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Response of Rhizosphere Soil Microbial Community Structure to the Natural Succession of Saline-Alkali Tolerant Vegetation on the Songnen Plain

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Abstract: Soil salinization and alkalization is a key factor limiting the sustainable development of global agriculture. The natural succession of vegetation in saline-alkali soil is an effective method for improving of saline-alkali soil, and microorganisms play an important role in vegetation growth. This study analysed the microbial community structure and diversity of saline-alkali tolerant vegetation rhizosphere soil in northeastern China where natural succession of saline-alkali tolerant vegetation has occurred, using high-throughput sequencing technology. We found that the community structures of bacteria and eukaryotic microorganisms were substantially different with the vegetation natural succession. The bacterial community changed from Actinobacteria-dominated to Proteobacteria-dominated communities. The eukaryotic microorganismal community generally transitioned from alga-dominated (Streptophyta and Ochrophyta) to alga-dominated (Streptophyta and Ochrophyta) and fungi-dominated (Ascomycota) to fungi-dominated (Ascomycota) communities. Based on the difference analysis of microbial community compositions and basic physicochemical properties with the natural succession of vegetation, we found that the bacterial groups that played a key role in the natural succession of the vegetation were Actinobacteria and Proteobacteria and that the eukaryotic microbial groups were Ascomycota, Nematoda, and Streptophyta. The results provide theoretical guidance for microbial inoculation to promote plant succession and improve low-yield saline-alkali soils.

Keywords: High-throughput Sequencing; Technology; Natural Succession; Soil Salinization and Alkalization.

1. Introduction

Soil salinization is one of the main factors limiting the sustainable development of global agriculture. This problem existed before the emergence of humans and agriculture, but it has become increasingly serious with the development of human agricultural practices such as irrigation [1]. Almost half of the world's irrigated lands and approximately 20% of its cultivated lands are affected by salinization [2]. The intensification of soil salinization on cultivated lands may have a catastrophic impact on global agriculture, likely resulting in a 50% loss of cultivated lands by 20503.

The Songnen Plain (43°30'– 48°40'N, 121°30'–127°00'E) located in northeastern China is an important commodity grain production base, and its animal husbandry industry is also substantially developed [4]. However, with the disturbance of human activities in recent decades, areas of saline-alkali soils on the Songnen Plain have increased, resulting in the loss of large-scale cultivated lands [5]. The Songnen Plain is one of the world's three major soda saline-alkali soil areas, which accumulates higher sodium carbonate (NaCO3) and sodium bicarbonate (NaHCO3) and has a higher pH than that in other areas [6]. Given the high salinity and pH stress, most crops and plants are unable to grow, but some saline-alkali tolerant plants can live in this extreme environment; in addition, their natural succession has occurred. The succession of the dominant species of the vegetation community in soda saline-alkali soil is as follows. Initially, Suaeda salsa were planted as pioneer species in the bare land. S. salsa and Puccinellia tenuiflora dominated the community during the early successional stage. As the succession proceed, other gramineous plants emerged as companion species in the above plant community. Then, the plant community generally transitioned from dominance by S. salsa and P. tenuiflora to dominance by Aneurolepidium chinense and other gramineous plants during the middle phase of succession. Finally, A. chinense dominated the climax community. Thus, it is important to study the adaption and succession mechanisms of saline-alkali tolerant plants to extreme environments, and the application of the adaptation and succession mechanisms of these plants on saline-alkali soil has important practical significance for increasing the world's available cultivated land resources. To date, there have been many studies that have made progress on understanding the breeding of saline-alkali plants, improving molecular genetics, identifying specific saline-alkali tolerance genes, and understanding the saline-alkali tolerant mechanisms of plants [7-10].

In extreme soil ecosystems, the limiting factors for plant growth are usually mineral elements [11]. Typically, plants release nutrients such as carbon (C) and nitrogen (N) through their roots to the rhizosphere microecosystem, affecting the metabolism of some microorganisms. Soil microorganisms, in turn, decompose soil organic matter, plant litter, and animal residues into nutrients such as nitrogen (N) and phosphorus (P), which strongly affect plant growth [12]. In the interaction between plants and microorganisms, soil exists as a medium. The interaction among plants, soil and microorganisms forms a microecosystem. Therefore, research on the functions of microorganisms in microecosystems is important.

The processing of isolating and identifying microorganisms based on pure culture technology have resulted in successful progress in terms of soda saline-alkali soil [13-15]. However, these studies mostly analysed the characteristics of certain species of microorganisms and scarcely analysed the changes in microbial community

structure during vegetation natural succession because many microorganisms in the soil cannot be easily cultivated [16]. With the increase in and development of sequencing technology, researchers have been able to use this technology to study belowground microbial communities more comprehensively and accurately than before. Based on this technology, Chao et al. studied the dynamic changes in soil bacterial communities during secondary succession of abandoned farmland on the Loess Plateau, China [11], Wang and Guo analysed the response of soil fungal communities to the succession of saline vegetation in the Yellow River Delta, China [17], and Zhao et al. studied the dynamic changes in soil microbes during the succession of mountain vegetation in the southwestern karst area of China [18]. However, there is little research on the response of soil microbial communities to the natural succession of vegetation in soda saline-alkali soils in northeastern China.

In this study, we used the rhizosphere soil of saline-alkali tolerant vegetation from the soda saline-alkali soil on the Songnen Plain as an example, and Illumina Hiseq high-throughput sequencing technology was used to analyse the differences in the microorganismal community diversity and abundance of the rhizosphere soil of the main vegetation at different stages of the vegetation succession process. Our goals were to study the distribution of soil bacteria and eukaryotic microorganisms in soda saline-alkali soils and their response to the natural succession of saline-alkali tolerant vegetation. The results may provide a theoretical reference for microbial improvements in low-yield soda saline-alkali soil.

2. Materials and Methods

2.1. Study Site and Soil Sampling

Soils were collected from the Alkali Soil Natural Environment Science Center (46°27′N, 125°22′E, 150 m a.s.l.) of Northeast Forestry University (Anda practice base) [4], which built in 2002 and is a long-term experimental base. The plot is approximately 5 km northeast of Anda city, Heilongjiang Province, northeast China. The ecosystem in this area is considered to have experienced the most severe secondary salinization in China. The site has a northern temperate continental semi-arid monsoon climate. The annual average temperature is 3.2 °C, and the annual average precipitation is 418.7 mm. The soil is a typical calcium black soil with high carbonate, bicarbonate, and pH values. The dominant saline-alkali tolerant vegetation types in the study area consist of *Suaeda salsa* and *Puccinellia tenuiflora*, and *Aneurolepidium chinense*. The main soluble salts that stress plant growth in soda saline-alkali soil are sodium carbonate and sodium bicarbonate. According to the degree of tolerance to sodium carbonate, sodium bicarbonate and pH, *Suaeda salsa* are severe saline-alkali tolerant plants and *Puccinellia tenuiflora* and *Aneurolepidium chinense* are moderate saline-alkali tolerant plants.

To study the effects of the roots of newly grown plants on rhizosphere microorganisms, the rhizosphere soil of various newly grown plants was taken at the experimental base on May 20, 2016. We selected three vegetation rhizosphere soils that dominated each succession stage. Four kinds of soil samples below were collected: soil with almost no vegetation cover roots (AS), rhizosphere soil of the annual herb *S. salsa* with weak (S), the rhizosphere soil of the perennial herb *P. tenuiflora* with more developed roots (P), and the rhizosphere soil of the perennial grass *A. chinense* with the most developed roots (A). After the removal of visible stones, plant roots, and debris on the ground surface, a soil sample without vegetation cover was collected at a depth of 0–20 cm by a five-point sampling method. Each rhizosphere soil sample of saline-alkali tolerant vegetation was collected as follows: all vegetation in 9 random plots (20 cm x 20 cm) was uprooted, and the loose soil was removed by shaking. Then, the soil that adhered to the root was removed, and three soil samples were mixed as one sample. Three replicates of each soil sample. All samples were placed in an ice box and then transported to the laboratory. The pretreated soil samples were divided into two parts. One part was stored at -80°C until the molecular biology analysis, and the other part was air-dried and sieved (2 mm) for basic physicochemical properties analysis.

2.2. Determine Basic Physicochemical Properties of the Soil Samples

The electrical conductivity (EC) of the soil samples was determined using a conductivity test pen (ST10C-B, OHAUS, USA) according to the instructions. The pH of the soil sample was determined by a pH metre (FE28, METTLER TOLEDO, Switzerland) in 1:2.5 soil:ultrapure water suspensions. The ammonium (NH_4^+) content was determined by the indophenol blue colorimetric method [19]. Nitrate (NO_3^-) was determined using an ion chromatograph (883 basic IC plus, Metrohm, Switzerland). The contents of carbonate (CO_3^{2-}) and bicarbonate (HCO_3^-) were determined with the double indicator neutralization titration method [20].

2.3. DNA Extraction

The total DNA of the soil microbes was extracted from a 0.5 g soil sample using the ISOIL for Beads Beating soil DNA extraction kit (Nippon Gene, Toyama, Japan) according to the kit instructions. A 2% agarose gel electrophoresis was conducted to examine the integrity of the extracted DNA. The extracted DNA was diluted to 1 ng/μL using sterilized water, and the diluted DNA was used as the PCR amplification template. The concentration and quality of the extracted and diluted DNA were assessed using a Microvolume UV-Vis Spectrophotometer (NanoDrop one, Thermo Fisher Scientific, USA).

2.4. PCR Amplification and Illumina Hiseq high-throughput Sequence

The hypervariable V4 region of the 16S rRNA gene from the bacteria was amplified with the primer set 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') [21]. The hypervariable V4 region of the 18S rRNA gene from the eukaryotic microorganisms was amplified with the primer set 528F (5'-GCGGTAATTCCAGCTCCAA-3') and 706R (5'-AATCCRAGAATTTCACCTCT-3') [22]. The above primers were tagged with barcodes for each amplification. All PCRs were carried out inμL 30 μL reactions consisting of μL15 μL of Phusion® High-Fidelity PCR Master Mix with GC Buffer and Phusion® High-Fidelity DNA polymerases (New England Biolabs), 0.2 μM of forward and reverse primers, and approximately 10 ng of template DNA. The PCR procedure was as follows: 95 °C for 5 min; 25 cycles of 95 °C for 30 s, 55 °C for 30 s, 72 °C for 40 s; and 72 °C for 10 min [23]. A 2% agarose gel electrophoresis was conducted to examine the PCR amplification (mixed with loading buffer) effect. Then, the successful amplification PCR products (with a bright main

strip) were extracted and purified with the GeneJET Gel Extraction Kit (Thermo Scientific). According to the concentration of purified PCR products in triplicate, these products were mixed at equimolar ratios for high-throughput sequencing on the Illumina Hiseq platform (Novogene, Tianjin, China).

2.5. Statistical Analysis

The raw reads obtained by high-throughput sequencing of the sample were subjected to barcode and primer removal, split, filtration, and chimaera removal, and effective sequences for the subsequent analysis were obtained. The effective sequences with greater than 97% similarity were classified into the same operational taxonomic units (OTUs) using 'Uparse' (v7.0.1001) [24]. Species annotation analysis based on OTU representative sequences was performed using the Mothur method and the SSUrRNA database [25] of SILVA (http://www.arb-silva.de/) [26] to obtain taxonomic information and calculate the community composition of each sample at each classification level. OTU abundance information was homogenization using a standard sequence number corresponding to the sample with the least sequences. Subsequent analyses of alpha diversity and beta diversity were all performed based on these output homogenization data. QIIME software (Version 1.7.0) was used to calculate the Chaol index, the Shannon index, the Simpson index [27,28], and library coverage. Rarefaction curves were drawn using R software (Version 2.15.3). The principal component analysis (PCA) diagram was constructed using the ade4 package and the ggplot2 package of R software (Version 2.15.3). Statistical analyses, such as the means, standard deviation, one-way analysis of variation (ANOVA), and a multiple comparison of least significant difference (LSD), were performed using SPSS Statistics17.0.

2.6. Accession Numbers

The raw sequences obtained in this study have been deposited in the Sequence Read Archive (SRA) of NCBI and the accession numbers of the prokaryotic and eukaryotic microorganisms are PRJNA596089 and PRJNA596096, respectively.

3. Results

3.1. Saline-alkali Soil Basic Physicochemical Properties

The basic physicochemical properties of different soil samples are shown in Table 1. Some basic physicochemical properties of soil samples during the vegetation natural succession were substantially different (Table 1). Soil EC can represent the total soluble salt content of soda saline-alkali soil [17]. The soil EC at 25 ℃ ranged from 0.89±0.01 to 1.21±0.01 ms cm^{-1}. In the succession of vegetation P to A, the EC decreased significantly ($P<0.05$) With the natural succession of vegetation, the contents of soil CO_3^{2-}, HCO_3^-, and pH had a downward trend. During S to P succession, pH decreased significantly ($P<0.05$), and the moderate saline-alkali tolerant plant *P. tenuiflora* had the lowest pH. During the process of severe saline-alkali tolerant plant *S. salsa* colonized bare land, CO_3^{2-} decreased significantly ($P<0.05$), and the moderate saline-alkali tolerant plant *P. tenuiflora* had the lowest CO_3^{2-} contents. During the succession AS to S to P, HCO_3^- decreased significantly ($P<0.05$), and the moderate saline-alkali tolerant plant *A. chinense* had the lowest HCO_3^- contents. With the natural succession of vegetation, soil NO_3^- and NH_4^+ contents did not change significantly, but the content had an upward trend, and the moderate saline-alkali tolerant plant *A. chinense* had the highest NO_3^- and NH_4^+. The large differences about the physicochemical properties during the natural succession suggested that the colonization and succession of saline-alkali tolerant vegetation played an important role in physicochemical properties. Since the changes in soil physicochemical properties are not only affected by plant root metabolism but also related to rhizosphere soil microorganisms, we next analysed the change of community structure of the soil microorganisms in the vegetation natural succession.

Table 1. Saline-alkali soil basic physicochemical properties.

Sample names	AS	S	P	A
EC (25 ℃, ms cm^{-1})	1.01±0.01c	1.07±0.02b	1.21±0.01a	0.89±0.01d
pH	10.46±0.44a	10.05±0.17a	9.40±0.26b	9.47±0.23b
CO_3^{2-} (mg kg^{-1})	276.1±44.50a	164.96±21.12b	128.82±14.34b	158.21±45.09b
HCO_3^- (mg kg^{-1})	476.30±44.56a	271.47±39.88b	154.75±32.52c	145.13±22.86c
NO_3^- (mg kg^{-1})	0.12±0.08a	0.73±0.58a	0.6±0.56a	0.91±0.02a
NH_4^+ (mg kg^{-1})	0.7±0.21a	0.7±0.39a	0.88±0.36a	0.91±0.11a

AS, without vegetation cover; S, *S. salsa*; P, *P. tenuiflora*; A, *A. chinense*

The value is the mean ± standard deviation with three replicates. Different letters in the same line indicate significant differences based on one-way ANOVA followed by an LSD test ($p < 0.05$)

3.2. Sequencing Library Information and Analysis of Richness and Diversity of Microorganisms

High-throughput sequencing library information and microbial community richness and diversity are shown in Table 2. The sequence library coverage of all samples was more than 98.8%, indicating that the microbial gene sequences in the four soil samples were almost always detected. The sequencing results almost reflect the true situation of the soil microorganisms in the soda saline-alkali soils. At the same time, the rarefaction curve tended to be flat, which indicated that the amount of sequencing data increasingly became reasonable and that more sequence will only produce a small number of OTUs.

The OTUs of the bacteria decreased with the vegetation natural succession, especially that in the moderate saline-alkali tolerant plants *A. chinense*, which had the lowest OTUs. The Chao1 index indicated that the richness of bacteria decreased with the vegetation natural succession from AS to

S to P, especially in the moderate saline-alkali tolerant plants *P. tenuiflora*, which had the lowest bacterial richness. The Shannon index indicated that the diversity of bacteria decreased with the vegetation natural succession, especially in the moderate saline-alkali tolerant plants *A. chinense*, which had the lowest bacterial diversity. These results were also supported by the rarefaction curves of the bacteria. The above results suggest that the diversity and richness of the soil bacterial community responded to the natural succession of saline-alkali-tolerant vegetation.

The OTUs of the eukaryotic microorganisms decreased significantly during the process of severe saline-alkali tolerant plant *S. salsa* colonized bare land, but then increased significantly with the succession from S to P to A, especially the moderate saline-alkali tolerant vegetation *A. chinense*, which had the highest OTUs ($P<0.05$). The Chao1 index indicated that the richness of eukaryotic microorganisms decreased during the process of severe saline-alkali tolerant plant *S. salsa* colonized bare land, but then increased with the succession from S to P to A ,especially the moderate saline-alkali tolerant vegetation *A. chinense*, which had the highest richness The Shannon index indicated that the diversity of the eukaryotic microorganisms decreased significantly during the process of severe saline-alkali tolerant plant *S. salsa* colonized bare land, but then increased with the succession from S to P to A, especially the moderate saline-alkali tolerant vegetation *A. chinense*, which had the highest diversity ($P<0.05$). These results were also supported by the rarefaction curves of the eukaryotic microorganisms. The above results suggest that the diversity and richness of the soil eukaryotic microorganism community responded to the natural succession of the saline-alkali tolerant vegetation.

Table 2. Sequencing library information and analysis of the diversity and richness of the microbial communities.

	Samples	OTUs	Chao1 index[a]	Shannon index[a]	Coverage[a]
Bacteria (16S rDNA)	AS	3,040.33±668.67a	2,992.24±652.73a	8.62±0.54a	98.80%±0.26%
	S	2,908.67±795.64a	2,831.87±761.06a	8.59±0.80a	98.90%±0.35%
	P	2,528±698.96a	2,463.37±664.84a	8.08±0.62a	99.03%±0.31%
	A	2,507.33±275.95a	2,502.41±495.25a	7.82±0.34a	99%±0.46%
Eukaryotic microorganisms (18S rDNA)	AS	477±12.49ab	483.60±34.76a	5.80±0.67a	99.83%±0.06%
	S	382.67±43.73b	419.62±44.32a	3.73±0.92b	99.77%±0.06%
	P	490.67±81.62ab	501.01±86.01a	5.77±0.29a	99.77%±0.06%
	A	515.33±76.71a	539.35±106.27a	6.41±0.28a	99.8%±0.1%

[a] Calculation-based OTU (97% sequence similarity). AS, without vegetation cover; S, *S. salsa*; P, *P. tenuiflora*; A, *A. chinense*.

The value is the mean ± standard deviation with three replicates. Different letters in the same column indicate significant differences based on one-way ANOVA followed by an LSD test ($p < 0.05$). The Chao1 index is used to show the microbial community richness. The higher the value is, the higher the community richness. The Shannon index is used to indicate the microbial community diversity. The higher the value is, the higher the community diversity.

3.3. Microbial Community Composition Changes with Vegetation Natural Succession

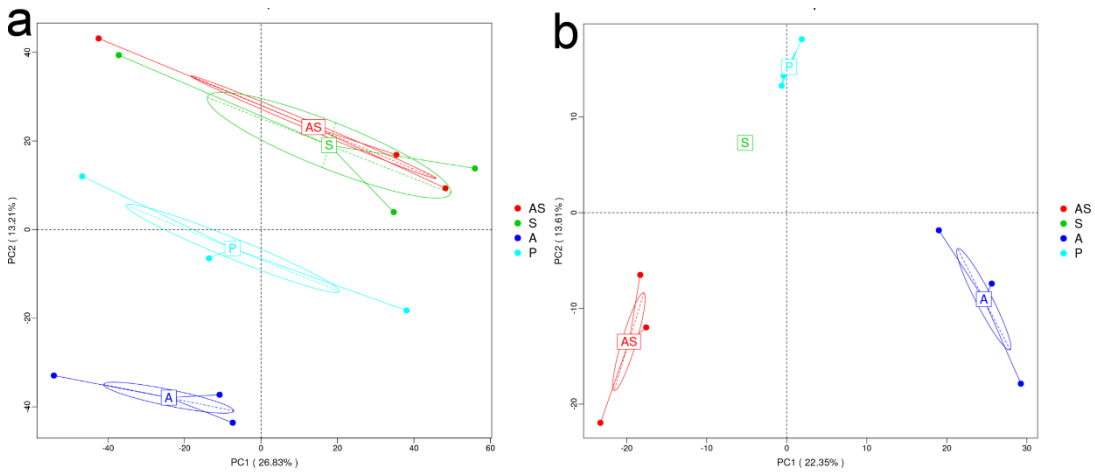


Figure 1. Principal component analysis (PCA) of microbial community composition based on OTUs. Bacterial community composition (**a**) and eukaryotic microorganism community composition (**b**). AS, without vegetation cover; S, *S. salsa*; P, *P. tenuiflora*; A, *A. chinense*; b, bacteria; and e, eukaryotic microorganisms. The abscissa and the ordinate represent the first principal component and the second principal component, respectively. The percentage on the axis indicates the contribution of the first or the second principal component to the difference between the different samples. Each point represents one sample, and the same sample is represented by the same colour.

To analyse the differences in the microbial community compositions in soda saline-alkali soil, PCA based on the

OTU level was constructed (Fig. 1a and 1b). The contribution rates of the first principal component (PC1) and the second principal component (PC2) were 26.83% and 13.21%, respectively (Fig. 1a). Based on the distance, the differences in the bacterial community structure between different samples increased gradually with the vegetation natural succession proceed (Fig. 1a). The result suggested that the natural succession of vegetation led to differences increasing in bacterial community composition between different succession stages.

The contribution rates of the first principal component (PC1) and the second principal component (PC2) were 22.35% and 13.61%, respectively (Fig. 1b). Based on the distance, it can be seen that the soil eukaryotic microbial community composition differences decreases (AS→S→P) and then increases (P→A) with the natural succession of vegetation.

To study the differences in microorganisms between different successional stages in soda saline-alkali soil, the changes in the relative abundance of the soil microorganisms at the phylum level were constructed (Fig. 2a). The TOP 10 phylum of bacteria was Proteobacteria (33.32% on average), followed by Actinobacteria (21.61%), Gemmatimonadetes (13.90%), Firmicutes (9.24%), Bacteroidetes (5.40%), Acidobacteria (4.35%), Chloroflexi (2.21%), and Nitrospirae (1.46%). The relative abundance of some bacteria between different successional stages in the TOP 10 phylum level showed substantial differences (Fig. 3), such as Proteobacteria, Actinobacteria, Gemmatimonadetes, Firmicutes, Bacteroidetes, and Chloroflexi.

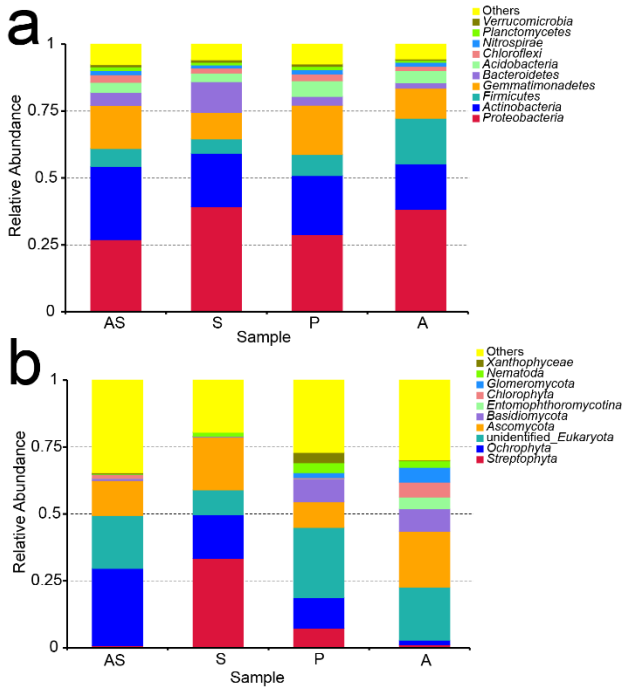


Figure 2. Changes in the relative abundance of the soil microorganisms in the TOP 10 phylum level. Bacterial community composition (**a**) and eukaryotic microorganism community composition (**b**). AS, without vegetation cover; S, *S. salsa*; P, *P. tenuiflora*; and A, *A. chinense*. Different colors indicate different species (see the legend of the figure)

The dominant TOP 10 phylum of eukaryotic microorganisms was unidentified_Eukaryota (18.77% on average), followed by Ascomycota (15.82%, fungi), Ochrophyta (14.52%, algae flora), Streptophyta (10.67%, algae), Basidiomycota (4.50%, fungi), Nematoda (1.95%, metazoa), Glomeromycota (1.88%, fungi), and Chlorophyta (1.84%, algae). The relative abundance of some eukaryotic microorganisms between successional stages in the TOP 10 phylum level showed substantial differences (Fig. 3b), such as unidentified_Eukaryota, Ascomycota, Ochrophyta, Streptophyta, Nematoda, Glomeromycota, Xanthophyceae, and Chlorophyta.

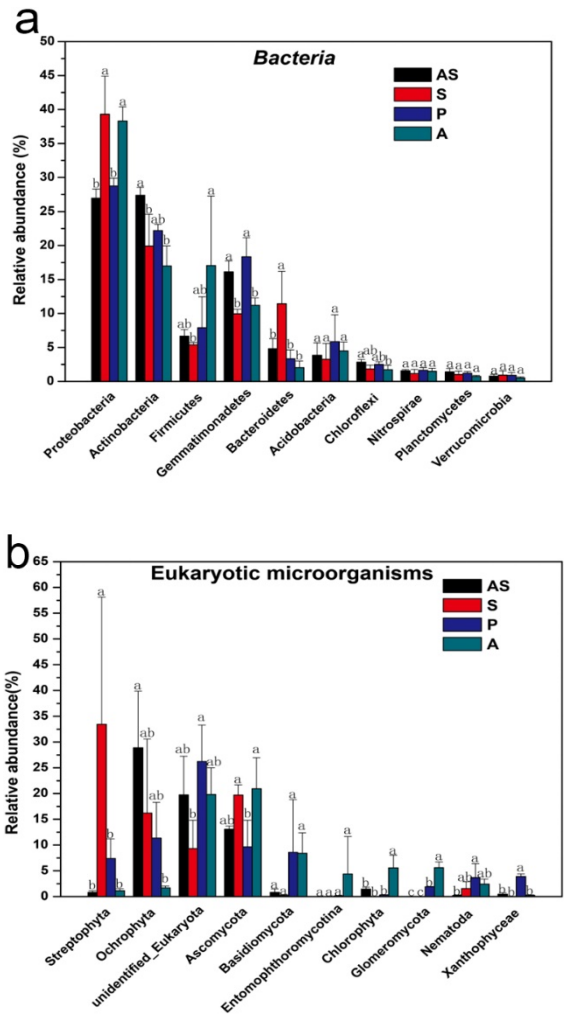


Figure 3. Changes in the relative abundance of the soil microorganisms in the TOP 10 phylum level. Bacterial community composition (**a**) and eukaryotic microorganism community composition (**b**). AS, without vegetation cover; S, *S. salsa*; P, *P. tenuiflora*; and A, *A. chinense*. The same sample is represented by the same colour (see the legend of the figure). The values are the mean ± standard deviation (n = 3). Different letters indicate significant differences ($P < 0.05$)

To further analyse the dominant phylum, the changes in the major classes between different successional stages were constructed (Fig. 4). Among the Proteobacteria, Gamma-, Alpha-, Delta-, Beta- and Epsilon-classes were found in all samples, and unidentified Proteobacteria were found in all samples (Fig. 4a). Alphaproteobacteria occupied 14.37% of all bacteria and dominated this phylum. The relative abundance of Alphaproteobacteria, Gammaproteobacteria, and Deltaproteobacteria changed significantly with the succession of vegetation ($P<0.05$). Gammaproteobacteria, Deltaproteobacteria, Betaproteobacteria, and

Epsilonproteobacteria were contained in 12.85%, 4.45%, 1.52%, and 0.0086% of all bacteria, respectively. Acidimicrobiia were dominant in Actinobacteria, many unidentified Actinobacteria (in 7.2% of bacteria) were found, and the relative abundance of Acidimicrobiia, Thermoleophilia, Nitriliruptoria changed significantly with the succession of vegetation ($P<0.05$). The classes in Gemmatimonadetes were all unidentified (13.9%). Bacilli dominated Firmicutes. Cytophagia dominated Bacteroidetes, and the relative abundance of Cytophagia, Flavobacteriia, and Sphingobacteriia changed significantly with the succession of vegetation ($P<0.05$). Holophagae dominated Acidobacteria, and unidentified-Acidobacteria were found (3.6%). Anaerolineae dominated Chloroflexi, and unidentified Chloroflexi were found (1.82%), and the relative abundance of Ardenticatenia changed significantly with the succession of vegetation ($P<0.05$) Nitrospira was the only species found in Nitrospirae.

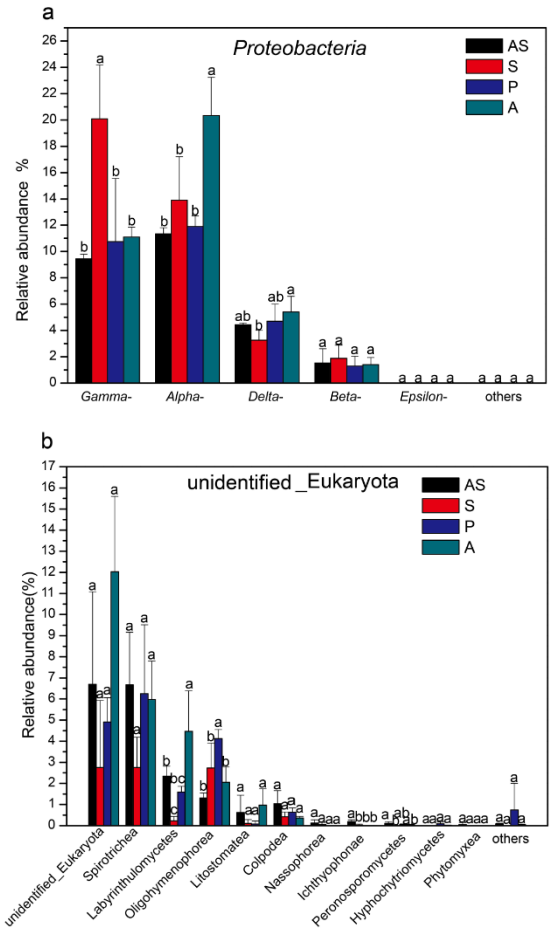


Figure 4. Relative abundance of *Proteobacteria* (**a**) unidentified *Eukaryota* (b) and at the class level. AS, without vegetation cover; S, *S. salsa*; P, *P. tenuiflora*; and A, *A. chinense*. The same sample is represented by the same colour (see the legend of the figure). The values are the mean ± standard deviation (n = 3). Different letters indicate significant differences ($P < 0.05$).

Except for Phytomyxea, the other classes were found in all the samples. Among unidentified Eukaryota, Spirotrichea occupied 6.59% of all the eukaryotic microorganisms and dominated this phylum. The relative abundance of Labyrinthulomycetes, Oligohymenophorea, Ichthyophonae, and Peronosporomycetes changed significantly with the succession of vegetation ($P<0.05$). Unidentified Eukaryota, Labyrinthulomycetes, Oligohymenophorea, Litostomatea, Colpodea, Nassophorea, Ichthyophonae, Peronosporomycetes, Phytomyxea, and Hyphochytriomycetes accounted for 5.41%, 2.15%, 2.55%, 0.44%, 0.62%, 0.048%, 0.06%, 0.053%, 0.026%, and 0.013%, respectively, of the soil microorganisms. Dothideomycetes dominated Ascomycota, the relative abundance of Dothideomycetes, Sordariomycetes, Eurotiomycetes, Pezizomycetes, Saccharomycetes, and Laboulbeniomycetes changed significantly with the succession of vegetation ($P<0.05$). Chrysophyceae dominated Ochrophyta, and a higher relative abundance of unidentified Ochrophyta was found in this phylum. Liliopsida dominated Streptophyta and the relative abundance changed significantly with succession ($P<0.05$)., and unidentified Streptophyta were found in this phylum. Agaricostilbomycetes dominated Basidiomycota and the relative abundance changed significantly with succession ($P<0.05$)., and unidentified Basidiomycota were found in this phylum. Nematoda consisted of Chromadorea and Enoplea, Chromadorea were dominant in the phylum, and the relative abundance of Chromadorea changed significantly with the succession of vegetation ($P<0.05$). Glomeromycetes were only found in Glomeromycota, and the relative abundance of Glomeromycetes changed significantly with the succession of vegetation ($P<0.05$). Chlorophyceae dominated Chlorophyta, and the relative abundance of Chlorophyceae and Pedinophyceae changed significantly with the succession of vegetation ($P<0.05$).

4. Discussion

In our study, after analysing the differences in the basic physicochemical properties (Table 1), we found that the plants dominated by *S. salsa* may play an intermediate transition role in the change in physicochemical properties. With the success of *S. salsa* colonization, we found that pH and the CO_3^{2-} and HCO_3^- concentrations decreased, while the NO_3^- concentration increased, which provided advantageous conditions for the natural succession of subsequent vegetation. The pH, CO_3^{2-}, and HCO_3^- contents at the S decreased by approximately 3.9%, 40.25%, and 43%, respectively, compared to those at the AS This result indicated that intermediate transition plants *S. salsa* could not only decrease the most critical limiting factors for plant growth in saline-alkali soils, such as pH and carbonate and supercarbonate contents, but also increase the content of nitrogen (such as NO_3^-) as one of the key factors for plant growth. This conclusion is similar to some research results. For example, some studies found that *S. salsa* can tolerate high salt environments during germination and seedling stages [29]. Therefore, some studies have suggested that the pioneer plant *S. salsa* can be used to restore saline soil [30]. Our findings supported this finding and provided a theoretical basis for planting *S. salsa* to improve soda saline soil.

The PCA of the microbiota showed strong changes in the community compositions between different types of soda saline-alkali soil (Fig. 1). The differences in the bacteria were very clear as natural succession proceeded. These results were inconsistent with the findings of Kuramae et al., who found that there was little difference in soil bacteria and archaea between different successional stages in the secondary succession of chalk grass [31], but these results were consistent with the results of Lozano et al., who reported that bacteria showed a clear separation along the succession time of secondary succession in a dry environment [32]. The differences in the eukaryotic microorganisms were also clear

among the successional sites, which was similar to the findings by Wang and Guo, where the differences in fungal community structure were substantial in the salt-tolerant vegetation in the Yellow River Delta [17]. Our results showed that the microbial communities in the AS and S were more similar to each other than those in the P and A, indicating the transitional role of the pioneer plant *S. salsa* in the rhizosphere microbial community of the soda saline-alkali soil.

The bacterial community compositions principally consisted of *Proteobacteria*, *Actinobacteria*, *Gemmatimonadetes*, *Firmicutes*, *Bacteroidetes*, and *Acidobacteria* (Fig. 2). The results were similar to findings for other soil ecosystems at the phylum level [11,33,34]. The relative abundance of *Proteobacteria*, *Actinobacteria*, *Gemmatimonadetes*, *Firmicutes*, *Bacteroidetes*, and *Chloroflexi* had significant changes in the process of vegetation succession ($p<0.05$). In our study, *Proteobacteria* were the most abundant phylum regardless of succession stages (Fig. 2a), and the relative abundance of *Proteobacteria* increased significantly ($p<0.05$) in S with pioneer plant *S. salsa* colonizing on AS soil (Fig. 3a). *Proteobacteria* have been found to increase in number as succession age increased in reclaimed and revegetated coal mine spoils in China [35]. Banning et al. also found that the relative abundance of *Proteobacteria* increased during forest ecosystem succession in Australia [36]. These results indicated that *Proteobacteria* may play an important role in vegetation succession. In this study, the relative abundance of *Actinobacteria* significantly decreased ($P<0.05$) with the successful colonization of *S. salsa* on AS soil (Fig. 3a). Some members of *Actinobacteria* play an important role in the cycling of soil carbohydrates, amino acids and organic acids [32,34], and this K-strategist species can create nutritious microecosystem soil conditions. In contrast, most members of soil *Proteobacteria* are copiotrophic or R-strategists and become abundant when they are in nutrient-rich environments [37,38]. This scenario was consistent with the increase in the basic physicochemical properties of NO_3^- and NH_4^+ in the vegetation-covered soil in this study (Table 1). In our study, the microbial community changed from being *Actinobacteria* dominant to *Proteobacteria* dominant as natural succession proceeded, suggesting an improvement in soil nutrients and a transition in the dominant bacterial communities from slow-growing oligotrophic groups to fast-growing copiotrophic groups.

The soil eukaryotic microorganisms communities at the phylum level mainly consisted of unidentified *Eukaryota*, *Ascomycota* (fungi), *Ochrophyta* (algae), *Streptophyta* (algae), and *Basidiomycota* (fungi) (Fig. 2b). With the natural succession of vegetation, the relative abundance of *Streptophyta*, *Ochrophyta*, *Ascomycota*, *Chlorophyta*, *Glomeromycota*, *Nematoda*, and *Xanthophyceae* had significant changes ($P<0.05$). (Fig. 3b). Except for the unidentified *Eukaryota*, the eukaryotic communities generally transitioned from alga-dominated (*Streptophyta* and *Ochrophyta*) to alga-dominated (*Streptophyta* and *Ochrophyta*) and fungus-dominated (*Ascomycota*) to fungus-dominated (*Ascomycota*) (Fig. 2b), which is similar to the findings of Jing et al., where the number of *Streptophyta* tended to decline as revegetation proceeded on the Loess Plateau of China [39]. In contrast to the findings of Zhang et al., who found that the abundance of *Ascomycota* decreased over approximately 110 years throughout secondary succession in the Qinling Mountains of China [40]. The reason for this difference may be due to succession time and vegetation types: (1) the succession time of the sample in our study was far less than 110 years; (2) the plants we studied were all herbs, but the study plot of Zhang et al. consisted of shrubland and forest in a later successional stage [40]. Most *Ascomycota* are saprophytic fungi, which are important decomposers in soil ecosystems and play an important role in improving soil C cycling and promoting plant nutrient uptake [41,42]. Therefore, the colonization of the pioneer plant *S. salsa* and the increase in the abundance of *Ascomycota*, which can promote degradation of plant litter and the C cycle in soil, could create nutritious microecosystem soil conditions. Photosynthesis in algae utilizes carbon dioxide in the environment. In our study, we found that *Streptophyta* (algae) significantly increased in the S with the successful colonization of *S. salsa* on AS soil., and the contents of soil CO_3^{2-} and HCO_3^- decreased significantly during the same process (Table 1) ($P<0.05$). This result indicated that *Streptophyta* is primarily responsible for the reduction in soil CO_3^{2-} and HCO_3^-. These results suggest that as natural succession proceeds, the degree of soil salinization improves, which may occur because with the success of *S. salsa* colonization, the roots of this plant can enrich more algae with photosynthesis (such as *Streptophyta*), which in turn reduces soil CO_3^{2-} and HCO_3^-. Studies have found that some Nematoda can reduce crop production [43], but some types can increase crop yields or plant growth by driving soil nutrient cycling [44,45]. In our study, we found that the relative abundance of Nematoda increased significantly ($P<0.05$) during the succession from AS to S to P (Fig. 3b). This result indicated that an increase in Nematoda leads to an increase in soil nutrient content and provides a prerequisite for the succession of more developed root plants.

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