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Evaluation of the microbial community in various saline alkaline-soils driven by soil factors of the Hetao Plain, Inner Mongolia

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Soil microbial communities play a crucial role in maintaining diverse ecosystem functions within the saline-alkali soil ecosystems. Therefore, in this study, we collected various saline-alkaline soils from across the Inner Mongolia Hetao irrigation area. The soil chemical properties were analyzed, and the microbial diversity of bacteria and fungi was measured using 16 S rRNA and ITS rRNA amplicon sequencing. The dynamic relationship between the soil microbial community and soil factors was analyzed using the ABT (Aggregate Enhanced tree) model, the co-occurrence network, and the structural equation model. The results indicated that electrical conductivity (EC) was the biggest driving force of various saline-alkaline soils, affecting the community structure of bacteria (22.80%) and fungi (21.30%). The soil samples were categorized into three treatment levels based on their EC values: the low-salinity group (L, EC: 0–1 ms/cm, $n = 10$), the medium-salinity group (M, EC: 1–2 ms/cm, $n = 8$), and the high-salinity group (H, EC > 2 ms/cm, $n = 6$). The results demonstrated a negative correlation between microbial abundance and salinity-alkalinity, while revealing an enhanced interrelationship among species. The alterations in bacterial (12.36%) and fungal (22.92%) communities in various saline-alkali soils were primarily driven by saline-alkali ions, which served as the principal direct factors. The negative correlation between EC and SOM exhibited the highest magnitude, whereas the positive correlation between soil organic carbon and EC demonstrated the greatest strength. Therefore, it was further substantiated that EC played a pivotal role in shaping the distinct microbial communities in saline-alkali soils.

Keywords Bacterial diversity, Electrical conductivity, Fungal diversity, Key microbial groups, Saline-alkali soi, Hetao Plain

Salinization of soil is a prominent factor that contributes to soil degradation, which is extensively prevalent across the world and poses a global environmental predicament. Based on the incomplete statistics from UNESCO and FAO[1], the global saline-alkali land area is approximately 960 million ha, and continuously expanding by 1.5 million ha every year[2], which is seriously damaging and weakening soil productivity. Therefore, paying attention to saline-alkali soil and improving salinization is critical to preventing soil degradation. The Hetao Plain of the Yellow River basin in Inner Mongolia is located in northwest China and is the largest irrigation area in Asia[3,4]. Furthermore, this region lies in an arid and semiarid area, which has witnessed high evaporation of water and low rain capacity. In addition, the Yellow River is used for irrigation all year round in the Hetao Plain[5]. The problem of soil secondary salinization is extremely prominent. The area of saline and alkaline land in the Hetao Plain is about 345 thousand ha[6]. Thus, soil salinization and alkalization have developed into a very serious situation. Soil salinization reduces grain production by 60,000 tons every year in the local areas[7], which incurs huge economic losses and seriously hinders the sustainable development of agriculture.

Beneficial microorganisms can decompose and transport salt and alkali ions, and their secondary metabolites can absorb salt and alkali ions, which is an effective measure for improving saline-alkali soil. However, the effective application of this measure relies on a comprehensive understanding of the soil's inherent physical

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and chemical properties and its microbial conditions. The mounting evidence increasingly demonstrates that alterations in the external environment exert a profound impact on the community structure and diversity of soil microorganisms. Moreover, the enhancement of ecological conditions exhibits a close association with soil microbial diversity, encompassing improvements in soil quality, material degradation, multielement interconversion, and substantial enzyme production[8,9]. Therefore, some scholars have proposed that soil microbial diversity serves as a crucial indicator, reflecting, to a certain extent, the dynamic changes in the environment[10]. Numerous studies have demonstrated that soil salinization not only profoundly disrupts the physical structure of soil and diminishes its nutrient content but also exerts a substantial adverse influence on the diversity of soil microbial communities[11–13]. Zhang and Meng[14,15] conducted a meta-analysis of various saline-alkaline soil microorganisms, and the results showed that pH was the key influencing factor of soil microbial community diversity. Past studies have demonstrated that soil electrical conductivity (EC) induces fundamental alterations in microbial diversity[16–18]. The response of bacteria and fungi, which are crucial components of the soil microflora, to saline-alkali soil environments has been extensively studied. However, there is a lack of comprehensive research on the key soil factors that influence bacterial community diversity in different saline-alkali soils. In addition, the current research on microorganisms in saline-alkali soil within the Hetao irrigation area primarily focuses on investigating the impact of tillage practices and crop types on the microbial communities[19,20], as well as on exploring beneficial microorganisms associated with certain plant roots[21]. The microbial diversity composition in various saline-alkali soils within the yellow irrigation area of the Hetao Plain has scarcely been documented. Therefore, the investigation of the relationship between the soil microbial community and soil properties in the Hetao Irrigation District of Inner Mongolia can facilitate our comprehensive understanding of the underlying factors to generate soil salinization.

In this study, the chemical characteristics and microbial diversity of different saline-alkali soils were determined using low, medium, and high saline-alkali soils, and the key roles of different soil factors in shaping the microbial communities were investigated. Our study revealed the differences and dynamic changes of microbial community composition in different saline-alkali soils in Hetao irrigation area, Inner Mongolia. At the same time, the relationship between soil factors and microbial community was clarified. The present findings are expected to identify the key environmental drivers of differences between the bacterial and fungal communities so as to further understand the continuously changing characteristics of bacterial and fungal communities in saline-alkali soils.

Materials and methods

Site description

The study area was located in the Hetao Plain (40°13'-42° 28'N, 105°12'-109° 53'E) of the Inner Mongolia region, China. It is a typical mid-temperate continental monsoon climate, with an annual sunshine duration of 3,100-3,300 h. The annual average temperature is 6.5℃[22,23]. The average annual evaporation of 2,068 mm. The average annual precipitation of 183 mm, mainly from July to September. The soil type was mainly irrigation-warping soils, and organic matter content was low. Nitrogen and phosphorus were deficient, and Na^+, K^+, Cl^- and SO_4^{2-} ions were excess in the soil[24].

Site selection and soil sampling

Soil samples were collected from four representative counties of Bayannaoer City in Inner Mongolia (Fig. 1). The 24 soil samples were obtained. GPS positioning information and vegetation species were recorded. Soil samples were collected by a three-point sampling method with a side length of 5 m equilateral triangle, and the soil depth of the collected samples was 0–20 cm. After three soil samples were obtained, the samples were mixed evenly and divided into two parts. Part A of the soil samples was screened by a 2 mm screen after drying and used to determine the soil's chemical characteristics. The other portion was stored in a −80 °C refrigerator for high-throughput sequencing of soil microorganisms.

Soil chemical properties determination

Soil sample and water were mixed at 1:5, then standing, soil pH value was measured by pH meter (PH-3 C, REX, Shanghai). Soil available nitrogen (AN) was determined by using the alkaline diffusion method. Soil available phosphorus (AP) was determined by sodium bicarbonate extraction using the molybdenum-antimony anticolorimetric method. Soil available potassium (AK) was determined using a flame photometer. The assay was performed by leaching the soil with a neutral ammonium acetate solution and then measuring the potassium content in the leachate using a flame photometer. Soil organic carbon (SOM) was determined by using the potassium dichromate volumetric method and the external heating method[25]. The EC of the saturated soil extract was measured with an EC meter (DDS-307 A, REX, Shanghai). The CO_3^{2-}, HCO_3^-, Cl^-, and SO_4^{2-} of the soil were determined by potentiometric titration. The air-dried soil samples were ground and sieved, distilled water was added to shake evenly, and then phenolphthalein indicator was added. The ion concentration in the soil sample was calculated based on the amount and concentration of acid consumed during the titration. Ca^{2+} and Mg^{2+} cations were determined by EDTA titration. This method used ethylenediamine tetraacetic acid (EDTA) to react with calcium ions to form a stable complex, and then the content of calcium ions was determined by titration. The Na^+ and K^+ ions were determined by the differential method. Water soluble salts in the soil are washed with ethanol and glycol-ethanol solutions to reduce interference with the determination (spectrophotometer, TAS-990, Persee, Shanghai).

DNA extraction, Illumina Miseq sequencing, and data processing

The Soil DNA kit (Omega Bio-tek, Norcross, GA, U.S.) was used for the extraction of total microbial community DNA from 0.25-g soil samples. Subsequently, electrophoresis on a 1% agarose gel was performed to assess the

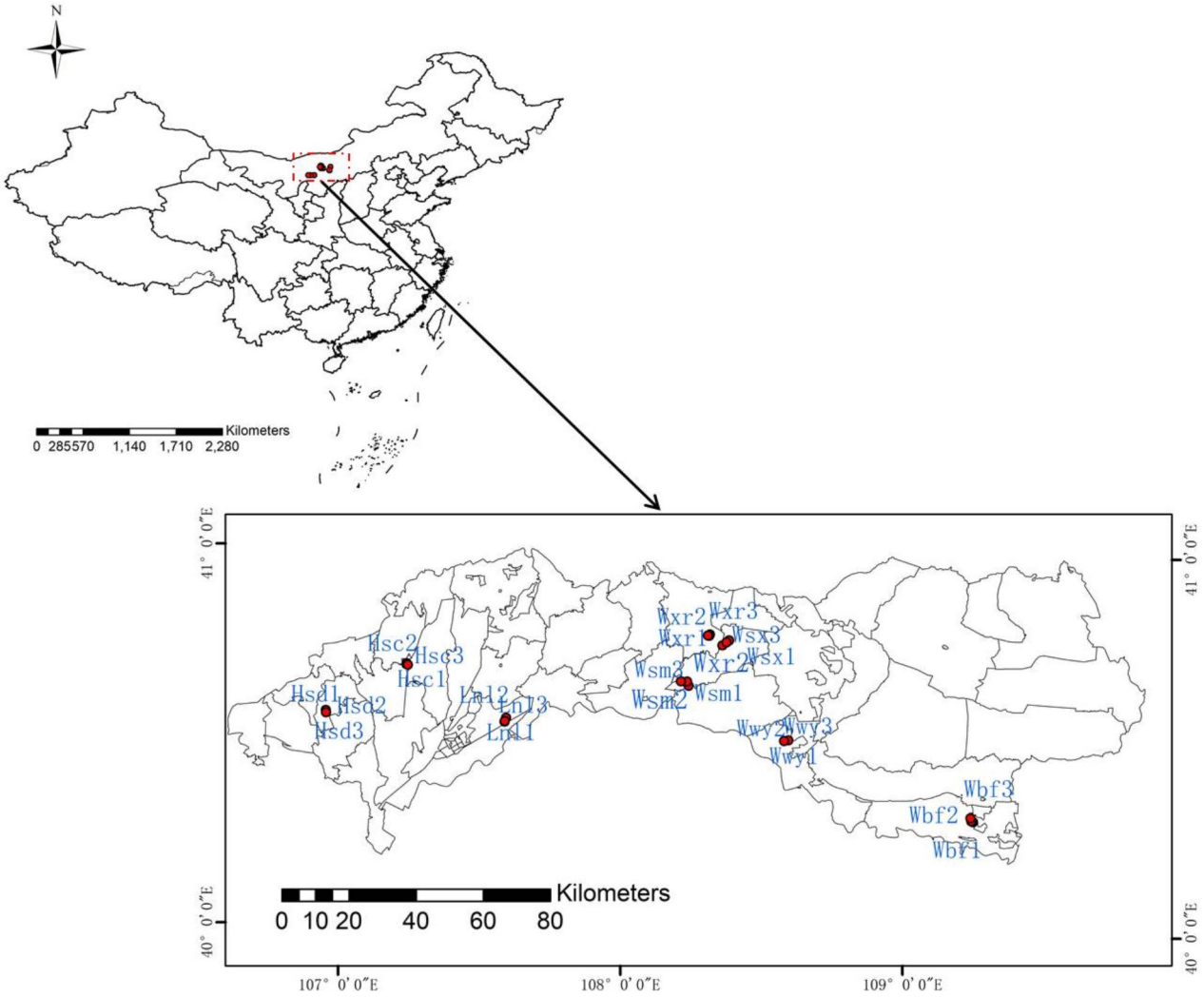

Fig. 1. Distribution of soil sample collection sites. Mainly concentrated in Bayannaoer City Wulateqianqi County, Wuyuan County, Hangjinhouqi County and Linhe District.

quality of the extracted DNA. The concentration and purity of the DNA extract solution (final volume 100 µL) were determined using a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The primers 338 F (5′-actCCtacGGgaggCAGCAGCAG-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) were used to amplify the V3-V4 region of the bacterial 16 S rRNA gene. Primers ITS1F (5′-CTTGGTCATTT AGAGGAAGTAA-3′) and ITS2R (5′-GCTGCGTTCTTCATCGATGC-3′) were used for amplifying the ITS2 region of the fungal. Sequencing was conducted using the NEXTflexTM Rapid DNA-Seq Kit (Bioo Scientific, USA) and Illumina′s Miseq PE300 platform (Meiji Inc, Shanghai, China), following the recommendations of the testing party.

Sequencing data were analyzed as follows: the original sequencing sequence quality was controlled by fast[26] software and the sequence were spliced by FLASH software[27]. After filtering DNA sequences using quality files, the remaining sequences were trimmed to remove sarcodes and forward primers. The low-quality (quality score < 20, length < 300 bp) sequences were excluded. The sequencing data were pretreated to remove chimeras from the datasets. After optimizing the sequences, the UPARSE [28] pipeline was used to generate an operational taxonomic unit (ASV) Table [29]. The identity threshold to bin the sequences into ASVs was 97%, and the most abundant sequence from each ASV was selected as the representative sequence for that ASV. The taxonomy of each ASV representative sequence was analyzed using the RDP Classifier[30] against the 16 S rRNA database using a confidence threshold. All sequencing data were stored in the NCBI Sequence Access Archive (SRA) database (accession number: PRJNA1064468 and PRJNA893343).

Statistical analysis

The sampling test (Fig. 1) was completed using the ArcGIS 10.2 software. The specific steps were done in the drawing software "Desktop" in the ArcGIS toolbox. Analysis of variance was performed for each soil chemical characteristic index using Spass software. The α-diversity index (Chao index, Shannon index, Simpson index)

and sparse curve of soil sample microorganisms were calculated using QIIME software. Differences in microbial community composition were analyzed using Principal Coordinate Analysis (PCoA) based on the Bray-Curtis dissimilarity matrix. Aggregate Enhanced tree (ABT) analysis[31] was performed using the gbm plus package to determine the relative effects of environmental variables on bacterial community composition (PCoA axis 1). The relative contributions of soil salt and alkali ions and soil chemical properties to microbial community variation were determined using variation distribution analysis (VPA).

The relationships and interactions between bacterial species under different conductivity gradients were analyzed using the network analysis method. All pairs' Pearson's correlation coefficients were calculated by the mother (version 1.29.2) for network analysis. The Pearson's correlation coefficient was further filtered at a cut-off point absolute 0.6–0.93. The BH method[32] was used for multiple hypothesis correction with edges of P-value adjustment values less than 0.05 and was used to further improve the accuracy of the network. Gephi visualized the interactive network through the Fruchterman-Reingold layout[33]. The average clustering coefficient, average path length, and network modularity were also calculated[34] using Gephi software. The active species that interact most actively with other species are called network central hubs[35,36]. According to the network analysis, the top 10 network hubs were selected from different salinity treatments. The structural equation model (SEM) was used to evaluate the relationship between the abundance of bacteria and fungi in different saline-alkali soils and soil chemical properties. To streamline the structure of the SEM, a Pearson's correlational analysis (two-tailed test) was conducted on these soil variables to help identify highly significant soil factors. Finally, the variables were incorporated into AMOS 17.0 (SPSS, Chicago, IL, USA) to construct the structural equation model.

Results

Chemical properties analysis of the soil samples

The chemical properties of 24 soil samples with varying degrees of salinization were analyzed and are presented in (Supplementary Table 2). In all of the soil samples studied, the pH value was 8.27–10.51, and the CV was 6.89%. The CVs of AN, AP, AK, SOM, HCO_3^-, CO_3^{2-}, Na^+, K^+, and EC were 45.37%, 59.36%, 42.06%, 41.575%, 42.69%, 94.53%, 79.86%, 49.79%, and 69.86%, respectively. The CV of Cl^-, SO_4^{2-}, Ca^{2+}, and Mg^{2+} were 139.27%, 132.03%, 154.56%, and 119.54%, respectively. The results indicated that each index of the soil samples had obvious variation, except for the pH (Table 1), which might have affected the microbial community structure.

Bacterial and fungal community analyses

The effects of soil chemical factors on the bacterial and fungal communities were assessed by using the ABT model (Fig. 2). The largest driving force in different saline alkali soil bacteria was EC, which accounted for 22.80%, followed by AK (19.42%) and SOM (13.20%). Among the fungi, EC emerged as the predominant driving force (21.30%), closely followed by AK (17.27%) and SOM (12.11%). The samples were initially categorized into three groups based on their EC level, namely the low-salinity group (L), medium-salinity group (M), and high-salinity group (H). The EC for L processing ranged from 0 to 1 ms/cm ($n=10$), the EC for M processing ranged from 1 to 2 ms/cm ($n=8$), and the EC for H processing exceeded 2 ms/cm ($n=6$). The PCoA test results revealed a significant correlation between bacteria ($R=0.5835$, $p<0.001$) and fungus ($R=0.4121$, $p<0.001$). However, the bacterial and fungal communities in different saline-alkali soils exhibited clear isolation (Fig. 3), indicating that the L, M, and H groups, which were categorized based on the EC levels, were highly representative.

Changes in the microbial alpha diversity

The alpha diversity analysis revealed significant variations in the composition of the top ten bacterial and fungal species across different saline-alkaline soils at the phylum level (Fig. 4). The abundance of bacterial species

Indicators	Maximum value	Minimum value	Average	Standard deviation	Variation coefficient (%)
pH	10.51	8.27	8.96	0.62	6.89
AN	73.05	2.68	36.40	16.52	45.37
AP	59.14	6.28	23.81	14.14	59.36
AK	365.85	87.79	206.83	87.00	42.06
SOM	9.04	0.36	5.18	2.15	41.57
Cl^-	4.48	0.13	0.66	0.91	139.27
SO_4^{2-}	7.55	0.13	1.61	2.13	132.03
HCO_3^-	0.79	0.14	0.34	0.14	42.69
CO_3^{2-}	0.71	0.08	0.17	0.16	94.53
Ca^{2+}	2.70	0.60	0.49	0.76	154.46
Mg^{2+}	0.79	0.01	0.18	0.22	119.54
Na^+	1.49	0.05	0.41	0.33	79.86
K^+	0.25	0.03	0.12	0.06	49.79
EC	4.10	0.20	1.37	0.96	69.86

Table 1. The coefficient of chemical characteristics of the collected soil samples. AN is soil available nitrogen, AP is soil available phosphorus, AK is soil available potassium, SOM is soil organic carbon, EC is soil electrical conductivity, Cl^-, SO_4^{2-}, HCO_3^-, CO_3^{2-}, Ca^{2+}, Mg^{2+}, Na^+ and K^+ is soil salt and alkali ions.

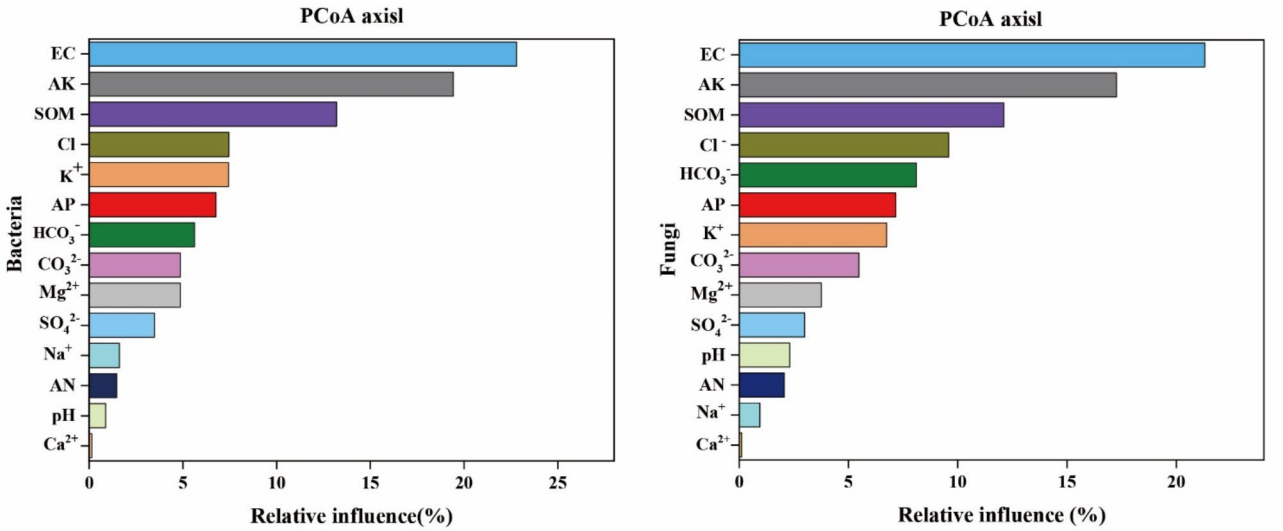

Fig. 2. Relative variable driving force of different soil chemical factors affecting bacterial and fungal composition based on ABT model (%).

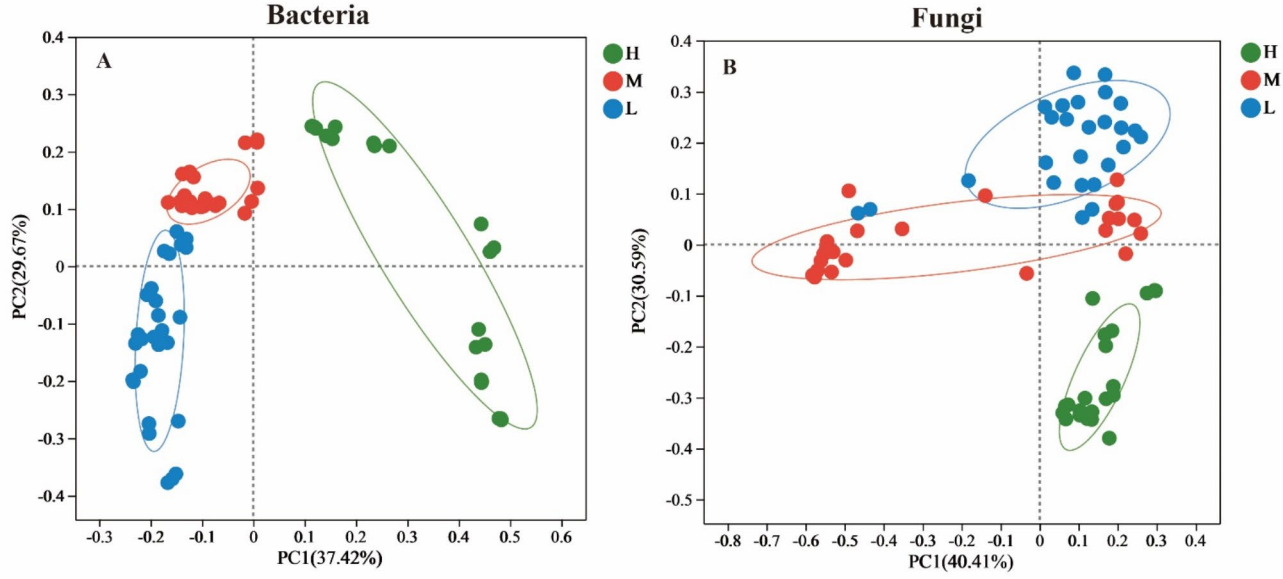

Fig. 3. The principal co-ordinates analysis (PCoA) analysis of bacterial (A) and fungal (B) communities in various saline-alkaline soils at the ASV level. The values are the means of three replicates, error bars represent standard errors (L: $n = 10$; M: $n = 8$; H: $n = 6$). ** represents $p < 0.01$ level significant, and * represents $p < 0.05$ level significant. The L, M, and H treatments represent EC of 0–1, 1–2, and > 2 ms/cm, respectively.

was higher, whereas the fungal diversity was lower. In the Hetao irrigation area of the Inner Mongolia region, *Proteobacteria*, *Actinobacteriota*, and *Chloroflex*i are the predominant bacterial groups in various saline-alkaline soils. The relative abundance of *Proteobacteria* was 22% in L, 26% in M, and 30% in H (Supplementary Table 3). The relative abundance of *Actinobacteriota* was 19% in L, 19% in M, and 17% in H. The relative abundance of *Chloroflexi* was 18% in L, 16% in M, and 11% in H. The top three fungal communities at the phylum level were Ascomycota, Basidiomycota, and *Mortierellomycota*. The prevalence of Ascomycota was significantly higher in various saline-alkali soils, accounting for 83% in H, 86% in M, and 76% in L. The number and species diversity of bacterial and fungal communities in various saline-alkali soils were analyzed. The Chao and Shannon, indices of the bacteria and fungi exhibited a negative correlation with increasing salinity (Fig. 5), and the number of the Simpson index bacterial and fungal microbial communities was positively correlated with salinity. The treatments exhibited notable variations ($p < 0.05$). The species and quantity of bacteria and fungi indicated the highest level in L, whereas H exhibited the lowest level. The findings revealed that the abundance and diversity of bacteria and fungi were the lowest in the high saline-alkali soil.

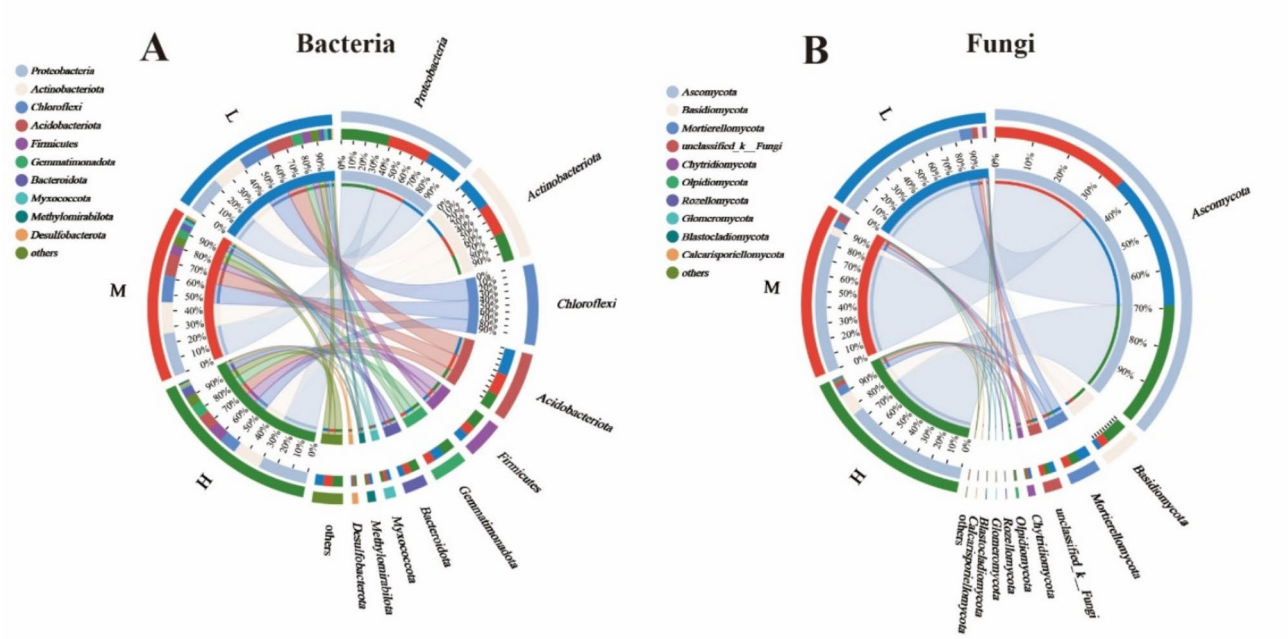

Fig. 4. Based on the Circos map of bacterial (**A**) and fungal (**B**) communities in various saline-alkaline soils at the phylum level, the community richness was top 10.

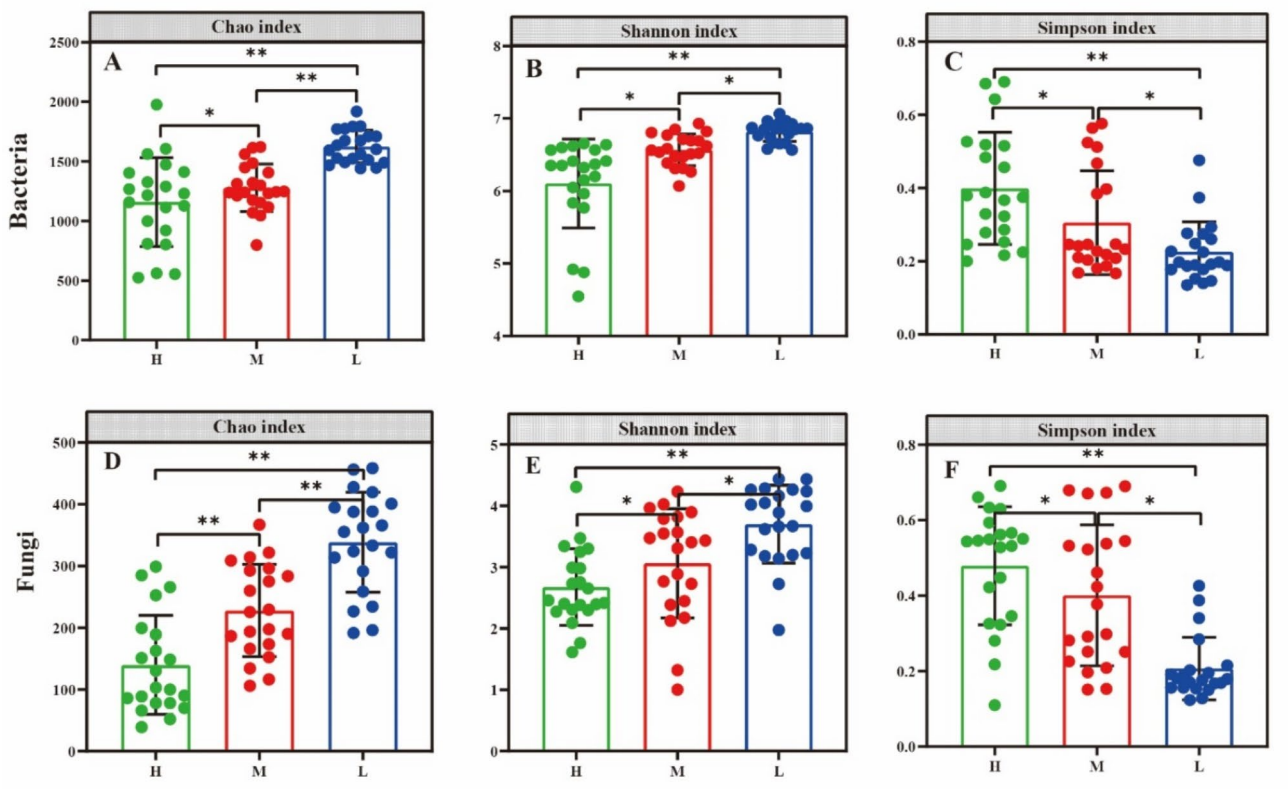

Fig. 5. The Chao index (**A**, **D**), Shannon index (**B**, **E**) and Simpson index (**C**, **F**) of bacteria and fungi in three salinity rates. Chao is used to estimate the index of the number of species (such as ASVs) contained in the sample. Shannon and Simpson were used to estimate microbial diversity in a sample.

Microbial co-occurrence networks

The microbial communities in the Hetao saline-alkali soil of Inner Mongolia exhibit a high degree of complexity and diversity, thus warranting the selection of the top 200 bacterial and fungal species (Fig. 6). The results of network correlation analysis revealed significant variations in the network parameters of bacteria and fungi across various saline-alkaline soils ($p < 0.05$). The bacterial network of L treatment had 177 points and 1,173 edges, and the modularity was 0.398 with 8 modules (Table 2). The M treatment had 192 points and 1,324 edges, and the modularity was 0.457 with 8 modules. The H treatment had 199 points and 2,371 edges, and the modularity was 0.668 with 6 modules. The fungal network of L treatment had 185 points and 631 edges, and the modularity was 0.551 with 10 modules. The M treatment had 191 points and 1,249 edges, and the modularity was 0.564 with 8 modules. The H treatment had 194 points and 1,253 edges, and the modularity was 0.729 with 6 modules. The network points, network edges, and modularity coefficients exhibit an upward trend with increasing salinity, while the number of modules shows a decreasing pattern.

The 10 nodes with the largest number of edges were defined as network hubs, which were active mediators in the bacterial community network (Supplementary Table 4). The *Acidobacteriota*, *Proteobacteria*, and *Chloroflexi* taxa represent the central nodes of bacterial communities in diverse saline-alkaline soils, including 70% network hubs at the phylum level. Half of the network hubs in the L treatment belonged to *Acidobacteriota* and *Proteobacteria*, such as ASV198, ASV692, ASV209, ASV149, ASV1596. The hubs of M treatment had similar phylogenetic classifications to the L treatment, with half assigned to *Chloroflexi* and *Proteobacteria*, such as ASV1411, ASV90, ASV1423, ASV2436, ASV9. However, at the highest salinity, the 40% hubs were classified as Proteobacteria, for instance, ASV2325, ASV81, ASV47, ASV608. The findings demonstrate that *Acidobacteriota* thrives in low to moderate salinity environments, whereas the majority of *Proteobacteria* species exhibit adaptability to high salinity conditions. The *Ascomycota* and *Mortierellomycota* taxa represent the central nodes of fungal communities in diverse saline-alkaline soils, including 90% network hubs at the phylum level. The *Ascomycota* community comprised 66.67% of the overall network hub. Therefore, at the three saline-alkali treatment, more than half hubs were classified as *Ascomycota*. The findings suggest that *Ascomycota* exhibits adaptability to diverse soil environments with varying salinity and alkalinity levels, and it represents the predominant fungal community in saline-alkali soils of Hetao Plain in Inner Mongolia.

Relationship between soil factors and soil microbial communities

We investigated the relationship between environmental factors and the core flora by analyzing heat maps of bacterial and fungal communities and the soil characteristics in different saline-alkaline soils (Fig. 7). The abundance of bacteria and fungi exhibited a significant correlation with the chemical properties of the soil ($p < 0.5$). The bacterial dominant phylum of *Acidobacteriota*, *Proteobacteria*, and *Chloroflexi* exhibited significant correlations with AP, AK, Cl^-, SO_4^{2-}, HCO_3^-, CO_3^{2-}, Ca^{2+}, Mg^{2+}, Na^+, and EC (Supplementary Fig. 3). The fungal dominant phylum of *Ascomycota*, *Basidiomycota*, and *Mortierellomycota* exhibited significant correlations with pH, AP, AK, HCO_3^-, CO_3^{2-}, Ca^{2+}, Mg^{2+}, Na^+, K^+, and EC (Supplementary Fig. 4). The variation distribution analysis (VPA) revealed that the predominant drivers of bacterial and fungal community changes in different saline-alkaline soils were the soluble salt ion components, accounting for 12.36% (bacterial) and 22.92% (fungal), respectively.

Contrasting determinants of bacterial and fungal beta diversities

The SEM was employed to elucidate the potential mechanisms of soil factors influencing the spatial distribution of the microbial community structure (Fig. 8). The data of the model was $\chi^2/df = 2.807$, $p = 0.207$, CFI = 0.891, GFI = 0.915, and RMSEA = 0.048. These findings demonstrated a significant impact of fungal and bacterial richness in various saline-alkali soils ($p < 0.001$). The negative correlation coefficients of EC with bacterial and fungal communities in different saline-alkali soils were 0.91 and 0.86, respectively. The positive correlation coefficients of SOM with the bacterial and fungal communities were the largest at 1.09 and 0.84, respectively. The test further demonstrated that EC exerted the greatest influence on the structure of bacterial and fungal communities, thereby exhibiting a significant negative correlation on the bacterial and fungal community composition. The bacterial and fungal communities of the saline-alkali land in the Inner Mongolia Hetao Plain were directly influenced by soil salt and alkali ions, whereas the soil nutrients indirectly affected these communities.

Discussion

Driving factors of microbial community differences in various saline-alkali soils

The results demonstrated significant alterations in soil chemical properties associated with variations in salinity and alkalinity, potentially influencing the microbial community structure[37]. The presence of varying levels of salt and alkali content, as well as the spatial and temporal distribution in saline-alkali soil, give rise to diverse microbial community structures[38,39]. We found that EC was the largest driver of bacterial (22.80%) and fungal (21.30%) communities in different saline-alkali soils, followed by AK(19.42, 17.27%), and SOM (13.20%, 12.11%). According to the soil electrical conductivity (EC) content, the samples were initially categorized into three treatment levels: low salinity group (L, EC: 0–1 ms/cm, $n = 10$), medium salinity group (M, EC: 1–2 ms/cm, $n = 8$), and high salinity group (H, EC > 2 ms/cm, $n = 6$). The group results were simultaneously validated using PCoA, revealing a distinct separation of soil bacterial and fungal communities among each treatment ($p < 0.001$). It shows that the L, M and H groups divided according to EC levels are highly representative, and EC has been preliminarily identified as the biggest driving force affecting the community structure of bacteria and fungi in different saline-alkali soils[40].

The objective of the Center for Microbial Ecology is to investigate the pivotal environmental factors influencing the variability of microbial community diversity[41,42]. The soluble salt ions were the main driving

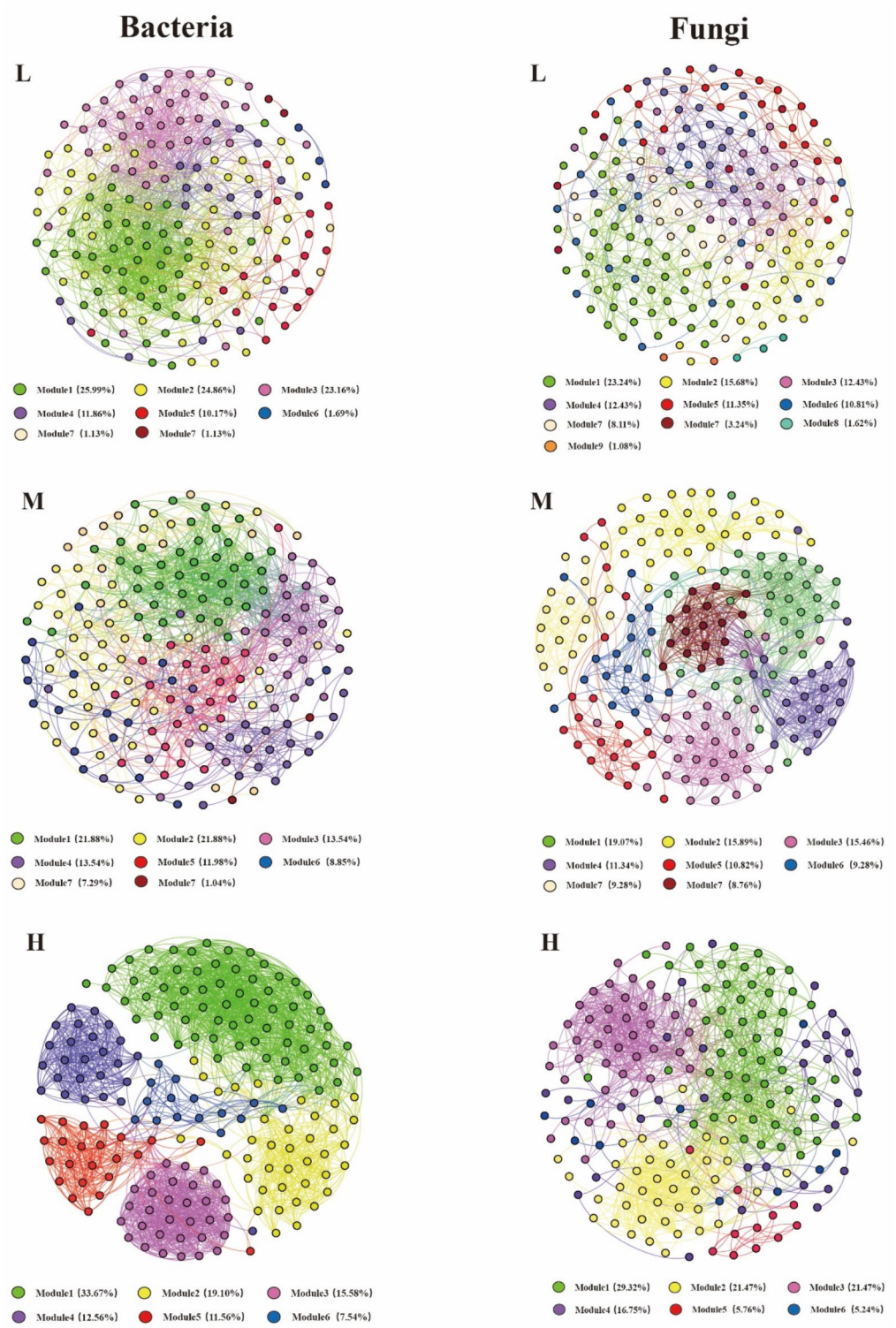

Fig. 6. Networked analysis of bacteria and fungi treated with different salts and alkalis based on Pearson correlation. The different color edges belong to different modules. The bacteria and fungi of (L) Low-salinity treatment (EC: 0–1) with modularity resolution of 0.398,0.551 (bacteria, fungi) and 8,10 modules; (M) middle-salinity treatment (EC: 1–2) with modularity resolution of 0.457,0.551 and 8,8 modules; and (H) high-salinity treatment (EC: >2) with modularity resolution of 0.668,0.729 and 6,6 modules. Figure legend is the proportion of modules in different processing.

Toplogical features	L	M	H
Bacteria Network metrics			
Nodes	177	192	199
Edges	1324	1173	2371
Modularity	0.398	0.457	0.668
Network diameter	7	6	6
Average degree	14.96	12.219	23.829
Graph density	0.085	0.064	0.12
Average path length	2.838	2.925	2.97
Average clustering coeffcient	0.483	0.435	0.761
Fungi Network metrics			
Nodes	185	191	194
Edges	631	1249	1253
Modularity	0.551	0.564	0.729
Network diameter	9	10	9
Average degree	6.822	13.079	12.918
Graph density	0.037	0.069	0.067
Average path length	3.66	3.427	4.024
Average clustering coeffcient	0.402	0.537	0.673

Table 2. Topological characteristics of co-occurrence networks of soil microbial communities in various saline-alkaline soils. (corresponding to Fig. 6).

factors for bacterial (12.36%) and fungal (22.92%) community changes, and soluble salt ions had a greater effect on fungi. It can be inferred that the bacterial and fungal communities in the saline-alkali land of Hetao Plain, Inner Mongolia are directly influenced by soil salt and alkali ions, while basic soil properties indirectly affect these communities[17]. The EC had the largest total negative correlation to bacterial and fungal communities, while SOM had the largest total positive correlation to bacterial and fungal communities in the SEM. Thus, the electrical conductivity (EC) of soil is determined by the combined presence of various cations and anions in the leaching solution[43]. Therefore, on a larger spatial scale, EC can provide a more comprehensive assessment of the salt and alkali content in various saline-alkali soils compared to pH measurements. It has been reported that soil environmental factors have different effects on microbial community diversity in the case of large geographical distance[44]. The relative significance of biotic and abiotic factors in soils may additionally be contingent upon the breadth and scale of ecological gradients[45]. Our results were similar to the study of Wang et al.[46]. (2020) who reported that the EC as a key factor drives the composition of the bacterial community under alkaline conditions in saline-alkali soil of Songnen Plain. Consequently, electrical conductivity (EC), being a pivotal indicator of soil properties, exhibits strong predictive capability for discerning alterations in microbial community structure across diverse saline-alkali soils[47].

Some scholars had found that soil pH was a key regulator of microbial community distribution, which could affect the community dynamics and ecological processes of bacteria and fungi in different saline-alkali soils[48]. However, our study revealed that the diversity, abundance, and community structure of bacteria and fungi in various saline-alkali soils did not exert a significant influence on pH levels (Fig. 3). The variation coefficient of pH value is the smallest in different saline-alkali soils, and pH value may not be the main limiting factor for microbial communities in saline-alkali soil (Table 2). Although the pH value is an important factor affecting microbial community structure in different saline-alkali soils, our results are not inconsistent with the mainstream theory[16]. The reason for this conclusion may be that in the same salt-alkali land or within a smaller spatial range, changes in pH can significantly affect the structure of microbial communities and other soil factors. However, at larger spatial scales, soil environmental differences are very large, and soil saline and alkaline ions are the key factors affecting soil microbial communities[49,50].

Changes of bacterial and fungal community diversity in different saline-alkali soils

Numerous studies have substantiated the impact of environmental factors on alterations in microbial community structure, encompassing soil salinity, soil nutrients, and altitude[51,52]. This study showed that the species types of bacterial communities were more abundant in different saline-alkali soils at the gate level, while the fungal richness was less. The communities with a high abundance of bacteria include *Proteobacteria*, *Actinobacteriota*, *Chloroflexi*, *Acidobacteriota* and *Firmicutes*. Among them, the abundance of *Proteobacteria* increases in correlation with salinity, whereas the abundance of *Actinobacteriota* and *Chloroflexi* decreases as salinity levels rise. The three most prominent fungal communities in terms of species diversity were *Ascomycota*, *Basidiomycota*, and *Mortierellomycota*. The *Ascomycota* community exhibits absolute dominance in low, middle, and high saline-alkali soils, with proportions of 83%, 86%, and 76% respectively. Meanwhile, the Chao and Shannon indices of bacteria and fungi in different saline-alkali soils exhibited a decline concomitant with increasing salinity levels. The observed treatments exhibited statistically significant differences ($p < 0.05$). We found that the degree of soil high salinity significantly restricted the number and species of bacteria and fungi, and the richness of some core

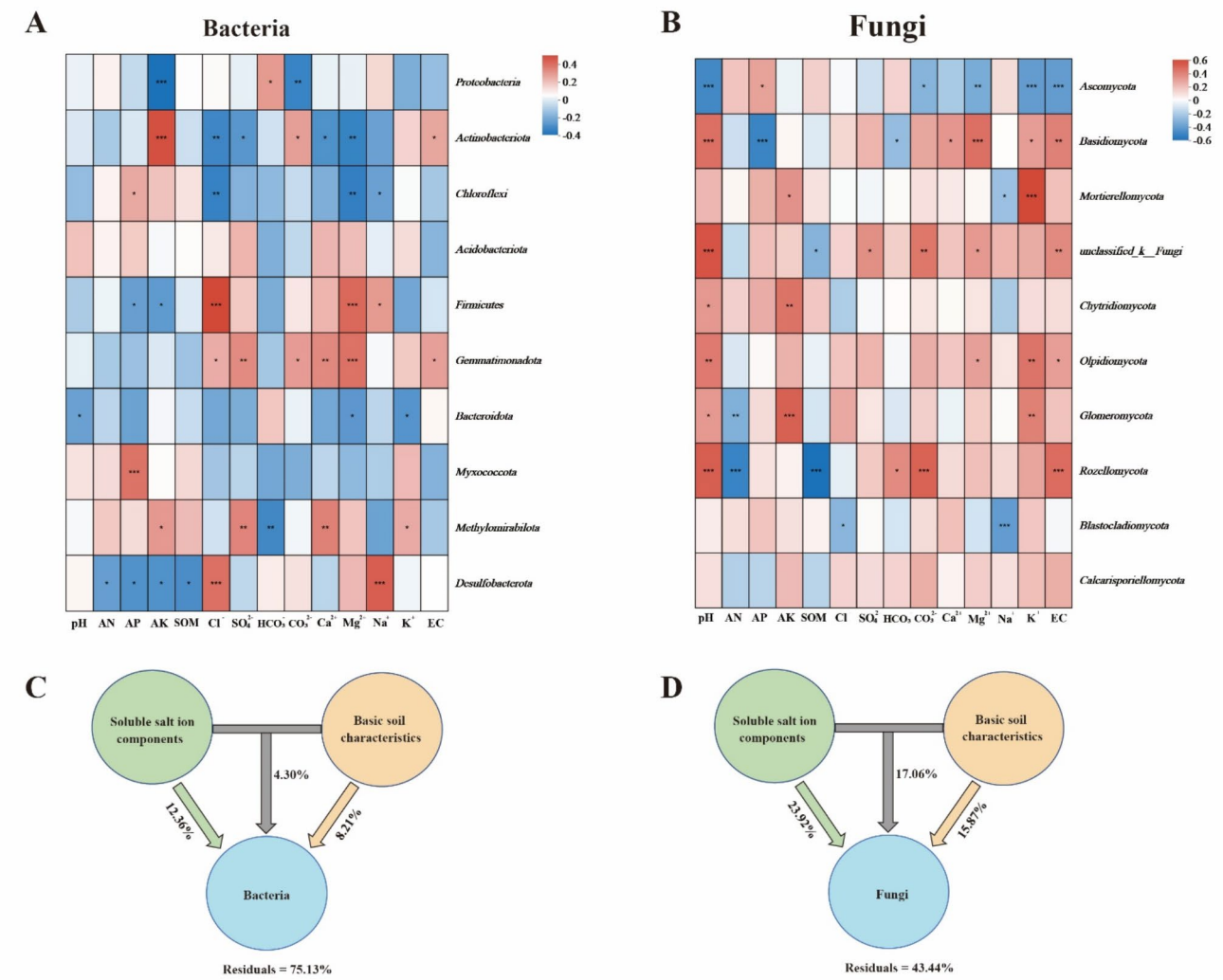

Fig. 7. Correlation heat map between soil physicochemical properties and bacterial (A) and fungal (B) community (phyla level) of different saline-alkaline soils. Variation partitioning analysis (VPA) of the effects of soluble salt ion components and basic soil characteristics on bacterial (C) and fungal (D) communities. Soluble salt ion components include Cl^-, SO_4^{2-}, HCO_3^-, CO_3^{2-}, Ca^{2+}, Mg^{2+}, Na^+, K^+, EC. Basic soil characteristics include pH, available nitrogen (AN), available phosphorus (AP), available potassium (AK), soil organic matter (SOM).

communities would change with the change of salt and alkali content. The results showed that the composition of microbial community structure reflected their ecological strategies to some extent, which was consistent with the diversity changes of different saline-alkali soils[53].

The application of cooccurrence network analysis provides a mathematical perspective to validate the principle of microbial community aggregation, enabling the characterization of network properties and interrelationships among soil microbial species[54,55]. Therefore, based on modularity analysis in network analysis, this study revealed a positive correlation between salinity and the characteristics of network points, network edges, and modularity coefficients in the bacterial and fungal networks. Additionally, it was observed that the number of modules decreased with increasing salinity. The improvement in salinity will lead to the creation of more ecological niches; however, competition still persists as a limiting factor for microbial community richness[56].

Another important action of network analysis in the study of microbial ecology was to identify the core bacterial community of maintain the soil ecosystem[57]. The *Acidobacteriota*, *Proteobacteria*, and *Chloroflexi* taxa represent the central nodes of bacterial communities in diverse saline-alkaline soils, including 70% network hubs at the phylum level. The *Ascomycota* and *Mortierellomycota* taxa represent the central nodes of fungal communities in diverse saline-alkaline soils, including 90% network hubs at the phylum level. The *Ascomycota* community comprised 66.67% of the overall network hub. The *Acidobacteriota*, a saprophytic bacterium, has been found in several studies to be capable of degrading inorganic carbon and playing a crucial role in the soil carbon cycle[58]. However, under conditions of increasing saline-alkali content, its relative abundance tends to decrease, indicating soil environment deterioration and reduced substrate degradation ability. The *Proteobacteria* belongs to a rapidly proliferating group of eutrophic bacteria, and its abundance positively

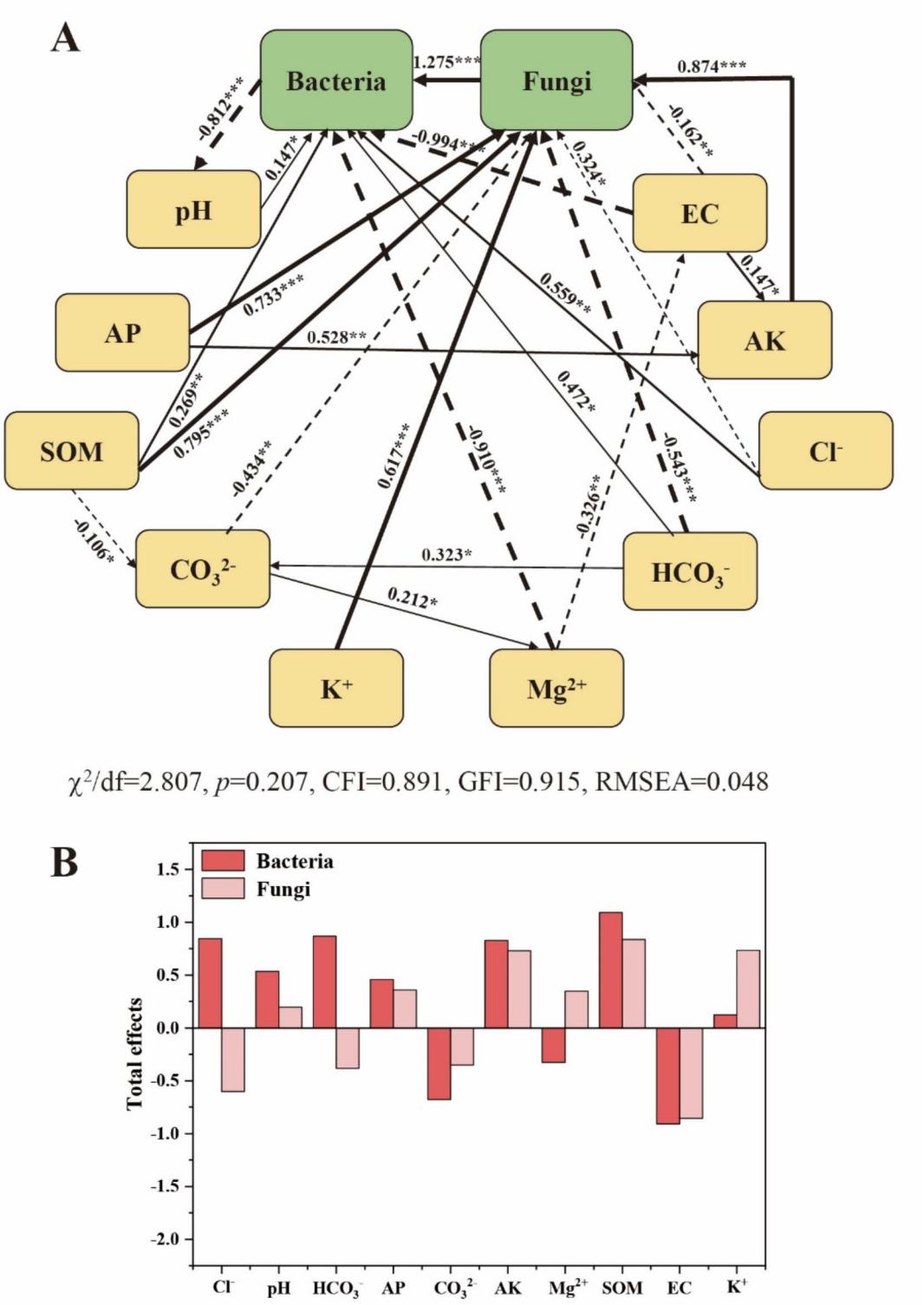

Fig. 8. Structural equation model (SEM) with the direct and indirect effects of different saline-alkaline soils on bacterial and fungal community changes. Adjacent number that is labeled in the same direction as the arrow represents path coefficients and the width of the arrow is in proportion to the degree of path coefficients. The solid line represents the positive correlation, and the dashed line shows the negative correlation. Significance levels are denoted with $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$. Standardized total effects (direct plus indirect effects) calculated by the SEMs (A) are displayed in B respectively. The available nitrogen (AN), available phosphorus (AP), available potassium (AK), soil organic matter (SOM).

correlates with salinity levels, suggesting a potential shift in the nutrient structure composition of the microbial community[59]. The *Chloroflexi* is believed to exist in large quantities in highly saline soils and has been shown to play a leading role in the production of soil halophilic enzymes and the metabolism of pollutant remediation[44]. For the main fungal phylum, Ascomycota was involved in the litter decay process in arid environments[60]. The analysis of soil microbial diversity, community structure and co-occurrence network showed that the higher the salinity of saline-alkali soil, the lower the microbial species and quantity. However, the increased connectivity and interrelationships between species indicate that more cooperation between microorganisms is needed to adapt to harsh soil conditions in high-salinity environments.

Ecological implications of microbial community variation in saline-alkali land in Hetao irrigation area of Inner Mongolia

This study confirmed that the community structure of bacteria and fungi in different saline-alkali soils in Hetao irrigation area of Inner Mongolia was significantly different. The underground ecological process and nutrient transfer rate in the irrigated area are relatively vulnerable compared to conventional cultivated land due to long-term utilization of Yellow River water for irrigation[6]. Therefore, the nutrient cycling in saline soil and the interaction facilitated by soil microorganisms impede the ecosystem's capacity for nutrient renewal, leading to notable variations in microbial-driven ecosystem processes across different spatiotemporal contexts[8]. The varied responses of bacterial and fungal communities to environmental factors elucidated in this study further validate the diversity of functional roles within microbial communities. The results demonstrated that the microorganisms exhibiting high abundance in diverse saline-alkali soils, including *Acidobacteriota*, *Proteobacteria*, *Chloroflexi*, *Ascomycota* and *Mortierellomycota*, play a pivotal role in facilitating material decomposition, nutrient transport and transformation. Therefore, the soil microorganisms in saline soil ecosystems within irrigated areas exhibit characteristics pertaining to community construction mechanisms and spatial-temporal distribution, with their community structure being influenced by both temporal and environmental factors. The metabolic and ecological functions of different microbial groups in saline land ecosystems in irrigated areas can vary depending on the specific environmental conditions.

Conclusions

Our study found that soil electrical conductivity (EC) was the largest driving force regulating the composition of microbacteria (22.80%) and fungi (21.30%) communities in different saline-alkali soils at large spatial scales. Simultaneously, it effectively predicts the variations in microbial community structure across diverse saline-alkali soils, serving as a pivotal indicator of soil factors. There were significant differences in bacterial and fungal diversity and community structure in different saline-alkali soils. The co-occurrence networks analysis showed that the network points, network edges and modularity coefficients of bacteria and fungi increased with the increase of saline-alkali content, while the number of modules decreased with the increase of saline-alkali content. The findings indicated that enhanced microbial cooperation was imperative for acclimatizing to the high saline-alkali soil environment. The *Acidobacteriota*, *Proteobacteria* and *Ascomycota* of the network hubs were the core communities of different saline-alkali soils. Among them, the *Acidobacteriota* was well-suited for thriving in environments with low to medium salinity, whereas the *Proteobacteria* and *Ascomycota* were capable of flourishing in high salinity conditions. They were good microbial indicator communities that can reflect the change of saline-alkali soil environment. The correlation between microbial diversity and soil environmental factors will helpful to analyze the construction of microbial communities in different saline-alkali soils and provide theoretical support for the improvement of saline-alkali soils by beneficial microorganisms in the future.

Data availability

Sequence data that support the findings of this study have been deposited in the National Center for Biotechnology Information with the primary accession code PRJNA1064468 and PRJNA893343.All sequencing data were stored in the NCBI Sequence Access Archive (SRA) database (accession number: PRJNA1064468 and PRJNA893343).

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References

1. Zhang, Y. T. et al. Characterization of soil salinization and its driving factors in a typical irrigation area of Northwest China. *Sci. Total Environ.* **837**, 155805 (2022).
2. Li, H. et al. Current states and challenges of salt-affected soil remediation by cyanobacteria. *Sci. Total Environ.* **669**, 258–272 (2019).
3. Ren, F. et al. Yield-compatible salinity level for growing cotton (*Gossypium hirsutum* L.) under mulched drip irrigation using saline water. *Agricultural Water Management* **250**, 106–859 (2021).
4. Zhang, H. Y. et al. Late Quaternary tectonic uplift and Yellow River evolution create high arsenic aquifers in the Hetao Basin, China. *Geoscience Frontiers* **15**, 101888 (2024).
5. Zhang, Y. T. et al. Sustainable agricultural water management in the Yellow River Basin. *China. Agricultural Water Management* **288**, 108473 (2023).
6. Negacz, K. et al. Saline soils worldwide: Identifying the most promising areas for saline agriculture. *J. Arid Environ.* **203**, 104775 (2022).
7. Rao, Y. et al. Mechanisms of plant saline-alkaline tolerance. *Journal of Plant Physiology* **281**, 153916 (2023).
8. Liu, B. et al. Effects of saline-alkali stress on bacterial and fungal community diversity in Leymus chinensis rhizosphere soil. *Environmental Science and Pollution Research* **29**, 70000–70013 (2022).
9. Bencherif, K. et al. Impact of soil salinity on arbuscular mycorrhizal fungi biodiversity and microflora biomass associated with Tamarix articulata Vahll rhizosphere in arid and semi-arid Algerian areas. *Science of the Total Environment* **533**, 488–494 (2015).

10. Yang, H. T. et al. Nutrients addition decreases soil fungal diversity and alters fungal guilds and co-occurrence networks in a semi-arid grassland in northern China. *Science of The Total Environment* **926**, 172100 (2024).
11. Bi, B. Y. et al. Dynamic changes of soil microbial community in *Pinus sylvestris* var. mongolica plantations in the Mu Us Sandy Land. *J. Environ. Manag* **287**, 112306 (2021).
12. Chi, Z. F. et al. Soil organic matter and salinityas critical factors affecting the bacterial community and function of *Phragmites australis* dominated riparian and coastal wetlands. *Sci. Total Environ* **762**, 143156 (2021).
13. Fierer, N. Embracing the unknown: Disentangling the complexities of the soil microbiome. *Nat. Rev. Microbiol* **15**, 579–590 (2017).
14. Zhang, C. et al. Elaeagnus angustifolia can improve salt-alkali soil and the health level of soil: Emphasizing the driving role of core microbial communities. *Journal of Environmental Management* **305**, 114401 (2022).
15. Meng, H. et al. Pedogenesis of typical zonal soil drives belowground bacterial communities of arable land in the Northeast China Plain. *Scientific Reports* **13**, 14555 (2023).
16. Cao, H. et al. Effect of pH on the surface charges of permanently/variably charged soils and clay minerals. *Scientific Reports* **14**, 23169 (2024).
17. Che, R. et al. Increase in ammonia-oxidizing microbe abundance during degradation of alpine meadows may lead to greater soil nitrogen loss. *Biogeochemistry* **136**, 341–352 (2017).
18. Zeng, J. et al. Nitrogen fertilization directly affects soil bacterial diversity and indirectly affects bacterial community composition. *Soil Biol. Biochem* **92**, 41–49 (2016).
19. Sun, G. F. et al. Water-heat-salt effects of mulched drip irrigation maize with different irrigation scheduling in Hetao Irrigation District. *Transactions of the Chinese Society of Agricultural Engineering* **33**, 144–152 (2017).
20. Judith, K. C. et al. Effects of tillage practices on soil microbiome and agricultural parameters. *Science of The Total Environment* **705**, 135791 (2020).
21. Alicia, M. C. et al. The impact of crop diversification, tillage and fertilization type on soil total microbial, fungal and bacterial abundance: A worldwide meta-analysis of agricultural sites. *Agriculture, Ecosystems & Environment* **329**, 107867 (2022).
22. Guo, S. S. et al. Analysis on spatial-temporal evolution of soil salinity and its driving factors in Hetao Irrigation District during recent 30 years. *China Rural Water and Hydropower* **167**, 159–162 (2016).
23. Yang, J. S. et al. Research on ecological management and ecological industry development model of saline-alkali land in the Hetao Plain, China. *Acta Ecologica Sinica* **36**, 7059–7063 (2016).
24. Peng, Z. Y. et al. Water and salt movement under partial irrigation in Hetao Irrigation District, Inner Mongolia. *Journal of Hydraulic Engineering* **47**, 110–118 (2016).
25. Bao, S. D. *Soil Agrochemical Analysis* (China Agriculture Press, 2020).
26. Chen, S. et al. Fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics* **34**, 1884–1890 (2018).
27. Sheng, Y. H. et al. Molecular determinants of response kinetics of mouse M1 intrinsically-photosensitive retinal ganglion cells. *Scientific Reports* **11**, 23424 (2021).
28. Callahan, B. J. et al. Ultra-accurate microbial amplicon sequencing with synthetic long reads. *Microbiome* **9**, 120 (2021).
29. Li, Z. Y. et al. New insights into biologic interpretation of bioinformatic pipelines for fish eDNA metabarcoding: A case study in Pearl River estuary. *Journal of Environmental Management* **369**, 122136 (2024).
30. Guo, X. Y. et al. Succession of the microbial communities and function prediction during short-term peach sawdust-based composting. *Bioresource Technology* **332**, 125079 (2021).
31. De'ath, G. Boosted trees for ecological modeling and prediction. *Ecology* **88**, 243–251 (2007).
32. Chen, X. Y. & Sarkar, S. K. Benjamini–Hochberg procedure applied to mid *p*-values. *Journal of Statistical Planning and Inference* **205**, 34–45 (2020).
33. Juan, G. R. et al. Visualizing large knowledge graphs: A performance analysis. *Future Generation Computer Systems* **89**, 224–238 (2018).
34. Zhou, H. et al. Network analysis reveals the strengthening of microbial interaction in biological soil crust development in the Mu Us Sandy Land, northwestern China. *Soil Biology and Biochemistry* **144**, 107782 (2020).
35. Barberán, A. et al. Using network analysis to explore co-occurrence patterns in soil microbial communities. *ISME J* **6**, 343–351 (2012).
36. Tarcísio, D. S. et al. Mutualistic interaction network structure between bird and plant species in a semi-arid Neotropical environment. *Acta Oecologica* **118**, 103897 (2023).
37. Cui, Q. et al. Biochar and effective microorganisms promote Sesbania cannabina growth and soil quality in the coastal saline-alkali soil of the Yellow River Delta, China. *Sci. Total Environ* **756**, 143801 (2021).
38. Guo, J. et al. Deciphering soil bacterial community structure in subsidence area caused by underground coal mining in arid and semiarid area. *Appl. Soil Ecol* **163**, 103916 (2021).
39. Toju, H. et al. Core microbiomes for sustainable agroecosystems. *Native Plants* **4**, 247–257 (2018).
40. Rath, K. M. et al. Linking bacterial community composition to soil salinity along environmental gradients. *ISME J.* **13**, 836–846 (2019).
41. Shao, T. Y. et al. Industrial crop Jerusalem artichoke restored coastal saline soil quality by reducing salt and increasing diversity of bacterial community. *Appl. Soil Ecol* **138**, 195–206 (2019).
42. Li, N. et al. Effects of planting *Melia azedarach* L. on soil properties and microbial community in saline-alkali soil. *Land Degrad. Dev* **32**, 2951–2961 (2021).
43. Shi, Y. et al. Effects of combined biochar and organic fertilizer on nitrous oxide fluxes and the related nitrifier and denitrifier communities in a saline-alkali soil. *Sci. Total Environ* **686**, 199–211 (2019).
44. Cui, Y. X. et al. Diversity patterns of the rhizosphere and bulk soil microbial communities along an altitudinal gradient in an alpine ecosystem of the eastern Tibetan Plateau. *Geoderma* **338**, 118–127 (2019).
45. Yang, T. et al. Soil fungal diversity in natural grasslands of the Tibetan Plateau: Associations with plant diversity and productivity. *New Phytol* **215**, 756–765 (2017).
46. Wang, S. et al. Exploring soil factors determining composition and structure of the bacterial communities in saline-alkali soils of Songnen Plain. *Frontiers in Microbiology* **10**, 2902 (2020).
47. Li, M. C. et al. Decoupling soil community structure, functional composition, and nitrogen metabolic activity driven by salinity in coastal wetlands. *Soil Biology and Biochemistry* **198**, 109547 (2024).
48. Tripathi, B. M. et al. Soil pH mediates the balance between stochastic and deterministic assembly of bacteria. *ISME J* **12**, 1072–1083 (2018).
49. Zhao, Q. et al. The influence of soil physicochemical properties and enzyme activities on soil quality of saline-alkali agroecosystems in Western Jilin Province, China. *Sustainability* **10**, 1529 (2018).
50. Xia, J. B. et al. Forest and grass composite patterns improve the soil quality in the coastal saline-alkali land of the Yellow River Delta, China. *Geoderma* **349**, 25–35 (2019).
51. Ren, H. et al. Biochar and rhizobacteria amendments improve several soil properties and bacterial diversity. *Microorganisms* **8**, 502–519 (2020).
52. Zhang, Z. M. et al. Diversity of microbial community structure in the spermosphere of saline-alkali soil in Shandong area. *Acta Microbiol Sin* **57**, 582–596 (2017).
53. Gong, X. W. et al. Linkages of microbial community structure and root exudates: Evidence from microbial nitrogen limitation in soils of crop families. *Science of The Total Environment* **881**, 163536 (2023).

54. Nuccio, E. E. et al. Climate and edaphic controllers influence rhizosphere community assembly for a wild annual grass. *Ecology* **97**, 1307–1318 (2016).
55. Liu, S. W. et al. Ecological stability of microbial communities in Lake Donghu regulated by keystone taxa. *Ecological Indicators* **136**, 108695 (2022).
56. Pringault, O. et al. Consequences of contaminant mixture on the dynamics and functional diversity of bacterioplankton in a southwestern Mediterranean coastal ecosystem. *Chemosphere* **144**, 1060–1073 (2016).
57. Zhu, C. et al. N-fertilizerdriven association between the arbuscular mycorrhizal fungal community and diazotrophic community impacts wheat yield. *Agric. Ecosyst. Environ* **254**, 191–201 (2018).
58. Zeng, Q. C. et al. Soil bacterial community response to vegetation succession after fencing in the grassland of China. *Sci. Total Environ* **609**, 2–10 (2017).
59. Zhang, C. et al. Soil bacterial community dynamics reflect changes in plant community and soil properties during the secondary succession of abandoned farmland in the Loess Plateau. *Soil Biol. Biochem* **97**, 40–49 (2016).
60. Luisa, M. M. et al. The enzyme patterns of Ascomycota and Basidiomycota fungi reveal their different functions in soil. *Applied Soil Ecology* **196**, 105323 (2024).

Author contributions

Xiaoyu Zhao wrote primary and revised versions of manuscript and main data analysis. Julin Gao and Xiaofang Yu conceived project idea, and design, and provided suggestions for revising the paper. Bizhou Zhang, Sainan Zhang, Jiangan Guo, Dongbo Li recorded and measured the test data. Dongbo Li and Jiangan Guo collected soil sample. Qinggeer Borjigin, Jiawei Qu critically, Qiang Li reviewed the full manuscript content. All authors read and approved the final manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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