (Atkinson et al., 2010). In the current study, compared to the CK treatment, the SR, BR, and SB treatments enhanced the SOM, TN, TP, TK, and CEC. This demonstrated that adding biochar to the soil and incorporating straw waste are two efficient ways to enhance the soil's physical qualities.

Only returning the original straw and treating the soil with biochar somewhat increased the variety of the bacterial community in comparison to CK. Furthermore, the bacterial populations of straw return and manage the nearby soils (Jindo et al., 2012; Maarastawi et al., 2018). These slight variations in pH, Total C, N, P, and K, as well as accessible N, P, and K were likely to be involved in these small effects on straw return. In straw return soil, only bulk density was significantly reduced. It is probable that the bulk density was insufficient to cause a significant shift in the bacterial population, as was the case with the bacterial communities found in the soil used to grow *Panax ginseng* (Bai et al., 2019).

Microbiological communities create a sophisticated ecological network in the natural world. The interaction between species may be predicted using the ecological network. Symbiosis and copolymerization were favorably connected in microorganisms that shared a mutually advantageous relationship, but biased symbiosis and predation were negatively correlated in those that were in a competitive relationship (Faust and Raes, 2012). The biochar treatment (BR and SB) in this study boosted nutrients such carbon, nitrogen, and phosphorus in comparison to the control (CK), boosting bacterial growth and metabolism and enhancing bacterial diversity. On farmland soil after applying biochar in northeast China, our prior findings indicated that Proteobateria, Bacteroidetes, Acidobacteria, and Actinobacteria were the dominating bacterial species (Ding and Li, 2022). In this study, an interaction network between the application of biochar and non-biochar treatments was built to examine the impact of biochar application on bacterial co-occurrence patterns in farmed soil. The findings

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demonstrated that after the application of biochar, the co-occurrence mode of microorganisms considerably altered. Following the application of biochar, bacterial interaction dramatically increased as compared to the CK that did not get biochar treatment. The interaction network's nodes rapidly increased as the network grew more intricate (Gundale and DeLuca, 2006). The complexity and stability of the microbial ecological network in the farming ecosystem increased with the availability of nutrients. High community stability is a crucial component in ensuring ecological function. Additionally, the unique porous structure of biochar can shield bacteria and lessen the harm done by its rivals. Studies have revealed that after applying biochar, the effects of soil pH and NH_4^+-N on bacteria were dramatically increased (Zhou et al., 2017). While changes in NH_4^+-N and soil acidity both supported bacterial growth, they also had an impact on the bacterial community and interaction network (Lauber et al., 2008). The modular structure of the interaction network treated with biochar was more complicated than the control group's, with more nodes and interactions, a higher network score, and mostly bacterial nodes. These modules did not closely adhere to taxonomic classification; for example, interactions between microorganisms did not rely on their taxonomic classification.

Conclusions

We found that crop straw products function in shifting soil bacterial communities and the greatest influence was observed in biochar addition soil, which supports our hypothesis. However, the differences in soil physiochemical properties with crop straw products addition contributed to the changes in diversity and structure of bacterial communities. There were different responses of bacterial genera to straw products and considerably more genera in response to biochar. These findings indicated that biochar is the most potent method of crop straw management in shifting soil bacterial community dynamics. Further interpretations of the bacterial functional genus associated with biochar properties will be useful to clarify the potential use of biochar to manage crop straw.

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