The rhizosphere bacterial community associated with five halophytes in arid saline land (#25925)

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The rhizosphere bacterial community associated with five halophytes in arid saline land

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Background. Soil salt content is naturally an important stress factor for plants and microbiomes in saline soil environments. Ebinur Lake Nature Reserve is located at the western margin of the Gurbantunggut Desert of northwest China, which has a large area of salinized environments and a high diversity of halophytes. This study aimed to investigate the bacterial diversity and community structure in bulk and rhizosphere soils related to five halophytic plant species to gain insight into the effects of both plant species and soil salt content on bacterial community structure. **Methods.** Bacterial 16S rDNA V3–V4 region was amplified and sequenced using the Illumina Miseq platform of 15 bulk and 15 rhizosphere samples. The bacterial community diversity and structure were compared between rhizosphere and bulk soils, as well among the rhizosphere of five plants. **Results.** The bacterial richness and diversity in halophyte rhizospheres were significantly higher than those in bulk soils, and the bacterial structure between them also differed significantly. Phyla Proteobacteria and Firmicutes, and genera *Exiguobacterium*, *Citrobacter*, *Acinetobacter* and *Pseudomonas* were abundant groups in bulk soil, whereas their relative abundance in rhizosphere communities was significantly lower. Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes, Planctomycetes and Acidobacteria were the most abundant phyla in the rhizosphere, and *Halomonas, Exiguobacterium, Gracilimonas, Citrobacter, Acinetobacter, Pseudomonas, Deferrisoma, Aliifodinibius, Thioprofundum* and *Gp10* were the most abundant genera. ANOSIM analysis showed that there were significant differences in rhizosphere community structure between five halophytes ($P = 0.001$), and a total of 9 phyla, 17 classes, 93 genera and 293 OTUs differed significantly. Apart from the differences, similarities were found in that 647 OTUs and most of the abundant genera were shared in the rhizosphere bacteria of five halophytes. **Discussion.** Halophytic plants were shown to have significant effects on soil bacterial communities. The similarities and dissimilarities among rhizosphere communities

of five halophytic plants indicates that rhizosphere effect and salinity were the two most important factors in shaping the bacterial community structure in saline lands.

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ABSTRACT

Background. Soil salt content is naturally an important stress factor for plants and microbiomes in saline soil environments. Ebinur Lake Nature Reserve is located at the western margin of the Gurbantunggut Desert of northwest China, which has a large area of salinized environments and a high diversity of halophytes. This study aimed to investigate the bacterial diversity and community structure in bulk and rhizosphere soils related to five halophytic plant species to gain insight into the effects of both plant species and soil salt content on bacterial community structure.

Methods. Bacterial 16S rDNA V3–V4 region was amplified and sequenced using the Illumina Miseq platform of 15 bulk and 15 rhizosphere samples. The bacterial community diversity and structure were compared between rhizosphere and bulk soils, as well among the rhizosphere of five plants.

Results. The bacterial richness and diversity in halophyte rhizospheres were significantly higher than those in bulk soils, and the bacterial structure between them also differed significantly. Phyla Proteobacteria and Firmicutes, and genera *Exiguobacterium*, *Citrobacter*, *Acinetobacter* and *Pseudomonas* were abundant groups in bulk soil, whereas their relative abundance in rhizosphere communities was significantly lower. Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes, Planctomycetes and Acidobacteria were the most abundant phyla in the rhizosphere, and *Halomonas, Exiguobacterium, Gracilimonas, Citrobacter, Acinetobacter, Pseudomonas, Deferrisoma, Aliifodinibius, Thioprofundum* and *Gp10* were the most abundant genera. ANOSIM analysis showed that there were significant differences in rhizosphere community structure between five halophytes ($P = 0.001$), and a total of 9 phyla, 17 classes, 93 genera and 293 OTUs differed significantly. Apart from the differences, similarities were found in that 647 OTUs and most of the abundant genera were shared in the rhizosphere bacteria of five halophytes.

Discussion. Halophytic plants were shown to have significant effects on soil bacterial communities. The similarities and dissimilarities among rhizosphere communities of five halophytic plants indicates that rhizosphere effect and salinity were the two most important factors in shaping the bacterial community structure in saline lands.

Keywords: Halophyte, Rhizosphere, Bacterial community, diversity

INTRODUCTION

The rhizosphere represents one of the most diverse habitats on the planet (Hinsinger et al. 2009). Rhizosphere microbiomes receive carbon metabolites from the plant through root exudates (Bais et al. 2006). In turn, they convert nutrients into more usable forms for assimilation by plants (Zhang et al. 2009) or into secreted growth regulators, such as growth-promoting hormones and volatile organic compounds to promote plant growth (Palaniyandi et al. 2014; Vaishnav et al. 2015). Some beneficial microbes enhance pathogen resistance, water retention, and the drought and salt resistance ability of plants (Lee et al. 2015; Ngumbi & Kloepper 2016).

Salinization is an important land degradation problem, and high salinity limits plant growth and crop productivity. Due to natural processes such as mineral weathering, dust and precipitation or artificial processes such as irrigation (Oosterbaan 1988), salts accumulate in soils, leading to saline soils and increasing the difficulty for plants to absorb soil moisture. Halophytes are salt-tolerant plants that can grow in saline soil, such as those found in saline semi-deserts, mangrove swamps, marshes, sloughs and seashores. Dominant halophytes play a significant role in carbon sequestration, nutrient mineralization, nutrient cycling and improving micro-environment (Chaudhary et al. 2015).

Interestingly, salt tolerance of halophytes is connected with plant associated microbiomes (Ruppel et al. 2013). To date, many halophilic bacteria have been isolated from halophyte roots, soil and desert habitats, such as species affiliated with genera such as *Halomonas*, *Halobacillus*, *Brevibacterium, Bacillus*, *Stenotrophomonas*, *Alkalimonas*, *Staphylococcus* and *Methylibium* (Ramadoss et al. 2013; Sgroy et al. 2009; Shi et al. 2012a; Siddikee et al. 2010; Zhou et al. 2012), which represents a distinct difference from bacterial composition in nonhalophytic rhizosphere. Analysis of plant-associated halophilic bacteria is important to learn about their ecological functions, and how these organisms evolved mechanisms of saline adaptation, which could yield potential uses in biotechnology (Ruppel et al. 2013).

It has been demonstrated that plant species have important effects on rhizosphere microbial diversity and structure. Different halophytic plant species or genotypes tend to influence distinct root associated bacterial communities (Chaudhary et al. 2015). For instance, Actinobacteria, Firmicutes, and Proteobacteria are the most abundant bacteria phyla in the rhizosphere of *Aster tripolium* (Szymanska et al. 2016), while Acidimicrobiales, Myxococcales and Sphingomonadales are enriched in *Halimione portulacoides* and *Sarcocornia perennis* (Oliveira et al. 2014). On the genus level, *Bacillus* dominates in the rhizosphere soil of *Aster tripolium*

(Szymanska et al. 2016), while *Puccinellia limosa* are dominated by *Halomonas* and *Nesterenkonia* (Borsodi et al. 2015). However, it is reported that the plant effect on rhizosphere community structure is minor compared to environmental factors, such as soil salinity (Borruso et al. 2014).

Ebinur Lake Nature Reserve was located at the western margin of the Gurbantunggut Desert, Xinjiang, China. The Reserve has a typical continental climate and is dry and windy, with an annual average precipitation of 105 mm and an evaporation of 1315 mm. The soil in the Reserve is highly salinized and alkalized, and the average electrical conductivity (EC) and pH value of the 0–10 cm soil layer are 5.41 mS/cm and 8.77, respectively, with an average water content of 7.19% (Zhang et al. 2014). There is a great diversity of halophytes in the Reserve area, such as: *Populus euphratica, Tamarix ramosissima, Haloxylon ammodendron, Halostachys caspica, Halocnemum strobilaceum, Suaeda galuca, Halidium floiatum, Kalidium capsicum, Lycium ruthenicum, Salicornia europaea.*

Previous studies on rhizosphere microbial community of halophytes contribute greatly to our understanding of the rhizosphere bacterial community structure, as well as isolation and identification of a wide range of halophilic bacteria, gaining new insights into their ecological functions and their potential effects on salt tolerance and adaptation of plants in saline or hypersaline environment. Rhizosphere microbial community structures are influenced by various factors such as plant species, soil properties, and growth stage and geographic environments (Pii et al. 2016; Rodriguez-Blanco et al. 2015; Song et al. 2017; Tian & Gao 2014). In different ecosystems, the effect of different factors on bacterial community structure may vary. Though some studies focused on bacterial community of halophytes, current knowledge on bacterial community structure in rhizospheric soils of halophytes is relatively limited to glycophytes or crops. Therefore, more studies on the structure and functionality of halophytes associated with the bacterial community in natural saline soils are needed to gain a better and thorough knowledge of their roles in ecosystem function (Berg & Smalla 2009).

In present study, we focus on bacterial communities associated with five halophytes (i.e. *Lycium ruthenicum*, *Limonium gmelinii, Kalidium foliatum*, *Halostachys caspica*, and *Halocnemum strobilaceum*) in arid areas of northwest China. The rhizosphere bacterial community diversity and structure was evaluated via a Illumina MiSeq sequencing approach, comparing bacterial communities of rhizosphere with bulk soils, as well differences in rhizosphere community structure between the five plant species, exploring the relationship between both plant species and soil salt content with respect to rhizosphere bacterial community structure in saline habitats.

MATERIALS & METHODS

Study areas and sample collection

The soil samples were collected from the Ebinur Lake Wetland Nature Reserve, Xinjiang, China (44.595°N, 83.552°E) during the summer, following previously established protocols (Chaudhary et al. 2015; Edwards et al. 2015). Three healthy individuals per species were selected randomly and sampled. In total, 30 samples, including 15 rhizosphere and 15 bulk soil samples, were collected. These 15 plant individuals were distributed within a radius of about 1 km surrounding the coordinate mentioned above. The collected soil samples were immediately transported to the laboratory on ice. Root fragments remaining in the rhizosphere and bulk soils were carefully removed and the samples were then divided into two portions, one part stored at room temperature for chemical analysis, and the other at −20°C for DNA extraction.

DNA extraction, amplification, and sequencing

Total genomic DNA was extracted using the E.Z.N.A™ Mag-Bind Soil DNA Kit (OMEGA). Extracted DNA was detected by 1.0% agarose gel and quantified using a Nanodrop 2000 spectrophotometer (Nanodrop Technologies, Wilmington DE). The bacteria 16S rDNA V3–V4 region was amplified and sequenced for analysis. PCR products were visualized using electrophoresis on 1.5% agarose gels and purified using VAHTS™ DNA Clean Beads (Vazyme, Nanjing, China). Finally, about 10 ng of DNA from each sample was sequenced with the Illumina MiSeq platform by the Sangon Technology Co., Ltd. (Shanghai, China).

Sequence preprocessing and OTU assignment

Quality control was conducted following (Schmieder & Edwards 2011). Chimeric sequences were identified by UCHIME (Edgar et al. 2011) and discarded. Sequences matching plant organelle DNA were also removed. Sequences were assigned to OTUs at 97% similarity level. Taxonomic results of representative OTUs were annotated according to their RDP classifier (Wang et al. 2007) and applied to BLAST against the Silva and NCBI databases (Quast et al. 2013). OTUs with an RDP classification threshold below 0.8 or with identity and coverage lower than 90% were denoted unclassified.

Statistical analysis

Richness of OTUs were calculated using the *vegan* package version 2.3-0 (Dixon 2003) in R software version 3.2.2. Rarefaction analysis was performed in mother 1.30.1 (Schloss et al. 2009). Species accumulation curves, estimation of diversity and richness indices (Shannon index, Chao1 index, coverage), principal components analysis (PCA) and non-metric multi-dimensional scaling (NMDS) analysis were performed with the *vegan* package, while a heat map was constructed using the *gplots* package. Differences of bacterial community structure between bulk and rhizosphere, as well as among rhizosphere communities were tested by ANOSIM (Clarke 1993) based on Bray–Curtis dissimilarities within the *vegan* package. The significance of difference regarding soil physic-chemical properties or richness of bacterial species was

159 determined by ANOVA analysis performed by SAS 9.4. PEARSON correlations of soil chemical
160 properties and between bacterial diversity were performed in Graphopad Prism version 7.0.

161 **RESULTS**

162 **Soil properties**

163 The average soil moisture content (SWC, %) of bulk soil was 16.40 ± 4.57, Electrical
164 conductance (EC) and pH was 6.30 ± 1.21 and 8.14 ± 0.27. The mean total organic carbon
165 (TOC), soil organic matter (SOM), total nitrogen (TON) and available phosphorus (AP) were
166 8.05 ± 4.15 g/kg, 13.87 ± 7.15 g/kg, 0.48 ± 0.23 g/kg and 0.82 ± 0.14 g/kg, respectively. Soil of
167 *Halostachys caspica* and *Halocnemum strobilaceum* had higher EC and lower TOC, SOM, and
168 TON compared to that of other plant-associated soils ($P < 0.05$). The AP content in soil of
169 *Halocnemum strobilaceum* was significantly lower than that of other samples ($P < 0.05$) (Table
170 1).

171 **Diversity of bacterial community**

172 In total, 1.83 Gb of raw data was obtained from all samples, and after sequence quality control,
173 1.18 Gb of clean reads was used in further analysis. The sequence data were available from the
174 NCBI Sequence Read Archive database under accession number SRP129060.

175 The sequencing coverage of all samples ranged from 91% to 95%. Rarefaction curves were
176 shown to stabilize with increasing sequence numbers (Fig. 1A), suggesting that the bacterial
177 communities were reasonably well-characterized. Species accumulation curves almost reached a
178 plateau, and as the OTUs did not significantly increase with increasing sample size, this indicates
179 that the sample size was sufficient for data analysis (Fig. 1B). A total of 109–1397 chimeras and
180 4–1450 reads matching plant DNA sequences in each sample were identified. After removal of
181 chimeras, plant sequences and singletons, a total of 1315341 reads were obtained from soil
182 samples, with an average of 43760 ± 5886 sequences for each rhizosphere sample and 43930 ±
183 4428 for each bulk sample. The sequences were grouped into 8087 OTUs, with an average of
184 782 ± 323 OTUs detected in 15 bulk soil bacterial communities, whereas 1013 ± 55 to 2036 ±
185 428 OTUs were identified in the rhizosphere bacterial communities of five halophytes, with an
186 average of 1692 ± 475. OTUs for the *Halocnemum strobilaceum* samples were significantly
187 lower than that of other four species ($P < 0.01$), both for rhizosphere and bulk soils. Interestingly,
188 the OTUs in bulk soils of *Lycium ruthenicum* were significantly higher than those of the other
189 four species (Table 2).

190 In general, the rhizosphere community diversity was significantly higher than that of bulk
191 soils (ANOVA $P < 0.01$). The Shannon index of *Halostachys caspica* and *Halocnemum*
192 *strobilaceum* were significantly lower than that of other three species ($P < 0.01$), especially with
193 respect to the diversity and richness of *Halocnemum strobilaceum*, which was significantly lower

than that of other species ($P < 0.01$). By contrast, the diversity among bulk soils was not significantly different ($P > 0.05$) (Table 2).

The rhizosphere microbial communities were clearly separated from bulk soil in PCA (Fig. 2A) and NMDS analysis (Fig. 2B), indicating that there was a significant difference between the community composition in rhizosphere and bulk soil communities (ANOSIM, R = 0.961, $P = 0.001$). In PCA analysis, examination of axis 1 and 2 explained 97% of the variance in the data, indicating that the relative abundance of most OTUs were different between rhizosphere and bulk soils. The bulk samples were aggregated together, indicating high similarities for bacterial communities of bulk soils. Conversely, the aggregation degree of rhizosphere samples was lower than that of bulk samples. The rhizosphere communities of *Halocnemum strobilaceum*, *Halostachys caspica* and *Kalidium foliatum* could be separated from each other; however, *Limonium gmelinii* and *Lycium ruthenicum* could not be clearly separated.

Bacterial community structure

A total of 36 phyla, 61 classes, 201 families and 617 genera were identified in all samples. In the bulk soil samples, Proteobacteria and Firmicutes were the dominant phyla. At class level, Gammaproteobacteria and Bacilli were the dominant group. At lower rank, genera of *Exiguobacterium*, *Citrobacter*, *Acinetobacter*, *Pseudomonas* and *Bacillus* were the most abundant genera (Fig. 3). For rhizosphere soils, Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes, Planctomycetes, Acidobacteria, Candidatus Saccharibacteria, Verrucomicrobia, Chloroflexi were the most abundant phyla. Gammaproteobacteria, Bacilli, Actinobacteria, Alphaproteobacteria, Sphingobacteriia, Deltaproteobacteria, Planctomycetia, Flavobacteriia, Cytophagia were the most abundant classes. *Halomonas, Exiguobacterium, Gracilimonas, Citrobacter, Acinetobacter, Pseudomonas, Deferrisoma, Aliifodinibius, Thioprofundum, Gp10, Marinobacter, Geminicoccus, Fodinicurvata, Nitriliruptor, Aciditerrimonas* and *Planococcus* were the most abundant genera.

Differences between rhizosphere and bulk soil samples

A significant difference was observed in rhizosphere samples compared to the bulk soils ($P < 0.001$). The abundant groups in bulk soil communities decreased in rhizosphere communities, and some low abundant groups were enriched significantly and became abundant (Fig. 3).

At phylum level, the relative abundance of Proteobacteria and Firmicutes decreased in rhizosphere soils, especially Firmicutes, which decreased significantly ($P < 0.001$); whereas, phyla Actinobacteria, Bacteroidetes, Planctomycetes, Acidobacteria, Candidatus, Saccharibacteria, and Verrucomicrobia, Chloroflexi were enriched in rhizosphere soil communities. Classes Gammaproteobacteria and Bacilli reduced significantly in rhizosphere bacterial communities compared to bulk soils ($P < 0.01$), whereas Actinobacteria, Alphaproteobacteria, Sphingobacteriia, Deltaproteobacteria, Planctomycetia, Flavobacteriia,

Cytophagia were enriched significantly ($P < 0.01$) and became abundant groups. The similar pattern also observed at lower taxonomic rank (family and genus), and number of abundant groups increased in rhizosphere samples compared to bulk soils. Genera *Exiguobacterium, Citrobacter, Acinetobacter, Halomonas* and *Pseudomonas* decreased significantly in abundance, but *Gracilimonas, Deferrisoma, Aliifodinibius, Thioprofundum, Gp10, Marinobacter, Geminicoccus, Fodinicurvata, Nitriliruptor, Aciditerrimonas* and *Planococcus* were enriched significantly ($P < 0.01$) (Fig. 3).

Community structure difference among the rhizosphere of five halophytes

ANOSIM analysis (999 permutations) showed that no significant difference of relative abundance at each taxonomic group was found among the bulk soil samples (ANOSIM phylum R = −0.11, $P = 0.859$; class R = −0.093, $P = 0.816$; family R = −0.05, $P = 0.651$; genus R = −0.049, $P = 0.629$), indicating that the bulk soil had similar bacterial community structure.

Significant differences were found among five plant species associated bacterial communities: phylum (R = 0.622, $P = 0.001$), class (R = 0.59, $P = 0.001$). The differences were even higher at lower rank: family (R = 0.828, $P = 0.001$) and genus (R = 0.865, $P = 0.001$). Nine phyla, 17 classes, and 93 genera were significantly ($P < 0.05$) different among bacterial communities associated with five plant species (Table S1). Significant differences ($P < 0.05$) were observed for phyla of Proteobacteria, Firmicutes, Acidobacteria, Bacteroidetes Verrucomicrobia, as well as most of the classes, such as Gammaproteobacteria, Bacilli, Actinobacteria, Alphaproteobacteria, Sphingobacteriia, Deltaproteobacteria, Planctomycetia, Flavobacteriia, Cytophagia. At genus level, *Exiguobacterium, Citrobacter, Pseudomonas, Halomonas, Gracilimonas, Deferrisoma, Gp10, Geminicoccus, Planococcus, Blastopirellula, Pelagibius, Pontibacter* also differed significantly among the communities associated with five plants ($P < 0.05$) (Fig. 4).

At the OTU level, a total of 293 OTUs showed significantly distinct community structures with five halophytes. These top 50 OTUs were mainly assigned to phyla Proteobacteria, Firmicutes, Acidobacteria, Bacteroidetes, classes Gamma-, Delta- and Alpha-proteobacteria, Bacilli, Actinobacteria and Flavobacteriia, and genera *Halomonas, Exiguobacterium, Geminicoccus, Citrobacter, Pseudomonas, Gracilimonas, Deferrisoma, Pontibacter*, etc. (Fig. 5), which were consistent with the results of phylum, class and genus shown in Fig. 4. Although significant differences were found, similarities among them were also presented. Venn analysis showed that 647 OTUs were shared by the rhizosphere communities associated with the five plants (Fig. 6), and the most of the abundant genera were also shared, with varied richness.

DISCUSSION

Bacterial community structure in saline soil compared to other environments

Soil salinity has important effects on plant community's composition, diversity and distribution pattern (Xi et al. 2016), and high soil salinity places severe stress on growth and basic survival for glycophytes. Therefore, in saline habitats, halophytic plants are the dominant vegetation (Naz et al. 2010). Also, salinity is also a major factor influencing soil bacterial diversity and community structure (Fang et al. 2016; Pavloudi et al. 2016), and the abundance, composition, and diversity of microbial communities in saline or hypersaline terrestrial environments is usually low (Jiang et al. 2007).

In Ebinur lake region, we found a very low diversity of bacterial communities compared to forest, grassland and agricultural areas (Rampelotto et al. 2013), maize crop soil (Garcia-Salamanca et al. 2013) and even saline soil (Canfora et al. 2014), but consistent with "extreme" hypersaline soils (Canfora et al. 2015). We also observed that the bulk soil surrounding *Halocnemum strobilaceum* and *Halostachys caspica* had lower diversity and richness than that of other samples. The Pearson correlation analysis show that EC is negatively, but not significantly, correlated with community diversity (Table S2). Overall, the bacterial communities of bulk soils in the saline environments studied here have high similarities. For instance, the abundant genera composition is very low, with bulk soils studied here comprised of only four genera *Exiguobacterium*, *Citrobacter*, *Acinetobacter* and *Pseudomonas.* Moreover, their high abundance is attributed by a minority of OTUs, with one OTU (OTU2) for *Acinetobacter*, two (OTU 0 and 4979) for *Citrobacter*, and four for *Exiguobacterium* (OTU 2, 4726, 4738 and 11635) and *Pseudomonas* (OTU 6, 6794, 11705 and 13215).

Canfora et al. (Canfora et al. 2015) reported that Proteobacteria and Actinobacteria were dominant phyla in natural saline soils of Sicily (Italy), whereas we found that Proteobacteria and Firmicutes were the abundant phyla. However, dominance of Gammaproteobacteria and Firmicutes (Bacilli) is consistent with previous studies on hypersaline soils (Borsodi et al. 2013; Tang et al. 2011), confirming the importance of these two taxa in saline or hypersaline environments. Distinct from non-saline habitats, halophilic bacteria are the most common group in saline environments, because salinity can reduce soil respiration (Asghar et al. 2012; Setia et al. 2011) and strongly affects microbial community composition favoring Archaea and halophilic bacteria (Rousk et al. 2011). Several salt-tolerant bacteria belong to *Bacillus, Halomonas, Stenotrophomonas, Alkalimonas, Salinibacter*, etc. have been isolated from a wide range of saline soils (Abou-Elela et al. 2010; Borsodi et al. 2015; Shi et al. 2012b; Zhou et al. 2012), which were also detected in the present study.

Bacterial community structure between rhizosphere and bulk soils

The rhizosphere effect (Morgan & Whipps 2001) is an important driving force in shaping microbial community structure. The rhizosphere habitat is more favorable for microorganisms (Li et al. 2014); therefore, rhizosphere microbiota is much higher in richness and diversity as

compared to surrounding soils (Avis et al. 2008). It was also found that the diversity and richness of bacterial communities associated with the five halophytes were significantly higher in rhizosphere soils than bulk soils. Furthermore, the rhizosphere samples were clearly distinct from bulk samples, indicating that halophilic rhizosphere bacterial communities structures are significantly different from respective bulk soil communities.

Importantly, the richness of abundant groups (i.e. gammaproteobacteria and Firmicutes) in bulk soils was reduced in rhizosphere soils, which was especially the case for Firmicutes, mostly represented by class Bacilli, which was reduced by about 80%. The reduction of Firmicutes in rhizosphere soil has also been reported in many cases, and for example was almost entirely excluded from the rhizosphere communities in barley (Bulgarelli et al. 2015). It is reported that *Bacillus* is the dominant genusin rhizosphere soil (Borsodi et al. 2015; Szymanska et al. 2016), whereas we found that *Exiguobacterium* was the most abundant genus, followed by *Planococcus* and *Bacillus*. Although abundance of Bacilli decreased significantly, the richness of *Bacillus* was relative stable, and its abundance in rhizosphere communities did not significantly differ compared to bulk samples, with respective mean values of 0.61% vs. 0.8%. Although Gammaproteobacteria decreased in rhizosphere soils, as observed via a decrease in the genera *Pseudomonas, Citrobacter,* and *Acinetobacter*, it was still the most abundant class, as reported in many plant-associated bacterial communities (Mukhtar et al. 2017). However, genera of *Halomonas, Thioprofundum, Marinobacter, Marinimicrobium, Haliea, Methylohalomonas, Microbulbifer* are enriched.

Pseudomonas and *Bacillus* are the two predominant bacterial species in important plant growth promoting rhizobacteria (PGPR) communities, and have multiple functional activities, such as phosphate solubilization and phytopathogens inhibition (Prashar et al. 2014). The retention of sizable abundance in the rhizosphere of these two genera and their richness reduction compared to bulk soils may be caused by plant effects and competition among bacteria species in saline soils.

In contrast with the decrease in abundance of gammaproteobacteria and Firmicutes, richness of alphaproteobacteria and deltaproteobacteria increased and became abundant groups due to enrichment of *Geminicoccus, Fodinicurvata, Rhodoligotrophos,* and *Pelagibius*, and *Deferrisoma*, respectively. Actinobacteria became abundant due to enrichment of genera *Nitriliruptor*, *Aciditerrimonas* and *Jiangella*. Alphaproteobacteria and Actinobacteria were found to be more abundant in the saline lands (Tkavc et al. 2011). *Actinobacteria* play an important role in the biogeochemical cycling of nutrients via solubilization of phosphorous (Franco-Correa et al. 2010). Bacteroidetes and Acidobacteria were abundant in rhizosphere communities attributed by enrichment of *Gracilimonas and Aliifodinibius*, and *Gp10*, respectively. Their abundance in rhizosphere communities compared to bulk soils might be correlated with the

337 availability organic matter. It has been reported that addition of carbon resources increases the
338 tolerance of microbes to osmotic stress, because adaptation to osmotic stress requires a high
339 amount of energy to synthesize organic osmolytes (Hagemann 2011).

340 **Rhizosphere microbial community difference among halophytes**

341 Generally, microbial composition of halophytes differs from that of glycophytes (Mukhtar et al.
342 2017) in that plenty of halotolerant or halophilic bacteria can be commonly identified in
343 halophyte rhizospheres (Ruppel et al. 2013). In this study numerous halophilic bacteria were
344 determined to have been enriched in rhizosphere soils, such as *Salinimicrobium, Halomonas,*
345 *Geminicoccus, Pelagibius, Microbulbifer, Planococcus* (*Planomicrobium*), *Rubrivirga,*
346 *Arenicella, Bacillus*, and *Mesorhizobium.* The enrichment of these halophilic bacteria in
347 halophyte rhizospheres is influenced by multiple factors. First, halophilic bacteria are adapted to
348 saline environments and their growth is salt dependent. Second, they require substrates to
349 produce energy for growth and reproduction. Rhizosphere soil is rich in organic matters that can
350 be easily degraded and assimilated by these organisms. Due to limited sample size, nutrient
351 contents of rhizosphere samples were not estimated; however, the TOC and TON in rhizosphere
352 soils associated with *Limonium gmelinii* and *Lycium ruthenicum* are much higher than that of
353 bulk soils (approximately 4 to 8 times, data not shown). Another important factor influencing
354 halophilic enrichment is possible mutualistic plant–microbe interactions, as these bacteria can
355 degrade root exudates for root assimilation and help plant growth. Other examples of benefit
356 conferred by these species include degradation of complex hydrocarbons by some *Planococcus*
357 and *Microbulbifer* members (See-Too et al. 2017), while *Mesorhizobium* is known to fix
358 nitrogen (Ardley et al. 2012). Additionally, *Bacillus* members are known to be generally
359 effective for suppressing disease (Okubo et al. 2016). Moreover, functional interactions between
360 plants and microorganisms contribute to salt stress tolerance of halophytes (Mukhtar et al. 2017).

361 Microbial community composition are plant specific (Lundberg et al. 2012), even varying
362 with cultivar (genotype) within the same plant species, which can shape different rhizobacterial
363 community structures (Andreote et al. 2009; Poli et al. 2016), and previous studies showed that
364 this also applies for halophytes. For instance, *Bacillus spp*. dominates in the rhizosphere bacterial
365 community of *A. tripolium* (90.9%) (Szymanska et al. 2016), and *Puccinellia limosa* is
366 dominated by *Nesterenkonia*, while *Bacillus* and *Halomonas* are the most abundant genera in
367 rhizosphere of *Bolboschoenus maritimus* (Borsodi et al. 2015). Similarly, *Halanaerobiales* was
368 the most abundant taxon found in all the different samples of *Phragmites australis* in a
369 hypersaline pond (Borruso et al. 2014).

370 It was also observed that there were significant differences in rhizosphere communities
371 among five halophytes both from ANOSIM results and community composition. We found that
372 *Halomonas* was the most abundant genera in rhizosphere communities, with a mean richness

value of 9.4%. *Halomonas* was the predominant genera in communities in *Halocnemum strobilaceum*, accounting for 32.4% of the total OTUs, which was significantly higher than that in the other four plant-associated communities. The high relative prevalence of *Halomonas* in *Halocnemum strobilaceum* (Al-Mailem et al. 2010), as well in *Bolboschoenus maritimus* and *Puccinellia limosa* rhizosphere communities (Borsodi et al. 2015); however, its abundance in the other four plants was significantly lower. Additionally, OTUs affiliated with *Fodinicurvata* (OTU9), *Gracilimonas* (OTU15), *Salegentibacter* (OTU55), *Haliea* (OTU52), *Mesorhizobium* (OTU51), and *Nitratireductor* (OTU201) were observed to have higher abundance in the *Halocnemum strobilaceum* rhizosphere community. Meanwhile, OTUs assigned to *Exiguobacterium*, *Citrobacter*, *Acinetobacter*, *Pseudomonas*, *Aciditerrimonas*, *Rhodoligotrophos* were significantly higher in the rhizosphere of *Halostachys caspica* than that of in other plants. OTUs affiliated with genera of *Marinimicrobium, Streptomyces, Jiangella* were found to be more abundant in *Kalidium foliatum* than in other species, and OTUs affiliated with *Deferrisoma* and *Geminicoccus* were much higher in *Limonium gmelinii* than those in other four species. OTUs in genera *Blastopirellula*, *Pelagibius* and *Roseibacillus* are most abundant in *Lycium ruthenicum* rhizosphere communities.

Although the dissimilarities of rhizosphere communities associated with five plant species are clear and notable, we noticed apparent similarities among them. PCA and NMDS showed *Halocnemum strobilaceum, Halostachys caspica* and *Kalidium foliatum* could be separated from each other, *Limonium gmelinii* and *Lycium ruthenicum* could not be clearly separated, indicating a relatively high similarity between them. Venn analysis revealed that 647 OTUs were shared by the rhizosphere communities associated with the five plants, and the most of the abundant genera were also shared. These findings suggest a convergence of halophyte rhizosphere bacterial community composition, which might be an adaptive consequence of the five halophytes long-term evolution in the same saline environment. It is reported that rhizosphere micro-organisms associated with halophytes, especially some PGPR, play an important role in halophytic plants to alleviate salinity stress and thrive in saline environments (Ali et al. 2015).

CONCLUSION

Through analysis of the bacterial community in the rhizosphere and bulk soil of five halophytes, it was found that the bacterial community diversity and composition in saline soil of Ebinur Lake region were very low, and this was significantly more pronounced in bulk soil than the rhizosphere. Furthermore, the bacterial community structure in rhizosphere soils was significantly different from that of bulk soils, and there was significant differences between rhizosphere bacterial communities according to ANOISM analysis as well. However, similarities were also observed, in that a large number of OTUs and most of the abundant genera were

shared by the five halophytes rhizosphere bacteria, providing evidence that rhizosphere effects can be less influential compared to environmental factors. In a hypersaline habitat, salinity could play a stronger role with respect to the rhizosphere effect in shaping the microbial communities (Li et al. 2013). Considering the similarities and dissimilarities among rhizosphere communities of five halophytic plants, the work presented here demonstrates that rhizosphere effect and salinity are the two most important driving forces in shaping the bacterial community structure in saline soils.

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ADDITIONAL INFORMATION AND DECLARATIONS

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Competing Interests

The authors declare that they have no competing interests.

Data Availability

This 16S rRNA sequencing data has been deposited at NCBI Sequence Read Archive (SRA) database under accession no. SAMN08336887 - SAMN 08336916.

Supplemental information

Table S1. Species that are significantly differentiated among five plant-associated bacterial communities at the phylum, class, family and genus levels.

Table S2. Pearson correlation analysis of soil chemical properties, with bacterial diversity indices in bulk soils.

FIGURE LEGENDS

Figure 1 Rarefaction curves (A) and species accumulation curve (B) for bacterial OTUs clustering at 97% sequence similarity of all samples associated with five halophytic plants.

Figure 2 Principal component analysis (PCA) (A) and non-metric multi-dimensional scaling (NMDS) (B) constructed with OTUs in bacterial community of all samples.

Figure 3 Relative abundance of the most abundant phyla, classes and genera in bacterial communities of bulk and rhizosphere soils.

Figure 4 Phyla, classes and genera that were significantly ($P < 0.05$) different among the five plant-associated bacterial communities.

Figure 5 Heatmap depicting most abundant OTUs that were significantly differentiated ($P < 0.05$) among bacterial communities associated with the five halophytes.

Figure 6 Venn diagrams of numbers of OTUs shared among the rhizosphere samples of five halophytic plants

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Figure 1

Rarefaction curves (A) and species accumulation curve (B) for bacterial OTUs clustering at 97% sequence similarity of all samples associated with five halophytic plants.

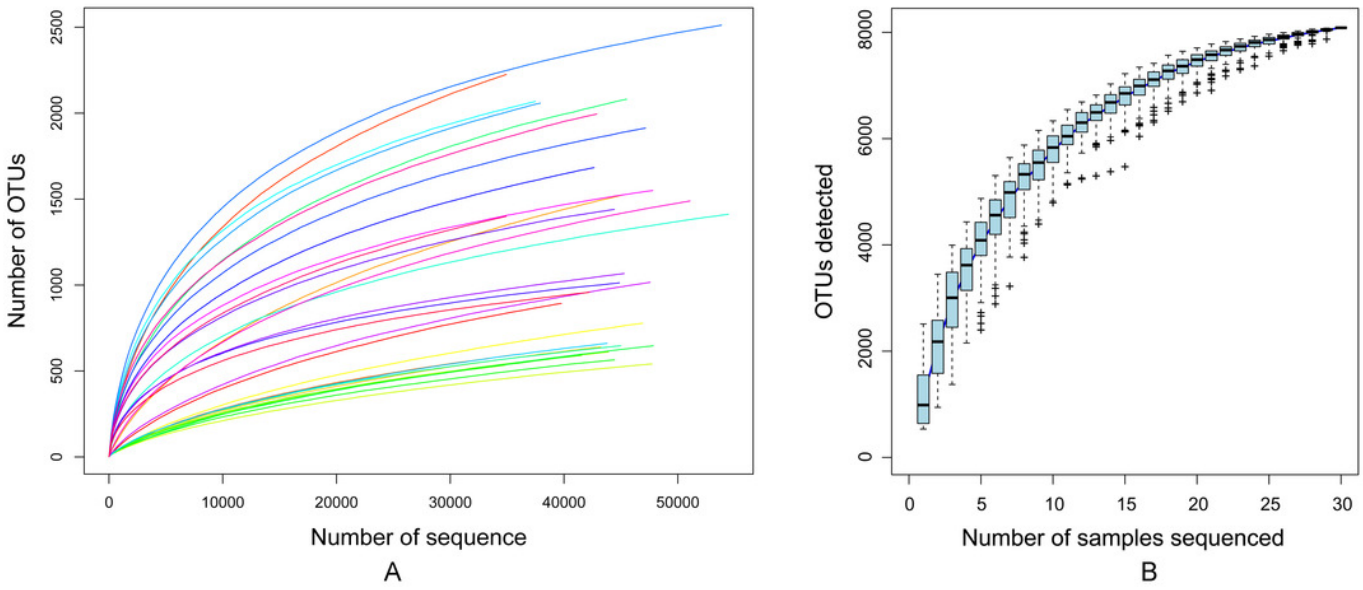


Figure 2

Principal component analysis (PCA) (A) and non-metric multi-dimensional scaling (NMDS) (B) constructed with OTUs in bacterial community of all samples.

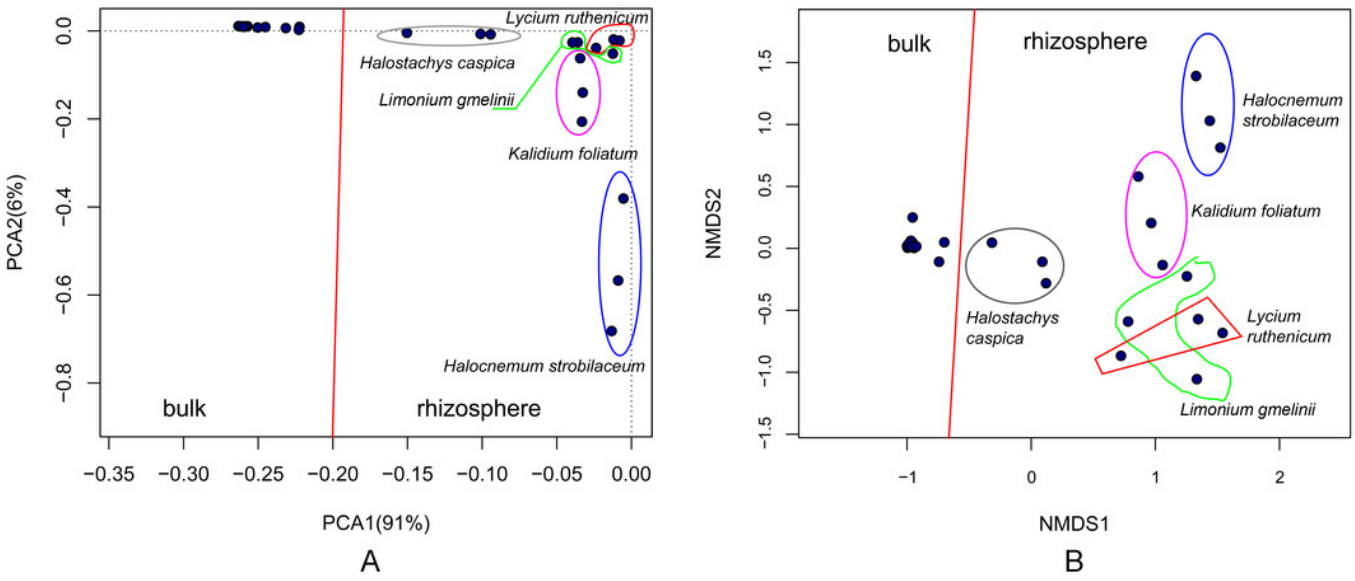
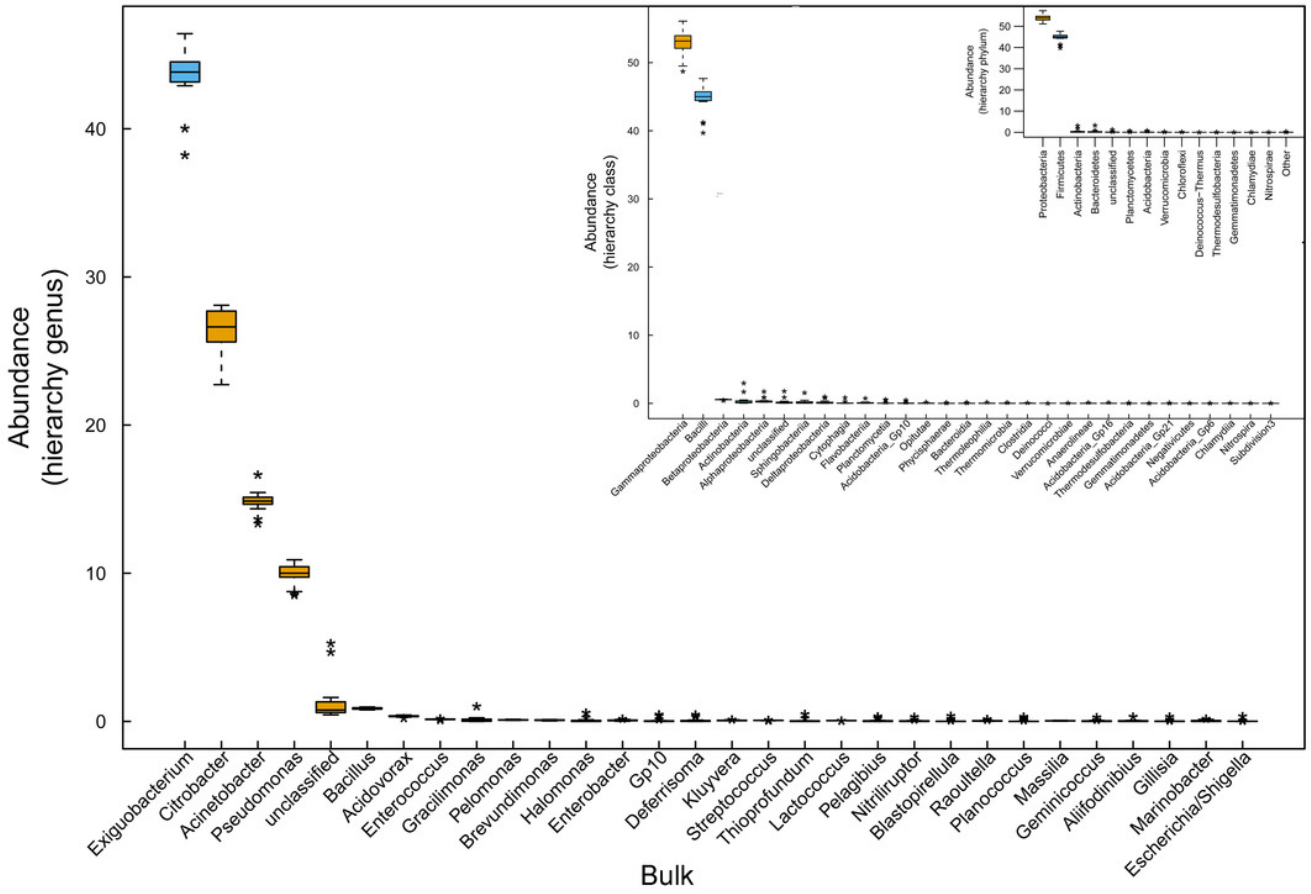


Figure 3

Relative abundance of the most abundant phyla, classes and genera in bacterial communities of bulk and rhizosphere soils.


Abundance
(hierarchy genus)
Exiguobacterium
Citrobacter
Acinetobacter
Pseudomonas
unclassified
Bacillus
Acidovorax
Enterococcus
Gracilimonas
Pelomonas
Brevundimonas
Halomonas
Enterobacter
Gp10
Deferrisoma
Kluyvera
Streptococcus
Thioprofundum
Lactococcus
Pelagibius
Nitriliruptor
Blastopirellula
Raoultella
Planococcus
Massilia
Geminicoccus
Alifodinibius
Gillisia
Marinobacter
Escherichia/Shigella
Bulk
Abundance
(hierarchy class)
Gammaproteobacteria
Bacilli
Betaproteobacteria
Actinobacteria
Alphaproteobacteria
unclassified
Sphingobacteriia
Deltaproteobacteria
Cytophagia
Flavobacteriia
Planctomycetia
Acidobacteria_Gp10
Opitutae
Physicisphaerae
Bacteroidia
Thermoleophilia
Thermomicrobia
Clostridia
Deinococci
Verrucomicrobiae
Anaerolineae
Acidobacteria_Gp16
Thermodesulfobacteria
Gemmatimonadetes
Acidobacteria_Gp21
Negativicutes
Acidobacteria_Gp6
Chlamydiia
Nitrospira
Subdivision3
Abundance
(hierarchy phylum)
Proteobacteria
Firmicutes
Actinobacteria
Bacteroidetes
unclassified
Planctomycetes
Acidobacteria
Verrucomicrobia
Chloroflexi
Deinococcus−Thermus
Thermodesulfobacteria
Gemmatimonadetes
Chlamydiae
Nitrospirae
Other

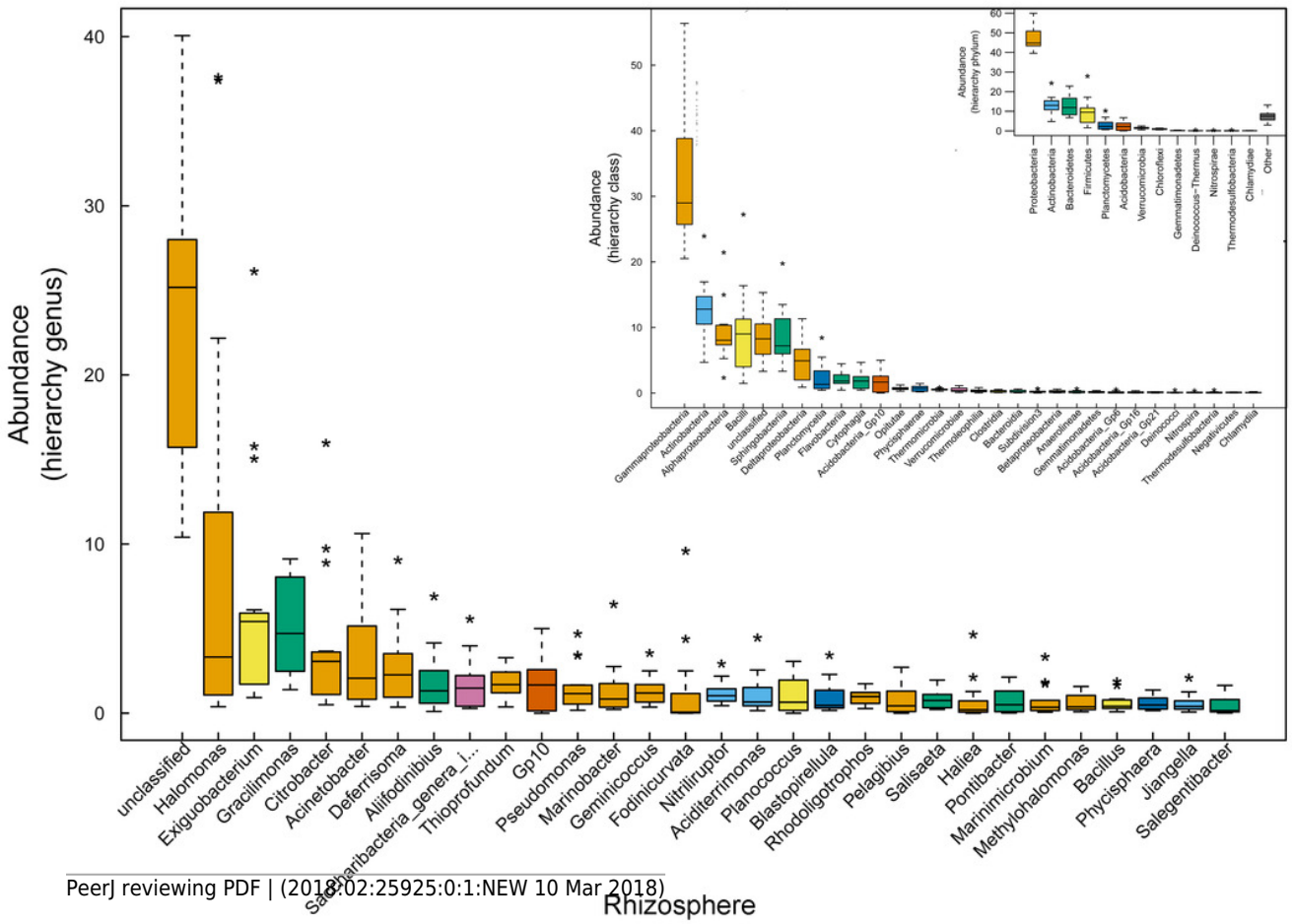

Abundance
(hierarchy genus)
unclassified
Halomonas
Exiguobacterium
Gracilimonas
Citrobacter
Acinetobacter
Deferrisoma
Alifodinibius
Salinaribacteria_genera_i...
Thioprofundum
Gp10
Pseudomonas
Marinobacter
Geminicoccus
Fodinicurvata
Nitriliruptor
Aciditerrimonas
Planococcus
Blastopirellula
Rhodoligotrophos
Pelagibius
Salisaeta
Haliea
Pontibacter
Marinimicrobium
Methylohalomonas
Bacillus
Phycisphaera
Jiangella
Salegentibacter
Rhizosphere
Abundance
(hierarchy class)
Gammaproteobacteria
Actinobacteria
Alphaproteobacteria
Bacilli
unclassified
Sphingobacteriia
Deltaproteobacteria
Planctomycetia
Flavobacteriia
Cytophagia
Acidobacteria_Gp10
Opitutae
Physicisphaerae
Thermomicrobia
Verrucomicrobiae
Thermoleophilia
Clostridia
Bacteroidia
Subdivision3
Betaproteobacteria
Anaerolineae
Gemmatimonadetes
Acidobacteria_Gp6
Acidobacteria_Gp16
Acidobacteria_Gp21
Deinococci
Nitrospira
Thermodesulfobacteria
Negativicutes
Chlamydiia
Abundance
(hierarchy phylum)
Proteobacteria
Actinobacteria
Bacteroidetes
Firmicutes
Planctomycetes
Acidobacteria
Verrucomicrobia
Chloroflexi
Gemmatimonadetes
Deinococcus−Thermus
Nitrospirae
Thermodesulfobacteria
Chlamydiae
Other

Figure 4

Phyla, classes and genera that were significantly ($P < 0.05$) different among the five plant-associated bacterial communities.

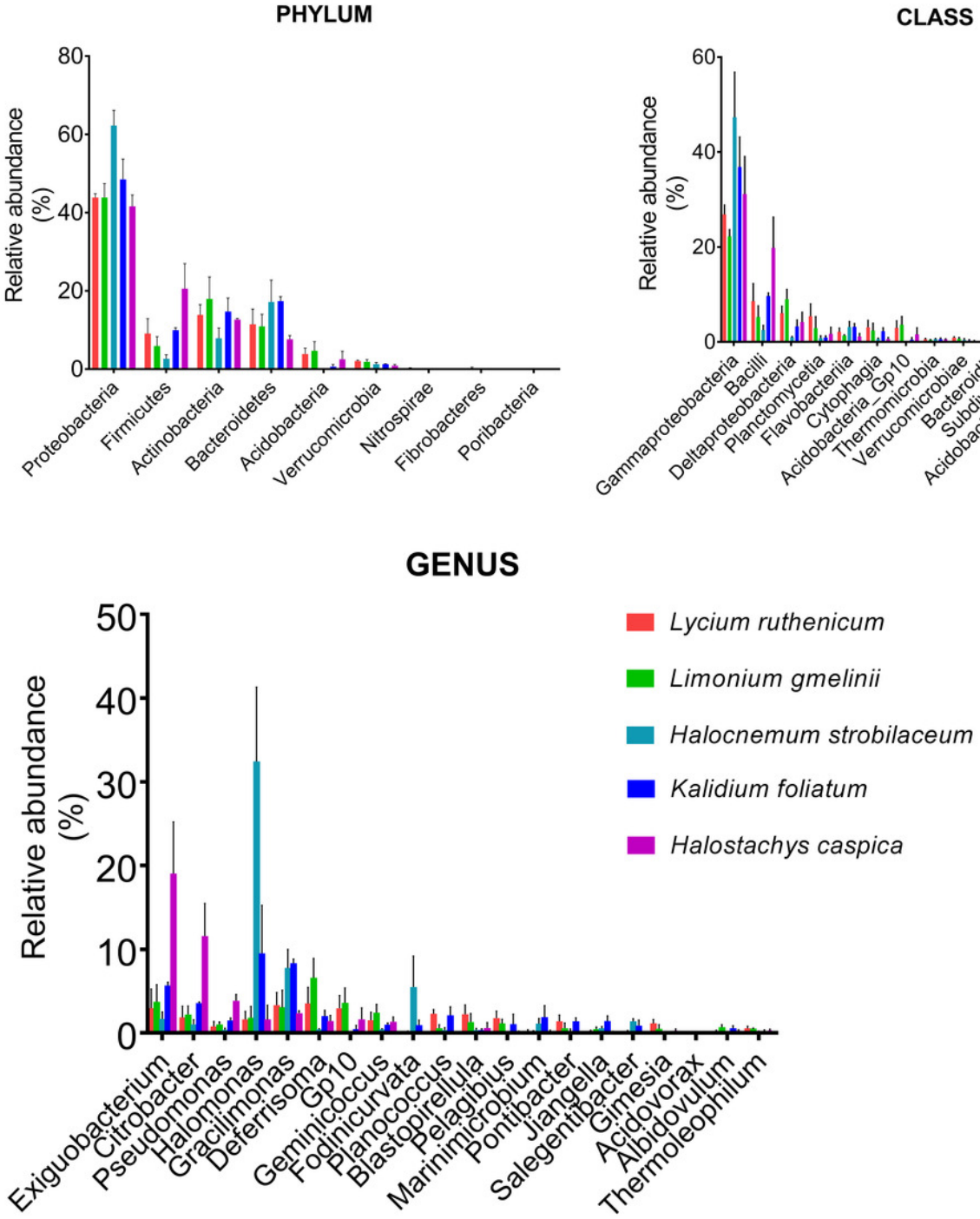


Figure 5

Heatmap depicting most abundant OTUs that were significantly differentiated ($P < 0.05$) among bacterial communities associated with the five halophytes.

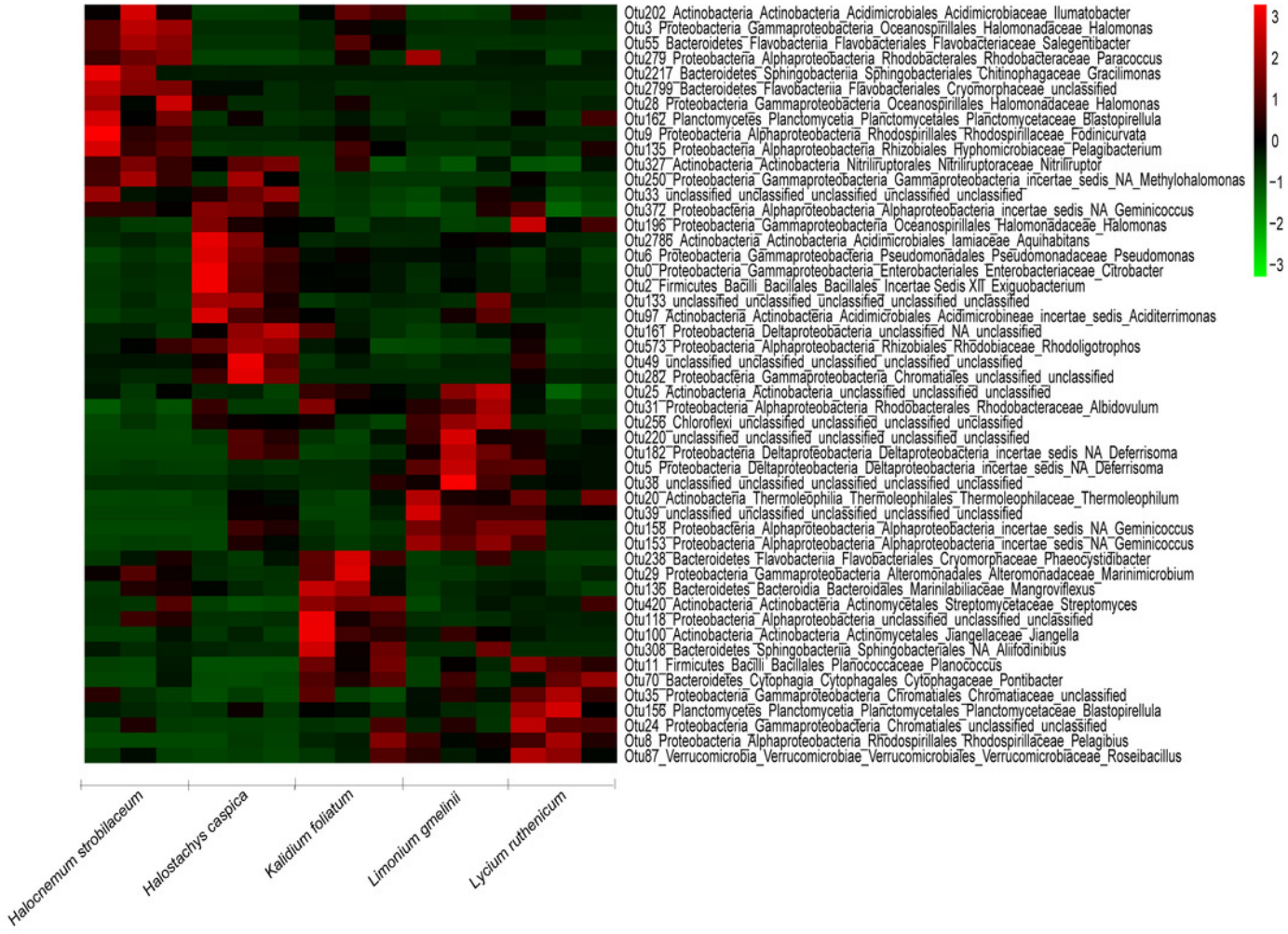


Figure 6

Venn diagrams of numbers of OTUs shared among the rhizosphere samples of five halophytic plants

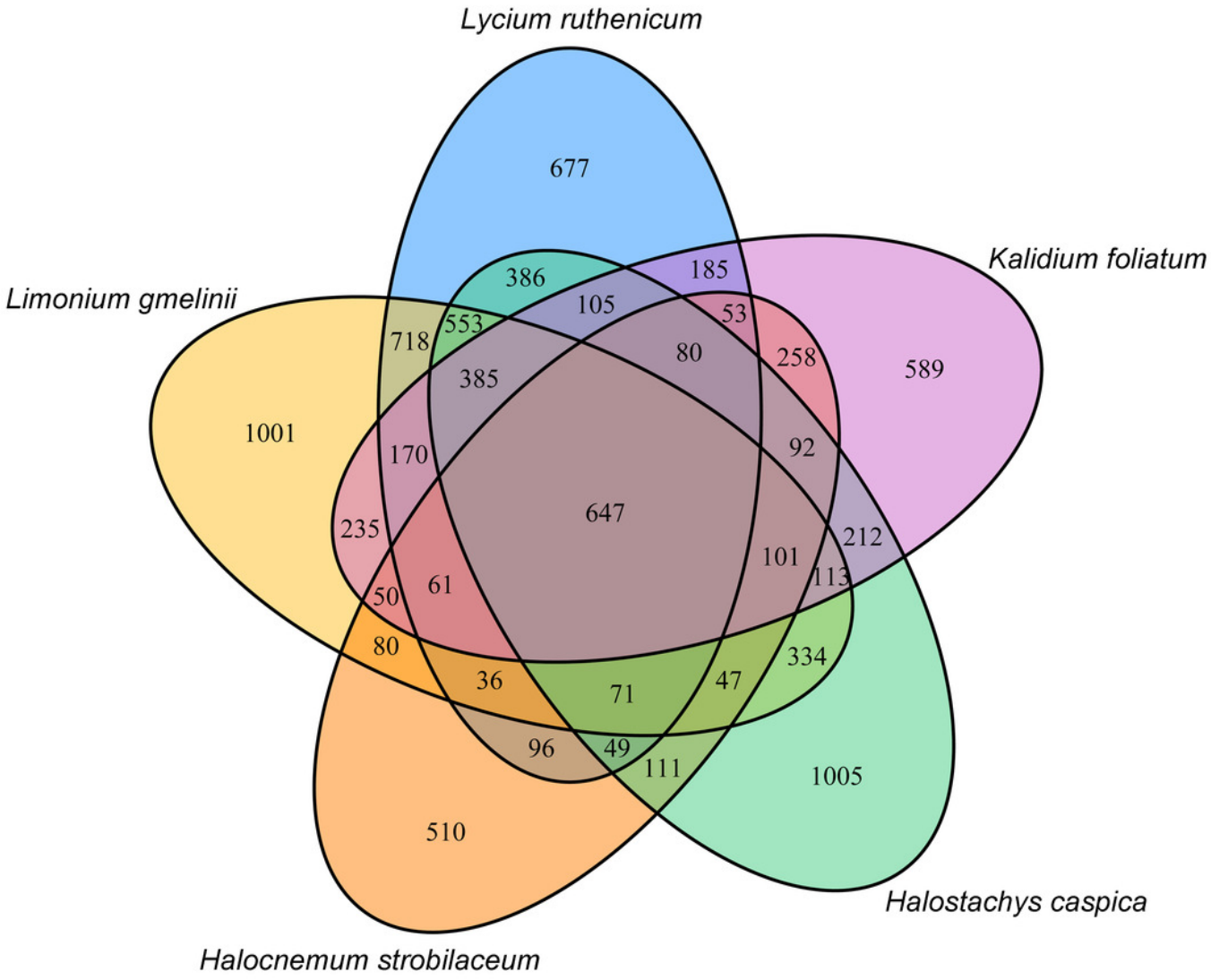


Table 1(on next page)

Soil characteristics in the rhizosphere and bulk soil associated with five halophytes

Table 1 Soil characteristics in the rhizosphere and bulk soil associated with five halophytes.

	TOC (g/kg)	SOM (g/kg)	TON (g/kg)	AP (g/kg)	pH	EC (mS/cm)	SWC (%)
Lycium ruthenicum	9.14 ± 3.43a	15.75 ± 5.92a	0.58 ± 0.24a	0.89 ± 0.15a	8.23 ± 0.37	5.56 ± 1.26b	19.73 ± 2.18a
Limonium gmelinii	10.78 ± 1.60a	18.59 ± 2.77a	0.60 ± 0.10a	0.80 ± 0.08a	8.33 ± 0.24	6.61 ± 0.91ab	17.02 ± 3.51a
Kalidium foliatum	11.27 ± 5.66a	19.43 ± 9.76a	0.64 ± 0.25a	0.92 ± 0.08a	8.02 ± 0.25	5.65 ± 0.53b	16.45 ± 6.11a
Halostachys caspica	5.53 ± 0.95b	9.53 ± 1.63b	0.34 ± 0.[illegible]3ab	0.82 ± 0.06a	8.05 ± 0.16	6.78 ± 1.42ab	10.42 ± 2.57b
Halocnemum strobilaceum	3.15 ± 1.09b	5.43 ± 1.88b	0.21 ± 0.02b	0.62 ± 0.06b	8.05 ± 0.30	7.14 ± 1.46a	17.71 ± 3.16a
mean	8.05 ± 4.15	13.87 ± 7.15	0.48 ± 0.23	0.82 ± 0.14	8.14 ± 0.27	6.30 ± 1.21	16.40 ± 4.57

Values are mean ± standard deviation (n = 3).

Different letters indicate significant differences among five halophytes ($P < 0.05$).

Table 2(on next page)

Alpha diversity indices of bacterial communities in rhizosphere and bulk soils.

Table 2 Alpha diversity indices of bacterial communities in rhizosphere and bulk soils.

	Rhizosphere				Bulk			
Species	Seq number	OTUs number	Shannon index	Chao 1 index	Seq number	OTUs number	Shannon index	Chao 1 index
Limonium gmelinii	47887 ± 5635	2036 ± 428a	5.54 ± 0.49a	2668 ± 331a	43295 ± 3573	770 ± 127b	1.95 ± 0.1b	1347 ± 170b
Lycium ruthenicum	39430 ± 3002	2040 ± 40a	5.77 ± 0.23a	2659 ± 18a	47961 ± 2942	1343 ± 284a	2.43 ± 0.37a	2063 ± 397a
Kalidium foliatum	42386 ± 6677	1463 ± 78b	5.07 ± 0.11b	2020 ± 28b	41614 ± 4845	635 ± 33b	1.75 ± 0.03b	1127 ± 80b
Halostachys caspica	44963 ± 9774	1906 ± 434a	4.66 ± 0.80b	2603 ± 507a	44619 ± 3157	601 ± 42b	1.70 ± 0.03b	1197 ± 207b
Halocnemum strobilaceum	44133 ± 1655	1013 ± 55b	4.13 ± 0.29b	1346 ± 104b	42159 ± 6628	562 ± 43b	1.71 ± 0.05b	1007 ± 53b
mean	43760 ± 5886	1692 ± 475*	5.03 ± 0.73*	2260 ± 583*	43930 ± 4428	782 ± 323	1.91 ± 0.32	1348 ± 429

Values are the means ± SD (n = 3).

Different letters indicate significant differences among five halophytes ($P < 0.05$).

* denotes parameters are significantly different between rhizosphere and bulk soil samples ($P < 0.01$).