Drivers on The Halophytes' Rhizosphere Bacteria Community and Functions in North China Salinized Areas

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Abstract

Soil salinity is a serious environmental issue in arid China. Soil bacteria play a fundamental role in soil systems and respond rapidly to environmental changes. However, the responses of soil bacterial community to the different halophytes remains poorly understood. We investigated rhizosphere soil bacterial community changes under different halophytes in north China using high-throughput sequencing. Three typical halophytes were *Leymus chinensis* (LC), *Puccinellia tenuiflora* (PT), *Suaeda glauca* (SG). The dominant phyla were Proteobacteria, Actinobacteria, and Chloroflexi across three halophytic vegetation. These bacteria have important assistance for halophytes adapt to saline soil. PICRUSt forecasts demonstrated that energy metabolism, amino acid metabolism and carbohydrate metabolism are main bacterial functions in halophyte vegetation soil, and the abundance of metabolism these bacterial functions in SG was significantly higher than that in LC and PT. The pH value of different halophyte rhizosphere soils has a more significant effect on bacterial diversity than EC and soil trophic status, and soil water content (SWC) was important effect factors leading to differences in bacterial functions. These results give us a deeper understanding of the diversity and functional differences of rhizosphere soil bacterial communities in the typical halophytic vegetation of northern China.

Introduction

Soil salinity is one of the rising environmental issues causing considerable yield losses worldwide especially in arid and semiarid regions[1]. It damages the soil structure, reduces soil quality and limits the growing of crops[2]. Halophytes, such as *Suaeda glauca* (*Bunge*), *Puccinellia tenuiflora*, *Tamarix chinensis Lour* and *Leymus chinensis*, are plant species that grow well in saline soil due to their saline-alkali tolerance features[3-4]. They contribute enormously to the developing countries supply of food, fuel, fiber and fodder[5]. Saline soils are thought harsh environments for life, but such environments survive active and diverse microorganisms[6]. Soil microorganisms are crucial to the maintenance of ecosystem functions due to their contributions to soil structure formation and stability, organic matter decomposition, and nutrition cycling[7]. Soil microbial communities from salinity environment are more complex than soils under neutral pH or moderate salinity conditions[8]. Although most studies on halophytes have concentrated solely on phytoremediation of saline land and heavy metal contaminated soils by planting halophytes, little known microbially mediated decomposition processes in the rhizosphere soil under different halophytes[9-10].

The rhizosphere is a critical interface supporting the exchange of nutrients between plants and their associated soil environment[11]. It had long been recognized that bacteria are the most abundant and diverse group of microbes in the soil, and major drivers of biogeochemical cycles and participate in maintaining ecosystem functioning[12]. Many studies indicated that halotolerant rhizobacteria isolated from halophytes enhance salt tolerance in their host plants[13]. For example, Marasco et al. (2016) reported that rhizobacteria colonized in the *Salicornia strobilacea* rhizoplane is capable of improving plant growth[14]. Kearl et al. (2019) found that several bacteria communities, such as *Halomonas*, *Bacillus*, and *Kushneria*, have been observed to improve growth of alfalfa under saline conditions[15]. Likewise, several studies have been published on beneficial effects of bacterial application on wheat growth under salt conditions[16-17]. But, more information on the bacterial community present in the rhizospheric of various halophyte species is needed before these halotolerant rhizobacteria can be applied in salinity affected agriculture soil[18].

Pyrosequencing is a novel technique to expand our understanding of the bacterial community composition, diversity and in relation to their environments[19]. Simultaneously, PICRUSt has been widely used to predict the bacterial functions based on the16S rRNA gene[20]. Here, we investigated the effect of various halophyte species on soil bacterial communities in rhizospheric soil using high-throughput sequencing in north China. The objectives of this study were to: (i) investigated the soil properties changes under different halophyte species, (ii) reveal the bacterial community composition, diversity and predicted functions in soils from different halophyte species, (iii) to determine the possible factors in shaping bacterial community changes in these rhizospheric soils.

Material And Methods

Site description, experimental design and sampling. Soil samples were collected from three typical salinized areas in the north of China (40°28'37"-44°34'19", 85°54'03"-123°17'45"). The three typical salinized areas are located in Tumochuan Plain in Inner Mongolia, Songnen Plain in Jilin Province, and Manasi River Basin in the Xinjiang. The dominant halophyte is *Leymus chinensis (Trin.) Tzvel* in Tumochuan Plain, *Puccinellia tenuiflora* (*Griseb.*) *Scribn. et Merr* in Songnen Plain, and *Suaeda glauca* (*Bunge*) *Bunge* in Manasi River Basin (more than 85% of species present). *Leymus chinensis (Trin.) Tzvel* can tolerate cold, drought and alkali, has good nutritional value and high palatability. It has potential value as animal forage[57]. *Puccinellia tenuiflora* (*Griseb.*) *Scribn. et Merr* is monocotyledonous halophyte and an alkali tolerant species that can survive in highly alkaline soil[58]. *Suaeda glauca* (*Bunge*) *Bunge* is a rigid, annual, 100 cm high grass occurring in alkali conditions such as coastal region, wastelands, canal banks, and fields and tender plants are delicious and edible[59]. A summary of each treatment is given in Table 1. Soil samples were collected in the middle of May 2019. Within an area of approximately 35 ha, we randomly selected nine plots (replicates) in each samples, each plot 3 m×3 m within monospecific population of each halophyte species for sampling. With the use of soil corer (5 cm diameter), soil samples were collected from the roots of plants with the same canopies by using sterile brushes[60]. Soil samples of each replicate were put in individual plastic bags and transported to the laboratory. After visible stones and plant residues were removed, soil samples were sieved (2 mm) the separated into two subsamples, one portion was air-dried for the determination of chemical analysis, and the reminder was stored in a -20 °C refrigerator for molecular analysis.

Soil properties determination. Soil pH was measured with a compound electrode (INESA Scientific PHSJ-3F) using a soil to water ratio of 1:2.5. Soil electrical conductivity (EC) were tested using a 1: 5 soil water suspension. The soil water content (SWC) was measured after drying in an oven at 105°C for 24 h. Soil organic matter (SOM) was determined by oxidizing organic C with potassium dichromate (K_2Cr_2O7), and alkali hydrolysable nitrogen (AN) was measured by alkaline hydrolysis. The available potassium (AK) was measured by flame photometry after 1 mol L^{-1} $CH_3CO_2NH_4$ neutral extraction[61], and available phosphorus (AP) was extracted by $NaHCO_3$ and measured by molybdenum blue colorimetry.

DNA extraction and PCR amplification. Soil DNA was extracted from fresh soil sample (0.5 g) using Soil DNA Kit (Omega Bio-tek, Norcross, GA, U.S.), according to the manufacturer's protocols. The quality of extracted DNA was assessed by 1% agarose gel electrophoresis and substandard samples were extracted again until all the samples passed the quality control. All extracted DNA samples were stored at -20 °C for further analysis. The V4-V5 hypervariable regions of the soil bacterial 16S rRNA gene were subjected to high-throughput sequencing by Majorbio Pharmaceutical Technology Co., Ltd. (Shanghai, China) using PE300 sequencing platform (Illumina, Inc., CA, USA). The V4-V5 bacterial 16S rRNA gene were amplified by PCR using the primers pair 515 F/907R. The PCR program was as follows: denaturation at 95°C for 30 s; annealing at 5 °C for 30 s; extension at 72°C for 45 s; 27 cycles; holding at 72°C for 10 min and storing at 10°C. In order to guarantee the accuracy of the analysis results, Quantitative Insights into Microbial Ecology (QIIME software) (version1.9.0) was used for sequence filtering, and then the chimeric sequence was removed using Mothur software to obtain a high-quality sequence for subsequent analysis[62]. The Usearch program was used to cluster eligible sequences into operational classification units (OTUs) with a cut-off of 97% similarity. The representative sequence of each OTU is compared with the reference database.

Pyrosequencing data processing and statistical analysis. Venn diagrams were used to describe compare the similarities between soil bacterial communities at OTU level. According to the species abundance of each sample in the OTU list, Soil bacterial richness indices (Chao and Ace) and diversity index (Shannon) were calculated by using Mothur software[63]. We used nonmetric multidimensional scaling (NMDS) based on unweighted UniFrac distance to reveal changes in soil bacterial community structures[64]. Linear discriminant analysis (LDA) coupled with effect size measurements (LEfSe) analysis was conducted to search for statistically different biomarkers between groups[65]. The influences of halophytic vegetations and soil properties on soil bacterial community structures were examined by Mantel test and distance-based redundancy analysis (db-RDA). Spearman correlation coefficients were calculated and tested for significance between the dominant OTUs and soil properties. PICRUSt (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) was performed to

predict the relative abundance of main metabolic function genes[20]. The functional genes were predicted from the Kyoto Encyclopedia of Genes and Genomes (KEGG) catalogue[37]. We explored redundancy analysis (RDA) to understand the relationship between relative abundance of predicted functional genes and soil properties. SPSS version 20.0 (SPSS Inc., Chicago, USA) was utilized to data statistics and analysis of variance to determine the difference between soil properties, bacterial community and bacterial function spectrum. R was used to perform redundancy analysis. Venn diagram, cladogram, spearman correlation heatmap and abundance of KEGG heatmap were drawn using R[66]. The specific functional differences were performed using Origin version 9.0 (Microcal Software, Inc., Northampton, MA, USA).

Results

Soil properties. The changes of rhizosphere soil properties under different halophytes were shown in Table 2. Significant differences of soil pH were found between soil samples from the different halophytes ($P < 0.05$). PT had the highest soil pH, whereas LC had the lowest pH. Soil EC significantly ($P < 0.05$) varied from 0.45 to 1.44 ($mS \cdot cm^{-1}$) across the soil samples where the LC treatment showed highest values. There was a significant variation in SOM under different halophytes rhizosphere soil ($P < 0.05$). The highest SOM content was found in samples from LC, which was 84.31% higher than that of SG. SWC followed the order PT > LC > SG, and there was no significant difference between LC and PT ($P > 0.05$). There was a significant variation in available nutrients, which ranged from 7.28 to 17.05 mg kg^{-1} for available phosphorous (AP), from 115.2 to 240.73 mg kg^{-1} for available potassium (AK), and from 115.2 to 240.73 mg kg^{-1} for available nitrogen (AN), respectively ($P < 0.05$).

Soil bacterial diversity. Venn diagrams indicated that the sum of total observed OTUs in the soil samples from three halophytes rhizosphere was 10,154 (Fig. 1a), and 1,413 OTUs were shared by all three groups. The numbers of OTUs co-occurred in LC, PT and SG were 1 232, 522 and 931, respectively. To determine whether different halophytes are associated with alteration of soil bacterial community structure, we profiled the overall structural changes of bacterial community by using NMDS based on unweighted UniFrac dissimilarities (Fig. 1b). NMDS ordinations showed soil samples in LC are closely accumulated together, samples in PT and SG separated with LC along NMDS axis1 ($P < 0.01$). Coverage percentage of soil samples under three halophytes exceeded 98%, indicating that the sequencing results can be used for further analysis (Fig. 2). LC had the highest Shannon index, but there was no significant difference in LC and SG ($P > 0.05$). The ACE index of samples under different halophytes had the same trends with Chao index, the highest values were recorded in LC, but there was no significant difference in SG and LC ($P < 0.05$)

Soil bacterial community composition. A total of 38 bacterial phyla were procured from soil samples. *Proteobacteria, Actinobacteria, Chloroflexi, Gemmatimonadetes, Acidobacteria, Bacteroidetes, Planctomycetes,* and *Firmicutes* were dominant phyla through entire soil samples (Fig. 3a). However, no significant difference was observed between the LC, PT and SG. To further compare the differences in bacterial community composition between rhizosphere soil under different halophytes, we conducted the relative abundance of bacteria (> 0.02%) at the class level (Fig. 3b). The dominant classes were *Actinobacteria*, *Alphaproteobacteria, Gemmatimonadetes, Gammaproteobacteria* and *Anaerolineae* in LC, PT and SG. Statistical analysis was performed from the phylum to the class level under the LEfSe tool. Groups were shown in cladograms, and LDA scores of 3 or greater were confirmed by LEfSe (Fig. 3c). *Planctomycetes, Planctomycetacia* and *Rhodothermia* were significantly enriched in LC. *Rokubacteria, Deltaproteobacteria, Anaerolineae, NC10, Ignavibacteria* and *Thermodesulfovibrionia* were significantly enriched in PT. *Cyanobacteria, Chloroflexia, Oxyphotobacteria, Nitrospira, Nitrospirae, Deinococci, Deinococcus_Thermus* and *Gitt_GS_136* were significantly enriched in SG.

Soil potential bacterial functions. We used PICRUSt to predict the soil bacterial community function in the rhizosphere soil under three halophytes (Fig. 5). The classification of KEGG functions at pathway Level 1 and pathway Level 2 was performed in Figure 4. The result showed that the predicted functions mainly the bacterial metabolism, and the relative abundance of three halophytes was above 60% at pathway Level 1. Carbohydrate metabolism, amino acid metabolism, energy metabolism, nucleotide metabolism and lipid metabolism were main metabolic functions at pathway Level 2. Interestingly, the carbohydrate metabolism, amino acid metabolism and energy metabolism were observed to account for more than 7% at pathway Level 2

throughout the three halophytes. Further statistical tests revealed the relative abundance of carbohydrate metabolism, Lipid metabolism and xenobiotics biodegradation and metabolism were highest in SG (Fig. 5). Nucleotide metabolism, Replication and repair, Folding, sorting and degradation and Transcription were no significant difference between LC and PT ($P > 0.05$), which was significantly higher than that in SG. Cell motility and Transport and catabolism in LC and SG, which was significantly higher than that in PT (Fig. 5; $P < 0.05$).

Correlation between bacterial communities and soil properties. We explored the influence of soil properties on bacterial community composition at OTU level through db-RDA analysis (Fig. S1; Mantel test, R = 0.492, $P = 0.001$). It is apparent from this chart that the two axes of CAP axis explain 37% of total variation, and soil EC, pH, SOM, AK and AN were longer arrows. The quantitative data between the soil properties and the bacterial communities analyzed by db-RDA at the OTU Leve l was shown in Table 3, Soil EC ($P = 0.001$), pH ($P = 0.001$), SOM ($P = 0.002$), AK ($P = 0.001$) and AN ($P = 0.001$) were closely correlated to the CAP axis. EC, pH, SOM and AN were good illustration that these soil properties were significantly correlated with the top 50 abundance of bacterial OTUs through further correlation analysis (Fig. 6; Table S1), and this coincides with the results of db-RDA analysis. To determine which soil properties affected the metabolic functions, we performed RDA analysis of soil properties and pathway Level 2's KEGG function. We found that these metabolic functions are significantly correlated with soil SWC ($P = 0.001$) and AK ($P = 0.001$; Table 4). On the other hand, carbohydrate metabolism, amino acid metabolism, nucleotide metabolism, lipid metabolism, xenobiotics biodegradation and metabolism, glycan biosynthesis and metabolism, translation, replication and repair, folding, sorting and degradation, transport and catabolism and signaling molecules and interaction were significantly correlated with soil SWC (Table S3; $P < 0.01$).

Discussion

Soil properties. Our study found that soil properties, such as pH, EC, SOM, were changed significant in rhizosphere soil of three halophytes. Soil pH in PT was significantly higher than that of SG and LC, the changes in soil pH could be attributed to the higher concentration of Na_2CO_3 in soil. Previous studies showed that saline-alkali soil increased soil pH by increasing soil Na^+, CO_3^{2-}, and HCO_3^-concentration[21-23]. Excessive soluble salt content leads to the EC in LC being significantly higher than that in SG and PT. The current study found that soil SOM content is significant differences in rhizosphere soil of three halophytes, and the highest SOM content was found in samples from LC. It is consistent with prior studies that have noted the influence of different halophytes type to soil organic matter[24]. This discrepancy could be attributed to different halophytes forms influencing organic matter input by plant debris input and rooting depth[25].

Soil bacterial community diversity of different halophytes. Soil bacterial diversity is critical to maintaining the soil ecosystems[26]. Yamamoto (2018) who reported the diversity of bacterial communities, depends on the halophytic plant species and the sampling site[13]. In our study, the soil samples from different regions with three halophytes are geographically distant, environment and climatic factors are different (Table 1). These factors can shape the diversity of rhizosphere soil bacterial community. The results of NMDS analysis confirm that soil bacterial community were significant difference in rhizosphere soil of three halophytes. Study has shown that the rhizosphere of plants has specific selectivity for bacteria that colonize the rhizosphere, which change the species richness and homogeneity, leading to differences in alpha diversity[27]. In addition, microbial community diversity also affected by different root exudates[28]. The total number of OTUs and bacterial diversity indices (including ACE, Chao, and Shannon) were the highest in LC. This is related to the higher SOM content in the LC rhizosphere soil, since accumulation of organic matter increases the chances of successful migration of soil bacteria community[29]. These consequences indicate the LC is more conducive to the restoration of soil bacterial diversity in saline-alkali conditions compared with PT and SG. This may provide basis for improvement the bacterial diversity by using halophyte in saline-alkali soils.

Soil bacterial community composition and potential functions of different halophytes. The rhizosphere microbiome plays an important role in promoting plant survival under adverse conditions in plant life[5]. *Proteobacteria, Actinobacteria, Chloroflexi, Gemmatimonadetes, Acidobacteria, Bacteroidetes, Planctomycetes, Firmicutes*, and *Cyanobacteria* were the dominant phyla in

this study. These phyla have also been recorded in other halophytes[30]. It is reported that most halophiles were observed in the *Proteobacteria*[31]. The important role of *Actinobacteria* secrete antimicrobial pathogens compounds and *Chloroflexi* decompose organic compounds has been demonstrated in previous studies[32-33]. Yamamoto et al. (2018) reported that *Proteobacteria* accounting for over 40% in samples from *Glaux maritima* and *Salicornia europaea*[13]. We found that the relative abundance of *Proteobacteria* was more than 20% in three halophytes rhizosphere soils (Fig. 3a). This indicates that three halophytes may affected by the salt stress, and enriched *Proteobacteria Actinobacteria and Chloroflexi* in the rhizosphere soil. Plant species certainly affect the structure of bacterial communities, and select specific microbial populations[34]. Specific selection leads to differences in the rhizosphere bacterial communities of halophytes, and confirmed in our research (Fig. 3c). These results indicate that bacteria have important assistance for halophytes adapt to saline soil, and the abundance bacteria is also affected by different halophytes vegetation types. Soil bacteria play a vital part in nutrient cycling, maintaining soil fertility, and carbon sequestration through amino acid and carbohydrate metabolism[35-36]. The relative abundance of bacterial metabolism was above 60% in soil from three halophytes rhizosphere. Carbohydrate metabolism, amino acid metabolism, energy metabolism, nucleotide metabolism and lipid metabolism were main metabolic functions at pathway Level 2. This finding is consistent with that of Cheng et al. (2018) and Chen et al. (2019) who found that amino acid metabolism and carbohydrate metabolism are the main metabolic functions by PICRUSt predictions[37-38]. These results suggested that amino acid metabolism and carbohydrate metabolism are main bacterial functions in soil, not only in halophyte vegetation soil but also in other soil types[39-41]. Chaudhary et al. (2018) indicated that the microbial functional gene abundance was affected by different halophyte vegetation types[42]. In our study, most of bacterial functions were significant difference in rhizosphere soil of three halophytes. This indicates that different halophytes in shaping different rhizosphere bacterial communities also caused differences in bacterial function.

Correlation between bacterial communities, functions and soil properties. Previous studies showed that soil properties are the most important factors leading to the changes in soil bacterial communities[43-46]. However, our results confirm that soil properties only explain 37% of total variation in the rhizosphere bacterial community. The remaining 60% of the variation may be caused by the rhizosphere exudates of different vegetation types, geographical location and climatic factors. The effect of root exudates on the bacterial community has been fully demonstrated[47]. The decomposition of root exudates and litter will change soil characteristics and promote the establishment of specific rhizosphere bacterial communities[48-49]. Geographical location and climatic factors not only affect vegetation types, but also affect the composition of plant rhizosphere or non-rhizosphere soil bacterial communities. The microbial biogeography is controlled primarily by edaphic variables, especially by pH [50]. Our results confirm that soil EC (P = 0.001), pH (P = 0.001) and SOM (P = 0.002) are significantly related to the soil bacterial community and are consistent with previous research[51-52]. Correlation analysis showed that ACE, Chao, and Shannon indices negatively correlated with pH, while the effect of EC and SOM on bacterial diversity in this study was not significant (Table S2). It is consistent with prior studies who reported that the soil bacterial diversity effected by pH[53-55]. The pH not only affects the abundance but also the bacteria diversity, this suggesting that soil pH is important factor in shaping soil bacterial diversity in rhizosphere soil of halophytes. Simultaneously, this influence of soil properties on bacterial functions is also reflected in the prediction function[34,49]. We found that the main metabolic functions significantly correlated with SWC (Table 4; Table S3; P < 0.001). This indicates that soil moisture regulated rhizosphere soil bacterial metabolic capacity of halophyte vegetation. This finding is consistent with that of Cui et al. (2018) who reported that soil moisture regulated microbial metabolism[56].

Conclusions

The dominant rhizosphere soil bacterial phyla under three halophytic vegetation were *Proteobacteria*, *Actinobacteria* and *Chloroflexi* in our study, and the abundance bacteria is also affected by different halophytic vegetation types. The bacterial diversity was significantly higher in LC and SG (P<0.05). Functional predictions, based on 16S rRNA gene by PICRUS, indicated that energy metabolism, amino acid metabolism, carbohydrate metabolism and fatty acid metabolism are main bacterial functions under soil samples from three halophytes. Soil pH, EC and SOC are significantly correlated with soil bacterial

communities (P<0.05), but bacterial diversity indices (ie. Ace, Chao and Shannon) were negatively correlated with soil pH (P<0.05). SWC strongly correlated with dominate functions. Together, soil pH is one of the key factors in shaping halophytic rhizosphere soil bacterial community composition and diversity, and SWC content is a possible factor affecting bacterial dominate predicted functions.

Declarations

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Author contributions statement

F.Y. and F.Z. initiated and designed the research. F.Y. and H.W. performed the experiments and collected the data. F.Y. analyzed the data and wrote the manuscript, F.Z. and Z.C. revised the manuscript. All authors reviewed and approved the final manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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Tables

Table 1. General description of geography at the three experimental sites. LC, *Leymus chinensis (Trin.) Tzvel.;* PT, *Puccinellia tenuiflora (Griseb.) Scribn. et Merr.;* SG, *Suaeda glauca (Bunge) Bunge.*

Treatments	LC	PT	SG
Dominat halophyte	Leymus chinensis (Trin.) Tzvel	Puccinellia tenuiflora (Griseb.) Scribn. et Merr	Suaeda glauca (Bunge) Bunge
Coordinates	40°28'37", 111°04'32"	45°59'12", 123°02'13"	44°37'53", 85°55'36"
Mean sea level (m)	1000	130	450
Climate	Temperate semi-arid continental monsoon climate	Temperate semi-arid continental monsoon climate	Temperate continental climate
Mean annual precipitation (mm)	400	399	300
Mean annual evaporation (mm)	1000-1100	662	1000
Mean annual temperature (°C)	6.3	5.2	6.5
Soil classification	Sandy soil	Meadow soil	Sandy soil

Table 2. *Soil physicochemical properties under three halophytes. LC, Leymus chinensis (Trin.) Tzvel.; PT, Puccinellia tenuiflora (Griseb.) Scribn. et Merr.; SG, Suaeda glauca (Bunge) Bunge. EC, electrical conductivity; SOM, soil organic matter; SWC, soil water content; AP, available phosphorus; AK, available potassium; and AN, available nitrogen. Values are means ± standard deviation (n = 4). Different lowercase letters into each column are significantly different (Tukey's test, P < 0.05).*

Treatments	pH	EC ($mS \cdot cm^{-1}$)	SOM ($g \cdot kg^{-1}$)	SWC	AP ($mg \cdot kg^{-1}$)	AK ($mg \cdot kg^{-1}$)	AN ($mg \cdot kg^{-1}$)
LC	9.22 ± 0.10^{c}	1.44 ± 0.42^{a}	36.13 ± 3.80^{a}	0.22 ± 0.02^{a}	17.05 ± 5.53^{a}	139.77 ± 8.57^{b}	75.60 ± 15.03^{a}
PT	10.53 ± 0.03^{a}	0.45 ± 0.04^{b}	20.45 ± 7.94^{b}	0.22 ± 0.05^{a}	7.28 ± 6.69^{b}	115.21 ± 22.81^{b}	43.28 ± 3.20^{b}
SG	9.54 ± 0.43^{b}	0.47 ± 0.08^{b}	5.67 ± 2.37^{c}	0.06 ± 0.05^{b}	8.84 ± 1.29^{b}	240.73 ± 80.39^{a}	35.18 ± 4.51^{b}

Table 3. Correlation between soil properties parameters and db-RDA axes. EC, electrical conductivity; SOM, soil organic matter; SWC, soil water content; AP, available phosphorus; AK, available potassium; and AN, available nitrogen. $^{**}P < 0.01$; $^{***}P < 0.001$.

Soil properties	CAP1	CAP2	R^2	*P* values
EC	0.998	-0.057	0.369	0.001 ***
pH	-0.796	-0.606	0.946	0.001 ***
SOM	0.971	-0.238	0.508	0.002 **
SWC	0.273	-0.962	0.171	0.110
AP	0.990	0.139	0.182	0.095
AK	-0.470	0.883	0.496	0.001 ***
AN	0.989	0.145	0.439	0.001 ***

Table 4. Significance of the soil physicochemical properties in explaining the KEGG function from RDA analysis. EC, electrical conductivity; SOM, soil organic matter; SWC, soil water content; AP, available phosphorus; AK, available potassium; and AN, available nitrogen. ** $P < 0.01$; *** $P < 0.001$.

Soil property	RDA1	RDA2	R^2	*P* values
EC	-0.199	0.979	0.023	0.815
pH	-0.514	0.858	0.137	0.167
SOM	-0.209	0.978	0.147	0.150
SWC	-0.837	0.547	0.446	0.001 ***
AP	-0.568	0.823	0.067	0.437
AK	0.998	-0.058	0.636	0.001 ***
AN	0.644	0.765	0.024	0.734

Figures

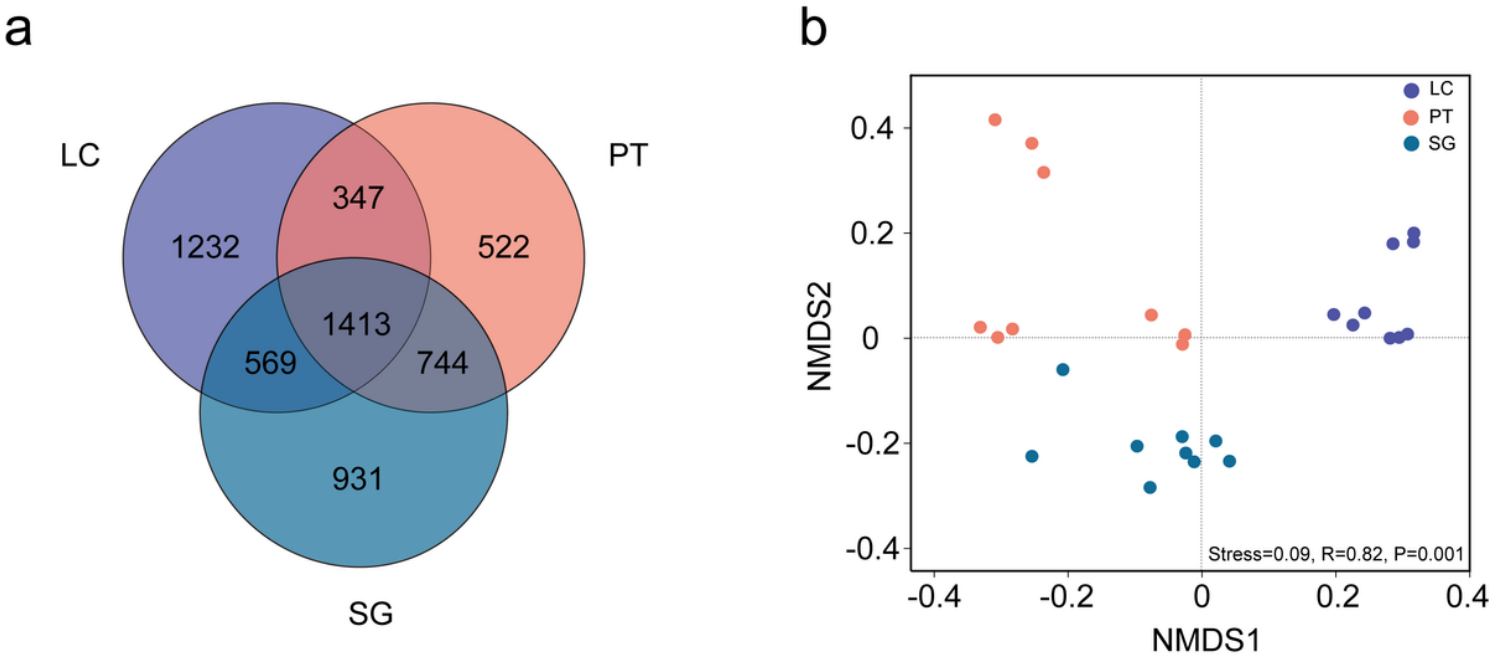

Figure 1

Venn diagram showing unique and shared OTUs under rhizosphere soil of three halophytes (a). NMDS analysis (b) based on the unweighted UniFrac distances at OTU level. LC, Leymus chinensis (Trin.) Tzvel.; PT, Puccinellia tenuiflora (Griseb.) Scribn. et Merr; SG, Suaeda glauca (Bunge) Bunge.

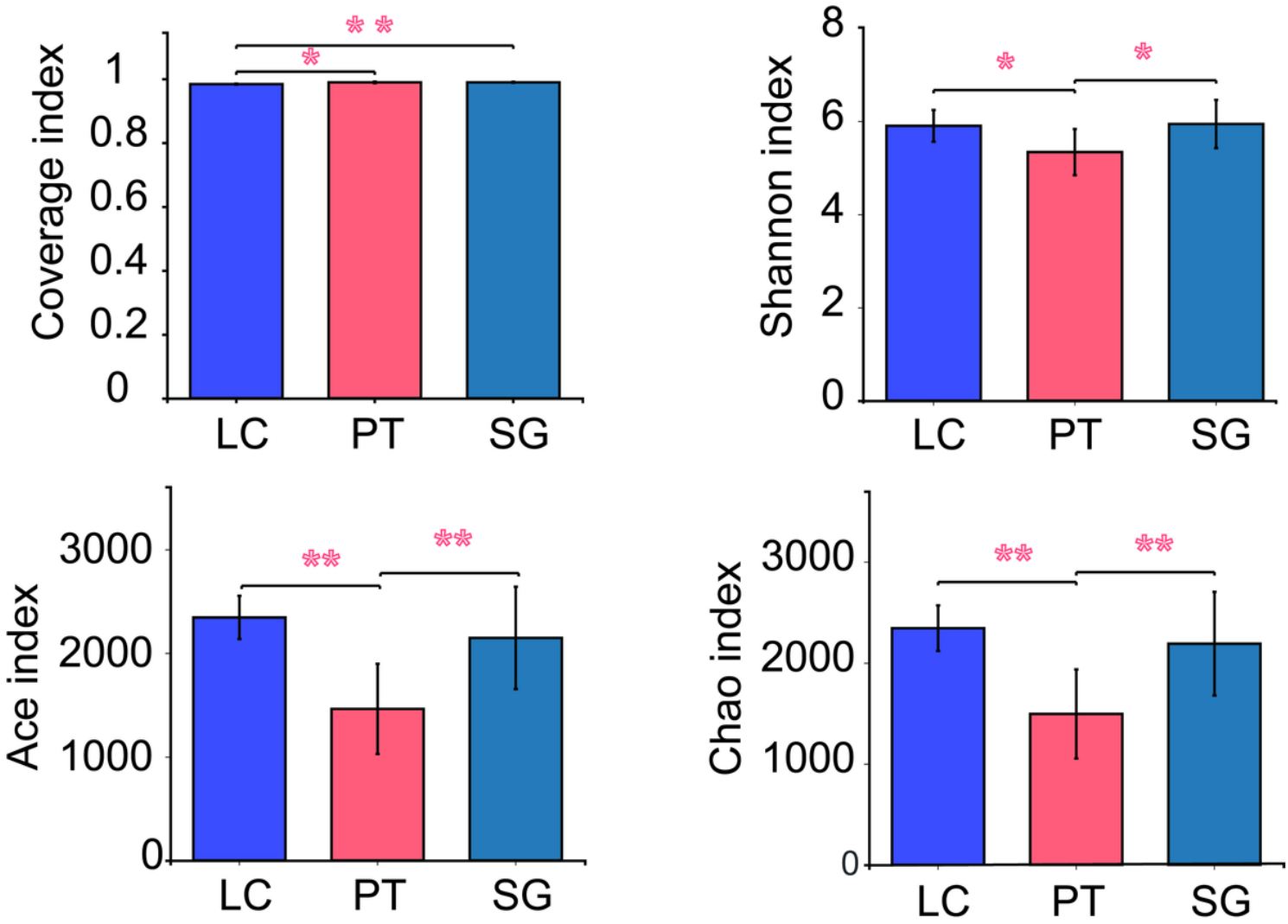

Figure 2

Histogram for α-diversity of the rhizosphere soil bacterial communities under three halophytes. LC, Leymus chinensis (Trin.) Tzvel.; PT, Puccinellia tenuiflora (Griseb.) Scribn. et Merr; SG, Suaeda glauca (Bunge) Bunge. (Tukey's test, *P < 0.05, **P < 0.01).

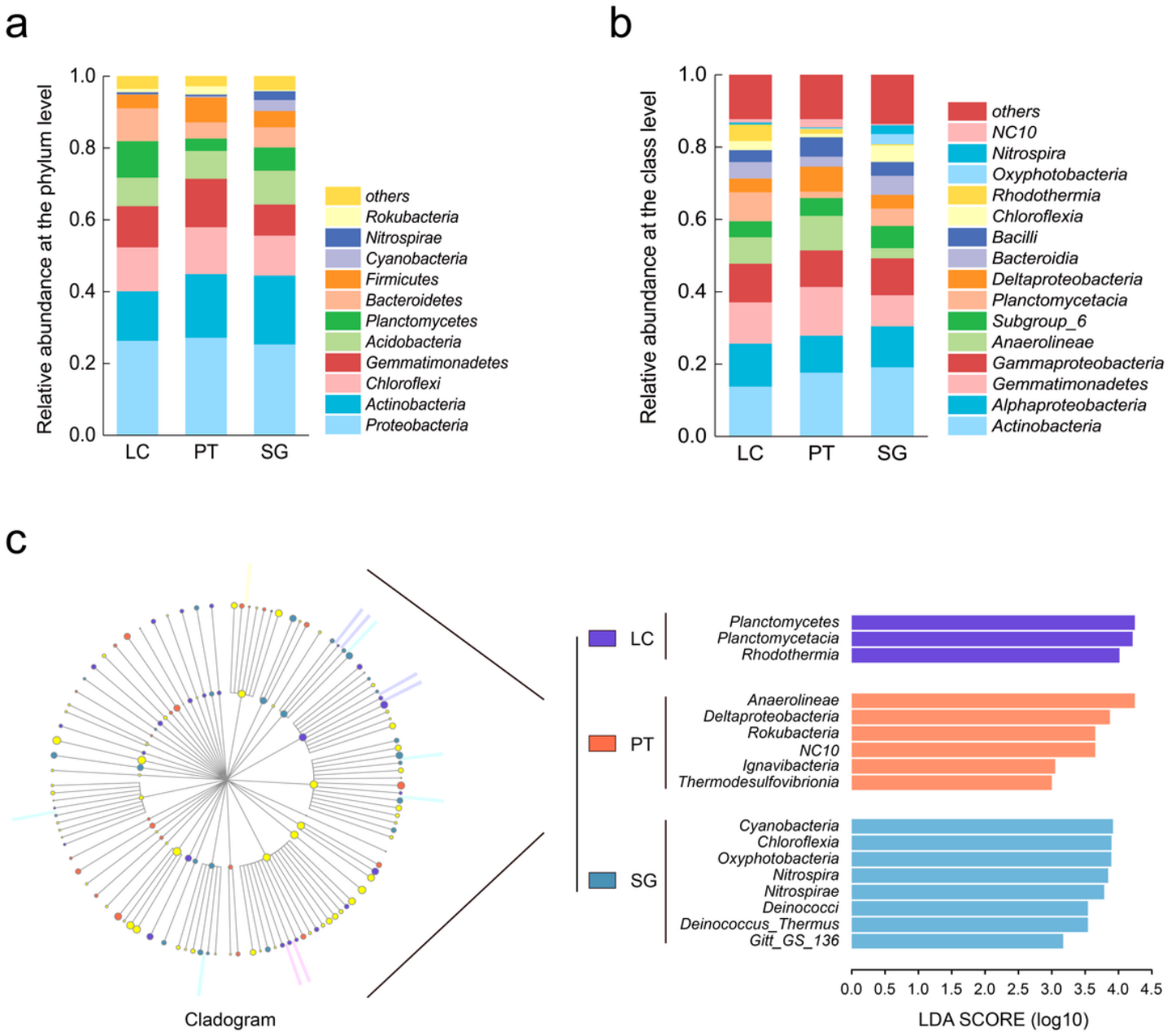

Figure 3

Relative abundance of rhizosphere soil bacterial communities at the phylum level (a) and class level (b) across three halophytes. Cladogram showing the phylogenetic distribution of the bacterial lineages associated with rhizosphere soil from the three halophytes; indicator bacteria with LDA scores of 3 or greater in bacterial communities (c). Different-colored regions represent different constituents (purple, LC; orange, PT; blue, SG). LC, Leymus chinensis (Trin.) Tzvel.; PT, Puccinellia tenuiflora (Griseb.) Scribn. et Merr; SG, Suaeda glauca (Bunge) Bunge. Circles indicate phylum levels from domain to class. The diameter of each circle is proportional to the abundance of the group.

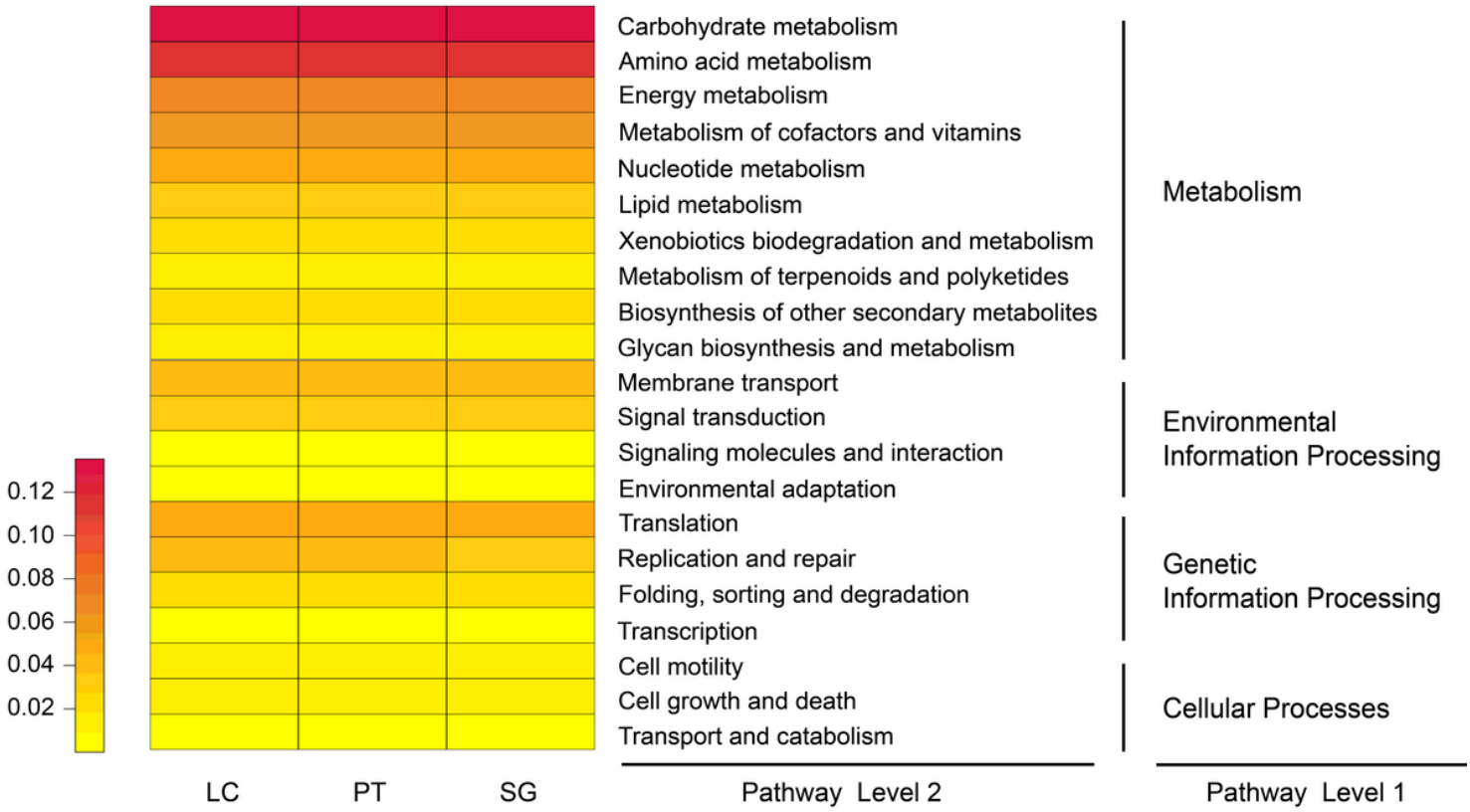

Figure 4

Predicted functions of the rhizosphere soil bacterial communities using PICRUSt at KEGG function classification. LC, Leymus chinensis (Trin.) Tzvel.; PT, Puccinellia tenuiflora (Griseb.) Scribn. et Merr; SG, Suaeda glauca (Bunge) Bunge. The changes in relative abundance of different functions were shown by the gradient of color patch. The legend is the value represented by the color gradient.

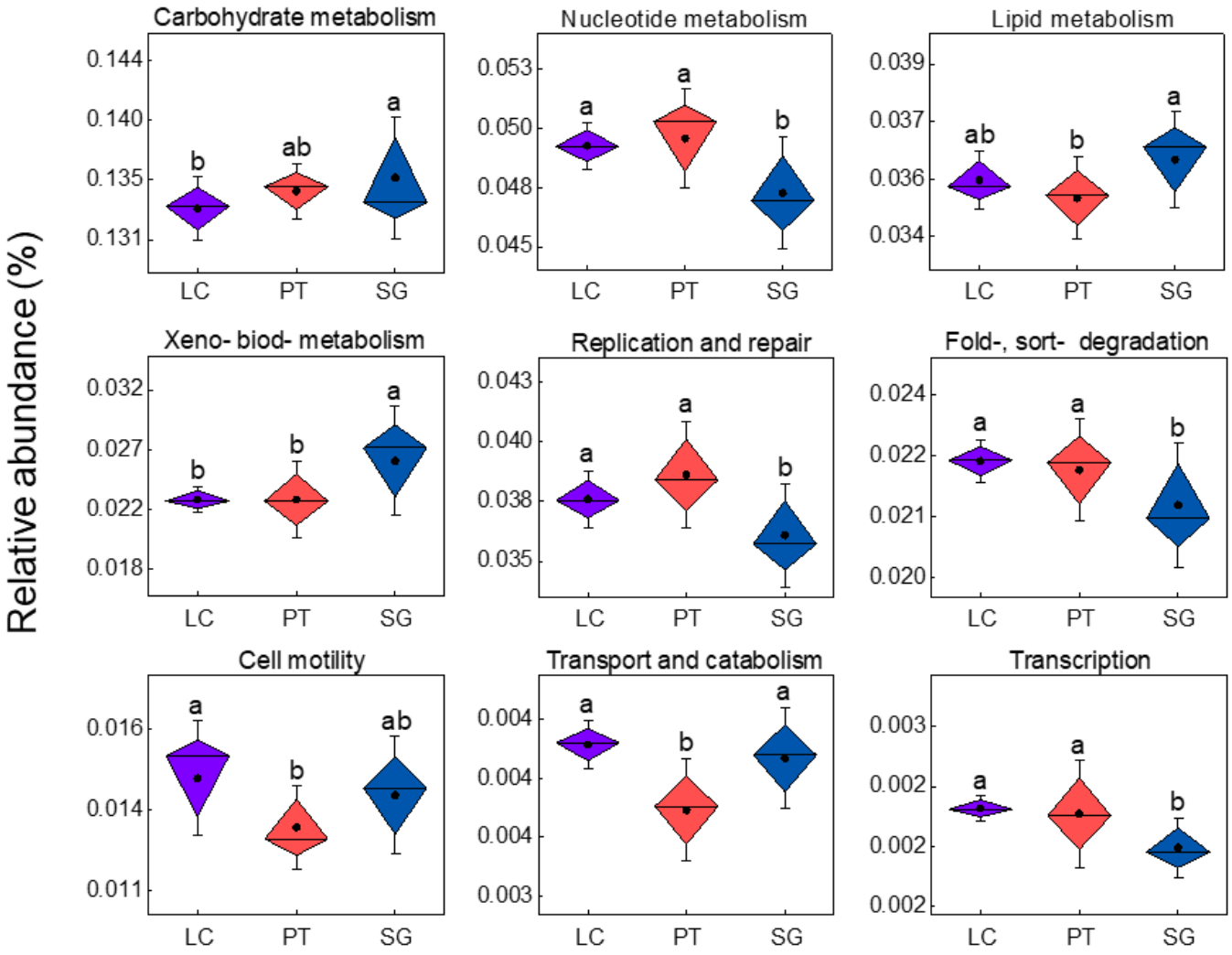

Figure 5

Box plots for metabolism functions of the rhizosphere soil bacterial communities under three halophytes. Carbohydrate metabolism, Nucleotide metabolism, Replication and repair, Lipid metabolism, Xenobiotics biodegradation and metabolism, Folding, sorting and degradation, Cell motility, Transport and catabolism and Transcription at pathway Level 2. Xeno- biod- metabolism: Xenobiotics biodegradation and metabolism; Fold, sort- degradation: Folding, sorting and degradation. LC, Leymus chinensis (Trin.) Tzvel.; PT, Puccinellia tenuiflora (Griseb.) Scribn. et Merr; SG, Suaeda glauca (Bunge) Bunge. Different lowercase letters indicate significant difference. Different lowercase letters indicate significant differences (Tukey's test, $P < 0.05$).

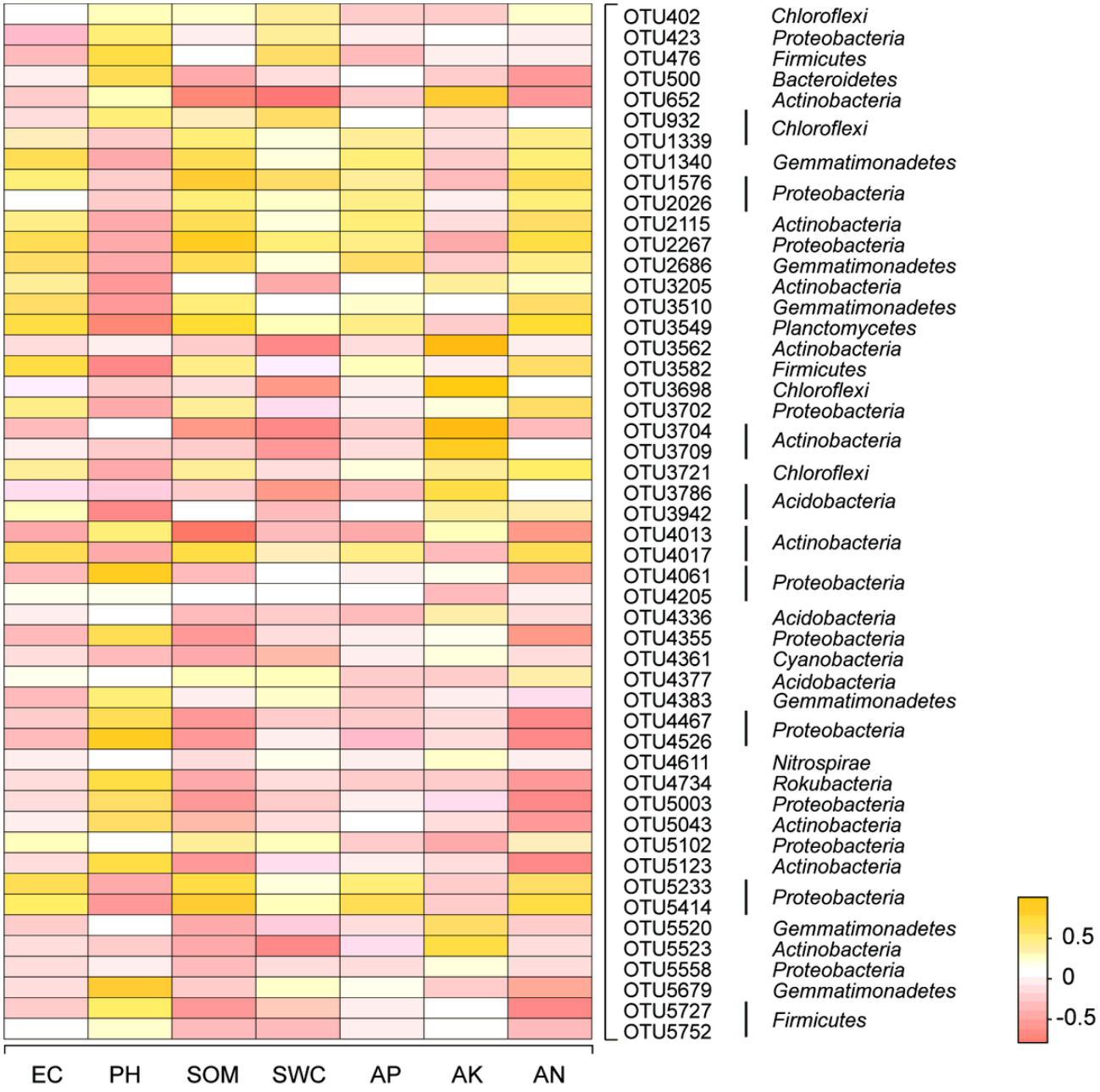

Figure 6

Correlation analysis between the top 50 OTUs of abundance and soil properties at the OTU level. The legend is the P value of spearman correlation represented by the color gradient. EC, electrical conductivity; SOM, soil organic matter; SWC, soil water content; AP, available phosphorus; AK, available potassium; and AN, available nitrogen.

Supplementary Files

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