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Original Research

Halophyte Planting Improves Saline-Alkali Soil and Brings Changes in Physical and Chemical Properties and Soil Microbial Communities

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Abstract

In this study, 5 kinds of halophytes were used to improve the saline-alkali soil, including *Panicum virgatum L.* (SG), *Achnatherum splendens (Trin.) Nevski* (AS), *Leymus chinensis (Trin.) Tzvel.* (CG), *Sphaerophysa salsula (Pall.) DC.* (SS), and *Sophora alopecuroides L.* (SA). The soil bacteria 16S RNA amplicon sequencing was performed by an Illumina Miseq platform. Planting of halophytes increases the content of organic matter, total nitrogen, total phosphorus, available phosphorus, and alkali-hydrolyzed nitrogen in the soil. *Sphaerophysa salsula (Pall.) DC.* (*Sphaerophysa salsula*) has the most significant improvement effect on saline-alkali land. In soil, *Proteobacteria* play a crucial role in the degradation of soil organic matter, which may be an important factor in improving saline-alkali soil. Besides, root exudates of halophytes might promote *Proteobacteria* growth, especially in *Sphaerophysa salsula (Pall.) DC.* and *Sophora alopecuroides L.* (*Sophora alopecuroides*). Halophyte planting has a significant impact on the restoration of saline-alkali soil.

Keywords: halophytes, saline-alkali soil, improvement, physical and chemical properties, soil bacterial community

Highlights

- Halophyte planting has a significant impact on the restoration of saline-alkali soil, and provide reference methods for the biological improvement of saline-alkali soils • halophyte planting has affected soil microbial structure, and the effect of *Sphaerophysa salsula (Pall.) DC.* is prominent.
- Proteobacteria plays a crucial role in the degradation of soil organic matter, which may be an important factor in improving saline-alkali soil.
- Halophytes interact with soil microorganisms to repair soil salinity.

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Introduction

Salt and Alkali Stresses are one of the major abiotic stresses on plants and have seriously affected agricultural productivity in most parts of the world [1, 2]. Especially in arid and semi-arid regions, soil salinization is a major threat to agriculture, where water scarcity and inadequate irrigation management severely reduce crop yield [3]. At present, the development of saline-alkali agriculture using salt-tolerant economic plants is a feasible way to utilize saline-alkali land [4-6]. In Northwest China, halophytes are important cash crops, and farmers have good forages planting practices. Therefore, it is of great significance to explore halophyte planting for the biological improvement of saline-alkali land. Some grasses, especially wild forages, often show durable survivability and have a strong resistance to stress. For instance, several legumes, including Medicago sativa (alfalfa) and M. truncatula, have cultivars that have adapted to saline soils [7]. There are precedents for the use of salt-tolerant plants to improve saline-alkali land, and the critical role of 'plant-microbe interaction' is pointed out [8-11]. When plants suffer from salt stress, a series of responses occur, and different plants respond differently [12]. While responding to stress, the plant root system also produces various secretions. Previous studies reported that a small change in the composition and quantity of root exudates causes major changes in the population of microorganisms in the root zone [13, 14]. Furthermore, the organisms in the soil can as well influence the plants by releasing regulatory substances [15]. In this way, a virtual circle is gradually established, and the saline-alkali land is greatly improved in the process. Wang et al. reviewed the biological improvement of saline-alkali soil reference system and noted that suitable varieties, suitable cultivation measures, and comprehensive evaluation system were the three critical aspects of evaluating improved methods [1]. As a supplement, this study will reveal the effects of halophyte planting on bacterial community structure in the saline-alkali land.

In this study, we evaluated the improvement effects of several pastures on saline-alkali soil. In addition, we also revealed the changes in soil microbial communities in rhizosphere soil and bulk soil after planting of different forages by high-throughput sequencing. This study will provide reference methods for the biological improvement of saline-alkali soils. In addition, it also provides a theoretical basis for the establishment of other biological improvement methods for saline-alkali soils.

Materials and Methods

Experimental Location

The present study was carried out on the saline alkali land in Ningxia Shizuishan City Pingluo County Qianjin Farm, Ningxia University Saline Land Improvement Test Station (E-106°28'; N-39°05'). The average annual precipitation at this location is 172.5 mm, which is mainly concentrated in July, August, and September. However, the annual mean surface evaporation is greater than the annual mean precipitation, which is approximately 1755 mm. Besides, the average sunshine hours are 2800-3200 h. The average annual temperature is 8.5ºC with diurnal difference of 8-15ºC. Frost-free period last 155 days per year and the annual average relative humidity is 56%. The pH value was measured using an INESA pH meter (Shanghai REX Instrument Factory, Shanghai, China). Total dissolved solids (TDS) were measured in situ using a portable SG3 conductivity meter (Mettler-Toledo Co., Shanghai, China). Total alkalinity (TA) was measured using a Metrohm auto-titrator (Metrohm Co., Herisau, Switzerland). The data were calculated according to Zhang's report [16]. The values of pH, TDS and TA was 9.1, 3.02, and 20.6%, respectively.

Experimental Design and Samples Collection

In the present study, 5 common halophyte species in Northwest China were selected to improve the saline-alkali soil, including *Panicum virgatum L.* (SG), *Achnatherum splendens (Trin.) Nevski* (AS), *Leymus chinensis (Trin.) Tzvel.* (CG), *Sphaerophysa salsula (Pall.) DC.* (SS), and *Sophora alopecuroides L.* (SA). The seeds (and seedlings) were obtained from Forage Experiment Station of Inner Mongolia Agricultural University. The bare ground with no pasture was set as control (CK). For the test areas, a total of three areas are divided, each area includes six blocks. The block is 6 meters long, 5 meters wide, and an area of 30 m^2. The area spacing is 1.5 m; block spacing is 1m. A 5-meter wide protection line was set up to isolate the test area.

The experiment was performed with a completely randomized block design with three replications. Three soil samples (5-cm diameter × 30 cm depth) were collected randomly in each plot before planting. The halophytes seeds (AS, SS and SA) and seedlings (SG and CG) were sown (20 cm row spacing) on the 17th and 18th of April in 2017, respectively. The experiment ended in July 2018. All seedlings were managed under the general field management methods, and no fertilization was done for the whole growing period.

Bulk soil and rhizosphere soil samples were collected from the experimental field. On July 25, 2018. Three replicates were randomly selected from each plot to collect rhizosphere soil, and then mixed as one sample. For bulk soil samples, a five-point sampling method was used to collect the soil samples. All the collected samples were transported in a dry ice sampling box and stored at -80ºC. Each sample was divided into two parts, one of which was used to determine the physical and chemical properties of soil according to Lu's methods [17], and the other was used

to analyze 16S RNA of soil microorganisms. Briefly, the soil organic matter (OM) content was determined using the potassium dichromate volumetric method; Total nitrogen (TN) was determined by Kjeldahl method; Total phosphorus (TP) was determined using sulfuric acid-perchloric acid digestion method; Alkaline hydrolyzed nitrogen (HyN) was determined by alkali-hydrolyzed reduction diffusing method; Available potassium (AK) was determined using the sodium bicarbonate extraction-molybdenum-antimony anti-spectrophotometric method; Available phosphorus (AP) was determined by Olsen method; Soil pH was measured with glass electrode in a 1:2.5 soil/water suspension. Exchangeable sodium percentage (ESP) were calculated directly, based on the concentrations of exchangeable cations; Total salt content was detected by heating drying method.

Finally, a total of 11 groups (33 soil samples) were used for sequencing, including the control group (CK); bulk soil groups of *Panicum virgatum L.* (marked as SG), *Achnatherum splendens (Trin.)* Nevski (marked as AS), *Leymus chinensis (Trin.) Tzvel.* (marked as CG), *Sphaerophysa salsula (Pall.) DC.* (marked as SS), and *Sophora alopecuroides L.* (marked as SA); and rhizosphere soil groups of *Panicum virgatum L.* (marked as SG-r), *Achnatherum splendens (Trin.) Nevski* (marked as AS-r), *Leymus chinensis (Trin.) Tzvel.* (marked as CG-r), *Sphaerophysa salsula (Pall.) DC.* (marked as SS-r), and *Sophora alopecuroides L.* (marked as SA-r). All the groups and their abbreviations were listed in Table 1

DNA Extraction and Sequencing

DNA was extracted from 1g soil of each sample using a PowerSoil DNA Isolation Kit (MOBIO, Carlsbad, CA, USA). Then the DNA concentration was quantified by a Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, CA, USA). The V3 and V4 region of the 16S rRNA gene was amplificated with 341F (5'-3': CCTAYGGGRBGCASCAG)/806R (5'-3': GGACTACHVGGGTWTCTAAT) primers. A 20 μL PCR reaction system was used containing 2 μL template DNA, 0.5μL of each forward- and reverse-primer, 10 μL PCR mix (2×, BioTeke, Wuxi, Jiang Su, China), and 7 μL dd H_2O. The PCR amplification was performed as follows: one cycle of 95ºC for 15 min, followed by 35 cycles of 94ºC for 30 sec, 55ºC for 30 sec, and 72ºC for 45 sec, and followed by a final extension at 72ºC for 5 min. PCR products from one sample were pooled and then gel purified (2% agarose gel) using a GeneJETTM Gel Extraction Kit (Thermo Fisher, Waltham, MA, USA). DNA quantification was performed using QuantiFluo (Promega, Lyon, France). DNA library construction was performed using the MetaVx™ Library Preparation kit (GENEWIZ, South Plainfield, Nan Jing, USA), and 16S rRNA gene sequencing was performed on the Illumina Miseq platform (Illumina, San Diego, USA; Pair-end 300 bp).

Bioinformatic Analysis

After the double end sequencing of DNA, the original data were saved in FASTQ format. The QIIME (V1.9.1, http://qiime.org/) software was then used to identify and eliminate the query sequence and USEARCH (V8.1.1861, https://www.drive5.com/usearch/download.html) was employed to check and remove the chimera sequences to obtain high-quality sequences. The number of high-quality sequences of each sample and the length distribution of the high-quality sequences in the whole sample were calculated using the R software (V3.2.0, https://www.r-project.org/). High-quality sequences with ≥97% nucleotide similarity were clustered into operational taxonomic units (OTUs) with QIIME as described previously [18]. Briefly, reads were demultiplexed and primers and barcodes were trimmed using QIIME's default settings. Following trimming, paired end sequence reads were merged with default QIIME settings and clustered into OTUs against the Silva_132 16S rRNA database (http://www.arb-silva.de/) which has taxonomic categories predicted to the species level. Shared OTU abundances were visualized using a Venn diagram. Rarefaction curves and rank-abundance curves were calculated in QIIME. Then, sequences were rarefied prior to the calculation of alpha diversity indexes using QIIME, including Chao1, Shannon, ace, and Simpson. Beta diversity (diversity between the samples) was measured using weighted UniFrac analysis (http://bmf.colorado.edu/unifrac/index.psp). The stacked bar graphics were prepared to represent OTU relative abundance metrics in R, using the gplots package. Heat maps were graphed in TBtools (V0.66839, https://github.com/CJ-Chen/TBtools) base on the dominant bacteria. Linear discriminant analysis (LDA) coupled with effect size (LEfSe) was performed using the LEfSe program (http://huttenhower.sph.harvard.edu/galaxy/root?tool_id=lefse_upload).

Statistical Analysis

Statistical analysis was performed following previous studies [19, 20]. SPSS 22.0 software was used for statistical data analysis. Data were expressed as the mean±SD and analyzed by one-way ANOVA followed by multiple comparisons with Duncan test. A value of $p<0.05$ was considered significant, and $p<0.01$ was considered highly significant.

Results

Effects of Different Grasses on Physical and Chemical Properties of Soil

Halophyte planting dramatically altered soil physicochemical properties. After planting, the values of TN, OM, AP, and HyN in the soil increased

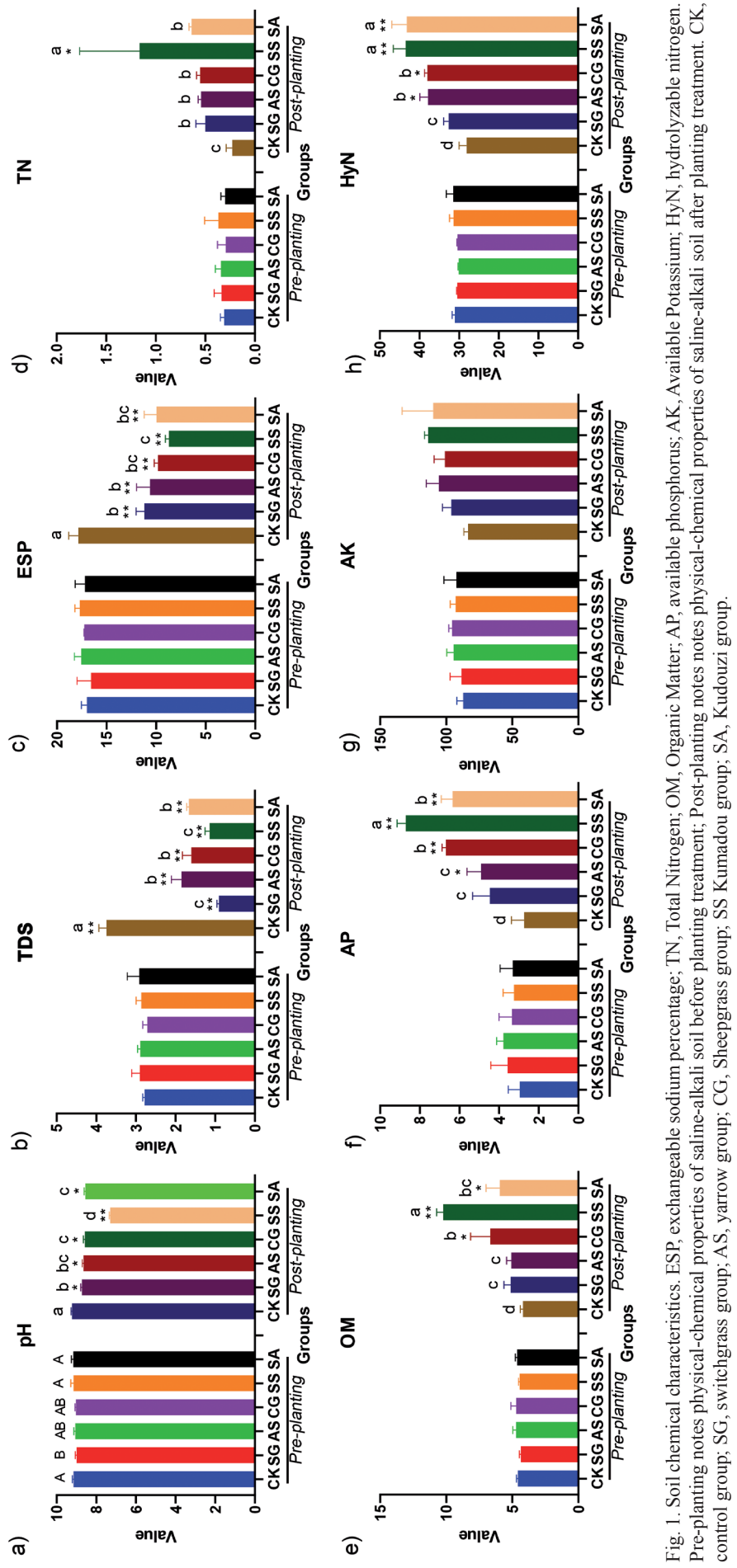

Fig. 1. Soil chemical characteristics. ESP, exchangeable sodium percentage; TN, Total Nitrogen; OM, Organic Matter; AP, available phosphorus; AK, Available Potassium; HyN, hydrolyzable nitrogen. Pre-planting notes physical-chemical properties of saline-alkali soil before planting treatment; Post-planting notes notes physical-chemical properties of saline-alkali soil after planting treatment. CK, control group; SG, switchgrass group; AS, yarrow group; CG, Sheepgrass group; SS Kumadou group; SA, Kudouzi group.

significantly; the pH, total salt content, and ESP were all significantly decreased. The land planted with *Sphaerophysa salsula (Pall.) DC.* (SS) had the highest values of TN, OM, AP, and HyN, which were significantly higher than the land before planting. Correspondingly, planting *Sphaerophysa salsula (Pall.) DC.* had the most significant effects on pH, total salt content, and ESP of the saline soils (Fig. 1). In addition, we found that halophyte planting treatment had no effects on AK content. The soil water content and porosity test results showed that forage grass planting significantly increased the AWC, total porosity, and capillary porosity (Fig. 2). Similarly, *Sphaerophysa salsula (Pall.) DC.* showed outstanding effects on saline-alkali land improvement, which had a higher value of AWC, total porosity, and capillary porosity than that of other halophyte planting treatments. *Panicum virgatum L.* (SG) and *Achnatherum splendens (Trin.) Nevski* (AS) planting decreased the value of non-capillary porosity, but *Leymus chinensis (Trin.) Tzvel.* (CG) and *Sophora alopecuroides L.* (SA) increased it.

Effects of Different Grasses on Enzyme Activities of Soil

As expected, the effect of forage application on enzyme activity in saline-alkali soils was extremely significant. Halophyte planting treatment led to a significant increase in all the tested enzyme activities. After *Sphaerophysa salsula (Pall.) DC.* treatment, the activities of ALP, NR, and Urease increased the most. *Sophora alopecuroides L.* treatment induced the activities of CAT and Invertase, which was significantly higher than the other treatments (Fig. 3). Among the halophytes, *Achnatherum splendens (Trin.) Nevski* showed a lower value of ALP, CAT, NR, and Urease activities. *Panicum virgatum L.* treatment showed a lower value of Invertase.

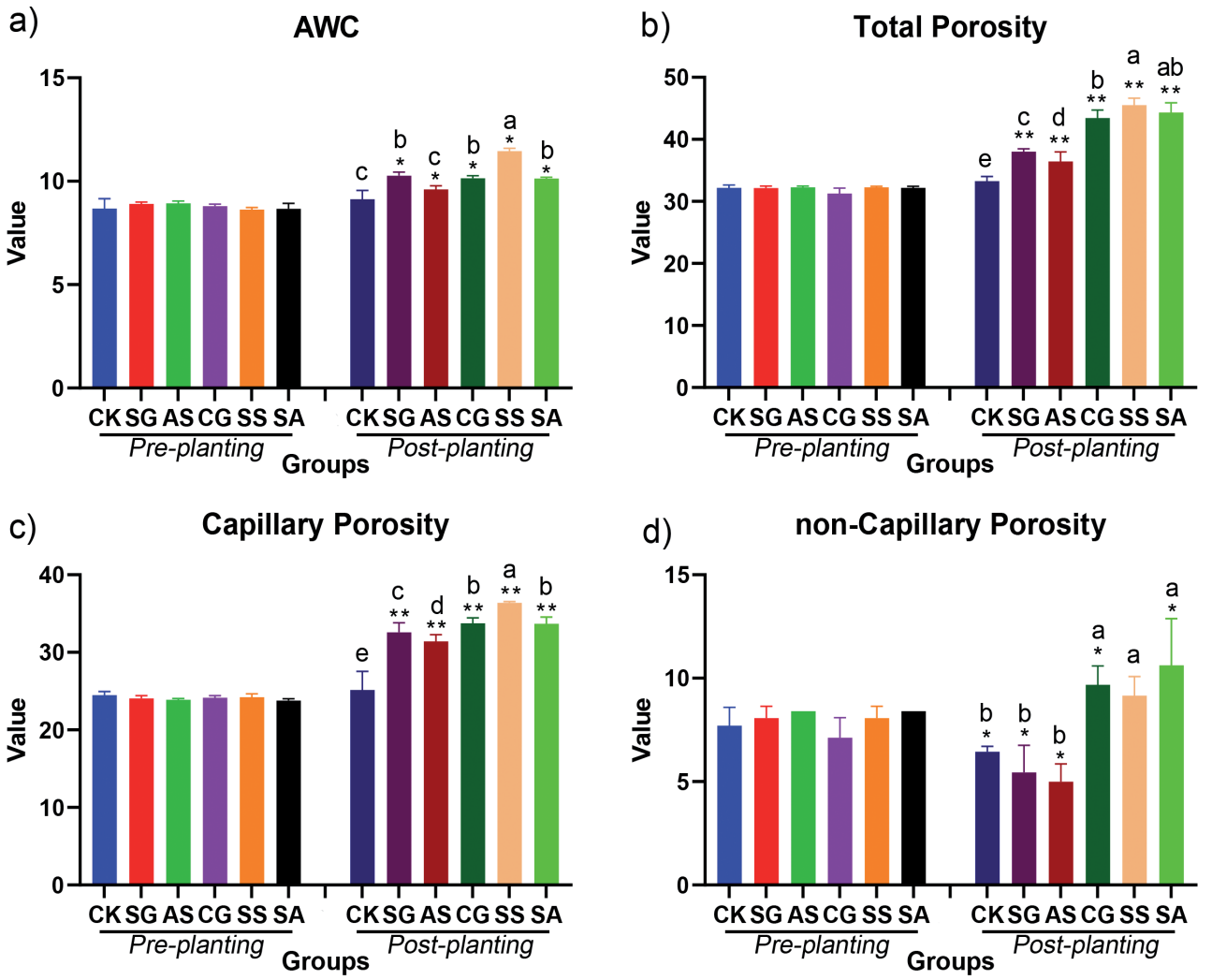

Fig. 2. Soil physical characteristics. AWC, absolute water content; Pre-planting notes physical-chemical properties of saline-alkali soil before planting treatment; Post-planting notes notes physical-chemical properties of saline-alkali soil after planting treatment. CK, control group; SG, switchgrass group; AS, yarrow group; CG, Sheepgrass group; SS Kumadou group; SA, Kudouzi group.

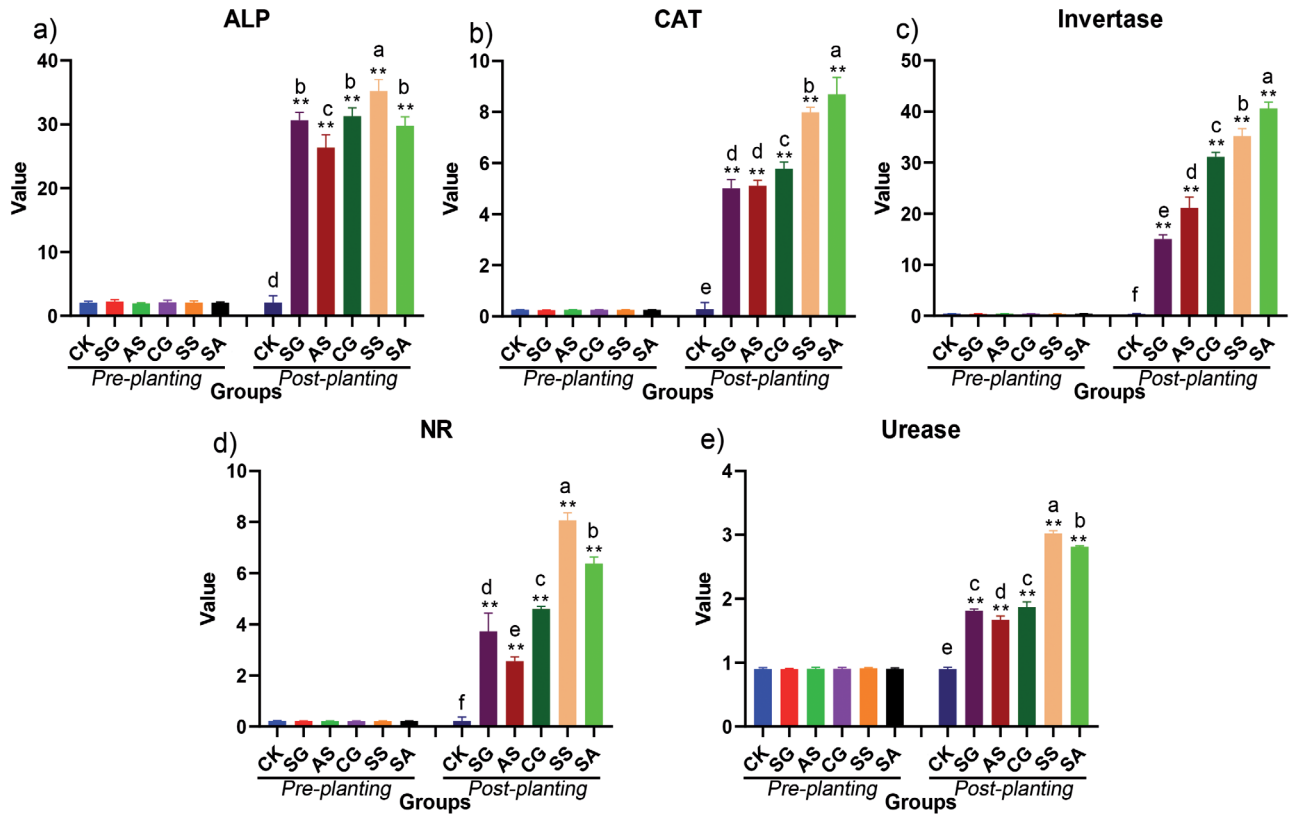

Fig. 3. Soil enzyme activities. ALP, alkaline phosphatase; CAT, catalase; NR, nitrate reductase; Pre-planting notes physical-chemical properties of saline-alkali soil before planting treatment; Post-planting notes physical-chemical properties of saline-alkali soil after planting treatment. CK, control group; SG, switchgrass group; AS, yarrow group; CG, Sheepgrass group; SS Kumadou group; SA, Kudouzi group.

Sequence Data Summary

Based on sequencing analysis of the V3 and V4 regions of the 16s rRNA gene, a mass of paired sequences with barcode and primer sequences were obtained. We identified 1,730,483 pair-end reads from the 33 soil samples, with an average length of 454bp. The average Q20, Q30, and GC contents were 91.31%,

Table 1. Groups and their abbreviations.

Group name	Abbre-viations
Control group	CK
Bulk soil of *Panicum virgatum L.*	SG
Bulk soil of *Achnatherum splendens (Trin.)* Nevski	*AS*
Bulk soil of *Leymus chinensis (Trin.) Tzvel*	GG
Bulk soil of *Sphaerophysa salsula (Pall.) DC*	SS
Bulk soil of *Sophora alopecuroides L.*	SA
Rhizosphere soil groups of *Panicum virgatum L.*	SG-r
Rhizosphere soil of *Achnatherum splendens (Trin.) Nevski*	AS-r
Rhizosphere soil of *Leymus chinensis (Trin.) Tzvel.*	GG-r
Rhizosphere soil of *Sphaerophysa salsula (Pall.) DC.*	SS-r
Rhizosphere soil of *Sophora alopecuroides L.*	SA-r

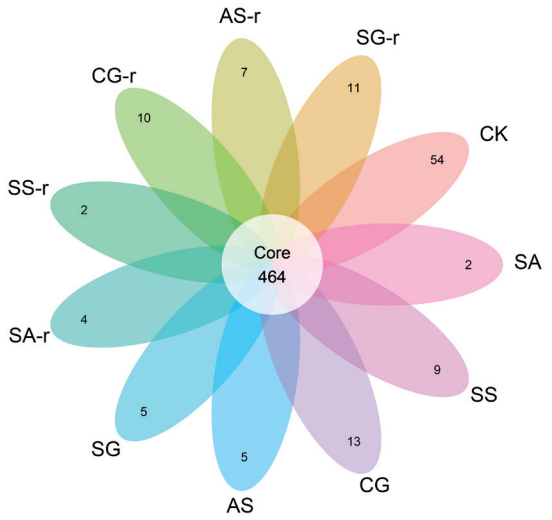

Fig. 4. Operational Taxonomic Units (OTU) based petal maps. CK, control group; SG, switchgrass group; AS, yarrow group; CG, Sheepgrass group; SS Kumadou group; SA, Kudouzi group; -r means rhizosphere soil samples.

86.95%, and 56.19%, respectively, and the clean reads of Q20 occupied over 95% of the total, suggesting high-quality sequencing (Table S1). In total, 3,460,966 sequenced reads were clustered into 2,318 OTUs, of which there were 464 core OTUs were identified in all the groups (Fig. 4).

Soil Bacteria Richness and Diversity

All the optimized reads were classified into OTUs under different taxonomic levels (Table 2). The rarefaction curves based on the OTUs of the bacterial community in soil reached saturation plateau, indicating that the sequencing depth was sufficient to represent

Table 2. Numbers of OTUs in different samples.

Sample	Phylum	Class	Order	Family	Genus	Species	Unclassified
CK_1	505	502	420	396	296	197	2
CK_2	451	447	397	363	275	185	1
CK_3	578	572	485	457	349	229	1
SG_r_1	1460	1453	1254	1135	919	543	0
SG_r_2	1367	1360	1178	1072	868	510	0
SG_r_3	1428	1422	1235	1113	904	539	2
AS_r_1	1367	1359	1166	1051	846	508	0
AS_r_2	1330	1323	1151	1040	837	505	0
AS_r_3	1436	1430	1235	1115	901	533	1
CG_r_1	1228	1224	1059	959	765	444	0
CG_r_2	1373	1366	1182	1058	861	507	0
CG_r_3	1116	1112	975	881	703	421	2
SS_r_1	1220	1219	1080	979	791	450	2
SS_r_2	1339	1334	1170	1052	865	502	2
SS_r_3	1375	1370	1194	1070	859	508	2
SA_r_1	1228	1294	1132	1021	824	480	2
SA_r_2	1337	1333	1178	1061	861	495	2
SA_r_3	1386	1381	1201	1086	881	519	1
SG_1	1491	1486	1285	1145	929	560	1
SG_2	1346	1339	1168	1053	842	500	1
SG_3	1376	1369	1179	1053	851	497	0
AS_1	1337	1330	1150	1035	828	480	1
AS_2	1258	1255	1096	988	795	472	0
AS_3	1423	1418	1226	1098	877	527	0
CG_1	1279	1275	1111	1009	808	476	0
CG_2	1420	1415	1226	1102	890	533	0
CG_3	1085	1080	936	851	675	403	0
SS_1	1505	1497	1306	1181	939	565	0
SS_2	1412	1407	1225	1098	893	516	0
SS_3	1374	1370	1189	1072	875	520	0
SA_1	1358	1352	1176	1052	854	486	0
SA_2	1404	1398	1208	1091	873	521	0
SA_3	1448	1443	1244	1121	907	534	0

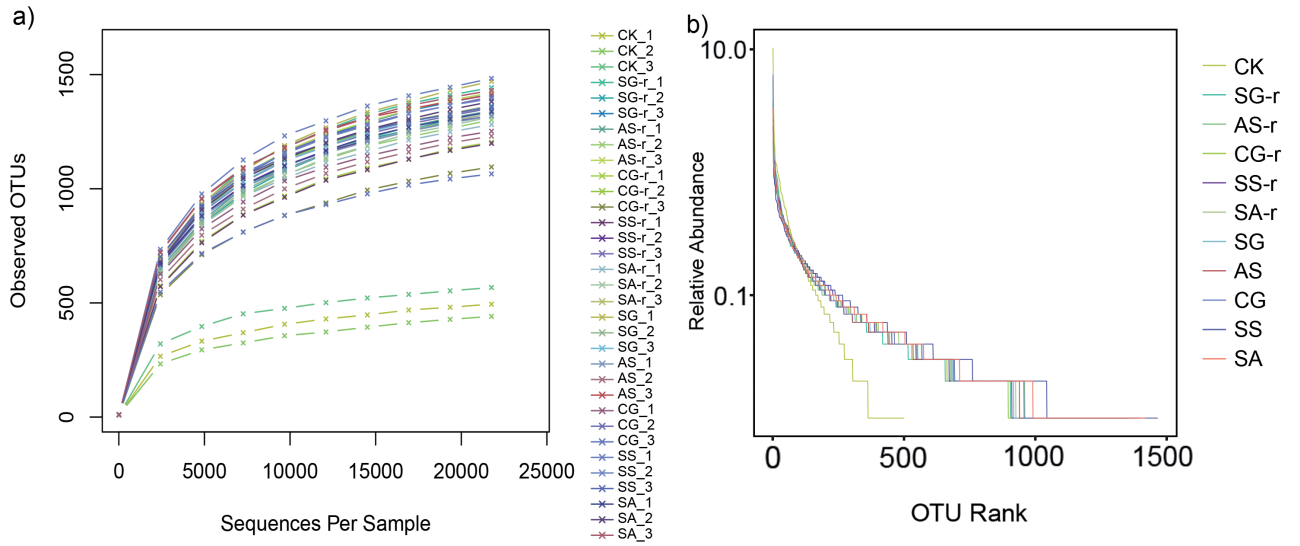

Fig. 5. Rarefaction curves and rank abundance curves of α-diversity. a) rarefaction curves plot, X-axis is number sequencing reads randomly chosen from a certain sample to obtain OTUs. b) Rank–abundance curves of soil bacteria. CK, control group; SG, switchgrass group; AS, yarrow group; CG, Sheepgrass group; SS Kumadou group; SA, Kudouzi group; -r means rhizosphere soil samples.

the majority of microbe species (Fig. 5a). The rank abundance curve for OTUs showed that the bacterial richness of halophytes treated groups was higher than that of CK (Fig. 5b). It was evident for the differences in the bacterial richness (i.e., the Chao 1 value) listed in Table 3.

Bacterial Community Structure of Bacteria

The taxonomic distributions of microbial communities were evaluated at different levels of classification. At the phylum level, the relative abundance of *Proteobacteria* and *Acidobacteria* phylum was increased by the halophyte planting treatment. However, the relative abundance of *Bacteroidetes, Gemmatimonadetes,* and *Actinobacteria* phylum were negatively regulated by the planting treatment (Fig. 6a). Seven dominant bacteria were identified, of which the relative abundance was shown in Fig. 6b). After halophyte planting, the abundance of *Proteobacteria* increased dramatically, followed by *Acidobacteria.* Interestingly, the relative abundance of all the increased bacteria in rhizosphere soil was higher than that in bulk soil; and the relative abundance of all the decreased bacteria in rhizosphere soil was lower than that in bulk soil (Fig. 6b). At the genus level, the dominant bacteria were *Gemmatimonadaceae*

Table 3. The [a]lpha diversity indexes in different groups.

	Ace	Chao 1	Shannon	Simpson	Goods_coverage
CK	634.77±42.14 [b]	677.99±48.87 [b]	6.696±0.32 [b]	0.975±0.0055 [b]	0.995±0.0000 [b]
SG_r	1631.46±21.85 [a]	1638.54±25.17 [a]	8.697±0.24 [a]	0.994±0.0015 [a]	0.988±0.0006 [a]
AS_r	1589.38±52.14 [a]	1601.24±35.25 [a]	8.674±0.11 [a]	0.994±0.0006 [a]	0.988±0.0000 [a]
CG_r	1434.60±109.48 [a]	1447.62±105.97 [a]	8.458±0.36 [a]	0.991±0.0040 [a]	0.990±0.0006 [a]
SS_r	1502.15±90.43 [a]	1523.29±102.52 [a]	8.712±0.28 [a]	0.994±0.0012 [a]	0.990±0.0006 [a]
SA_r	1536.32±57.68 [a]	1553.23±72.02 [a]	8.651±0.21 [a]	0.993±0.0015 [a]	0.989±0.0010 [a]
SG	1630.62±103.25 [a]	1655.94±106.32 [a]	8.717±0.19 [a]	0.994±0.0015 [a]	0.988±0.0010 [a]
AS	1535.50±49.75 [a]	1573.79±45.12 [a]	8.685±0.28 [a]	0.994±0.0015 [a]	0.989±0.0010 [a]
CG	1436.83±197.11 [a]	1459.82±208.07 [a]	8.471±0.31 [a]	0.990±0.0036 [a]	0.990±0.0017 [a]
SS	1606.31±80.76 [a]	1641.30±103.50 [a]	8.966±0.03 [a]	0.995±0.0010 [a]	0.989±0.0015 [a]
SA	1603.89±45.64 [a]	1628.28±70.38 [a]	8.857±0.15 [a]	0.995±0.0012 [a]	0.989±0.0006 [a]

Data with different letters are significantly different ($p<0.05$).

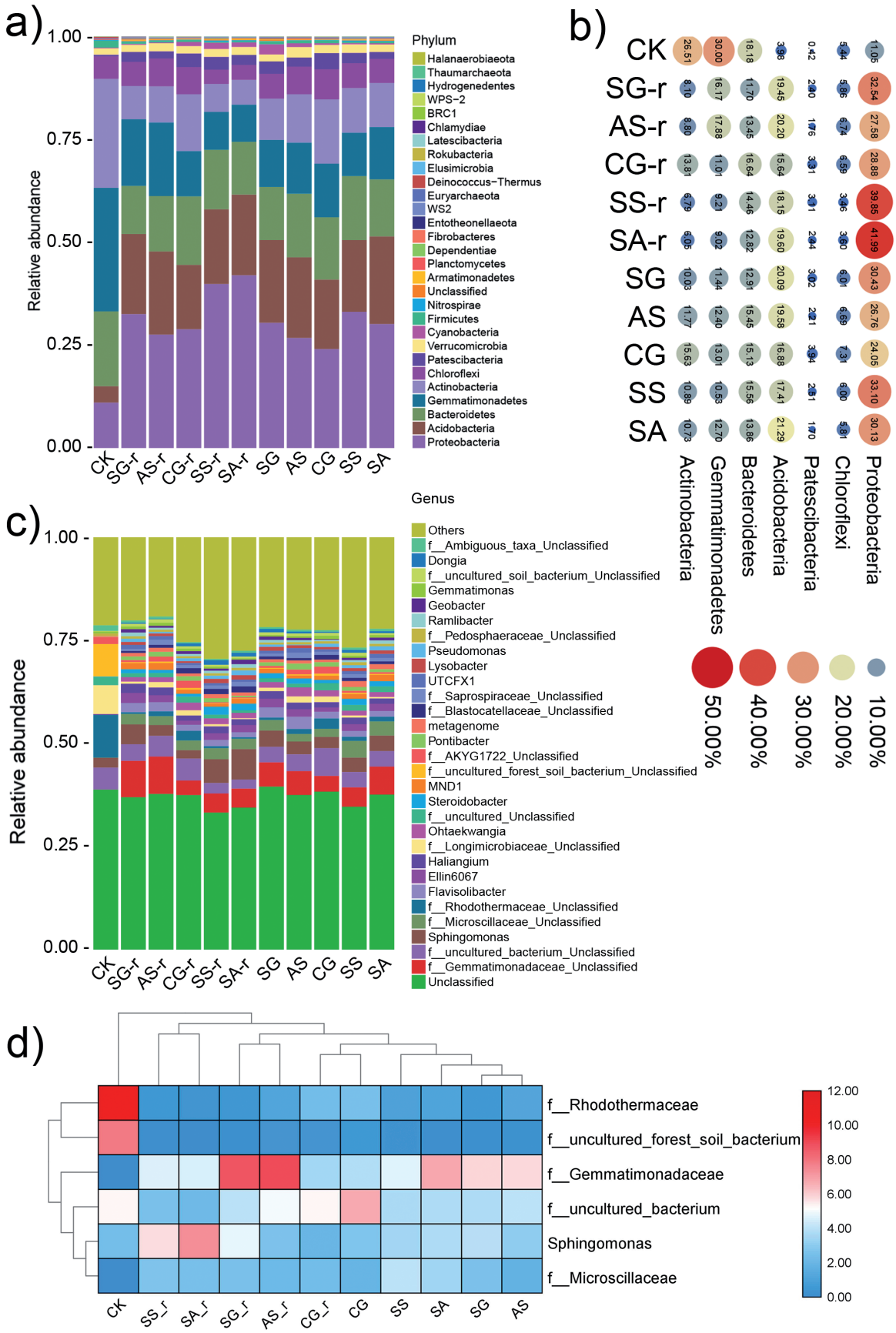

Fig. 6. Bacterial community structure. a, the relative abundance of bacteria at the phylum level; b, relative abundance of dominant bacteria at the phylum level; c, a, the relative abundance of bacteria at the genus level; d, relative abundance of dominant bacteria at the genus level. The larger the circle, the darker the color, the higher the proportion. CK, control group; SG, switchgrass group; AS, yarrow group; CG, Sheepgrass group; SS Kumadou group; SA, Kudouzi group; -r means rhizosphere soil samples.

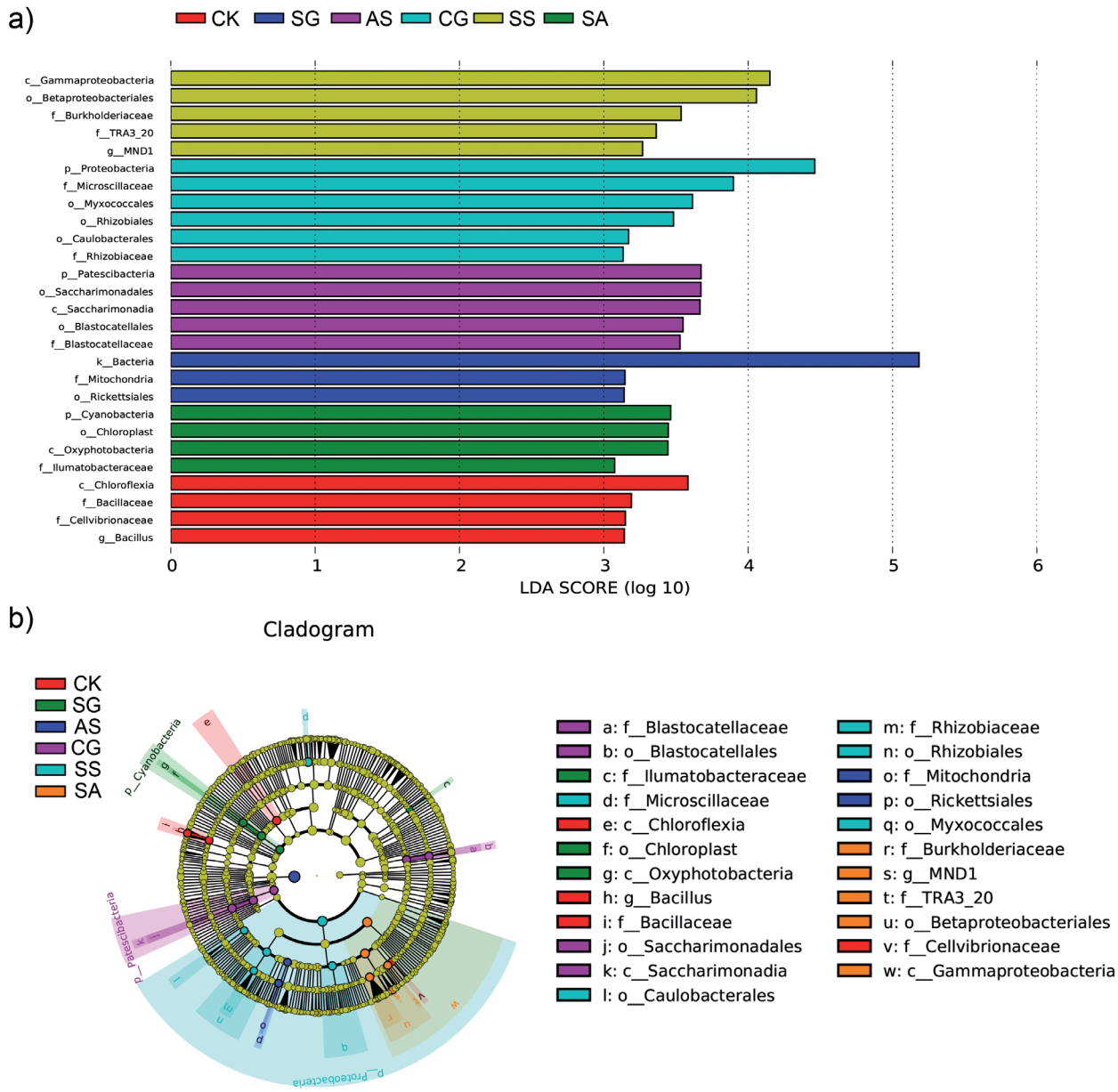

Fig. 7. Dominant bacteria in different classification level. The colored circles imply the dominant bacteria in relative abundance in different classification level. The larger the radius of the circles is, the higher the species in abundance. P: Phylum; C: Class; O: Order; F: Family; G: Genera.

(families), *Rhodothermaceae* (families), *forest_soil_bacterium* (families), *Sphingomonas* (families), and Microscillaceae (families) (Fig. 6c). The relative abundance of Rhodothermaceae (families) and forest_soil_bacterium (families) was decreased by the planting treatment, while Gemmatimonadaceae (families), Sphingomonas (families), and Microscillaceae (families) were increased (Fig. 6d).

Results of Linear Discriminant Analysis

The specific species that had a significant difference between groups was calculated using T-test and LEfSe (LDA Effect Size) analysis. At the phylum level, the relative abundance of *Bacillus, Ellin6067, Haliangium, MND1, Flavisolibacter,* and *Steroidobacter* showed a remarkable difference (p<0.001) in the CK group (Fig. S1). As for the bacteria with a remarkable difference between rhizosphere soil group and bulk soil, there were *Ellin6067, Haliangium, MND1, Steroidobacter*, etc. (Fig. S1). Using GraPhlAnl, the dominant members at different classification levels are displayed as the classification tree (Figs 7a and b). A total of 23 taxa that had a discrepancy in relative abundance were presented in CK and halophyte planting treated groups (e.g., *Gammaproteobacteria, Proteobacteria, Chloroflexia*, etc.). The cladogram in Fig. 7b) showed the core bacterial species with a significant difference in CK and halophyte planting treated groups.

Discussion

In this study, halophyte planting significantly reduced the soil pH value, total salt content, and ESP of the saline-alkali soil, which was similar to previous studies [21-24]. These studies, including ours, showed that planting halophytes had positive effects on the remediation of saline-alkali soil. In particular, the TN, OM, and AP in saline-alkali soil have significantly been improved after planting. The physical properties of saline-alkali soil are improved due to the interspersed roots of salt-tolerant plants [1]. After the cultivation of halophytes, we believe that *Sphaerophysa salsula (Pall.)* DC. had the greatest impact on soil physical properties, while Achnatherum splendens had the smallest. In addition, AWC, total porosity, and capillary porosity were also increased after halophyte planting. Su et al. reported that higher soil moisture and OM contents favoring biodegradation in soil and improving microbial activity and soil adsorption [25, 26]. Previous studies have shown that if the vegetation litter is reduced, soil OM cannot be replenished [27]. At the same time, wind erosion and freeze-thaw action continuously accelerate the mineralization of soil OM, which will greatly reduce soil fertility [28, 29]. The planting of halophytes had greatly supplemented the OM, which led to the improvement of fertility and salinity. Among all the planted halophytes, we were impressed by the improvement of *Sphaerophysa salsula (Pall.) DC.* on the saline-alkali soil. *Sphaerophysa salsula (Pall.) DC.* is a species of flowering plant in the legume family known by the common name alkali swainsonpea [30]. It is native to Asia, but it is known in many other parts of the world as an introduced species. It grows in cultivated land and disturbed habitat, easily tolerating alkaline substrates [30]. In addition, *Sphaerophysa salsula (Pall.) DC.* is rich in protein and oil. Compared with other halophytes, *Sphaerophysa salsula (Pall.) DC.* has more biomass and strong salt tolerance, which may be the main reason for its ability to improve saline-alkali soil.

Bacteria play important roles in the conversion of soil organic and inorganic matter. Bacteria diversity can be affected by many factors, including soil conditions, seasonal plants, and age [31]. The results of alpha diversity showed that the soil microbial species, abundance, and evenness had been greatly improved after halophyte planting, which was consistent with previous studies [32, 33]. We found that *Proteobacteria* was the most dominant bacteria after halophyte planting. Members of the phylum *Proteobacteria* can degrade a wide range of macromolecules [34], which are reported to compose the critical phyla in OM degradation [35]. The relative abundance of *Proteobacteria* in Rhizosphere was higher than that in bulk soil, which indicated that root exudates might promote *Proteobacteria* growth, especially in *Sphaerophysa salsula (Pall.) DC.* and *Sophora alopecuroides L.*. This interaction might be one of the reasons why *Sphaerophysa salsula (Pall.) DC.* and *Sophora alopecuroides L.* showed a better improvement on the saline-alkali soil. It is known that *Proteobacteria* dominates in the reactors treating high-nitrate wastewater, and many types of denitrifies are included in the phylum *Proteobacteria*. In this study, the value of TN and HyN was significantly increased by *Sphaerophysa salsula (Pall.) DC.* and *Sophora alopecuroides L.* planting. Moreover, the abundance of *Proteobacteria* is higher in these two groups than that in other groups. Beyond all doubt, the increase in TN and HyN was mainly due to the accumulation of *Proteobacteria* members. *Actinobacteria*, of which most members grow at a pH value of 5.79-5.82, is an essential indicator for soil pH [36]. In the soil with vegetation, *Acidobacteria* usually had a higher relative abundance [37]. In the present results, the soil pH was decreased, which lead to an increase of *Acidobacteria.* Moreover, the relative abundance of *Bacteroidetes, Gemmatimonadetes,* and *Actinobacteria* phylum were negatively regulated by the planting treatment. Lauber found a positive correlation between the pH value and the relative abundance of *Bacteroidetes* [38]. Also, Ganzert et al. noted that *Bacteroidetes* preferred higher values of soil pH and emphasized that pH is the main factor for shaping bacterial community [39]. Hence, we believed that the decrease in *Bacteroidetes* abundance was mainly due to the changes in pH. The previous study had shown that some taxa of *Gemmatimonadetes* are beneficial for maintaining or improving soil fertility [40]. In addition, researchers found that *Gemmatimonadetes* was a new phototrophic bacterial phylum, which played a crucial role in the oxidation of organic compounds and fixation of N_2 [41, 42]. The competition of other bacteria might cause a decrease in Gemmatimonadetes. Soil enzyme activity is one of the critical factors during nutrient cycling in soils [43-45], which were closely related to soil microorganisms and soil OM. In our results, we found that the halophyte planting treatment significantly increased the soil enzyme activities. This might be caused by the plant themself and their interactions with microorganisms. The sharp increase in enzyme activity also proved the practicality of halophyte planting practice for saline-alkali soil improvement.

Conclusions

Halophyte planting has significant impact on the restoration of saline-alkali soil. It increases the content of organic matter, total nitrogen, total phosphorus, available phosphorus, and alkali-hydrolyzed nitrogen in the soil. Meanwhile, the soil after cultivation has more water content and higher porosity, which provides a good foundation for the survival of microorganisms. The results of soil physical and chemical properties show that *Sphaerophysa salsula (Pall.) DC.* (*Sphaerophysa salsula*) has the most significant improvement effect

on saline-alkali land. Soil bacteria 16S RNA amplicon sequencing results showed that *Proteobacteria* plays a crucial role in the degradation of soil organic matter, which may be an important factor in improving saline-alkali soil. Besides, root exudates of halophytes might promote *Proteobacteria* growth, especially in *Sphaerophysa salsula (Pall.) DC.* and *Sophora alopecuroides L.* (*Sophora alopecuroides*). In addition, the abundance changes of several bacteria (e.g., *Actinobacteria*, *Bacteroidetes*) due to the decrease in pH. These results can provide reference methods for the biological improvement of saline-alkali soils. Also, it provides a theoretical basis for the establishment of other biological improvement methods for saline-alkali soils.

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Data Availability Statement

Our data has been uploaded, and the database address and login number are https://bigd.big.ac.cn/CRA003680.

Author Contributions

Xueqin Wang summarized the sequencing data, performed the data analysis and prepared the original manuscript.

Fengju Zhang and Bo Zhang attended discussion and revised manuscript.

Xing Xu designed the experiment and revised the manuscript.

All authors approved the final manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. XUEQIN WANG X.X., FENGJU ZHANG, KONG XIN Biological improvement of saline alkali soil reference system: A review. Sciences in Cold and Arid Regions, **10** (6), 516, **2018.**
2. MUNNS R. Comparative physiology of salt and water stress. Plant Cell Environ., **25** (2), 239, **2002**.
3. ZAKERY-ASL M.A., BOLANDNAZAR S., OUSTAN S. Effect of salinity and nitrogen on growth, sodium, potassium accumulation, and osmotic adjustment of halophyte Suaeda aegyptiaca (Hasselq.) Zoh. Arch. Agron. Soil Sci., **60** (6), 785, **2014**.
4. JIA T., WANG J., CHANG W., FAN X., SUI X., SONG F. Proteomics Analysis of E. angustifolia Seedlings Inoculated with Arbuscular Mycorrhizal Fungi under Salt Stress. Int. J. Mol. Sci., **20** (3), 788, **2019**.
5. PANTA S., FLOWERS T., LANE P., DOYLE R., HAROS G., SHABALA S. Halophyte agriculture: Success stories. Environ. Exp. Bot., **107**, 71, **2014**.
6. ROZEMA J., MUSCOLO A., FLOWERS T. Special Issue: Sustainable cultivation and exploitation of halophyte crops in a salinizing world. Environ. Exp. Bot., **92**, 1, **2013**.
7. LONG R., LI M., ZHANG T., KANG J., SUN Y., CONG L., GAO Y., LIU F., YANG Q. Comparative Proteomic Analysis Reveals Differential Root Proteins in Medicago sativa and Medicago truncatula in Response to Salt Stress. Front. Plant Sci., **7**, 424, **2016**.
8. ADESEMOYE A.O., OBINI M., UGOJI E.O. Comparison of plant growth-promotion with Pseudomonas aeruginosa and Bacillus subtilis in three vegetables. Braz. J. Microbiol., **39** (3), 423, **2008**.
9. BHATTI G.A., QURESHI P.N., QURESHI A., SULTANA K. Studies on heat shock response of wheat seedlings using E. coli GroEL antibodies. Pakphyton, **5**, 157, **2020**.
10. AFRASAYAB S., FAISAL M., HASNAIN S. Comparative study of wild and transformed salt tolerant bacterial strains on Triticum aestivum growth under salt stress. Braz. J. Microbiol., **41** (4), 946, **2010**.
11. LIU W.-G., LIU J.-X., YAO M.-L., MA Q.-F. Salt tolerance of a wild ecotype of vetiver grass (*Vetiveria zizanioides* L.) in southern China. Bot. Stud., **57** (1), 27, **2016**.
12. PARIDA A.K., DAS A.B. Salt tolerance and salinity effects on plants: a review. Ecotox. Environ. Safe., **60** (3), 324, **2005**.
13. LING N., RAZA W., MA J., HUANG Q., SHEN Q. Identification and role of organic acids in watermelon root exudates for recruiting Paenibacillus polymyxa SQR-21 in the rhizosphere. Eur. J. Soil Biol., **47** (6), 374, **2011**.
14. BADRI D.V., VIVANCO J.M. Regulation and function of root exudates. Plant Cell Environ., **32** (6), 666, **2009**.
15. IGIEHON N.O., BABALOLA O.O. Rhizosphere Microbiome Modulators: Contributions of Nitrogen Fixing Bacteria towards Sustainable Agriculture. Int. J. Env. Res. Pub. He., **15** (4), 574, **2018**.
16. ZHANG J., YANG G., JI C. The relationship between the vertical distribution of soil arthropod and soil factors in saline-alkali Lycium barbarum plantation. J. Agr. Sci., **37** (3), 11, **2016**.
17. LU R., Soil and Agricultural Chemistry Analysis Methods, Beijing: China Agricultural Science and Technology Press, **2002** [In Chinese].
18. TSUDA A., SUDA W., MORITA H., TAKANASHI K., TAKAGI A., KOGA Y., HATTORI M. Influence of Proton-Pump Inhibitors on the Luminal Microbiota in the Gastrointestinal Tract. Clin. Transl. Gastroen., **6** (6), e89, **2015**.
19. LU Z., DENG Y., VAN NOSTRAND J.D., HE Z., VOORDECKERS J., ZHOU A., LEE Y.J., MASON O. U., DUBINSKY E.A., CHAVARRIA K.L., TOM L.M., FORTNEY J.L., LAMENDELLA R., JANSSON J.K., D'HAESELEER P., HAZEN T.C., ZHOU J. Microbial gene functions enriched in the Deepwater Horizon deep-sea oil plume. ISME J., **6** (2), 451, **2012**.
20. YANG Y., WU L., LIN Q., YUAN M., XU D., YU H., HU Y., DUAN J., LI X., HE Z., XUE K., VAN NOSTRAND J., WANG S., ZHOU J. Responses of the functional structure of soil microbial community to livestock grazing in the

Tibetan alpine grassland. Glob Chang Biol., **19** (2), 637, **2013**.
21. XU Z., SHAO T., LV Z., YUE Y., LIU A., LONG X., ZHOU Z., GAO X., RENGEL Z. The mechanisms of improving coastal saline soils by planting rice. Sci. Total Environ., **703**, 135529, **2020**.
22. ZHANG T., ZHAN X., KANG Y., WAN S., FENG H. Improvements of soil salt characteristics and nutrient status in an impermeable saline–sodic soil reclaimed with an improved drip irrigation while ridge planting Lycium barbarum L. J. Soil. Sediment., **17** (4), 1126, **2017**.
23. LU S., WANG C., DU Y., WU Y., GAO Y., LIU S. Effects of rice planting years on physicochemical property and fungi community in soda saline-alkali soil. Journal of South China Agricultural University, **40** (1), 15, **2019**.
24. XIA J., REN J., ZHANG S., WANG Y., FANG Y. Forest and grass composite patterns improve the soil quality in the coastal saline-alkali land of the Yellow River Delta, China. Geoderma, **349**, 25, **2019**.
25. SU W., HAO H., DING M., WU R., XU H., XUE F., SHEN C., SUN L., LU C. Adsorption and degradation of imazapic in soils under different environmental conditions. PloS one, **14** (7), e0219462-e0219462, **2019**.
26. KRAUSS M., WILCKE W. Sorption Strength of Persistent Organic Pollutants in Particle-size Fractions of Urban Soils. Soil Sci. Soc. Am. J., **66** (2), 430, **2002**.
27. WU C., LI Y., CHEN M., LUO X., CHEN Y., BELZILE N., HUANG S. Adsorption of Cadmium on Degraded Soils Amended with Maize-Stalk-Derived Biochar. Int. J. Env. Res. Pub. He., **15** (11), 2331, **2018**.
28. SIX J., BOSSUYT H., DEGRYZE S., DENEF K. A history of research on the link between (micro)aggregates, soil biota, and soil organic matter dynamics. Soil Till. Res., **79** (1), 7, **2004**.
29. WAN T., TU W., XI H., LI Y., TANG X., YANG Y. Study on Vegetation and Soil Characteristics of Desertification Grassland in Northwest Sichuan. Acta Agrestia Sinica, **13**, 650, **2013**.
30. LI G., ZHAO P., SHAO W., Cash Crop Halophytes of China, in Sabkha Ecosystems: Volume VI: Asia/Pacific, B. Gul, et al., Editors. **2019**, Springer International Publishing: Cham. p. 497-504.
31. HRYNKIEWICZ K., BAUM C., LEINWEBER P. Density, metabolic activity, and identity of cultivable rhizosphere bacteria on Salix viminalis in disturbed arable and landfill soils. J. Plant Nutr. Soil Sci., **173** (5), 747, **2010**.
32. WANG F., LIANG Y., JIANG Y., YANG Y., XUE K., XIONG J., ZHOU J., SUN B. Planting increases the abundance and structure complexity of soil core functional genes relevant to carbon and nitrogen cycling. Sci. Rep-UK, **5** (1), 14345, **2015**.
33. XIAO C., YANG L., ZHANG L., LIU C., HAN M. Effects of cultivation ages and modes on microbial diversity in the rhizosphere soil of Panax ginseng. J. Ginseng Res., **40** (1), 28, **2016**.
34. CHOUARI R., LE PASLIER D., DAEGELEN P., GINESTET P., WEISSENBACH J., SGHIR A. Novel predominant archaeal and bacterial groups revealed by molecular analysis of an anaerobic sludge digester. Environ. Microbiol., **7** (8), 1104, **2005**.
35. RIVIÈRE D., DESVIGNES V., PELLETIER E., CHAUSSONNERIE S., GUERMAZI S., WEISSENBACH J., LI T., CAMACHO P., SGHIR A. Towards the definition of a core of microorganisms involved in anaerobic digestion of sludge. ISME J., **3** (6), 700, **2009**.
36. CHAUDHARY D.K., KHULAN A., KIM J. Development of a novel cultivation technique for uncultured soil bacteria. Sci. Rep-UK, **9** (1), 6666, **2019**.
37. LIN Y.-T., HU H.-W., WHITMAN W. B., COLEMAN D.C., CHIU C.-Y. Comparison of soil bacterial communities in a natural hardwood forest and coniferous plantations in perhumid subtropical low mountains. Bot. Stud., **55** (1), 50, **2014**.
38. LAUBER C.L., HAMADY M., KNIGHT R., FIERER N. Pyrosequencing-Based Assessment of Soil pH as a Predictor of Soil Bacterial Community Structure at the Continental Scale. Appl. Environ. Microb., **75** (15), 5111, **2009.**
39. GANZERT L., BAJERSKI F., WAGNER D. Bacterial community composition and diversity of five different permafrost-affected soils of Northeast Greenland. FEMS Microbiol. Ecol., **89** (2), 426, **2014**.
40. ZHANG Z., ZHANG P., LIN Q., CHA Z., LUO W. Response of bacterial communities in rubber plantations to different fertilizer treatments. 3 Biotech, **9** (8), 293, **2019**.
41. ZENG Y., FENG F., MEDOVá H., DEAN J., KOBLíŽEK M. Functional type 2 photosynthetic reaction centers found in the rare bacterial phylum Gemmatimonadetes. P. Natl. A. Sci., **111** (21), 7795, **2014**.
42. DUBBS J. M., ROBERT TABITA F. Regulators of nonsulfur purple phototrophic bacteria and the interactive control of CO_2 assimilation, nitrogen fixation, hydrogen metabolism and energy generation. FEMS Microbiol. Rev., **28** (3), 353, **2004**.
43. CAI Z., WANG J., MA J., ZHU X., CAI J., YANG G. Anaerobic Degradation Pathway of the Novel Chiral Insecticide Paichongding and Its Impact on Bacterial Communities in Soils. J Agr. Food Chem., **63** (32), 7151, **2015**.
44. ASAD M.A.U., LAVOIE M., SONG H., JIN Y., FU Z., QIAN H. Interaction of chiral herbicides with soil microorganisms, algae and vascular plants. Sci. Total Environ., **580**, 1287, **2017**.
45. CAI Z., RONG Y., CHEN J., WANG J., MA J., ZHANG W., ZHAO X. Effects of the novel cis-nitromethylene neonicotinoid insecticide Paichongding on enzyme activities and microorganisms in yellow loam and Huangshi soils. Environ. Sci. Pollut. R., **23** (8), 7786, **2016**.

Supplementary Material

Table S1. Sequence data summary.

Sample	Length(bp)	#Reads	Bases(bp)	Q20(%)	Q30(%)	GC(%)	#PE_reads	#Nochimera	AvgLen(bp)	GC(%)
CK_1	250	111934	27983500	86,81	80,5	58,55	55967	42884	454,57	58,23
CK_2	250	109290	27322500	93,02	89,01	58,71	54645	48270	454,42	58,7
CK_3	250	92426	23106500	93,12	89,24	57,85	46213	40817	453,48	57,86
SG_r_1	250	108074	27018500	92,45	88,49	56,13	54037	47457	455,79	56,01
SG_r_2	250	101772	25443000	92,35	88,35	56,33	50886	44672	456,38	56,21
SG_r_3	250	95816	23954000	92,5	88,62	55,91	47908	41797	454,73	55,8
AS_r_1	250	144822	36205500	90,97	86,36	56,92	72411	61945	454,95	56,77
AS_r_2	250	129576	32394000	92,18	88,1	56,51	64788	56345	454,75	56,43
AS_r_3	250	108660	27165000	92,36	88,35	56,09	54330	47632	456,54	55,95
CG_r_1	250	99866	24966500	91,98	87,84	56,3	49933	42952	452,49	56,18
CG_r_2	250	161552	40388000	92,48	88,59	55,77	80776	71014	455,03	55,66
CG_r_3	250	105318	26329500	87,69	82,03	56,26	52659	41312	452,68	56,03
SS_r_1	250	101630	25407500	88,37	83,2	54,84	50815	40551	451,51	54,65
SS_r_2	250	98192	24548000	88,3	82,98	55,5	49096	39340	454,96	55,24
SS_r_3	250	87466	21866500	85,68	79,52	55,89	43733	32043	454,62	55,55
SA_r_1	250	101460	25365000	88	82,58	55,68	50730	40540	455,17	55,39
SA_r_2	250	93802	23450500	88,17	82,89	55,06	46901	37675	452,58	54,88
SA_r_3	250	124930	31232500	87,39	81,78	55,53	62465	48827	454,47	55,22
SG_1	250	105962	26490500	93,58	90,1	55,87	52981	46888	453,74	55,82
SG_2	250	90334	22583500	92,78	89,02	55,65	45167	39545	454,32	55,56
SG_3	250	100158	25039500	93,57	90,06	56,13	50079	44544	455,07	56,05
AS_1	250	89306	22326500	93,67	90,2	56,03	44653	39610	453,92	56,01
AS_2	250	85168	21292000	93,29	89,69	56,03	42584	37495	454,4	55,99
AS_3	250	100870	25217500	93,62	90,15	55,94	50435	44971	454,6	55,86
CG_1	250	92324	23081000	92,46	88,48	56,24	46162	40187	452,01	56,18
CG_2	250	104168	26042000	92,55	88,64	56,02	52084	45868	455,5	55,88
CG_3	250	93616	23404000	92,43	88,42	56,53	46808	41130	452,45	56,5
SS_1	250	112828	28207000	91,17	86,73	56,03	56414	48037	453,03	55,92
SS_2	250	135620	33905000	92,62	88,77	55,64	67810	59665	455,33	55,52
SS_3	250	103198	25799500	92,74	88,88	55,6	51599	45795	453,79	55,5
SA_1	250	100618	25154500	92,37	88,37	55,73	50309	44402	455,65	55,58
SA_2	250	85164	21291000	90,85	86,14	56,84	42582	36519	453,59	56,55
SA_3	250	85046	21261500	91,65	87,31	56,12	42523	37086	454,57	55,78

CK, control group; SG, switchgrass group; AS, yarrow group; CG, Sheepgrass group; SS Kumadou group; SA, Kudouzi group; -r means rhizosphere soil samples; _1, _2 and _3 means biological replicates.

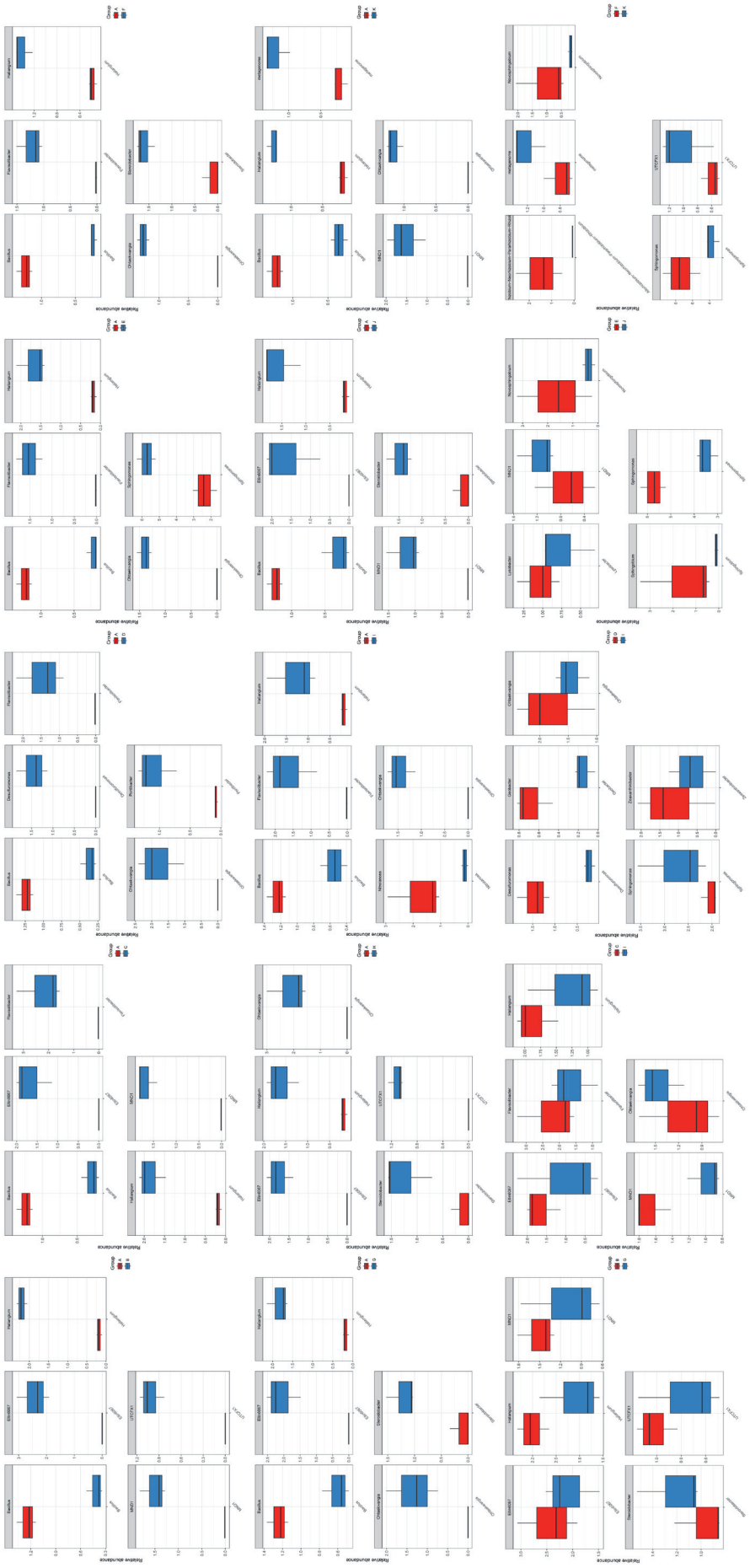

Fig. S1. Metastats analysis of bacteria abundance differences. The figure shows the abundance distribution of the top five bacteria with the largest difference between the two groups. The abscissa is the classification name of the five strains and the ordinate is the relative abundance of strains.