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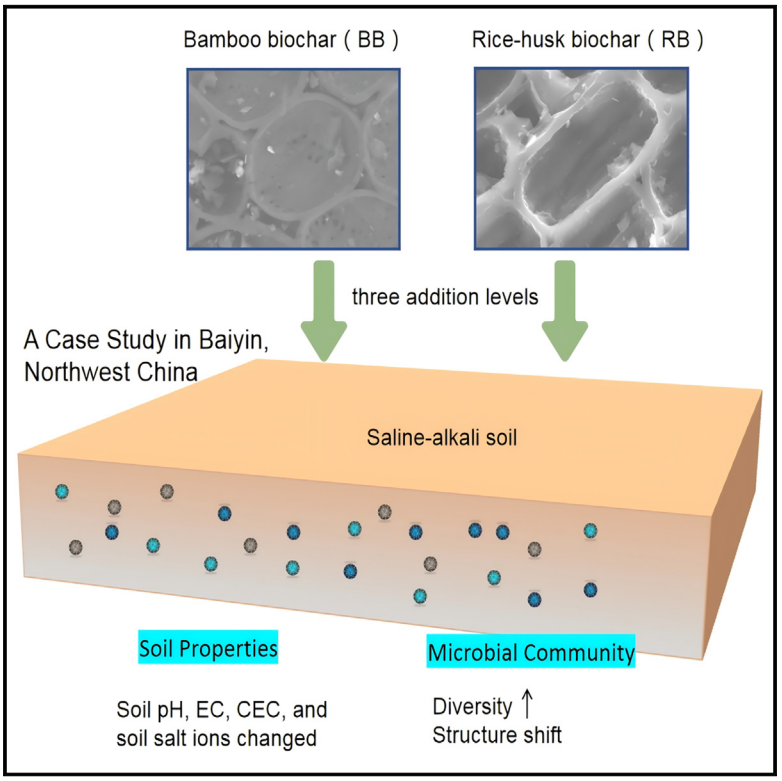
Effects of adding different types and amounts of biochar to saline alkali soil on its salt ions and microbial community in northwest China

Graphical abstract



Highlights

- Applying the different levels of RB and BB changed the soil pH, EC, CEC, and soil salt ions
- Adding soil carbon significantly changes soil microbial diversity
- RB-medium application optimally enhanced saline-alkali soil health in Baiyin

Authors

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In brief

Soil science; Microbiology; Soil chemistry; Soil biology

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Effects of adding different types and amounts of biochar to saline alkali soil on its salt ions and microbial community in northwest China

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SUMMARY

Currently, there are few reports on biochar applications when using different raw materials as well as addition levels to saline-alkali soil in northwest China. In this study, we tested the effects of rice-husk biochar (RB) and bamboo biochar (BB), each applied separately at three addition levels (2.5%, 5%, and 10%) on the pH, electrical conductivity (EC), cation exchange capacity (CEC), salt ions, and microbial communities of soil. Our experiment showed that applying the different levels of RB and BB changed the soil pH, EC, CEC, and soil salt ions. The RB-medium level application was evidently the best choice for improving the health of saline-alkali soil in the Baiyin region. While adding biochar negligibly altered the richness of soil microorganisms it did significantly increase their diversity. Accordingly, adding the appropriate amount and type of biochar to soil is crucial for saline-alkali land restoration in Baiyin, northwest China.

INTRODUCTION

Soil salinization refers to the phenomenon or process whereby soluble salts continuously accumulate on the surface of soil under natural or human action, impairing the growth of plants. In serious cases, it will evolve into land desertification, severely damaging local agricultural production and the ecological environment. About 1.12×10^9 hm^2 land globally is threatened by salinization, of which nearly 20% consists of irrigated soil whose proportion is increasing.[1,2] China is one of the countries most seriously affected by salinization. According to official statistics, the area of affected farmland has reached 8.2×10^7 hm^2 in China and its soil salinization problem as well as secondary salinization is worsening.[3,4]

Biochar, a solid formed by the thermal decomposition of organic matter at high-temperature under anoxic or hypoxic conditions, is mainly comprised of alkyl and aromatic compounds and is relatively stable; hence, biochar can persist for hundreds of years in soil.[5,6] The strong adsorption force, the high carbon content, and the well-developed pore structure of biochar all contribute to improving soil structure and enhancing crop yields.[7,8]

Besides changing the nutrient content of soil, the application of biochar can also reduce the leaching and volatilization of nutrients by modulating the physical and chemical properties and biological characteristics of the treated soil. By influencing enzyme activity and mineral nutrition, it can influence the growth and reproduction of microbes.[9,10] Meanwhile, due to the types and quantity of compounds contained in biochar and various biochar preparation processes, the ecological functions of biochar can differ greatly.[11]

Saline alkali soil is characterized by a poor structure, high salt content, and low organic matter content. The key to improving saline alkali soil is 3-fold: eliminate its salt ions, strengthen its structure, and augment its aeration and permeability. The unique properties of biochar can change soil permeability and water conductivity. For example, on the Hetao Plain, Inner Mongolia, using a soil column culture test it was shown that biochar promotes the loss of salt from saline alkali soil and improves the leaching efficiency.[12] In another study, biochar markedly increased the content of exchangeable Ca^{2+} and Mg^{2+} in saline alkali soil, while substantially decreasing its exchangeable Na content and improving the overall soil aggregate.[13]

Most research investigating the effects of adding biochar for soil improvement have focused on particular biochar or soil improvement in a specific region. And there are not many reports on using biochar to improve the saline alkali land in northwest China. Thus this paper studied how various types and quantities of biochar affected saline alkali soil's salt ions and microorganisms in northwest China after 180 days of cultivation. Using biochar to improve soil quality in saline alkali soil is an effective way to solve the urgent problem of soil salinization in northwest China, and these empirical findings provide a timely reference for solving that problem.

RESULTS

Effect of adding biochar on soil pH, EC, CEC, and salt ions

Two biochar types with different levels of addition significantly changed the pH, electrical conductivity (EC), cation exchange

Table 1. Soil physical and chemical properties of the six different biochar treatment combinations after 180 days

Treatment	pH	EC (ms/cm)	CEC (cmol/kg)	Na^+ (g/kg)	K^+ (g/kg)	Ca^{2+} (g/kg)	Mg^{2+} (g/kg)	Cl^- (mg/kg)	HCO_3^- (mg/kg)	SO_4^{2-} (g/kg)
LBB	9.19±0.041ab	5.66±0.11a	4.96±0.051a	11.23±0.16a	2.68±0.065a	40.97±0.93a	12.54±0.21a	3.14±0.23a	383.42±3.46a	6.07±0.057a
MBB	9.15±0.035b	5.35±0.032b	5.45±0.065b	8.71±0.17b	2.82±0.078b	36.47±0.76b	11.32±0.27b	4.76±0.22b	515.55±12.09b	6.73±0.11b
HBB	9.22±0.065ab	5.29±0.050bc	5.3±0.075bd	8.75±0.12bc	2.97±0.076c	37.74±0.58b	11.38±0.41b	4.03±0.18c	386.56±10.561a	8.25±0.13c
LRB	8.92±0.041c	5.2±0.057cd	6.65±0.13c	8.32±0.11e	2.13±0.071d	33.57±0.26c	11.24±0.33b	3.60±0.15d	446.37±14.61c	4.74±0.085d
MRB	8.73±0.031d	4.9±0.075e	6.82±0.12c	8.53±0.13f	2.45±0.081e	36.25±0.89b	11.43±0.36c	3.84±0.14cd	416.20±15.15d	4.57±0.16e
HRB	8.66±0.091d	5.1±0.025d	9.46±0.23e	8.88±0.16c	3.04±0.097c	40.38±0.56a	12.25±0.19d	4.54±0.29b	375.55±8.22e	5.56±0.079f
CK	9.28±0.043a	5.71±0.053a	5.16±0.15ad	9.18±0.15d	2.60±0.095f	41.49±0.90a	11.63±0.28c	4.62±0.19b	472.85±12.56f	5.73±0.10g

EC, electrical conductivity; CEC, cation exchange capacity; LBB, low level addition of bamboo biochar; MBB, medium level addition of bamboo biochar; HBB, high level addition of bamboo biochar; LRB, low level addition of rice-husk biochar; MRB, medium level addition of rice-husk biochar; HRB, high level addition of rice-husk biochar; CK, no biochar added (control). Values within the same column followed by different letters show significant differences (LSD, $p < 0.05$).

capacity (CEC), and salt ions of the soil (Table 1). Rice-husk biochar (RB) was able to significantly reduce the soil pH; both low level and high level addition of bamboo biochar (LBB and HBB) decreased it slightly, whereas only medium level addition of bamboo biochar (MBB) significantly lowered it. Soil EC was significantly reduced by MBB, HBB, LRB, MRB, and HRB, but the MRB treatment reduced it most, by 14.2% compared to control (CK). For CEC, applying RB at different levels significantly increased CEC, yet among the three BB treatments only MBB was capable of significantly augmenting CEC. The HRB treatment has the strongest effect on CEC, increasing it by 83.3%. Excluding the LBB treatment, which significantly increased the Na^+ content, all biochar treatment combinations significantly decreased its content, with the LRB treatment having the strongest impact (reducing Na^+ by 9.4%). Both MBB and HBB treatments significantly bolstered the K^+ content in soil, but the LRB and MRB treatments reduced it whereas the HRB treatment significantly increased it. With respect to Ca^{2+} in soil, the MBB, HBB, LRB, and MRB treatments all significantly reduced its content, but this was maximal under the LRB treatment (reduced by 19.1%). For the Mg^{2+} in soil, LBB and HRB treatments significantly augmented its content, yet MBB, HBB, and LRB significantly lessened it. Four treatments—LBB, HBB, LRB, and MRB—significantly lowered the Cl^- content of soil. They, along with the HRB treatment, likewise decreased the HCO_3^- content of soil in contrast to MBB that significantly increased it. The SO_4^{2-} content of soil was significantly augmented by the LBB, MBB, and HBB treatments but significantly reduced by the LRB, MRB, and HRB treatments. In this respect, HBB raised the SO_4^{2-} content by 43.9%, while MRB lowered it by 20.2%.

Effect of adding biochar on soil microbial community structure and diversity

Alpha diversity (α-diversity), in terms of a richness estimator (Chao1) and the Simpson diversity index, was evaluated for bacterial communities in the biochar-treated soil (Figure 1). The addition of biochar did not significantly change the Chao1 index of soil microorganisms, but it did increase their Simpson index, being greatest under the LBB treatment.

Figure 2 shows soil bacteria at the phylum level under the different biochar treatments. Evidently, *Acidobacteria* was the most abundant phylum in all samples, for which the ranking of predominant phyla (with a relative abundance >5%) was *Acidobacteria*, *Proteobacteria*, *Actinobacteria*, *Gemmatimonadetes*, *Verrucomicrobia*, and *Bacteroidetes* with relative abundances of 18.5%–30.8%, 14.9%–25.1%, 5.1%–31.7%, 8.9%–15.9%, 5.63%–7.5%, and 1.7%–9.4%, respectively. Relative abundances of the main bacterial genera in soils from the different treatments are shown in Figure 3A. This shows that, *Halomonas*, constituting a single species, had the highest relative abundance followed by *Lactobacillus*, *Marinobacter*, *Bacillus*, *Filobacillus*, and *Prevotella_1* (with a relative abundance >1%) in all treatments. In response to the MBB, HBB, LRB, MRB, and HRB treatments, the *Halomonas* increased significantly in soil when compared to the CK group. Under the MBB, HBB, and MRB treatments, the *Bacillus* genus significantly increased in soil but decreased under the LBB treatment, in comparison with the CK group.

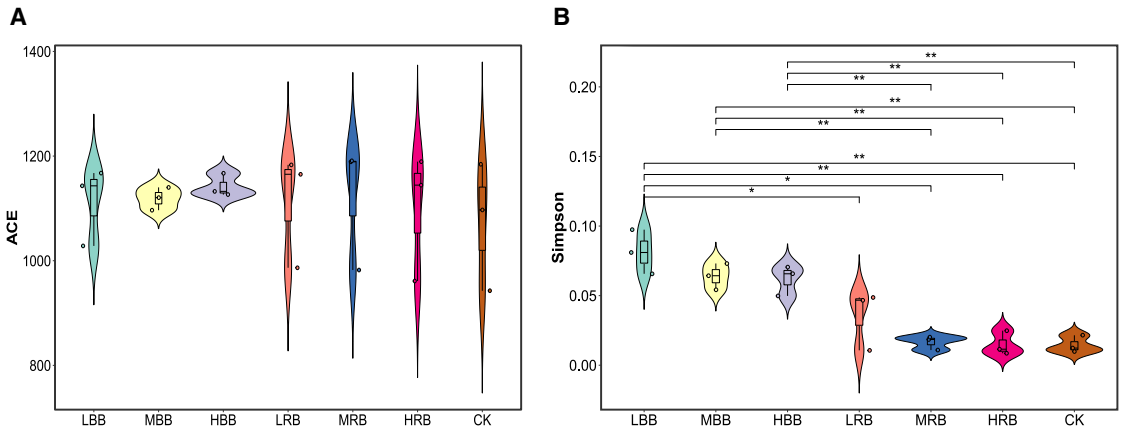

Figure 1. Boxplots of soil microbial diversity indices of soil bacteria for the six different biochar treatments and the control (CK) group

LBB, low level addition of bamboo biochar; MBB, medium level addition of bamboo biochar; HBB, high level addition of bamboo biochar; LRB, low level addition of rice-husk biochar; MRB, medium level addition of rice-husk biochar; HRB, high level addition of rice-husk biochar; CK, no biochar added. The * and ** respectively indicate a significant difference between the means at $p < 0.05$ and $p < 0.01$.

Regarding the fungi, relative abundances of the main genera in the biochar-treated soils are shown in Figure 3B. *Fusarium*, constituting a single species, had the highest relative abundance followed by *Cladosporium*, *Phallus*, and *Mortierella* (each with a relative abundance >3%) in all treatments. In the LRB treatment the genus *Cladosporium* was significantly increased in soil vis-à-vis the CK and highest among all the treatments. Multiple linear regression was then used to test relative microbial abundances as a function of soil physicochemical properties (Table 2). *Actinobacteria*'s model indicated that Mg^{2+} ions had pronounced, positive effects on its abundance, whereas the Ca^{2+}, SO_4^{2-}, and HCO_3^- ions in the soil negatively influenced it. The corresponding model for *Mucoromycota* revealed SO_4^{2-} positively affecting its abundance, whereas pH was a negative predictor of it.

Figures 4 and 5 shows the the correlations among soil pH, EC, CEC, salt ions, and the top-15 families of soil-dwelling bacteria and fungi. The changes in the relative abundance of *Nitriliruptoraceae* were significantly related to the soil K^+ content ($p < 0.001$) and Ca^{2+} content ($p < 0.01$). Significant positive correlations were found in the changed relative abundances of *Rhodobacteraceae* and *Rubidibacteraceae* with soil pH ($p < 0.01$). The relative abundances of *Balneolaceae* and *AKYG1722* were each correlated significantly with the soil Na^+ content ($p < 0.01$). *Halomonadaceae* and *Bacillaceae* had relative abundances closely related to soil SO_4^{2-} content ($p < 0.01$).

Concerning the fungi, changes in the relative abundance of *Pleosporaceae* were significantly correlated with the soil Ca^{2+} content ($p < 0.001$) and K^+ content ($p < 0.001$). The relative abundance of *Hypocreaceae* had a stronger significant correlation with soil EC ($p < 0.001$) than Ca^{2+} content ($p < 0.01$). For *Cladosporiaceae* and *Didymellaceae*, their relative abundances changed significantly with either the soil Ca^{2+} or K^+ content ($p < 0.01$). Finally, the relative abundance of *Aspergillaceae* was significantly associated with the soil Mg^{2+} content ($p < 0.01$).

DISCUSSION

Soil pH and salt ion content

The soil background values suggested the main cause of local salinization is the influence of Na^+, Ca^{2+}, Mg^{2+}, and SO_4^{2-} ions (Table 3). The results showed that applying two types of biochar with different levels of addition can significantly change the pH, EC, CEC, and salt ion content of soil. Compared with RB, BB has a weaker ability to reduce soil pH, probably due to its more alkaline nature. A difference in microstructure may also explain the different effects of the two types of biochar on CEC and EC. Compared with BB, the specific surface area and average pore size of RB treatment are both larger, which affects the CEC of that biochar (Table 4 and Figure 6). It is known that the specific surface area, hydrophobicity, and adsorption capacity of biochar depends on the raw material used to produce it.[14] We found that most biochar treatment combinations can significantly reduce the content of the Na^+ and Ca^{2+}, the major cations saline-alkali in soil. This is because biochar can adsorb soil ions via its own characteristics, such as CEC, organic carbon, specific surface area,[15] and the enhanced soil porosity by biochar—including its own contribution of pores along with the greater porosity caused by promoting microbial activity in the rhizosphere—may enable the leaching or storage of some soil salt ions.[16] At the same time, it can be seen that sometimes the biochar with a higher added level is not stronger at reducing salt ions' content, perhaps due to the fact that when the amount of biochar applied exceeds a certain level, the soil evaporates more, which in turn exacerbates salt accumulation on the surface.[17] Hence, from the perspective of reducing the main salt ions, MRB treatment is the best choice for the improvement of saline-alkali soil in the Baiyin region.

Microbial diversity

Adding biochar to soil did not significantly increase the richness of microorganisms, but it did significantly increased their

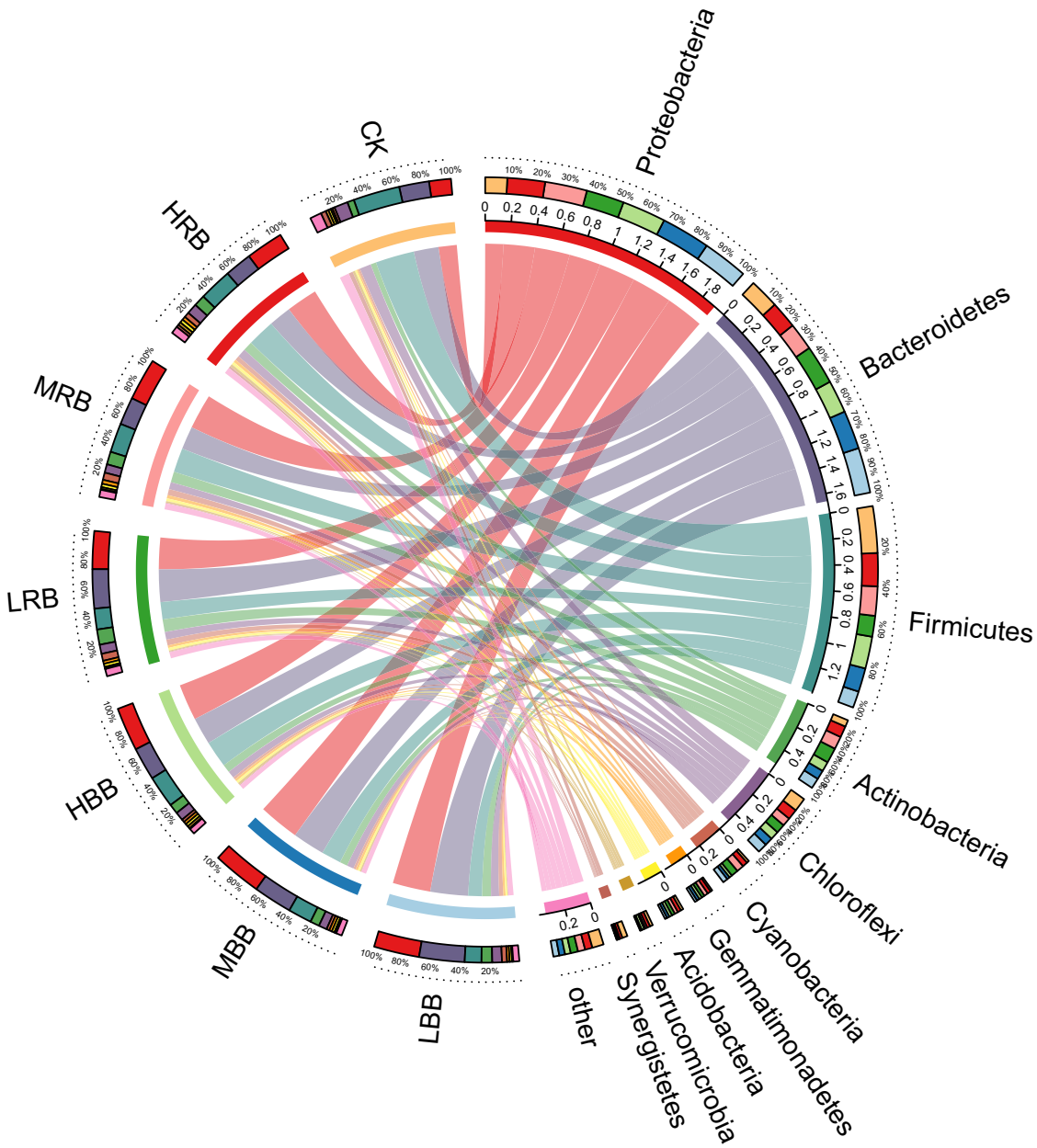

Figure 2. Relative abundances of bacterial phyla in soil from six different biochar treatments and the control (CK) group

LBB, low level addition of bamboo biochar; MBB, medium level addition of bamboo biochar; HBB, high level addition of bamboo biochar; LRB, low level addition of rice-husk biochar; MRB, medium level addition of rice-husk biochar; HRB, high level addition of rice-husk biochar; and CK, no biochar added.

diversity. The biochar's impact on soil pH is likely an important explanation.[18] Further, biochar will directly or briefly modify the microenvironment of soil microorganisms by improving key soil properties, namely porosity, water content, permeability, and temperature, thus increasing the diversity of those communities.[19] This result is consistent with other studies, as a global meta-analysis suggests that overall biochar has a limited impact on the proportion of major bacterial phyla, with only Acinetobacter and Gemmatimonas being significantly affected. But biochar can significantly change the diversity of soil bacteria.[20]

Soil microbial community and soil physicochemical properties

Biochar significantly changed the relative abundances of both bacteria and fungi at taxonomic rank of phylum, genus, or family. *Bacillus* possesses a biological control function, in that species of it are capable of forming a stable, extensive biofilm as well as secreting surfactin to protect plants from bacterial viruses.[21] The multiple regression results (Table 2) gave further insight into the relationships between soil microbial community and soil physicochemical properties. *Actinobacteria*'s model

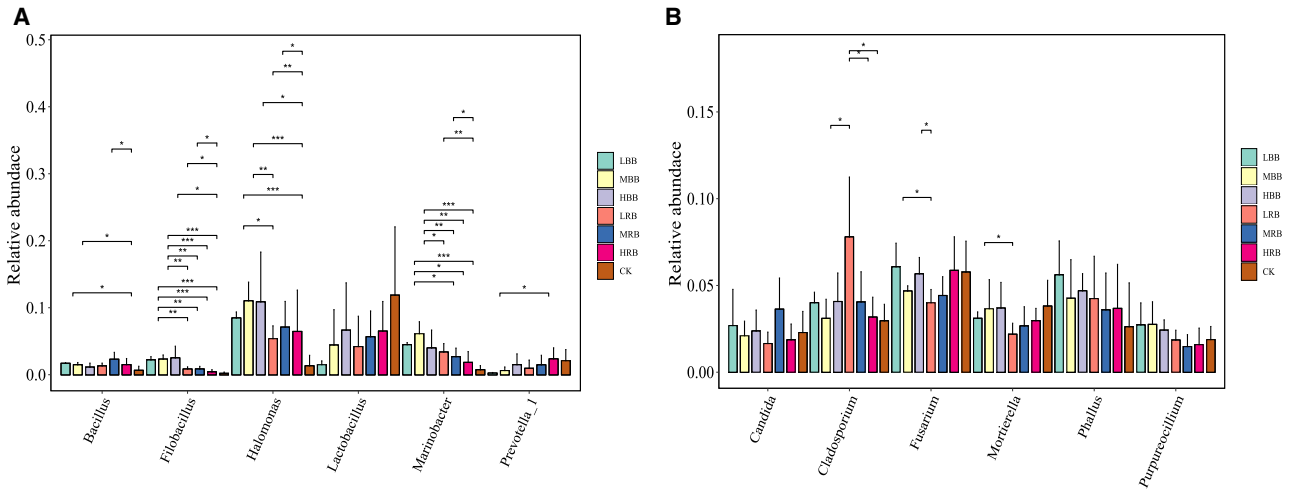

Figure 3. Relative abundances (means ± SEs, *n* = 5) of the main bacterial genera (a), and main fungal genera (b) in soil from six different biochar treatments and the control (CK) group

LBB, low level addition of bamboo biochar; MBB, medium level addition of bamboo biochar; HBB, high level addition of bamboo biochar; LRB, low level addition of rice-husk biochar; MRB, Medium level addition of rice-husk biochar; HRB, high level addition of rice-husk biochar; and CK, no biochar added.
The *, **, and *** respectively indicate a significant difference between means at $p < 0.05$, $p < 0.01$, and $p < 0.001$.

showed that Mg^{2+} ions positively influenced its abundance yet Ca^{2+}, SO_4^{2-}, and HCO_3^- ions negatively influenced its relative abundance. For *Mucoromycota*, soil SO_4^{2-} ions promoted its abundance yet pH diminished it. These results are consistent with other studies which showed that soil pH and heavy metals are drivers of soil bacterial composition because a changed pH affects the surface characteristics of microbial cells and also induce changes in their physiological and biochemical processes.[22,23] These likely led to differential shifts in the population growth rates of bacterial taxa and modified the structure of bacterial communities. Compared with the CK group, biochar reduced the relative abundance of *Fusarium*, which is the causal agent of lily blight and wilt diseases.[24] *Bacillus* enrichment coupled with *Fusarium* depletion in soil amended with biochar has been linked to a higher capacity of disease suppression.[24,25] Therefore, for local saline-alkali land management, how to prevent and control such diseases besides improving saline-alkali land is also a pertinent issue that warrants attention. Our results confirm that biochar is capable of reducing the main salt content, which relieves the problem of soil salinization. Considering the responses of soil bacteria and fungi analyzed at the family rank, the changes to soil pH, EC, CEC and salt ions have a great impact on soil microbial community. *Halophilic* microorganisms such as *Nitrilitruptoraceae* and *Balneolaceae* are very sensitive to changes in the concentrations of soil salt ions.[26,27] Most of the *Pleosporaceae* and *Didymellaceae* are phytopathogenic fungi,[28,29] and their relative abundances are significantly correlated with salt ions in our study. And these changes will have an impact on the utilization efficiency of water and different nutrients in the soil, as previous studies have shown, biochar addition could realize increase soil N fixation, and mitigate N losses.[30] Therefore, although our work here shows that a moderate biochar addition can significantly reduce the chief salt ions, how the microbial community is affected has more complex ways, which has both its advantages and disadvantages and requires long-term research and analysis to understand. Accordingly, adding the appropriate amount and type of biochar to soil is crucial for saline-alkali land restoration, and these results can provide theoretical basis for the ecological restoration of saline alkali soil in Baiyin City.

Table 2. Summary of the multiple regression models for the effects of environmental parameters on member taxa of the soil microbial community

Multiple linear regression equation	R^2	F	P
y (*Bacteroidetes*) = 0.273 + 0.055x(Na^+)–0.014x(Ca^{2+})	0.79	7.55	0.04
y (*Actinobacteria*) = 0.244–0.05x(SO_4^{2-})–0.007x(Ca^{2+})+0.015x(Mg^{2+})–0.0001x(HCO_3^-)	0.97	51.27	0.02
y (*Cyanobacteria*) = 0.686 + 0.007x(SO_4^{2-})–0.71x(pH)–0.045x(K^+)+0.007x(Na^+)	0.99	223.56	0.004
y (*Gemmatimonadetes*) = −0.20x(EC)+0.002x(Na^+)+0.00012x(Cl^-)–0.0002x(pH)–0.107	0.99	226.61	0.004
y (*Acidobacteria*) = 0.115–0.008x(pH)–0.001x(Na^+)–0.002x(EC)	0.95	20.05	0.01
y (*Mucoromycota*) = −0.006–0.001x(pH)+0.0004x(SO_4^{2-})	0.85	11.23	0.02
y (*Chytridiomycota*) = 0.0002x(Ca^{2+})–0.00015x(Mg^{2+})+0.00042x(CEC)–0.07	0.92	12.76	0.03

y, relative abundance of the bacterial or fungal phyla in soils; x, environmental parameters.

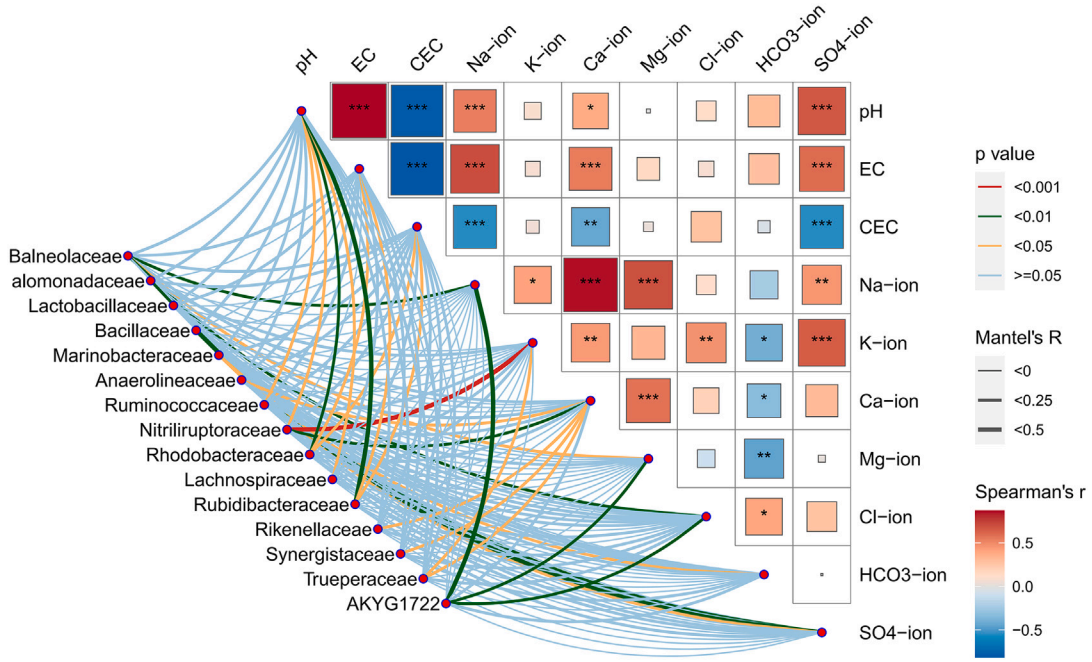

Figure 4. Mantel test of the main bacterial families in soils from six different biochar treatments and the control (CK) group

LBB, low level addition of bamboo biochar; MBB, medium level addition of bamboo biochar; HBB, high level addition of bamboo biochar; LRB, low level addition of rice-husk biochar; MRB, medium level addition of rice-husk biochar; HRB, high level addition of rice-husk biochar; and CK, no biochar added.

Limitations of the study

RB and BB clearly differ in their nutrient content and microstructural morphology. Using these two types of biochar with different levels of addition significantly changes the pH, EC, CEC, and salt ions in saline-alkali soil. Nonetheless, RB is better than BB for reducing the principal salt ions and MRB treatment is the

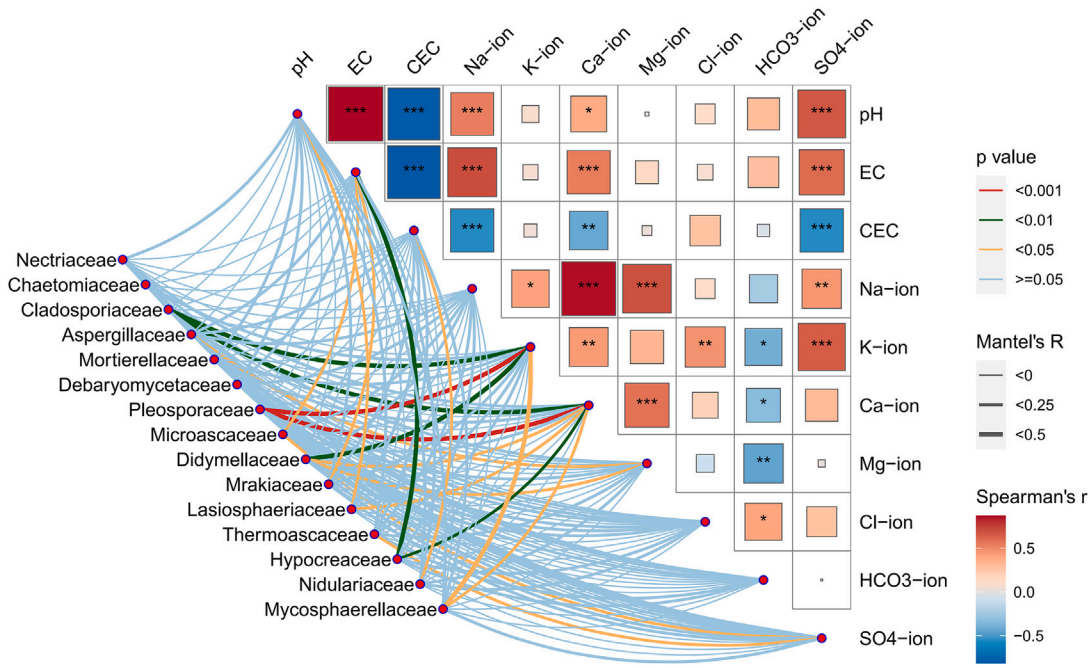

Figure 5. Mantel test of the main fungal genera (b) in soils from six different biochar treatments and the control (CK) group

LBB, low level addition of bamboo biochar; MBB, medium level addition of bamboo biochar; HBB, high level addition of bamboo biochar; LRB, low level addition of rice-husk biochar; MRB, medium level addition of rice-husk biochar; HRB, high level addition of rice-husk biochar; and CK, no biochar added.

Table 3. Background physical and chemical properties of the experimental saline-alkali soil used in this study's laboratory experiment

	TOC (%)	TN (g/kg)	pH	EC (ms/cm)	CEC (cmol/kg)	Na^+ (g/kg)	K^+ (g/kg)	Ca^{2+} (g/kg)	Mg^{2+} (g/kg)	Cl^- (mg/kg)	HCO_3^- (mg/kg)	SO_4^{2-} (g/kg)
Soil	3.32 ± 0.12	0.77 ± 0.024	9.29 ± 0.052	5.74 ± 0.043	5.18 ± 0.19	9.21 ± 0.24	2.62 ± 0.13	41.67 ± 0.98	11.61 ± 0.32	4.65 ± 0.16	480.13 ± 14.82	5.76 ± 0.17

TOC, total organic carbon; TN, total nitrogen; EC, electrical conductivity; CEC, cation exchange capacity; Values are the mean ± SE, n = 5 soil samples.

Table 4. Physical and chemical properties of the two types of biochars used in this study's laboratory experiment

Type	pH	Ash content (%)	BET (m^2/g)	C (%)	H (%)	O (%)	N (%)
RB	8.1	51.2	60.2514	31.28	2.22	65.51	0.44
BB	8.56	14.5	424.845	75.72	2.08	28.61	0.68

RB, rice-husk biochar; BB, bamboo biochar; BET, the Brunauer-Emmett-Teller method to determined specific surface area.

paramount choice in projects seeking to improve saline-alkali soil conditions in Baiyin. Biochar significantly changes their relative abundance of bacteria and fungal at the phylum, genus, and family ranks, especially that of some halophilic microorganisms. Our results suggest adding biochar to saline-alkali soil could serve as a helpful tool for saline-alkali land improvement in Baiyin, northwest China, but more long-term, well-replicated studies are needed to identify the advantages and disadvantages of ensuing changes in soil microbial structure.

RESOURCE AVAILABILITY

Lead contact

Further information and requests can be directed to the lead contact, Xia Zhao (zhaoxia@lzb.ac.cn).

Materials availability

This study did not generate new physical materials.

Data and code availability

Data

Qiu, Yang (2024), "Effects of adding different types and amounts of biochar to saline alkali soil on its salt ions and microbial community in northwest China", Mendeley Data, V2, https://doi.org/10.17632/wgr4g3nmc5.1.

Code

No code was generated in this study.

Other items

Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

ACKNOWLEDGMENTS

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AUTHOR CONTRIBUTIONS

Conceptualization, Y.Q.; methodology, L.Z.; investigation, Y.W. and X.Z.; resources, Y.Z.; writing-original draft, Y.Q.; writing-review and editing, Z.X.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- METHOD DETAILS

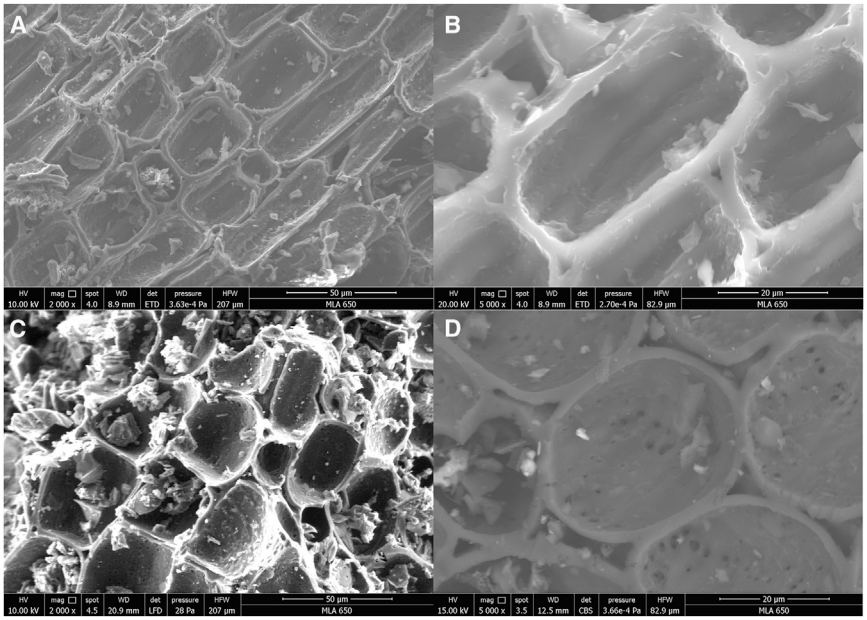

Figure 6. Microstructure (SEM) of the rice-husk biochar (RB) and bamboo biochar (BB) types used in the experiment

(A and B) show the SEM morphology of RB under 2000× and 5000× magnification, respectively; (C) and (D) show the SEM morphology of BB under 2000× and 5000× magnification, respectively.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals, peptides, and recombinant proteins		
Biochar	Zhejiang Biochar Engineering Technology Research Center	rice-husk biochar (RB) and bamboo biochar (BB)
Deposited data		
Data	Mendeley Data	https://data.mendeley.com/datasets/wgr4g3nmc5/1
Software and algorithms		
R language	The R Foundation	https://cloud.r-project.org/

METHOD DETAILS

Soil and biochar

The saline alkali soils used in the study came from the Baiyin municipality in Gansu Province, China (36°39′N, 104°33′E). Baiyin has an annual average precipitation of 210 mm and an annual evaporation of 2056 mm, and is situated within a temperate arid continental climate. Baiyin is part of the gully region of the Loess Plateau where the soil texture is loamy sandy soil and the saline-alkali soil is neutral saline alkali soil. This saline alkali soil was bulk-sampled from 0-20 cm layer from several soil pits. Once brought to the laboratory, all soil samples were sieved to remove any plant debris remaining in the soil using a 2-mm sieve. Background values of the saline-alkali soil used in this study are presented in Table 3.

Two types of biochar were provided by the Zhejiang Biochar Engineering Technology Research Center, namely, rice-husk biochar (RB) and bamboo biochar (BB). The raw material of RB come from 100% rice husk and the raw material source of BB 100% bamboo. The process flow includes drying and dehydration, extrusion molding, heating to 550°C for 30 minutes (RB) and 750°C for 3 hours (BB), and pass through a 0.25mm mesh sieve. They were selected for this study because they differ substantially in terms of their nutrient content, pH, and surface area characteristics (Table 4), in addition to their distinct microstructural morphology (Figure 6).

Experimental design

The laboratory experiment was a completely randomized design with five replicates consisting of saline alkali soils that received six treatment combinations of biochar type and amount: LBB, low level addition of bamboo biochar; MBB, medium level addition of bamboo biochar; HBB, high level addition of bamboo biochar; LRB, low level addition of rice-husk biochar; MRB, medium level addition of rice-husk biochar; and HRB, high level addition of rice-husk biochar; in addition, a control (CK) was set up (i.e., no biochar added). These were established to study ionic and microbial changes in response to differing biochar treatments.

Each fresh sample of saline-alkali soil was sieved through a 2-mm sieve, and 500 g of it was mixed with either biochar type and placed in a 1-L jar as follows. For each low-, medium-, or high-level treatment, the soil was uniformly mixed with 2.5%, 5%, or 10% mass fraction of the biochar, respectively. Soil moisture was adjusted to 10% at the beginning of the expeirment, which approximated the local soil moisture content. All treatment samples (35 jars in total) were incubated at 20°C for 180 days.

Sampling and measurement

Soil total organic carbon (TOC) was determined by the potassium dichromate method,[31] while total nitrogen (TN) was measured using an elemental analyzer (Vario Macro, Elementar, Germany). Soil pH was quantified according to ISO 10390 (ISO, 1994) using glass electrodes. Electrical conductivity (EC) was measured using a conductivity meter. To determine the cation exchange capacity (CEC), the ammonium chloride-ammonium acetate exchange method (LY/T 1243-1999).[32] The soil water-soluble salt content was calculated as follows: sodium ions (Na^+) and potassium ions (K^+) were determined by a flame photometer (JINGKE-FP640, China), based on the standard curve method; magnesium ions (Mg^{2+}) and calcium ions (Ca^{2+}) were determined by the EDTA semi-microtitration method; carbonate (CO_3^{2-}) and bicarbonate (HCO_3^-) ions were determined by the neutralization titration method; chloride ions (Cl^-) were determined by the $AgNO_3$ precipitate titration method; and sulfate ions(SO_4^{2-}) were determined by the $BaCl_2$-EDTA precipitate titration method.

Total amount of genomic DNA was extracted from each soil sample by using the PowerSoil DNA Isolation Kit (MoBio Laboratories, Inc., USA), as directed by the manufacturer. All extractions were stored at –80°C for subsequent analyses.

PCR amplification, sequencing, and statistical analysis

The 16S rRNA, ITS1 and ITS2 tag-encoded high-throughput sequencing was carried out externally, on the Illumina MiSeq platform, by the Biomarker Technologies Co., Ltd. (Beijing, China). Using a common primer pair, the 16S rRNA gene regions V3 and V4 were amplified (Forward primer, 5'- ACTCCTACGGGAGGCAGCA-3'; reverse primer, 5'- GGACTACHVGGGTWTCTAAT-3') along with adapter sequences and barcode sequences, while the 18S rDNA gene ITS1 region of fungi was amplified using a primer pair (Forward primer, 5′-CTTGGTCATTTAGAGGAAGGAAGTAAA-3′; reverse primer, 5′-GCTGCGTTCTTCATCGATGC-3′) with adapters sequences and barcodes. PCR products from the first step PCR were purified via VAHTSTM DNA Clean Beads at 95 °C for 5 min, followed by 15 cycles of 1 min at 95 °C, 1 min at 50 °C, and 1 min at 72 °C. A second round PCR was then performed in a 40μl reaction mixture which contained 20 μl 2×Phμsion HF MM, 8 μl ddH_2O, 10μM of each primer and 10μl PCR products from the first step. Finally, all PCR products were quantified by Quant-iT^{TM} dsDNA HS Reagent and pooled together. The reads first underwent quality-filtering by applying the method available in QIIME, whose default settings were then used for their Illumina processing. Next, the UPARSE pipeline was implemented to identify the operational taxonomic units (OTUs) at a 97% similarity threshold. For each OTU, a representative sequence was selected for its taxonomic assignment using the RDP Classifier. Estimated species richness was examined by running a rarefaction analysis; the Chao1 and Simpson indices of alpha diversity were obtained as described previously (Schloss et al., 2009).[33]

QUANTIFICATION AND STATISTICAL ANALYSIS

All statistical analyses were performed using R v4.3.1. Data were subjected to one-way analysis of variance (ANOVA) with Fisher's LSD (least significant difference) test, with statistical significance set at $p < 0.05$ (n = 5 soil samples per treatment). Results are expressed as mean ± SEM. Statistical details are reported in figure legends and results.