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Soil nitrogen transformation and functional microbial abundance in an agricultural soil amended with biochar

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ABSTRACT: Biochar soil amendments are attracting attention as one strategy to improve soil microbially ecological environment and regulate the soil nitrogen cycle. This study aimed to evaluate the effects of biochar application on agricultural soil improvement, nitrogen (N) mineralization and nitrification. The experiment was carried out on a typical farmland containing black soil and saline-alkaline soil in Northeast China. Four treatments were undertaken, including the control-treated black soil farmland (CS), the biochar-treated black soil farmland (BCS), the control-treated saline-alkali soil farmland (SAS), and the biochar-treated saline-alkaline soil farmland (BSAS). Basic physical and chemical properties, enzyme activity, and the contents of ammonium-nitrogen (NH_4^+-N) and nitrate-nitrogen (NO_3^--N) in the soil were subsequently determined. The co-occurrence networks of bacterial communities of the biochar and control treatment groups were constructed based on high-throughput sequencing data of the 16S rRNA genes. The results showed that the BCS and BSAS treatments significantly increased the contents of soil organic matter, total nitrogen, total phosphorus, and available phosphorus. The application of biochar significantly increased the NH_4^+-N contents in the black soil and saline-alkaline soil by 81.78 and 80.08 %, respectively, while significantly reducing the soil NH_4^+-N/NO_3^--N content, which promoted the transformation of NH_4^+-N into NO_3^--N. Subsequently, the released NH_4^+-N was transformed into NO_3^--N through nitrification. After the biochar application, the NO_3^--N contents in the black and saline-alkaline soils could be fixed. The biochar application significantly increased the abundance of *gdh*, AOA-*amo*A, AOB-*amo*A, *nir*K, *nir*S, *nos*Z, and *nif*H genes, with no significant difference in the abundance of *nap*A genes being found among different treatments. Microbes playing a key role in the co-occurrence network were Proteobateria, Acidobacteria, Bacteroidetes, Actinobacteria, and Chloroflexi. As compared with the CS and SAS treatments, under the BCS+BSAS treatment, the connectors, module hubs, connectedness, and clustering coefficient showed larger parameters, and the networks were more complex. The application of biochar gradually increased the nodes, edges, and average degree of the bacterial co-occurrence network, thus indicating that the interaction between microbial groups in the black and saline-alkaline soils post biochar application may be important in the biogeochemical cycle process in farmland soil.

Keywords: biochar, physio-chemical properties, soil microbial ecology, soil enzyme activity, co-occurrence pattern.

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INTRODUCTION

Nitrogen is an essential nutrient element, and 95 % of soil nitrogen is organic. After being converted into inorganic nitrogen through mineralization, plants absorb and utilize it (Qiu and Chen, 1995). Nitrification is another important way for soil nitrogen transformation, which is closely related to the further transformation of NH_4^+-N and the loss of soil nitrogen (Vitousek et al., 1997). In China, acidic soil is widely distributed in the south of the Yangtze River. Due to the nutrient deficient conditions in acidic soil, abundant nitrogen fertilizer must be applied to meet the growth of crops (Zhang et al., 2019). This resulted in severe soil acidification and loss of soil nitrogen (Xia et al., 2022). Therefore, an urgent solution is needed to improve the fertility of typical farmland soil types in northern China, increase nitrogen fertilizer utilization efficiency, and reduce soil nitrogen loss.

Biochar is the product of the high-temperature pyrolysis of agricultural wastes (straw, wood, livestock dung, etc.) under anoxic conditions (Chen et al., 2013a). Biochar prepared from different raw materials has different properties, including the surface structure and element composition (Wang et al., 2015). Due to its high stability and high C/N ratio, an increasing number of studies focused on how biochar affects nitrogen cycling (Yu et al., 2020). The mineralization of soil nitrogen is related to properties like soil pH and C/N ratio. Therefore, the biochar addition can affect the transformation of soil nitrogen by altering the soil physicochemical properties (Xia et al., 2021). Chen et al. (2016) found that the application of biochar could quickly stimulate soil microbial activity and increase the inorganic nitrogen fixation, thus inhibiting the mineralization and nitrification of soil nitrogen. On the other hand, Pan et al. (2016) found biochar addition promoted soil nitrification. This is possibly caused by the different types of soil and biochar. Additionally, enzymes in soil are catalysts participating in biochemical reactions, and their activities are closely related to the status of both soil nutrient cycling and nutrient content (Nannipieri et al., 2012). N-acetyl-D-(+)-glucosaminidase (NAG), ammonia monooxygenase (AMO), hydroxylamine oxidoreductase (HAO), and nitrous oxide reductase (NXR) are key enzymes in the processes of soil nitrogen mineralization and autotrophic nitrification (Qu et al., 2021). They can metabolize the N-terminal amino acid residues of proteins and polypeptides, with their activities representing the transformation and supply of soil nitrogen. The inorganic nitrogen absorbed by plant roots from the soil is mainly obtained from the enzymatic degradation products of soil microorganisms (Tatti et al., 2013). Nitrate reductase and nitrite reductase directly participate in the soil denitrification process. Urease hydrolyzed urea into inorganic nitrogen (such as NH_4^+) for its absorption and utilization by plant roots (Van Zwieten et al., 2010).

Co-occurrence networks are often used to characterize the coexistence and exclusion of microorganisms in response to external interference and help identify key microorganisms highly related to soil function and crop production (Banerjee et al., 2018). The network modules integrate complex high-dimensional species information, which is a collection of multiple highly related species sharing the same niche, and is regarded as an ecological cluster (Duran-Pinedo et al., 2011). Numerous studies have confirmed that biochar addition can change the microbial community structure and its enzyme activity, which is vital in soil ecological processes, including organic matter accumulation and nutrient transformation, thereby indirectly affecting the growth of plants (Kuzyakov, 2009). Although the impact of biochar on soil microorganisms is being increasingly focused on, how biochar affects the ecological network in microbial communities is still insufficient. Very few studies have focused on how biochar affects the interaction between bacterial communities in the black and saline-alkaline soils (Steiner et al., 2010).

This study aimed to assess the impact of biochar on the interaction between soil bacterial communities and the main driving factors, thereby providing a theoretical basis for farmland soil protection in Northern China.

MATERIALS AND METHODS

Site description

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 2021. The study site is classified as a typical temperate and monsoonal climate with a maximum potential rainfall of 550 mm and mean annual temperature is ≥10 °C.

Tested materials

Biochar

Biochar is a stable, carbon-rich product made from agricultural waste biomass, such as crop straw and peanut shells, via pyrolysis under low temperature and anoxic conditions. The tested biochar was commercially supplied by Liao Ning Golden Future Agriculture Technology Co., Ltd. The biochar presented pH 8.69 and N:P_2O_5:K_2O ratio equal to 8:11:15.

Soil samples

Soil samples were collected from the Modern Agricultural Demonstration Park at Heilongjiang Academy of Agricultural Sciences, and the soil type was black soil. Saline-alkali soils were collected from the Fanrong village in Zhaodong City, Heilongjiang Province (125° 34' 34" E~46° 23' 58" N). The soils in the sampled municipalities of Sucre are classified as Mollisols (IGAC, 2016). The main planting crop is soybean (*Glycine max*) in black soil and saline-alkali soil, which is continuously planted once a year. At the end of each April, agricultural machinery tillage is conducted once, and the soybean is sown. Farm fertilization and field management are performed according to the local practices. 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.

Experimental design

The experiment started on June 5 and lasted on September 10, in 2021. Pot experiments were performed using polypropylene plastic pots with a height and diameter of 0.30 m. Biochar and air-dried soil were well mixed and placed into the pot experiments. Four treatments were set as follows: (1) no biochar was added into the black soil (CS); (2) 40 g of biochar were added into 1 kg of black soil (BCS), i.e., 160 g biochar/pot; (3) no biochar was added into the saline-alkali soil (SAS); (4) 40 g of biochar were added into 1 kg saline-alkali soil (BSAS), i.e., 160 g biochar/pot. Samples were collected on the 60th day of culture. When sampling, a portion of fresh soil was stored in the refrigerator at -20 °C to determine soil NH_4^+-N, NO_3^--N, enzyme activity, and abundance of soil microorganisms. The remaining soil samples were air-dried, and separately ground, and sieved through 0.85 and 0.15 mm aperture sieves. Then they were stored in self-sealing bags to determine soil pH and physicochemical properties of organic matter.

Determination of soil physical and chemical properties

The ring knife method was used to determine the soil bulk density (Hu et al., 2017). Soil pH was determined in a 1:2.5 (w/v) ratio of air-dried soil to deionized water (Zhang

and Voroney, 2015). The methods of concentrated H_2SO_4 digestion and Kjeldahl were used to determine the total nitrogen content of the soil samples (Pan et al., 2021). Total phosphorus content of the soil samples was determined by $HClO_4$ and H_2SO_4 digestion molybdenum antimony anti-colorimetry (Bao, 2005). The soil's available nitrogen was measured using the Alkali-diffusion method (Deng et al., 2016). Determination of the available phosphorus 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 carbon content (Faina et al., 2012).

Determination of soil enzyme activity

Fluorescent microplate enzyme detection technology was used to determine the activities of the soil β-D-glucosidase (β-G), β-cellobiosidase (CBH), and NAG, by using the fluorescent substance 4-hydroxymethyl-7-coumarin (MUB) as the standard (Zhang et al., 2009). The sample well, blank control, negative control, quenching control, and reference control were set on the 96-well plate for each treatment. The fluorescence value was measured using the microplate reader after 4 h of incubation at 25 °C in the dark. The excitation and detection wavelengths were 365 and 450 nm, respectively. Activities of ammonia monooxygenase (AMO), hydroxylamine oxidoredutase (HAO), and nitrite oxidoredutase (NXR) in soil were determined using the ELISA kit from Jiangsu Meibiao Biotechnology Co., Ltd.

Soil urease (UE) activity was determined by urea colorimetry (Guan, 1986). One unit of enzyme activity was expressed as the milligram of ammonium ion produced by the hydrolysis of one gram soil at 37 °C for 24 h. The activities of soil denitrification enzymes (nitrate reductase and nitrite reductase) were measured by the benzenesulfonic acid-acetic acid-α-naphthylamine colorimetric method (Gao et al., 2019). One unit of enzyme activity of nitrate reductase (NR) was expressed as the milligram of NO_2^- produced by the reduction of 1 kg soil at 30 °C for 24 h. One unit of enzyme activity of nitrite reductase (NiR) was expressed as milligrams of NO_2^- reduced by the reduction of 1 kg soil at 30 °C for 24 h (Zeng et al., 2013). The activities of L-leucine aminopeptidase (LAP) and NAG in the soil were determined by the method from Bob Sinsabaugh Lab with some modifications (Sinsabaugh et al., 2000). Here, the crude enzyme solution was prepared using a buffer solution with a pH of 5. LAP uses 5 mM leucine p-nitroaniline as the substrate, whereas NAG used 2 mM pNP-β-Nacetylglucosaminide; and the control was also set. Their activities were determined using colorimetry with a microplate reader. The unit of enzyme activity was expressed as the amount (mg) of the substance hydrolyzed by the unit mass (g) of dry matter in the unit time (h). The soil NH_4^+-N and NO_3^--N were determined using indophenol blue colorimetry and phenol disulfonic acid colorimetry, respectively (Lu, 1999). The soluble organic nitrogen in the soil was extracted with K_2SO_4 0.5 mol L^{-1}, and it was determined using the Total Organic Carbon Analyzer (TOC-VcPH + TNM-1, Shimazu Inc., Kyoto, Japan) (Edwards et al., 2006). The biomass nitrogen of soil microorganisms was determined by the improved chloroform fumigation-K_2SO_4 extraction method. The soluble organic nitrogen in the extract was determined by Total Organic Carbon Analyzer (TOC-VcPH + TNM-1, Shimazu Inc., Kyoto, Japan). The microbial biomass nitrogen content was obtained by dividing the difference of organic nitrogen between the extracts of fumigated and non-fumigated soils by 0.54 (Yang et al., 2012).

DNA extraction and high-throughput assay

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) (Edwards et al., 2006). By using an ABI7500 fluorescence quantitative PCR instrument (Applied Biosystems, USA) and SYBR® Premium Ex Taq Kit (Takara, Japan), RT-qPCR was performed to analyze the abundance of microbial genes related to nitrogen cycle processes, such as ammoniation (*gdh*), nitrification (AOA-*amo*A and AOB-*amo*A), denitrification (*nir*S, *nir*K, and *nos*Z), nitrogen fixation (*nif*H), and nitrate dissimilatory reduction (*nap*A). The qPCR reaction system was 25 µL, including 1 µL DNA template, 12.5 µL SYBR® Premix Ex TaqTM, 0.5 µL forward and reverse primer each, 0.5 µL ROX Reference Dye II (50×), and 10× ddH_2O. The primer sequences for studying the functional genes in the nitrogen cycle are shown in table 1.

Statistical analysis

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. (2011). 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 for BCS and BSAS or CS and SAS was constructed using the CoNet plug-in in the Cytoscape 3.7.0 software.

Table 1. Primers of target gens of quantitative PCR

Targeted gene	Primer	Primer sequence(5'→3')	References
AOA-*amo*A	Arch-*amo*AF	5ATAGAGCCTCAAGTAGGAAAGTTCTA	Francis et al. (2005)
	Arch-*amo*AR	CCAAGCGGCCATCCAGCTGTATGTCC	
AOB-*amo*A	*amo*A-1F	GGGGTTTCTACTGGTGGT	Rotthauwe et al. (1997)
	*amo*A-2R	CCCCTCKGSAAAGCCTTCTTC	
*nir*K	*nir*K876	ATYGGCGGVCAYGGCGA	Braker et al. (1998)
	*nir*K1040	GCCTCGATCAGRTTRTGGTT	
*nir*S	*nir*SCd3aF	AACGYSAAGGARACSGG	Throbäck et al. (2004)
	*nir*SR3cd	GASTTCGGRTGSGTCTTSAYGAA	
*nos*Z	*nos*Z-F	CGCTGTTCITCGACAGYCAG	
	*nos*Z-R	ATGTGCAKIGCRTGGCAGAA	
gdh	*gdh* forward	CCACTTATTGCATTTACGTCAAAGA	Govindarajulu et al. (2005)
	gdh reverse	CCCAGTCATCTCAGCAAGAGAA	
*nif*H	*nif*HF	AAAGGYGGWATCGGYAARTCCACCAC	Rosch and Bothe (2005)
	*nif*HRb	TGSGCYTTGTCYTCRCGGATBGGCAT	
*nap*A	V17m	TGGACVATGGGYTTYAAYC	Bru et al. (2007)
	*nap*A4r	ACYTCRCGHGCVGTRCCRCA	

RESULTS

Soil physical and chemical properties

When biochar was applied, the physical and chemical properties of the soil changed (Table 2). Soi pH in SAS and BSAS was significantly higher than CS and BCS ($p<0.05$). The soil bulk density index of the SAS was the highest (1.38 Mg m^{-3}), while those with BCS were the lowest (1.25 Mg m^{-3}). The contents of soil organic carbon, total nitrogen, available nitrogen and available phosphorus were significantly increased in BCS treatment($p<0.05$). Compared with the CS and SAS, soil organic carbon for the biochar treatments BCS and BSAS increased significantly by 27.36 and 23.33 % ($p<0.05$). The rate of soil organic carbon to soil total nitrogen was higher in BSAS, and the corresponding soil porosity increased by 10.41 % more than SAS. In general, BCS treatment showed the highest organic carbon, total nitrogen, total phosphorus, available nitrogen, available phosphorus values are significantly ($p<0.05$) different compared with CS, BCS and BSAS values.

Effects of biochar on soil nitrogen mineralization and nitrification

The biochar treatment significantly changed the NH_4^+-N and NO_3^--N contents, with both showing an increasing trend in biochar-treated soil samples (Table 3). The NH_4^+-N content in the biochar-treated black and saline-alkaline soils increased by 81.78 and 80.08 %, respectively, compared to those without biochar treatment. In comparison, NO_3^--N increased by 91.55 and 91.36 %, respectively ($p<0.05$). After the biochar was applied to the soil, it was released as NH_4^+-N in the early stage. The biochar treatment significantly reduced the soil NH_4^+-N/NO_3^--N ($p<0.05$), which promoted the conversion of NH_4^+-N to NO_3^--N. The NH_4^+-N released was subsequently converted into NO_3^--N through nitrification, and this NO_3^--N in the black and saline-alkaline soils was then immobilized post-biochar application.

Table 2. Physical and chemical properties in the soil tested

Soil properties	CS	BCS	SAS	BSAS
Bulk density (Mg m^{-3})	1.29±0.03[(1)] b	1.25±0.04 b	1.38±0.14 a	1.37±0.28 a
pH(H_2O)	6.22±0.43 b	6.71±0.03 b	8.65±1.02 a	8.67±0.34 a
Organic carbon (g kg^{-1})	23.81±4.91 b	32.82±0.89 a	17.38±1.97 c	22.67±0.16 b
Total nitrogen (g kg^{-1})	4.55±0.27 a	4.84±0.31 a	3.89±0.03 b	4.04±0.17 b
Total phosphorus (g kg^{-1})	1.33±0.06 b	1.64±0.05 a	1.02±0.02 c	1.48±0.15 b
Available nitrogen (mg kg^{-1})	109.31±2.81 b	122.73±4.04 a	94.11±49.03 c	106.24±2.69 b
Available phosphorus (mg kg^{-1})	18.72±5.14 b	21.89±2.05 a	17.19±2.24 b	19.81±0.76 b
Rate of soil organic carbon to soil total nitrogen	15.36±1.18 c	17.83±1.87 b	19.52±0.65 b	21.79±0.94 a

[(1)] Mean ± standard erro (n = 3). Different letters indicate significant differences among treatments ($p<0.05$).

Table 3. Dynamic changes of soil ammonium nitrogen (A, NH_4^+-N), nitrate nitrogen (B, NO_3^--N), and the ratio of NH_4^+-N/NO_3^--N (C) in biochar application

Treatment	Ammonium nitrogen (NH_4^+-N)	Nitrate nitrogen (NO_3^-N)	Ratio of NH_4^+-N/NO_3^--N
	mg kg^{-1}		
CS	17.65±2.43[(1)] c	11.39±2.09 c	1.55±0.10 a
BCS	96.91±4.22 a	134.92±7.53 a	0.72±0.06 c
SAS	14.74±1.38 c	9.96±1.78 c	1.47±0.05 b
BSAS	74.01±2.59 b	115.31±8.65 b	0.64±0.19 c

[(1)] Mean ± standard erro (n = 3). Different letters indicate significant differences among treatments ($p<0.05$).

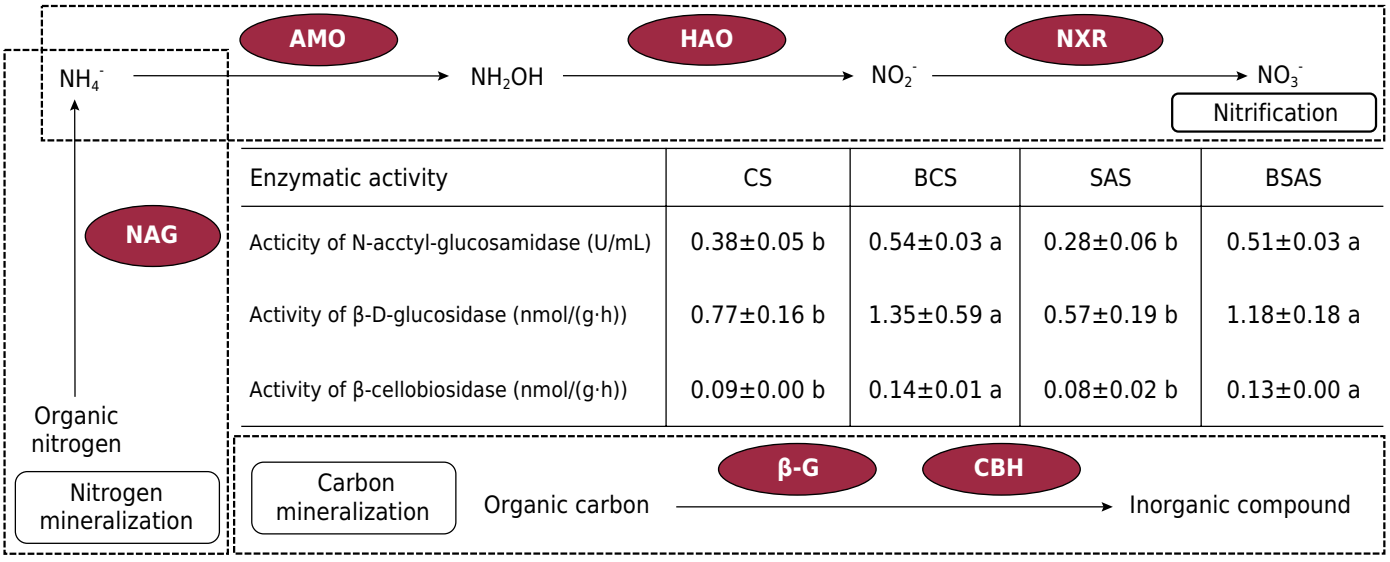
Effects of biochar application on soil nitrogen mineralization, nitrification and C-N cycle related enzymes activity

Soil nitrogen mineralization process of the biochar-treated soil as shown in figure 1. Application of biochar to stimulate the NAG activity. As compared with the CS and SAS treatments, BCS and BSAS treatments significantly increased the NAG activity by 29.62 and 45.09 %, respectively ($p<0.05$). In the soil nitrogen nitrification process, when compared with the CS treatment, the biochar treatment significantly reduced the activities of AMO, HAO, and NXR ($p<0.05$), with the BCS and BSAS treatments significantly reducing the activities of AMO by 9.01 and 12.71 %, HAO by 9.01 and 17.64 %, and NXR by 14.47 and 7.61 %, respectively. In the soil carbon cycle, as compared with the CS treatment, biochar treatment significantly improved the activities of β-G and CBH ($p<0.05$), with the BCS and BSAS treatments increasing the β-G activity by 42.96 and 51.69 %, and that CBH by 35.71 and 38.46 %, respectively.

Effects of major control factors on soil microbial properties

Biochar application had no significant effect on the functional gene *nap*A abundance in the nitrogen cycle (Figure 2), whereas it variably affected those of the remaining seven functional genes. The BCS and BSAS treatments promoted the abundance of multiple functional genes, including *gdh*, AOB-*amo*A, *nir*S, and *nir*K. Compared with CS and SAS treatments, the abundance of the *gdh* gene increased by 25.11 and 50.65 %, the AOB-*amo*A gene increased by 59.01 and 96.13 %, and that of *nir*K gene increased by 24.32 and 56.76 %, respectively, in the BCS and BSAS treatments. Moreover, the abundance of *gdh*, AOB-*amo*A, and *nif*H under the BSAS treatment was significantly higher than those under other treatments ($p<0.05$). The *nir*S gene abundance under BCS and BSAS treatments was significantly higher by 45.97 and 51.35 % than those under the CS and SAS treatments ($p<0.05$) respectively. The BCS treatment promoted

Enzymatic activity	CS	BCS	SAS	BSAS
Ammonia monooxygenase (U/mL)	526.76±15.03 a	479.25±21.35 b	468.11±12.09 b	408.57±18.56 c
Hydroxylamine oxidoredutase (U/mL)	114.73±9.42 a	106.91±5.04 b	90.15±11.84 b	74.24±9.52 c
Nitrite oxidoredutase (U/mL)	107.66±1.55 a	92.08±3.23 b	102.03±2.87 a	94.26±1.06 b



Enzymatic activity	CS	BCS	SAS	BSAS
Acticity of N-acctyl-glucosamidase (U/mL)	0.38±0.05 b	0.54±0.03 a	0.28±0.06 b	0.51±0.03 a
Activity of β-D-glucosidase (nmol/(g·h))	0.77±0.16 b	1.35±0.59 a	0.57±0.19 b	1.18±0.18 a
Activity of β-cellobiosidase (nmol/(g·h))	0.09±0.00 b	0.14±0.01 a	0.08±0.02 b	0.13±0.00 a

Figure 1. Activity of C and N cycle-related enzymes of soil in biochar application. Different letters indicate significant differences among treatments ($p<0.05$).

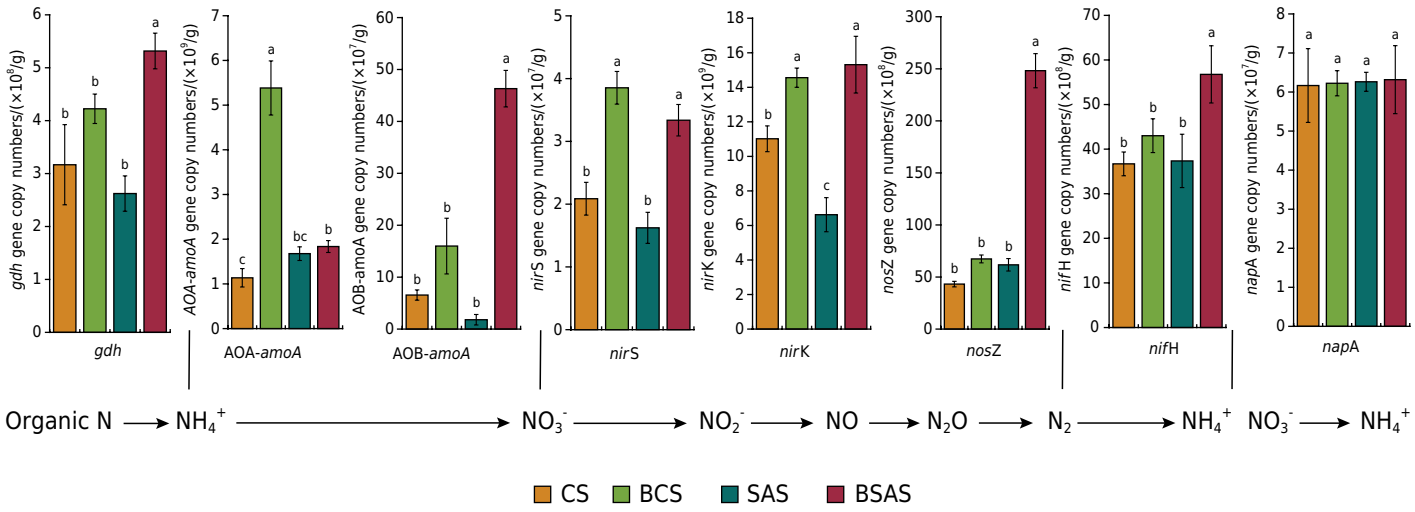


Figure 2. Effects of biochar application on the abundance of soil. Different letters above the bars indicate statistical differences among treatments at the significance level of $p<0.05$.

the functional gene AOA-*amo*A abundance, which was significantly increased by 78.99 % compared to the CS treatment. In contrast, no significant difference was found in functional gene AOA-*amo*A between the SAS and BSAS treatments ($p<0.05$). Compared to the CS, BCS, and SAS treatments, the abundance of the functional gene *nif*H under the BSAS treatment significantly increased by 35.34, 24.22, and 34.18 %, respectively ($p<0.05$).

Effects of biochar application on microbial co-occurrence patterns

It can be seen from figure 4 that the bacterial communities of the biochar-treated soil showed different patterns of co-occurrence networks. The co-occurrence networks of bacteria under CS+SAS (Figure 3a) were similar to those under BCS and BSAS (Figure 3b). The co-occurrence networks of bacteria with the treatments BCS and BSAS were relatively complex, thereby indicating that the biochar treatment had a greater impact on the co-occurrence network of the soil bacterial communities. Based on this observation, it was proposed that bacterial microorganisms, such as Proteobateria, Acidobacteria, Bacteroidetes, Actinobacteria, and Chloroflexi may be key species in biochar-treated black and saline-alkaline soil. The results of ZI and PI (Figure 3c and Figure 3d) suggested that the connectors and module hubs under the BCS+BSAS treatment were higher than those of the CS+SAS treatment. In the CS+SAS treatment, 573 main bacteria categories were recorded, including Proteobacteria (211), Acidobacteria (105), Bacteroidetes (85), Actinobacteria (62), Chloroflexota (38), Gemmatimonadota (23), Firmicutes (20), Nitrospirota (17), and Verrucomicrobiota (12). Furthermore, in the soil under BCS+BSAS treatment, 571 major categories were detected, mainly including Proteobacteria (207), Acidobacteria (93), Bacteroidetes (76), Actinobacteria (66), Chloroflexota (51), Gemmatimonadota phylum (29), Nitrospirota phylum (18), Saccharibacteria (17), and Verrucomicrobia phylum (14).

The co-occurrence networks of soil bacterial communities with the two treatments were compared, and the changes between the main topologies for each network are shown in table 4. Comparative analysis of the two networks indicated that the network under BCS+BSAS treatment was more complex, which was reflected in the lines connecting network nodes. Similarly, this was also reflected in the network topology connectivity. The BCS+BSAS treatment showed greater connectivity than the CS+SAS treatment. Additionally, the clustering coefficient under BS+SAS treatment was greater than that of CS+SAS treatment, which further proved the greater complexity of the network under BCS+BSAS treatment. With the increasing biochar addition, the nodes, edges, and

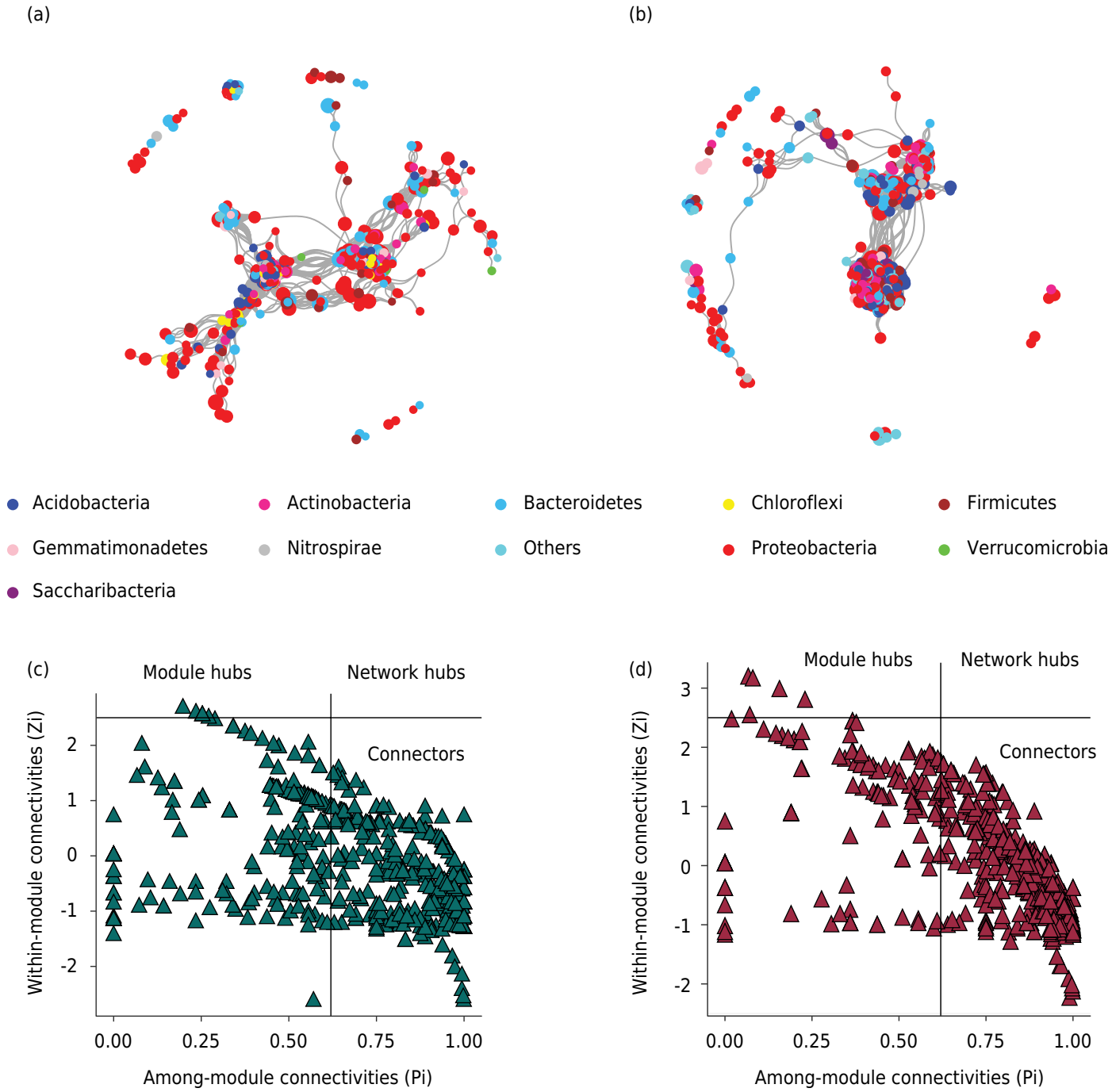


Figure 3. Co-occurrence network of soil bacteria dominant OTUs. In terms of the co-occurrence networks of the soil bacterial community under CS+SAS treatment (a) and BCS+BSAS treatment (b), the node size in each network was proportional to its relative abundance. The ZI-PI diagram showed the distribution of significant OTUs of soil bacteria based on their topological properties under the CS+SAS (c) and BCS+BSAS (d) treatments.

average degree of bacterial co-occurrence networks also increased gradually. Therefore, these results indicated that the biochar addition increased the complexity of bacterial networks in the black and saline-alkaline soils.

DISCUSSION

Biochar application improved the soil bulk density, pH(H_2O) value, and the contents of soil organic matter, total nitrogen, total phosphorus, available nitrogen, and available phosphorus in both the black and saline-alkaline soils. Biochar has the ability to improve

Table 4. Topological features of bacteria co-occurrence network in soils under biaochar application

Topological properties	CS+SAS	BCS+BSAS
No. of original OTUs	589	600
Nodes	578	591
Edges	17598	26206
Average degree	59.55	90.68
No. of clusters	9	10
clustering coefficient	0.63	0.86
Average path distance	3.74	4.01
Connectedness	0.16	0.10
Positive links/negative links	197.53	390.07

soil pH because the alkaline functional groups on the biochar surface determine its high pH (Van Zwieten et al., 2010). The ability of biochar to improve soil pH is related to its carbonate and organic acid contents (Wu et al., 2014). The application of biochar in saline-alkaline soil did not reach its optimum level, mainly due to the alkaline property of saline-alkaline soil in this test site. Biochar has a buffering capacity to acid and alkali, which prevented any significant difference in soil pH between treatments. Previous studies have shown that raw materials are one of the main factors affecting the properties of biochar (Zhao et al., 2018). Characteristics, including physicochemical properties and biochar surface structure prepared from different raw materials are the main factors contributing to significant differences in soil pH (Cong et al., 2020).

In this experiment, the soil bulk density of black and saline-alkaline soils exhibited a decreasing trend post biochar application, which was mainly due to the 1) porous and loose structure, 2) large specific surface area, 3) low density of biochar, and 4) the "dilution effect" generated in soil post application (Baiamonte et al., 2019). The addition of biochar significantly increased soil organic matter, total nitrogen, total phosphorus, available phosphorus, and available potassium due to the high content of carbon, mineral elements, and organic functional groups in biochar (Gao and Deluca, 2020). Effects of biochar on the physicochemical properties of the tested soil varied significantly, which may be related to the characteristics of the used biochar.

The pH and the content of mineral elements in the biochar matrix can stimulate the microbial and enzyme activities related to the soil nutrient cycle (Grunwald et al., 2017). The increase of soil organic carbon post biochar application was mainly due to the following reasons: 1) the carbon in the biochar formed by biomass pyrolysis mainly existed as an inert aromatic ring structure with very high carbon content, thereby increasing the soil organic carbon content by biochar addition; 2) biochar has strong adsorbability, which can adsorb the small organic molecules in soil and promote their polymerization to form soil organic matter (Hammer et al., 2014). Additionally, the porous structure of biochar provides attachment sites for the growth and reproduction of microorganisms, thereby providing a favorable habitat environment for microorganisms. The increase of soil microbial biomass further accelerated the decomposition and the release of soil organic nutrients, thus increasing the soil organic carbon content.

This study showed that the contents of soil total nitrogen, NH_4^+-N and NO_3^--N increased post biochar application, which differed from the results obtained by Nguyen et al. (2017) through meta-analysis. Nguyen et al. (2017) found that the high C/N characteristics of biochar and its introduced active substances promote soil mineral nitrogen fixation by microorganisms, thus reducing the nitrogen availability. Our result was consistent with those obtained by Song et al. (2017) and Liu et al. (2020), mainly because

biochar contained nitrogen. Additionally, the application of biochar could reduce nitrogen leaching and improve soil aeration, which inhibited microbial denitrification, thus reducing the formation and emission of N_2O and further increasing the total soil nitrogen content. In this study, biochar increased the NH_4^+-N and NO_3 N contents. Previous studies showed that biochar can stimulate the activity and quantity of soil microorganisms, which may increase the biological fixation of inorganic nitrogen in microorganisms, thus reducing the accumulation of mineral nitrogen (Nelissen et al., 2012). However, other studies believed that the biochar addition provides an unstable carbon source for soil microorganisms, which causes short-term soil nitrogen fixation (Bruun et al., 2012). Moreover, porous biochar can adsorb abundant polyphenols, which can be used as a carbon source by soil microorganisms and increase their demand for nitrogen.

Enzymes in the soil catalyze and drive the soil nutrient cycle (Li et al., 2015). Our study found that biochar stimulated the activities of β-G and CBH, which were related to carbon cycle in the black and saline-alkaline soil. This promotes the decomposition of soil organic carbon, which provides a substrate for microbial activities and helps improve the activity of soil microorganisms (Bian et al., 2016). The application of biochar usually increases the amount of soil nitrogen mineralization due to the "excitation effect" (Gan et al., 2003). The β-G and CBH are enzymes related to the decomposition of soil organic carbon, and their activities were significantly increased under BCS and BSAS treatments, which may provide more unstable carbon sources for nitrifying microorganisms, thus promoting nitrification. Dempster et al. (2012) found that biochar application-induced change in the physicochemical properties of soil affects the activity of nitrifying microorganisms. Related studies showed that the soil nitrification rate was related to the contents of organic matter, total nitrogen, available phosphorus, and available potassium, thus indicating the effect of different biochar on the soil nutrient content causes significant differences in the soil nitrification rate (Xu et al., 2014). Meanwhile, the biochar addition significantly impacted the key enzymes in the ammonia oxidation process, which further affects soil nitrification. Therefore, in this study, soil pH and nutrient content were the main factors affecting nitrification. Additionally, denitrification is an important way of nitrogen loss, and the application of biochar can affect it. Biochar may also increase the soil NO_3^--N content by inhibiting denitrification, thus increasing the nitrification rate. The NAG is an enzyme related to nitrogen mineralization, which degrades chitin in the soil and releases glucosamine (Zackrisson et al., 1996). The biochar addition significantly increases the NAG activity, which inhibits the soil nitrogen mineralization.

On the other hand, biochar can adsorb NH_4^+, which generates significant differences in the mineral nitrogen content of black and saline-alkaline soil, due to the different adsorption post-addition of biochar. The conclusions about biochar's effect on soil nitrification are debatable. The increase of soil pH promoted the transformation of soil NH_4^+-N into NO_3^--N, which favored nitrification (Chen et al., 2013b).

Soil nitrification can be divided into the ammonia oxidation and nitrosation stages. The first stage is the rate-limiting step of nitrification, in which two types of bacteria [ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA)] containing ammonia monooxygenase participate in the NH_3 oxidation (Yao et al., 2011). The second stage mainly depends on the *nxr* gene that encodes an enzyme for catalyzing the nitrification reaction in soil. Nitrification in soil is affected by many factors, like soil aeration conditions, texture, water content, temperature, pH, and fertilization (Sohi et al., 2010).

The biochar addition to soil significantly increased the soil pH. Studies have confirmed that soil pH was the main factor affecting the community structure, abundance, and diversity of ammonia-oxidizing archaea or ammonia-oxidizing bacteria, thereby affecting soil

nitrification (Nicol et al., 2008). Previous results showed that biochar application promoted soil nitrification by increasing the abundance of soil ammonia-oxidizing microbes (AOA, AOB), and the change in their abundance post biochar application was consistent with the results of this study (Song et al., 2014). In a 4-year-old field experiment, Ouyang et al. (2016) found that AOB was more sensitive than AOA to the nitrogen sources in the agricultural soil, thus playing a leading role in soil nitrification process. By using the stable isotope probe technology, Xia et al. (2011) found that AOB dominated ~76 % of agricultural soil nitrification.

Our results showed that AOA and AOB highly contribute to the total soil nitrification potential, thus indicating that both are participants in the soil ammonia oxidation process (Guo et al., 2017). This result demonstrated that AOA and AOB post-biochar treatment were important contributors to soil nitrification in the agricultural soil used in this study. However, AOB was more sensitive in responding to soil nitrification than AOA. The abundance of the *nir*K, *nir*S, and *nos*Z genes directly affects the denitrification process (Ji et al., 2020). In this study, the abundance of *nir*K and *nir*S genes is significantly increased by biochar treatment, thus indicating that *nir*K and *nir*S genotype denitrifying bacteria are more sensitive to high biochar application. Biochar could promote the reproduction of *nir*K and *nir*S genotype denitrifying bacteria. This is the same as the results of Liu et al. (2018), the application of biochar can increases the *nos*Z gene abundance to promote N_2O reduction, which shows that biochar has a great potential to reduce N_2O emission in the farmlands with black and saline-alkaline soil.

In the natural environment, microbial communities form a complex ecological network. Based on the ecological network, the interaction between species can be speculated. For microorganisms involved in mutually beneficial symbiosis, symbiosis and copolymerization were positively correlated, while for those in competition, biased symbiosis and predation were negatively correlated (Faust and Raes, 2012). In this study, compared with the control, the biochar treatment increased nutrients, like carbon, nitrogen, and phosphorus in farmland with black and saline-alkaline soil, promoting bacterial growth and metabolism and improving bacterial diversity. Our previous results suggested that Proteobateria, Bacteroidetes, Acidobacteria, and Actinobacteria were the dominant groups of bacteria in black and saline-alkaline soil after applying biochar (Ding and Li, 2022). In this study, the effects of biochar application on bacterial co-occurrence patterns in farmland soil were explored by constructing an interaction network between biochar application and non-biochar treatment in black and saline-alkaline soil. The results showed that the co-occurrence mode of microorganisms changed significantly post-biochar application. As compared with the control group without biochar treatment, the interaction of bacteria increased significantly after the biochar application. The nodes of the interaction network also increased significantly, with the network becoming more complex. This was consistent with the results of Gundale and DeLuca (2006), which showed that biochar application, can improve soil nutrient availability by altering the soil's physico properties (pH and water holding capacity). In the farmland ecosystem, the more abundant the available nutrients, the higher were the complexity and stability of the microbial ecological network. The high stability of the community is an important factor in ensuring ecological function. In addition, the special porous structure of biochar can protect bacteria and reduce the damage caused by its competitors. Studies have shown that the effects of soil pH and NH_4^+-N on bacteria were significantly enhanced post-biochar application (Zhou et al., 2017). The change of soil acidity favored the growth of bacteria, while the change of NH_4^+-N changed the bacterial community, thus affecting the interaction network (Lauber et al., 2008). As compared with the control group, the modular structure of the interaction network under biochar treatment was more complex, with a higher network score, more nodes and interactions, and most of them were bacterial nodes. These modules did not strictly follow the taxonomic classification, i.e., microorganisms showed interactions but did not depend on their classification.

Similarly, a study on a bacterial community by Burke et al. (2011) indicated that the microbial species composition among samples was quite different and shared a functional similarity of up to 70 %. This result demonstrated that the composition of bacterial communities was determined by functional genes rather than species classification, and it was proposed that species with similar nutrients or other ecological characteristics can occupy the same niche. In this study, adding biochar redistributed the farmland ecosystem resources, which could be the possible cause for the bacterial collinearity change.

CONCLUSION

Biochar changed the physicochemical properties of farmland soil with black and saline-alkaline soil, like pH, nutrient content, and enzyme activity, thus affecting the mineralization and nitrification of farmland soil nitrogen. Biochar promoted the mineralization and nitrification of nitrogen, immobilized NO_3^--N in farmland soil, and increased the nitrification rate. The AOA and AOB genes were most sensitive to biochar application, and the changes in their abundance affected the abundance of the communities of the overall nitrogen cycle in farmland soil. Therefore, biochar application significantly enriched the network interaction of bacterial communities in the farmland soil with black and saline-alkaline soil, while also strengthening the positive relationship among bacteria. In the future, we will study the long-term effects of biochar application as soil amendments may be a good practice to improve soil microbial ecosystem, soil health and quality and mitigate climate change.

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REFERENCES

Baiamonte G, Crescimanno G, Parrino F, De Pasquale C. Effect of biochar on the physical and structural properties of a sandy soil. Catena. 2019;175:294-303. https://doi.org/10.1016/j.catena.2018.12.019

Banerjee S, Schlaeppi K, van der Heijden MGA. Keystone taxa as drivers of microbiome structure and functioning. Nat Rev Microbiol. 2018;16:567-76. https://doi.org/10.1038/s41579-018-0024-1

Bao SD. Soil agrochemical analysis. Beijing: China Agriculture Press; 2005.

Bian XL, Zhao WL, Yue ZH, Wang HY, Jiao H, Sui HX. Research process of soil enzymes effect on carbon and nitrogen cycle in agricultural ecosystem. Chin Agr Sci Bulletin. 2016;32:171-8.

Braker G, Fesefeldt A, Witzel KP. Development of PCR primer systems for amplification of nitrite reductase genes (*nir*K and *nir*S) to detect denitrifying bacteria in environmental samples. Appl Environ Microb. 1998;64:3769-75. https://doi.org/10.1128/AEM.64.10.3769-3775.1998

Bru D, Sarr A, Philippot L. Relative abundances of proteobacterial membrane-bound and periplasmic nitrate reductases in selected environments. Appl Environ Microb. 2007;64:5971-4. https://doi.org/10.1128/AEM.00643-07

Bruun EW, Ambus P, Egsgaard H, Hauggaard-Nielsen H. Effects of slow and fast pyrolysis biochar on soil C and N turnover dynamics. Soil Biol Biochem. 2012;46:73-9. https://doi.org/10.1016/j.soilbio.2011.11.019

Burke C, Steinberg P, Rusch D, Thomas T. Bacterial community assembly based on functional genes rather than species. P Natl Acad Sci. 2011;108:14288-93. https://doi.org/10.1073/pnas.1101591108

Chen CR, Phillips IR, Condron LM, Goloran J, Xu ZH, Chan KY. Impacts of greenwaste biochar on ammonia volatilisation from bauxite processing residue sand. Plant Soil. 2013a;367:301-12. https://doi.org/10.1007/s11104-012-1468-0

Chen WF, Zhang WM, Meng J. Advances and prospects in research of biochar utilization in agriculture. Sci Agric Sin. 2013b;46:3324-33.

Chen YZ, Wang F, Wu ZD. Effect of biochar addition on pH and nitrogen transformation in acidic soil at tea plantations. Acta Tea Sinica. 2016;57:64-70.

Cong M, Zhang MY, Xia H, Jiang CC. Effect of biochar application on potassium content of different forms in red soil and the growth of pakchoi. J Huazhong Agricult University. 2020;39:22-8.

Dempster DN, Gleeson DB, Solaiman ZI, Jones DL, Murphy DV. Decreased soil microbial biomass and nitrogen mineralisation with *Eucalyptus* biochar addition to a coarse textured soil. Plant Soil. 2012;354:311-24. https://doi.org/10.1007/s11104-011-1067-5

Deng Z, Mo YF, Ong SP. Computational studies of solid-state alkali conduction in rechargeable alkali-ion batteries. NPG Asia Mater. 2016;8:e254. https://doi.org/10.1038/am.2016.7

Ding JN, Li X. Effects of biochar on microbial community diversity in rhizosphere soil of farmlands in northeast China. Appl Ecol Env Res. 2022;20:2801-16. https://doi.org/10.15666/aeer/2003_28012816

Duran-Pinedo AE, Paster B, Teles R, Chan KY, Downie A, Rust J, Cowie A. Correlation network analysis applied to complex biofilm communities. PLoS One. 2011;6:e28438. https://doi.org/10.1007/s11104-009-0050-x

Edwards KA, McCulloch J, Kershaw GP. Soil microbial and nutrient dynamics in a wet Arctic sedge meadow in late winter and early spring. Soil Biol Biochem. 2006;38:2843-51. https://doi.org/10.1016/j.soilbio.2006.04.042

Faina G, Ruth B, Ludwik H. Application of the Walkley-Black titration for the organic carbon quantification in organic rich sedimentary rocks. Fuel. 2012;96:608-10. https://doi.org/10.1016/j.fuel.2011.12.053

Faust K, Raes J. Microbial interactions: From networks to models. Nat Rev Microbiol. 2012;10:538-50. https://doi.org/10.1038/nrmicro2832

Francis CA, Roberts KJ, Beman JM, Santoro AE, Oakley BB. Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. P Natl Acad Sci USA. 2005;102:14683-8. https://doi.org/10.1073/pnas.0506625102

Gan JM, Meng Y, Zheng Z. Effects of fertilization on mineralization and nitrification of nitrogen in soil grown amomum under tropical rainforest. J Agro-Environ Sci. 2003;22:174-7.

Gao GF, Li PF, Zhong JX, Shen ZJ, Chen J, Li YT, Isabwe A, Zhu XY, Ding QS, Zhang S, Gao CH, Zhen HL. *Spartina alterniflora* invasion alters soil bacterial communities and enhances soil N_2O emissions by stimulating soil denitrification in mangrove wetland. Sci Total Environ. 2019;653:231-40. https://doi.org/10.1016/j.scitotenv.2018.10.277

Gao S, Deluca TH. Biochar alters nitrogen and phosphorus dynamics in a western rangeland ecosystem. Soil Biol Biochem. 2020;148:107868. https://doi.org/10.1016/j.soilbio.2020.107868

Govindarajulu M, Pfeffer PE, Jin H, Abubaker J, Douds DD, Allen JW, Shachar-Hill Y. Nitrogen transfer in the arbuscular mycorrhizal symbiosis. Nature. 2005;435:819-23. https://doi.org/10.1038/nature03610

Grunwald D, Kaiser M, Junker S, Marhan S, Piepho HP, Poll C, Bamminger C, Ludwig B. Influence of elevated soil temperature and biochar application on organic matter associated with aggregate-size and density fractions in an arable soil. Agr Ecosyst Environ. 2017;241:79-87. https://doi.org/10.1016/j.agee.2017.02.029

Guan SY. Soil enzyme and the research method. Beijing: China Agriculture Press; 1986.

Gundale MJ, DeLuca TH. Temperature and source material influence ecological attributes of ponderosa pine and Douglas-fir charcoal. Forest Ecol Manag. 2006;231:86-93. https://doi.org/10.2136/sssaj2005.0096

Guo JJ, Ling N, Chen H, Zhu C, Kong Y, Wang M, Guo S. Distinct drivers of activity, abundance, diversity and composition of ammonia-oxidizers: Evidence from a long-term field experiment. Soil Biol Biochem. 2017;115:403-14. https://doi.org/10.1016/j.soilbio.2017.09.007

Hammer EC, Balogh-Brunstad Z, Jakobsen I, Olsson PA, Stipp SLS, Rillig MC. A mycorrhizal fungus grows on biochar and captures phosphorus from its surfaces. Soil Biol Biochem. 2014;77:252-60. https://doi.org/10.1016/j.soilbio.2014.06.012

Hu H, Chen XJ, Hou FJ, Wu YP, Cheng YX. Bacterial and fungal community structures in Loess Plateau grasslands with different grazing intensities. Front Microbiol. 2017;8:606. https://doi.org/10.3389/fmicb.2017.00606

Instituto Geográfico Agustín Codazzi - IGAC. Suelos y tierras de Colombia, subdirección de agrología. Colombia: IGAC; 2016.

Ji C, Li SQ, Geng YJ, Miao YC, Ding Y, Liu SW, Zou JW. Differential responses of soil N_2O to biochar depend on the predominant microbial pathway. Appl Soil Ecol. 2020;145:103348. https://doi.org/10.1016/j.apsoil.2019.08.010

Kuzyakov Y, Subbotina I, Chen H, Bogomolova I, Xu X. Black carbon decomposition and incorporation into soil microbial biomass estimated by ^{14}C labeling. Soil Biol Biochem. 2009;2:210-9. https://doi.org/10.1016/j.soilbio.2008.10.016

Lauber CL, Strickland MS, Bradford MA, Fierer N. The influence of soil properties on the structure of bacterial and fungal communities across land use types. Soil Biol Biochem. 2008;9:2407-15. https://doi.org/10.1016/j.soilbio.2008.05.021

Li R, Liu Y, Chu GX. Effects of different cropping patterns on soil enzyme activities and soil microbial community diversity in oasis farmland. Chin J Appl Ecol. 2015;26:490-6.

Liu XR, Zhao GX, Zhang QW, Tian XP. Effects of biochar on nitrous oxide fluxes and the abundance of related functional genes from agriculture soil in the north China plain. Environ Sci. 2018;39:3816-25. https://doi.org/10.13227/j.hjkx.201711275

Liu ZQ, Lan Y, Yang TX, Zhang YX, Meng J. Effect of biochar application pattern on soil fertility and enzyme activity under limited fertilization conditions. J Agr Resou Environ. 2020;37:544-51. https://doi.org/10.13254/j.jare.2019.0143

Lu RK. Soil and agro-chemical analytical methods. Beijing: China Agricultural Science and Technology Press; 1999.

Mehlich A. Mehlich 3 soil test extractant: A modification of Mehlich 2 extractant. Commun Soil Sci Plan. 1984;15:1409-16. https://doi.org/10.1080/00103628409367568

Nannipieri P, Giagnoni L, Renella G, Puglisi E, Ceccanti B, Masciandaro G, Marinari SARA. Soil enzymology: Classical and molecular approaches. Biol Fert Soils. 2012;48:743-62. https://doi.org/10.1007/s00374-012-0723-0

Nelissen V, Rütting T, Huygens D, Staelens J, Ruysschaert G, Boeckx, P. Maize biochars accelerate short-term soil nitrogen dynamics in a loamy sand soil. Soil Biol Biochem. 2012;55:20-7. https://doi.org/10.1016/j.soilbio.2012.05.019

Nguyen TTN, Xu CY, Tahmasbian I, Che RX, Xu ZH, Zhou XH, Wallace HM, Bai SH. Effects of biochar on soil available inorganic nitrogen: A review and meta-analysis. Geoderma. 2017;288:79-96. https://doi.org/10.1016/j.geoderma.2016.11.004

Nicol GW, Leininger S, Schleper C, Prosser JI. The influence of soil pH on the diversity, abundance and transcriptional activity of ammonia oxidizing archaea and bacteria. Environ Microbiol. 2008;10:2966-78. https://doi.org/10.1111/j.1462-2920.2008.01701.x

Ouyang Y, Norton JM, Stark JM, Reeve JR, Habteselassie MY. Ammonia-oxidizing bacteria are more responsive than archaea to nitrogen source in an agricultural soil. Soil Biol Biochem. 2016;96:4-15. https://doi.org/10.1016/j.soilbio.2016.01.012

Pan FE, Hu JP, Suo L, Wang XQ, Ji YL, Meng L. Effect of corn stalk and its biochar on N_2O emissions from Latosol soil. J Agro-Environ Sci. 2016;35:396-402.

Pan YC, She DL, Chen XY, Xia YQ, Timm LC. Elevation of biochar application as regulator on denitrification/NH_3 volatilization in saline soils. Environ Sci Pollut Res. 2021;28:41712-25. https://doi.org/10.1007/s11356-021-13562-w

Qiu HC, Chen JZ. Nitrogen supply potential of upland soils. J Plant Nutr Fertil. 1995;1:33-9.

Qu TH, Li YF, Zhang SB, Li LL, Li YC, Liu J. Effects of biochar application on soil nitrogen transformation and N_2O emissions: A review. J Zhejiang A&F Univ. 2021;38:926-36. https://doi.org/10.11833/j.issn.2095-0756.20200549

Rosch C, Bothe H. Improved assessment of denitrifying, N_2-fixing, and total-community bacteria by terminal restriction fragment length polymorphism analysis using multiple restriction enzymes. Appl Environ Microb. 2005;71:2026-35. https://doi.org/10.1128/AEM.71.4.2026-2035.2005

Rotthauwe JH, Witzel KP, Liesack W. The ammonia monooxygenase structural gene amoA as a functional marker: molecular fine-scale analysis of natural ammonia-oxidizing populations. Appl Environ Microb. 1997;63:4704-12. https://doi.org/10.1128/aem.63.12.4704-4712.1997

Sinsabaugh RL, Reynolds H, Long TM. Rapid assay for amidohydrolase (urease) activity in environmental samples. Soil Biol Biochem. 2000;32:2095-7. https://doi.org/10.1016/S0038-0717(00)00102-4

Sohi SP, Krull E, Lopez-Capel E, Bol R. A review of biochar and its use and function in soil. Adv Agron. 2010;105:47-82. https://doi.org/10.1016/S0065-2113(10)05002-9

Song DL, Xi XY, Huang SM, Zhang SQ, Yuan XM, Huang FS, Liu Y, Wang XB. Effects of combined application of straw biochar and nitrogen on soil carbon and nitrogen contents and crop yields in a flnuvo-aquic soil. J Plant Nutr Fert. 2017;23:369-79.

Song YJ, Zhang XL, Ma B, Chang SX, Gong J. Biochar addition affected the dynamics of ammonia oxidizers and nitrification in microcosms of a coastal alkaline soil. Biol Fert Soils. 2014;50:321-32. https://doi.org/10.1007/s00374-013-0857-8

Steiner C, Glaser B, Teixeira WG, Lehmann J, Blum WE, Zech W. Nitrogen retention and plant uptake on a highly weathered central Amazonian Ferralsol amended with compost and charcoal. J Plant Nut Soil Sci. 2010;171:893-9. https://doi.org/10.1002/jpln.200625199

Tatti E, Goyer C, Zebarth BJ, Burton DL, Giovannetti L. Shortterm effects of mineral andorganic fertilizer on denitrifiers, nitrous oxide emissions and denitrification in long-term amended vineyard soils. Soil Sci Soc Am J. 2013;77:113-22. https://doi.org/10.2136/sssaj2012.0096

Throbäck IN, Enwall K, Jarvis Å, Hallin S. Reassessing PCR primers targeting *nir*S, *nir*K and *nos*Z genes for community surveys of denitrifying bacteria with DGGE. FEMS Microbiol Ecol. 2004;49:401-17. https://doi.org/10.1016/j.femsec.2004.04.011

Van Zwieten L, Kimber S, Morris S, Chan KY, Downie A, Rust J, Cowie A. Effects of biochar from slow pyrolysis of papermill waste on agronomic performance and soil fertility. Plant Soil. 2010;327:235-46. https://doi.org/10.1007/s11104-009-0050-x

Vitousek PM, Aber JD, Howarth RW, Likens GE, Matson PA, Schindler DW, Tilman DG. Human alteration of the global nitrogen cycle: Sources and consequences. Ecol Appl. 1997;7:737-50. https://doi.org/10.1890/1051-0761(1997)007[0737:HAOTGN]2.0.CO;2

Wang RF, Zhao LX, Shen YJ, Meng HB, Yang HZ. Research progress on preparing biochar and its effect on soil physio-chemical properties. J Agr Sci Techn. 2015;17:126-33.

Wu Y, Xu G, Lu YC, Shao HB. Effects of biochar amendment on soil physical and chemical properties: current status and knowledge gaps. Adv Earth Sci. 2014;29:68-79.

Xia H, Riaz M, Zhang MY, Liu B, Li Y, El-Desouki Z, Jiang C. Biochar-N fertilizer interaction increases N utilization efficiency by modifying soil C/N component under N fertilizer deep placement modes. Chemosphere. 2022;286:131594. https://doi.org/10.1016/j.chemosphere.2021.131594.

Xia H, Zhang MY, Liu B, Li YX, Cong M, Bumairemu R, Jiang CC. Effect of biochar on nitrogen use efficiency of crops: a Meta-analysis. J Huazhong Agr Univ. 2021;40:177-86.

Xia W, Zhang C, Zeng X, Feng Y, Weng J, Lin X, Jia Z. Autotrophic growth of nitrifying community in an agricultural soil. ISME J. 2011;5:1226-36. https://doi.org/10.1038/ismej.2011.5

Xu HJ, Wang XH, Li H, Yao HY, Su JQ, Zhu YG. Biochar impacts soil microbial community composition and nitrogen cycling in an acidic soil planted with rape. Environ Sci Technol. 2014;48:9391-9. https://doi.org/10.1021/es5021058

Yang YL, Wu FZ, He ZH, Xu ZF, Liu Y, Yang WQ, Tan B. Effects of snow pack removal on soil microbial biomass carbon and nitrogen and the number of soil culturable microorganisms during wintertime in alpine Abies faxoniana forest of western Sichuan, Southwest China. Chin J Appl Ecol. 2012;23:1809-16.

Yao H, Gao Y, Nicol GW, Campbell CD, Prosser JI, Zhang L, Singh BK. Links between ammonia oxidizer community structure,abundance and nitrification potential in acidic soils. Appl Environ Microb. 2011;77:4618-25. https://doi.org/10.1128/AEM.00136-11

Yu M, Liang S, Dai Z, Li Y, Luo Y, Tang C, Xu J. Plant material and its biochar differ in their effects on nitrogen mineralization and nitrification in a subtropical forest soil. Sci Total Environ. 2021;763:143048. https://doi.org/10.1016/j.scitotenv.2020.143048.7-8

Zackrisson O, Nilsson MC, Wardle DA. Key ecological function of charcoal from wildfire in the boreal forest. Oikos. 1996;77:10-9. https://doi.org/10.2307/3545580

Zeng ZB, Zhu B, Zhu XM, Liu XF, Wang Y. Effects of fertilization on N_2O emission and denitrification in purple soil during summer maize season in the Sichuan basin. Acta Pedol Sin. 2013;50:130-7.

Zhang H, Voroney RP. Effects of temperature and processing conditions on biochar chemical properties and their influence on soil C and N transformations. Soil Biol Biochem. 2015;83:19-28. https://doi.org/10.1016/j.soilbio.2015.01.006

Zhang LL, Wu ZJ, Chen LJ, Li DP, Ma XZ, Shi YF. A microplate fluorimetric assay for saccharіase activity measurement. Spectrosc Spect Anal. 2009;29:1341-4. https://doi.org/10.3964/j.issn.1000-0593(2009)05-1341-04

Zhang MY, Muhammad R, Zhang L, Xia H, Cong M, Jiang C. Investigating the effect of biochar and fertilizer on the composition and function of bacteria in red soil. Appl Soil Ecol. 2019;139:107-16. https://doi.org/10.1016/j.apsoil.2019.03.021

Zhao B, O'connor D, Zhang JL, Peng T, Shen Z, Tsang DC, Hou D. Effect of pyrolysis temperature, heating rate, and residence time on rapeseed stem derived biochar. J Clean Prod. 2018;174:977-87. https://doi.org/10.1016/j.jclepro.2017.11.013

Zhou J, Deng Y, Luo F, He ZL, Yang YF. Phylogenetic molecular ecological network of soil microbial communities in response to elevated CO_2. mBio. 2011;2:e00122-11. https://doi.org/10.1128/mBio.00122-11

Zhou X, Guo Z, Chen C, Jia Z. Soil microbial community structure and diversity are largely influenced by soil pH and nutrient quality in 78-year-old tree plantations. Biogeosciences. 2017;14:2101-11. https://doi.org/10.5194/bg-14-2101-2017