



Impact of soil salinity on the microbial structure of halophyte rhizosphere microbiome

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Abstract

The rhizosphere microbiome plays a significant role in the life of plants in promoting plant survival under adverse conditions. However, limited information is available about microbial diversity in saline environments. In the current study, we compared the composition of the rhizosphere microbiomes of the halophytes *Urochloa*, *Kochia*, *Salsola*, and *Atriplex* living in moderate and high salinity environments (Khewra salt mines; Pakistan) with that of the non-halophyte *Triticum*. Soil microbiomes analysis using pyrosequencing of 16S rRNA gene indicated that *Actinobacteria* were dominant in saline soil samples whereas *Proteobacteria* predominated in non-saline soil samples. *Firmicutes*, *Acidobacteria*, *Bacteroidetes* and *Thaumarchaeota* were predominant phyla in saline and non-saline soils, whereas *Cyanobacteria*, *Verrucomicrobia*, *Gemmatimonadetes* and the unclassified *WPS-2* were less abundant. Sequences from *Euryarchaeota*, *Ignavibacteriae*, and *Nanohaloarchaeota* were identified only from the rhizosphere of halophytes. Dominant halophilic bacteria and archaea identified in this study included *Agrococcus*, *Armatimonadetes gp4*, *Halalkalicoccus*, *Haloferula* and *Halobacterium*. Our analysis showed that increases in soil salinity correlated with significant differences in the alpha and beta diversity of the microbial communities across saline and non-saline soil samples. Having a complete inventory of the soil bacteria from different saline environments in Pakistan will help in the discovery of potential inoculants for crops growing on salt-affected land.

Keywords Soil microbiome · 16S rRNA gene · Pyrosequencing · Soil salinity · Halophilic bacteria · Haloarchaea

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Introduction

Salinization is one of the major problems that occur in more 100 countries of the world (Rengasamy 2006). Halophytes are plants that grow well in saline soil and water and compare well with forage crops like para grass, *Salsola stocksii*, *Kochia indica* and *Atriplex amnicola* (Ahmad et al. 2013). Therefore, these offer a better alternative where conventional crops cannot be raised and drainage is too expensive. They may contribute significantly to the developing world's supply of food, fiber, fuel and fodder (Dagla and Shekhawat 2005). Salinity also affects microbial diversity, which plays a role in maintaining biogeochemical cycles (Tripathi et al. 2006). Rhizosphere microbial communities from extreme environments (saline, acidic, arid) are more complex than soils having a neutral pH and exhibiting moderate salinity. The rich microbial diversity of halophyte rhizospheres helps these plants cope with high salinity and also tolerate drought (Naz et al. 2009).

Biodiversity has been recognized as the primary factor that affects ecosystem functioning. For example, higher microbial diversity increases the resistance and stability of soil under stress conditions and influences nutrient cycling (Wagg et al. 2014; Zhang et al. 2015). The rhizosphere microbiome plays an important role in plant health because it serves as a “second genome” to the plant (Berendsen et al. 2012). Studies of rhizosphere microbiomes have revealed their influence on chemical exudates responsible for production and secretion of signaling molecules by both microbes and plants (Curlango-rivera et al. 2013). Rhizobacteria promote plant growth by increasing the availability and uptake of carbon, nitrogen, and minerals from soil (Dodd and Perez-Alfocea 2012; Delmont et al. 2014). Acting through a variety of mechanisms, certain microbes provide a source of fixed nitrogen and/or increase the bioavailability of soluble phosphorous for the plant. Many microbes also control plant pathogens by producing antimicrobial, antifungal, and insecticidal compounds as well as help plants withstand salt, drought, and heat (Craita and Tom 2013; Rolli et al. 2015).

Halophilic bacteria are classified according to the degree of their salt tolerance: slight halophiles (0.2–0.85 M NaCl), moderate halophiles (0.85–3.4 M NaCl), and extreme halophiles (3.4–5.1 M NaCl) (Ventosa et al. 2008). Halophiles use compatible solutes to balance their cytoplasmic osmotic potential and to protect their cells against drought, low oxygen, and high temperature (Yancey 2005). These microbes often have novel enzymes that function under salt stress conditions such as proteases, xylanases, cellulases, and amylases with polyextremophilic properties (Delgado-García et al. 2014). Certain enzymes that halophiles synthesize are useful for bioremediation of pollutants in saline habitats (Dastgheib et al. 2011) or are important biomolecules, e.g., exopolysaccharides and phytohormones (Liszka et al. 2012). Abiotic stresses, e.g., temperature, pH, salinity, and drought, affect the phytomicrobiome, directly or indirectly, via the host. However, the overall microbial composition in saline habitats is affected more by salinity than by other abiotic stresses (Ma and Gong 2013).

Scientists have developed a wide range of methods to study microbial diversity, community structure, and functions to understand plant-microbe interactions and soil biology (Rincon-Florez et al. 2013; Mukhtar et al. 2017). High throughput sequencing approaches have been developed to understand the complexity of microbial communities in a wide range of environments. Continuing advances in sequencing technology make it possible to study the dynamics of microbial population by using metagenomics and metatranscriptomics. Such approaches may uncover not only the composition of the microbiome community, but also the functional traits that the microbes use to survive under harsh conditions.

The microbial community associated with the Khewra salt mines, an extremely saline environment, have not been previously explored. Hence, the rhizosphere microbiomes of different halophytes from Khewra mines were thoroughly evaluated by high-throughput sequencing of the 16S rRNA gene, covering the V3–V4 regions. The main objective of this study was to compare and evaluate the differences in microbial communities from non-saline environment (rhizosphere of *Triticum*), moderately saline environment (rhizosphere of *Urochloa* and *Kochia*) and highly saline environment (rhizosphere of *Salsola* and *Atriplex*, non-rhizospheric and lake-bank soil samples) in Pakistan.

Materials and methods

Soil sampling

The Khewra salt mine, which is characterized by crystals of pure halite, is the second largest salt mine in the world (Ahmad et al. 2007). It is surrounded by a vast salt range that covers a 150-mile area from east to west. Rhizospheric soil samples were collected from three sites: highly saline soil from Khewra salt mines (73°08'N, 32°37'E); moderately saline soil from the Biosaline Research Station (BSRS) of the Nuclear Institute for Agriculture and Biology (NIAB) in Faisalabad (73°07'N, 31°42'E), and non-saline soil from the fields near Forman Christian College in Lahore (74°34'N, 31°54'E). Rhizospheric soil samples from the halophytes, *S. stocksii* and *A. amnicola*, non-rhizospheric soil, and lake-bank saline soil samples were collected from the Khewra salt mines (Fig. S1). In addition, soil samples from under the canopy of *Urochloa mutica* and *K. indica* were collected from the BSRS site in Faisalabad. Lastly, *Triticum aestivum* rhizospheric soil was collected from the Lahore site (Supplementary Table S1). No specific permissions were required to collect plant and soil samples from the Khewra salt mines because it is a public land and natural resource. We also collected plant (*U. mutica* and *K. indica*) and soil samples from the experimental fields of the NIAB, Faisalabad and from Forman Christian College, Lahore (wheat soil samples). An area approximately 1.1 km from the Khewra salt mines was surveyed. The sampling area was selected according to land use and vegetation cover. The vegetation of this area is classified as sub-tropical, dry evergreen forest. *Suaeda*, *Salsola*, *Atriplex*, *Justica*, *Lantana*, and *Chrysopogon* are the dominant plant genera in this region. Samples of rhizospheric soil from *U. mutica*, *K. indica*, and *T. aestivum*, grown under field conditions, were collected by gently removing the plants and carefully removing the soil attached to the roots. For non-rhizospheric saline soil samples, the upper 8–10 cm of mineral soil were collected. Lake-bank soil samples were collected from the bank of a salt lake (Fig.

S2). At each site, soil samples of approximately 500 g each from four spatially separated plants from each species were collected at each site and placed in black sterile polythene bags. These samples were taken to the laboratory from the collection site in an ice box and stored at -80°C for pyrosequencing analysis.

Soil physicochemical characteristics

The physical and chemical properties of each soil sample were determined based on 400 g of dried and sieved soil. Electrical conductivity (dS/m) was measured by 1:1 (w/v) soil to water mixtures at 25°C (Adviento-Borbe et al. 2006). The pH was measured by making a 1:2.5 (w/v) soil to water suspension whereas moisture (%), temperature ($^{\circ}\text{C}$) and texture class were determined according to Anderson and Ingram (1993). Organic matter (C_{org}) was calculated by Walkley and Black method (1934), phosphorous (P) was estimated by extraction with sodium bicarbonate (Olsen et al. 1954), and calcium and magnesium were detected by atomic absorption spectrometry. Nitrate ions were measured by the Kjeldahl method and potential acidity (H+Al) was determined using an equation based on the pH in SMP buffer solution (pH SMP). Cation exchange capacity (CEC) is defined as the capacity to retain and release cations (Ca^{2+} , Mg^{2+} , K^{+} and Na^{+}), and sodium adsorption ratio (SAR) is the measure of the sodicity of soil, which is calculated as the ratio of sodium to magnesium and calcium.

DNA extraction and pyrosequencing

Metagenomic DNA was extracted from 0.5 g of soil using a FastPrep® instrument (MP Biomedicals, USA) according to the manufacturer's instructions. The concentration of metagenomic DNA was qualitatively determined on 0.8% (w/v) agarose gel and quantified using Nanodrop technology (NanoDrop 200c Thermo Scientific, USA). In total, 28 DNA samples seven rhizospheric and non-rhizospheric saline soil samples (with four replicates each) were used for amplification of 16S rRNA gene and pyrosequencing.

Amplification of 16S rRNA gene and pyrosequencing

The V3–V4 region of the 16S rRNA gene was amplified using primers F515 (5'-G TGCCAGCMGCCGCGG-3') and R907 (5'-CCGTCAATTCMTTTRAG TTT-3'), which were linked with unique identifier and adapter sequences (Supplementary Table S2). The detailed PCR conditions for amplicon sequencing were the same as described previously (Mirza et al. 2014). Briefly, a 50 μl PCR amplification reaction contained 1X buffer, 0.2 μM of each primer, 1.8 mM MgCl_2 , 200 μM deoxynucleoside triphosphates (dNTPs), 20 ng of template, and 1 μl FastStart high-fidelity

PCR system enzyme (Roche Applied Sciences). The PCR conditions were 3 min at 95°C , followed by 30 cycles of denaturation at 94°C for 45 s, primer annealing at 54°C for 45 s, extension at 72°C for 1 min, and a final extension for 7 min. Amplified PCR products were purified with Agencourt AMPure beads (Beckman Coulter, Brea, CA). Purified PCR products from different samples were pooled in equimolar concentrations. Pyrosequencing was performed on the mixture with the 454 GS FLX sequencer (454 Life Sciences) at the Utah State University Center for Integrated Biosystems.

Bioinformatics and statistical analyses

Sequences were processed and sorted using the default parameters in QIIME 1.3 (Caporaso et al. 2010). An offset of 10 nucleotides was set in order to remove the first 10 bases of each sequence, and high quality sequences with an average length of 375 bases were selected. A total of 63,450 high quality sequences were clustered into operational taxonomic units (OTUs) with 3% difference using UCLUST for 28 samples with sequence numbers ranging from 872 to 5457. For the identification of chimeric sequences, Chimera Slayer software was used (DeSantis et al. 2006). The cleaned sequences were analyzed using RDP classifier naïve Bayesian algorithm (Wang et al. 2007) with an 80% confidence threshold. For each taxonomic level (phylum, class, order, family and genus) Good's coverage was calculated by using 97% similarity cutoff (Good 1953).

Alpha and beta diversity were determined using QIIME alpha_rarefaction.py and beta_diversity_through_plots.py commands respectively. The rarefaction curves for observed OTUs and Shannon index were presented in Fig. S3. Alpha diversity was calculated at sequence depth of 2152 reads per soil sample, as alpha diversity indices are correlated with the number of sequences. The selected maximum sampling depth corresponded to minimum number of reads obtained from any of the remaining sequenced samples. Beta diversity was analyzed by using Principal component analysis (PCoA). A matrix was calculated using the weighted and unweighted UniFrac distances among samples at a sequence depth of 872 reads per soil sample. The robustness of the UPGMA tree was calculated with Jackknife test, based on 1000 replicates and the beta_diversity.py, UPGMA_cluster.py and tree_compare.py commands. The similarity percentages (SIMPER) was performed pairwise comparisons of groups of non-saline, moderately and highly saline soil samples and found the average contributions of each OUT to the average overall Bray-Curtis dissimilarity between soil samples through the decomposition of Bray-Curtis dissimilarity (Henderson and Seaby 2014). The decomposition of dissimilarity was calculated using Community analysis package 5 (CAP) for the difference of abundance of each

OTU in each soil sample (Henderson and Seaby 2014). To detect the taxonomic classifications that were significantly abundant in non-saline, moderately and highly soils, Wilcoxon's non-parametric rank-sum test and LDA using the LEfSe program was used (Segata et al. 2011). One-way ANOVA was applied to analyze differences among saline and non-saline soil samples. Detrended correspondence analysis (DCA) was used to measure the correlation between soil physicochemical properties and community structure. DCA was performed using PAST (PAleontological STatistics) 3.12 (Hammer et al. 2001). Spearman's rank correlation test explains the degree of dependence among the variables, but the coefficient does not suppose that the relationship among the variables is linear (Gautier 2001). Canonical corresponding analysis (CCA) was used to show overall patterns of microbial diversity in different soil samples by using PAST 3.12 (Hammer et al. 2001). To explain the differences in the composition of taxa inside the data matrix community, a heatmap (relative abundance matrix) was generated at class level using XLSTAT 7.0 software (Fahmy 2003). Variation partitioning was performed on the basis of canonical correspondence analysis and then applied on the community data to disentangle the specific effects of soil salinity on the microbial community structure. The variation in microbial diversity explained by the soil salinity was calculated using partitioning analysis (Borcard et al. 1992; Buttigieg and Ramette 2014) as implemented in the vegan R package with some modifications according to our data. To calculate the variation explained by one group (highly saline soils) within our dataset, we calculated the variation explained on the complete community matrix and compared this to a matrix from which all OTUs from highly saline soils had been removed. The difference in these variation partitionings was taken as the variation explained by highly saline soil group in the context of other groups, e.g., non-saline soil group. Sequence data analyzed through pyrosequencing was submitted to the NCBI Sequence Read Archive (SRA) under ID project PRJNA309754.

Results

Physicochemical characteristics of soil and microbial community structure

Soil samples from the seven different sites were characterized by a great spatial variability and covered a significant variation in soil salinity, pH, organic matter, vegetation type, texture class, CEC, and SAR. EC ($EC_{1:1}$) ranged from 1.11 ± 0.23 to 6.24 ± 1.21 dS/m, with the highest values in lake-bank soil samples and the lowest values in *Triticum* (non-halophyte) soil samples; pH values ranged from 7.65 ± 0.45 to 8.54 ± 0.57 , temperature from 22 ± 2

to 32 ± 3 °C, and moisture from 20 ± 3 to $35 \pm 4\%$. Total organic matter ranged from 29.29 ± 2.56 to 36.25 ± 4.87 g/Kg. The available P, K, Ca, and Mg contents were different in *Triticum* (non-halophyte) rhizospheric soil than in the other soil samples (Supplementary Table S3). CEC values ranged from 56.46 ± 4.51 to 81.15 ± 7.45 mg/dm³ and SAR values from 6.68 ± 2.56 to 13.45 ± 4.29 , with the highest values in non-rhizospheric saline soil samples and the lowest in *Triticum* soil samples. Differences in community structure among different soil samples were explained by DCA (Fig. 1). Soil sample replicates of *Urochloa* and *Kochia* and lake-bank showed greater variation in microbial community composition from site to site than other saline soil samples. At each site, certain bacterial and archaeal species prevailed better than others. The microbial communities expressed differently from point to point because of variations in environmental factors like salinity and pH. DCA also revealed that *Triticum* (non-halophyte) soil samples had significant differences in physicochemical characteristics compared to saline soil samples.

General analysis of pyrosequencing at phylum, class and genus level

Almost all reads (99%) were assigned at the domain level, 70–85% reads were classified at the phylum level and only 30–49% reads were classified to the genus level (Fig. S3). The maximum number of phyla (20) was identified from the rhizospheric soil sample of *Urochloa* and the minimum number of phyla (17) was from the rhizospheric soil samples of *Triticum*. Sequences assigned by the RDP classifier at the class level suggested an uneven distribution in soil samples from saline and non-saline environments (Fig. 2). A total of 56 different bacterial and archaeal classes were identified in this study, with the maximum number of classes (42) in non-rhizospheric saline soil samples and the minimum (30) in the rhizospheric soil samples of *Kochia*. Sequence analysis at the genus level showed that *Urochloa* soil samples had the maximum identifiable bacterial and archaeal genera (170) and the *Triticum* (non-halophyte) soil had the minimum identifiable bacterial genera (99) (Fig. 2).

Alpha and beta diversity patterns

Number of observed OTUs, alpha diversity (Shannon index), beta diversity, and phylogenetic diversity were calculated at 97% DNA similarity. The alpha diversity patterns were highly variable across the saline and non-saline soil samples (Fig. 3). All Chao1 values were greater than the observed OTUs, indicating that more OTUs could be retrieved by additional sequencing (Table S5). Rarefaction curves were obtained using only those sequences assigned to a genus level. The rarefaction curves did not reach saturation in all

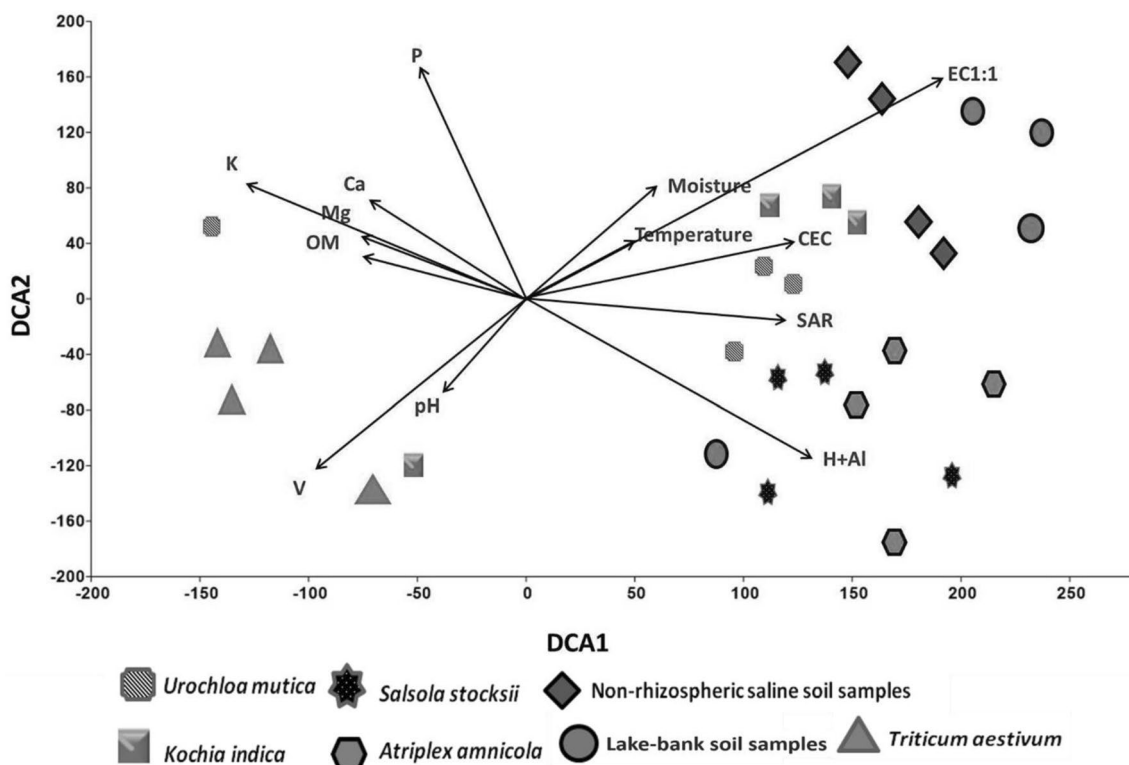


Fig. 1 DCA of the microbial communities from the rhizosphere of halophytes (*Urochloa*, *Kochia*, *Salsola*, and *Atriplex*) from moderately and highly saline and non-saline environments (*Triticum*) based on physical and chemical properties of the soils

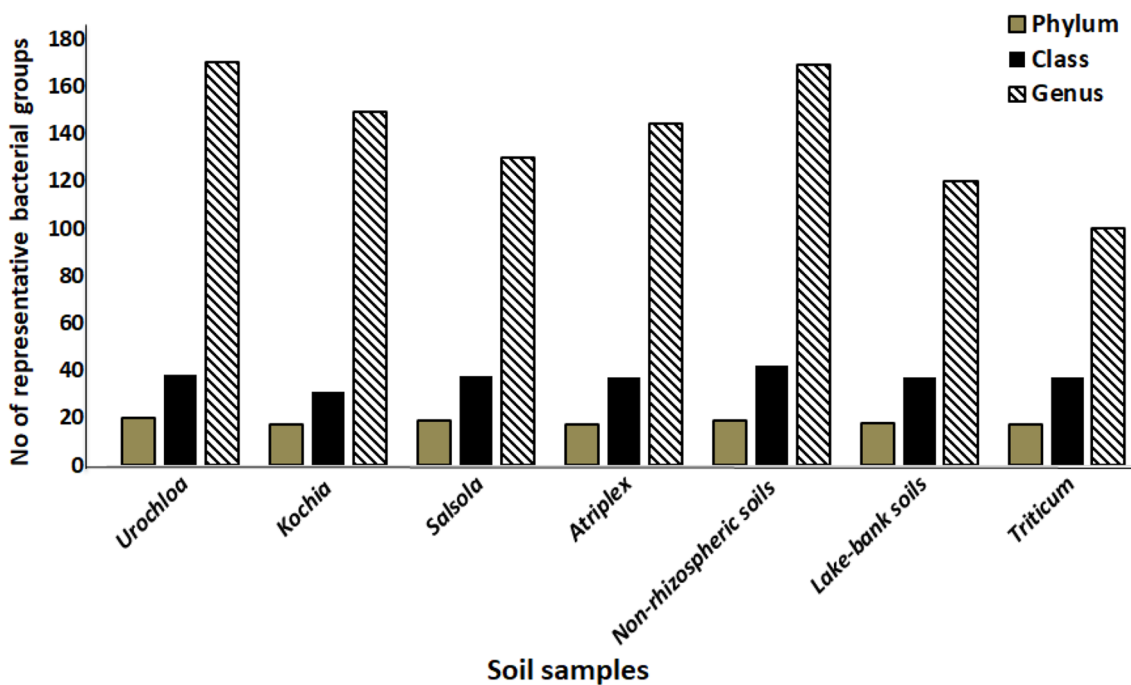


Fig. 2 Number of phyla, classes and genera detected through 16S rRNA gene pyrosequencing from the rhizosphere of halophytes, non-rhizospheric soil samples and non-halophyte (*Triticum*)

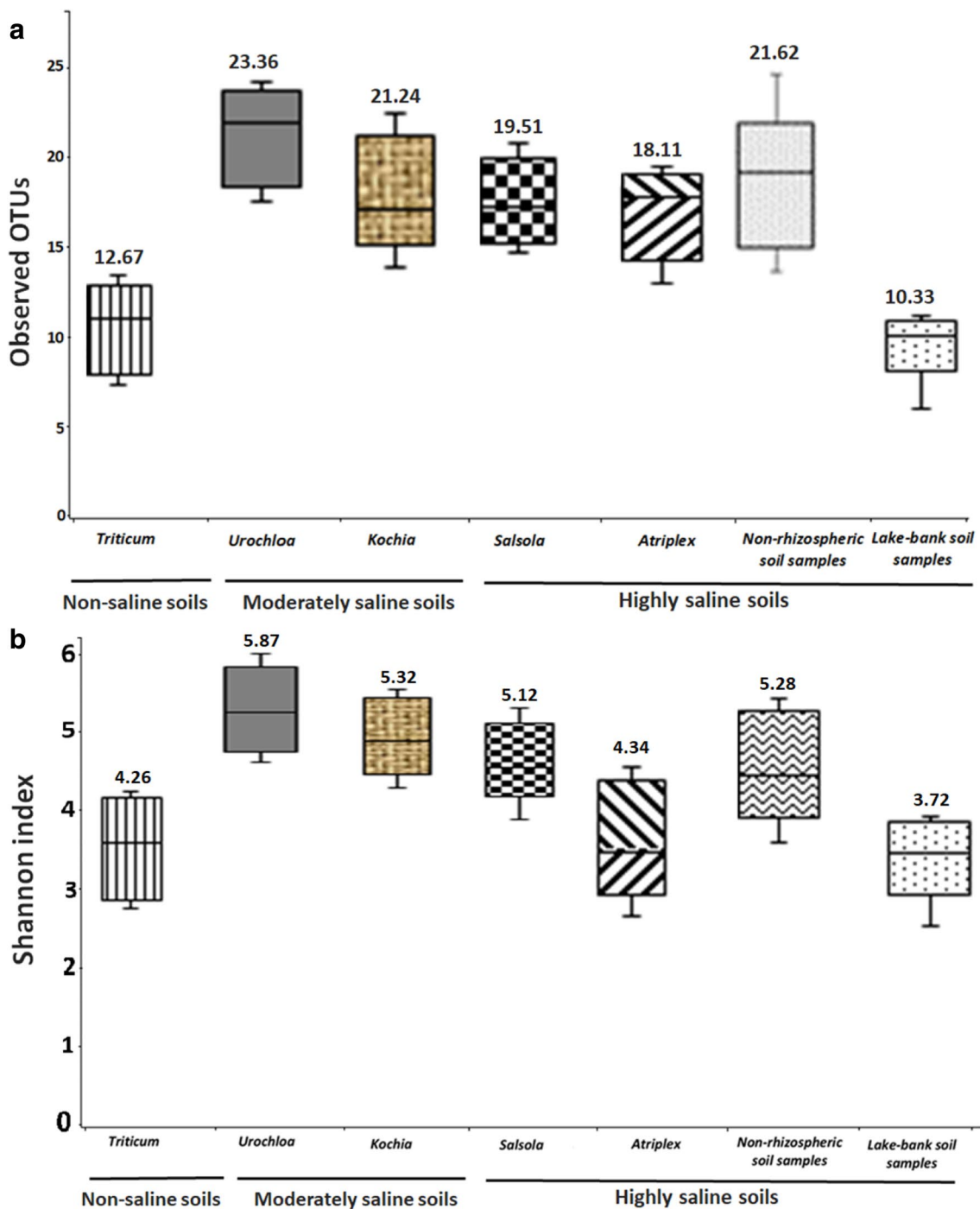


Fig. 3 Alpha diversity analysis. **a** Number of observed OTUs and **b** Shannon index. All the soil samples showed significant difference after a Mann–Whitney test with a confidence level of 99% ($P \leq 0.05$)

in each index. The average value for each group of soil samples ($n=4$) is shown next to each boxplot

the soil samples from non-saline, moderately and highly saline environments (Fig. S4). Results of observed OTUs and alpha diversity showed that *Urochloa* rhizosphere had more microbial diversity as compared to other halophytes and non-halophyte soil samples (Fig. 3). PCoA using unweighted UniFrac distances showed that non-saline soils

were clustered separately from moderately and highly saline soils (Fig. 4a). Similar results were observed in the UPGMA tree of unweighted UniFrac distances (Fig. 4b). Non-saline soils (*Triticum* rhizosphere) were grouped in one cluster, moderately saline soils (*Urochloa* and *Kochia*) and highly saline soils (*Salsola* and *Atriplex*, non-rhizospheric and

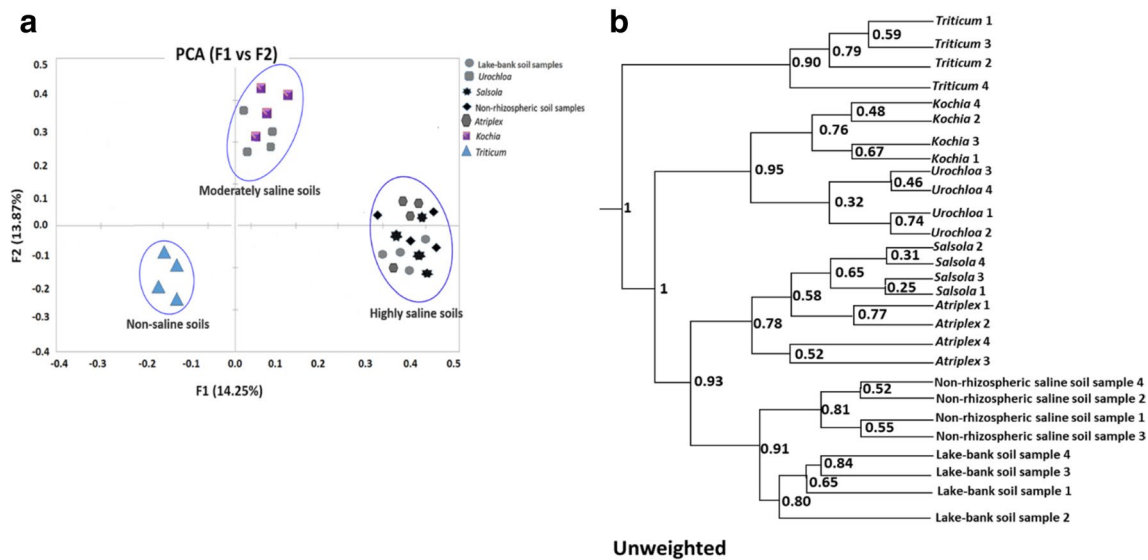


Fig. 4 Beta diversity analysis. Unweighted PCoA of UniFrac distances **a** non-saline soils, moderately and highly saline soils **b** UPGMA tree of unweighted UniFrac distances with Node values represents JS, using 1000 replicates

lake-bank) formed other two clusters (Fig. 4b). All the soil samples were analyzed in UPGMA tree of unweighted UniFrac at 100% jackknife support (JS). These results showed that microbial communities from moderately saline soils (*Urochloa* and *Kochia*) had more diversity as compared to other highly saline (*Salsola* and *Atriplex*, non-rhizospheric and lake-bank) and non-saline (*Triticum*) soil samples.

Microbial diversity comparisons between saline and non-saline soils

Rhizospheric soil microbial communities in moderately saline, highly saline and non-saline environments showed similar patterns of relative abundance in the major groups at the phylum level. A total of 24 bacterial and archaeal phyla were identified from saline and non-saline soil samples. The dominant phyla in all soil samples were *Actinobacteria* (16.14–36.34%), *Proteobacteria* (20.51–35.27%), *Firmicutes* (3.11–15.58%), *Acidobacteria* (1.11–5.51%), and *Bacteroidetes* (1.11–3.92%) (Fig. S5). Saline soils (*Urochloa*, *Kochia*, *Salsola* and *Atriplex*) showed greater abundance of *Actinobacteria* (7.23%), *Firmicutes* (4.53%), *Chloroflexi* (2.21%), *Thaumarchaeota* (1.26%), and *Euryarchaeota* (0.42%) as compare to non-saline soils (*Triticum*). Sequences of the 16S rRNA gene from *Proteobacteria* (5.22%), *Acidobacteria* (2.41%), and *Planctomycetes* (0.31%) were more abundant in the rhizosphere of *Triticum*. Sequences related to phylum *Chlamydiae* were found in the rhizosphere of *Urochloa* (0.14%), *Salsola* (0.084%), and lake-bank (0.11%) soil samples. Sequences belonging to the bacterial phylum *Hydrogenedentes* were detected only from the rhizosphere of *Urochloa* (0.14%) and *Salsola* (0.12%). Members of the

phylum *Ignavibacteriae* were identified from the rhizosphere of *Salsola* (0.047%) and non-rhizospheric (0.11%) saline soil samples. Sequences from the phylum *Nanohaloarchaeota* were detected only from the rhizosphere of *Salsola* (0.16%). Canonical correspondence analysis showed that microbial communities in the rhizosphere of *Urochloa* and *Kochia* (moderately saline environment) were similar. From highly saline environment, microbial communities were similar in the rhizosphere of *Salsola* and *Atriplex*, but lake-bank soil samples showed totally different microbial diversity as compared to other samples. Microbial community present in the rhizosphere of *Triticum* (non-halophyte) showed a great variation from the saline samples. For example, bacterial phyla *BRC1* and *Fusobacteria* were only present in the rhizosphere of wheat. This could be due to the differences in soil salinity at different sites (Fig. 5). Pearson correlation analysis was performed between different soil samples (halophyte and non-halophyte) and microbial diversity at the phylum level (Table 1). Our results showed that *Actinobacteria* significantly correlated with the rhizosphere of halophytes and *Proteobacteria* with the non-halophyte, whereas *Planctomycetes* correlated least with the rhizosphere of halophytes and *Bacteroidetes* with the non-halophyte (*Triticum*).

The relative distribution of bacterial groups at the class level showed significant differences among saline and non-saline soils. Different bacterial and archaeal classes were clustered and plotted with respect to the different soil sample values (saline and non-saline) in the heatmap (Fig. 6). The cluster structure showed a different distribution pattern in non-saline soils (*Triticum*) as compared to saline soils. The main group of bacterial and archaeal classes (*Actinobacteria*, *Alphaproteobacteria*, *Gammaproteobacteria*,

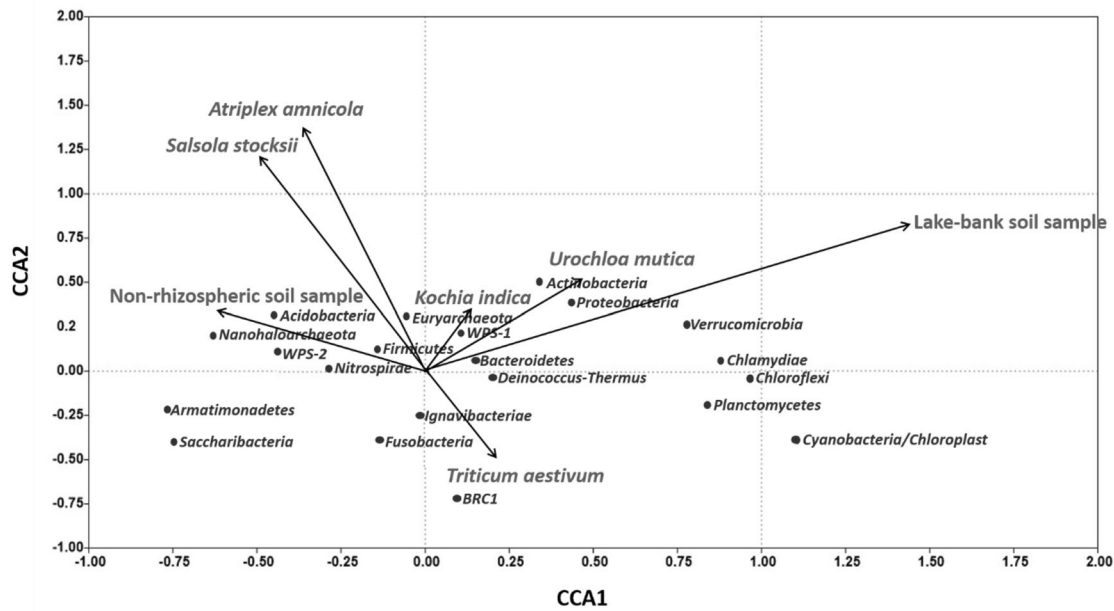


Fig. 5 CCA of the microbial communities from the rhizosphere of halophytes (*Urochloa*, *Kochia*, *Salsola*, and *Atriplex*) from moderately and highly saline and non-saline environments (*Triticum*) at the

phylum level. Among all the variables tested, these were significantly associated with the three different sites

Table 1 Pearson correlation analysis of the sequences classified as *Actinobacteria*, *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, *Acidobacteria* and *Planctomycetes* with the different saline and non-saline soil samples

Soil samples	Correlations	<i>Actinobacteria</i>	<i>Proteobacteria</i>	<i>Firmicutes</i>	<i>Bacteroidetes</i>	<i>Acidobacteria</i>	<i>Planctomycetes</i>
<i>U. mutica</i>	Positive correlations	148 ± 9	112 ± 8	57 ± 3	32 ± 2	68 ± 5	15 ± 1
	Negative correlations	64 ± 6	43 ± 4	23 ± 2	7 ± 1	24 ± 2	3 ± 1
	Total	212 ± 15	155 ± 12	80 ± 5	39 ± 3	92 ± 7	18 ± 2
<i>K. indica</i>	Positive correlations	112 ± 8	89 ± 5	56 ± 3	22 ± 2	33 ± 2	11 ± 1
	Negative correlations	32 ± 4	24 ± 4	15 ± 2	4 ± 1	12 ± 2	3 ± 1
	Total	144 ± 12	113 ± 9	71 ± 5	26 ± 3	45 ± 4	14 ± 2
<i>S. stocksii</i>	Positive correlations	145 ± 17	122 ± 8	87 ± 4	26 ± 2	35 ± 3	9 ± 1
	Negative correlations	24 ± 3	57 ± 6	22 ± 2	14 ± 2	5 ± 1	12 ± 2
	Total	169 ± 20	179 ± 14	109 ± 6	40 ± 4	40 ± 4	21 ± 3
<i>A. amnicola</i>	Positive correlations	111 ± 12	98 ± 5	58 ± 4	18 ± 1	26 ± 2	17 ± 2
	Negative correlations	56 ± 3	43 ± 3	45 ± 2	21 ± 2	8 ± 1	10 ± 1
	Total	167 ± 15	141 ± 8	103 ± 6	39 ± 3	34 ± 3	27 ± 3
Non-rhizospheric saline soil samples	Positive correlations	152 ± 12	102 ± 8	75 ± 4	27 ± 2	56 ± 5	9 ± 1
	Negative correlations	67 ± 5	44 ± 4	34 ± 3	16 ± 2	25 ± 2	4 ± 1
	Total	219 ± 17	146 ± 12	109 ± 7	43 ± 4	81 ± 7	13 ± 2
Lake-bank soil samples	Positive correlations	94 ± 9	128 ± 9	34 ± 2	24 ± 2	54 ± 4	17 ± 2
	Negative correlations	67 ± 5	54 ± 3	14 ± 1	19 ± 2	32 ± 2	11 ± 1
	Total	161 ± 14	182 ± 12	48 ± 3	43 ± 4	86 ± 6	28 ± 3
<i>T. aestivum</i>	Positive correlations	105 ± 9	146 ± 9	78 ± 5	6 ± 1	65 ± 3	13 ± 1
	Negative correlations	79 ± 4	65 ± 4	42 ± 3	5 ± 1	22 ± 2	5 ± 1
	Total	184 ± 13	211 ± 13	120 ± 8	11 ± 2	87 ± 5	18 ± 2

The values indicate the mean of four biological replicates (± SE) with significant (P < 0.05) Pearson correlations for each phylum

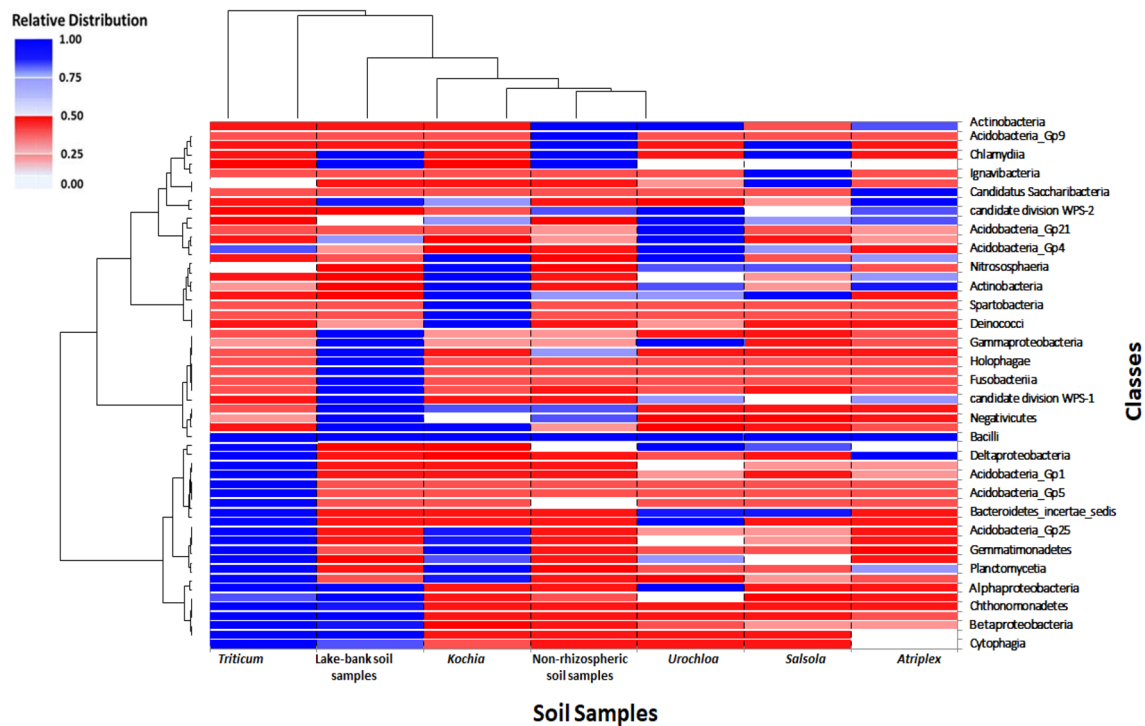


Fig. 6 The heatmap reports the normalized values of the taxonomic assignments at class level. Each value has been normalized following this criterion: $X_{ij}^{norm} = X_{ij} / \sum_{k=1}^N X_{ik}$, where X_{ij} is the occurrence of

the phyla 'j' in the soil sample 'i' and N is the number of saline and non-saline soil samples (in this study 7). Using this transformation, sequences assigned to each phylum can be compared in all soil samples independently from its order of magnitude

Deltaproteobacteria, *Bacilli*, *Sphingobacteriia* and *Nitrososphaeria*) shared similar composition and abundance among the different saline soils.

Soil salinity influences the halophyte microbiome (genus level)

Of 232 different phylotypes identified, 46 were commonly detected in all saline and non-saline soil samples. Our results also showed that 32 phylotypes were detected only from *Urochloa*, 18 from *Kochia*, 22 from *Salsola*, 30 from *Atriplex*, 30 from non-rhizospheric saline soil samples, 27 from lake-bank soil samples, and 27 from the rhizosphere of *Triticum* (Fig. S6). Total dissimilarity between pairs of non-saline, moderately and highly saline soils and the relative distribution of each bacterial or archaeal family to the observed dissimilarity was analyzed by using SIMPER test. Results of SIMPER analysis showed the difference in microbial diversity across non-saline and saline soil samples. Non-saline soils (*T. rhizosphere*) indicated the maximum dissimilarity, approximately 32% with moderately saline and 45% with highly saline soils (Fig. 7a). SIMPER analysis also showed that the overall differences between non-saline and saline soil samples were due to the detection of a wide range of taxa, each contributing a

relatively small percentage of the differences. For identification of specific bacterial genera that were significantly abundant in one site (non-saline soils) than the others (saline soils), LEfSe (LDA effective size) analysis was performed. The results of LEfSe analysis showed that 7 bacterial genera (*Bacillus*, *Azospirillum*, *Burkholderia*, *Enterobacter*, *Microbacterium*, *Aeromonas*, and *Mesorhizobium*) were more abundant in non-saline soils (*T. rhizosphere*) as compared to moderately and highly saline soils (Fig. 7b). Similarly, 13 bacterial genera were found to be abundant in moderately saline soils (*Urochloa* and *Kochia* rhizospheres) as compared to non-saline and highly saline soils. The top 5 bacterial genera based on LDA scores were *Virgibacillus*, *Arthrobacter*, *Klebsiella*, *Pseudomonas*, and *Azotobacter* from moderately saline soils (Fig. 7b). From highly saline soils (*Salsola* and *Atriplex*, non-rhizospheric and lake-bank soils), 7 bacterial genera (*Halobacillus*, *Aciditerrimonas*, *Methylobacillus*, *Dechloromonas*, *Patulibacter*, *Oceanobacillus*, and *Micrococcus*) were more abundant as compared to non-saline and moderately saline soils (Fig. 7b). These results also confirmed the abundance of bacterial phyla *Proteobacteria*, *Actinobacteria*, *Firmicutes*, and *Bacteroidetes*, as the top bacterial genera from all the soil samples were belonging to these phyla (Fig. 7b). From the rhizosphere of

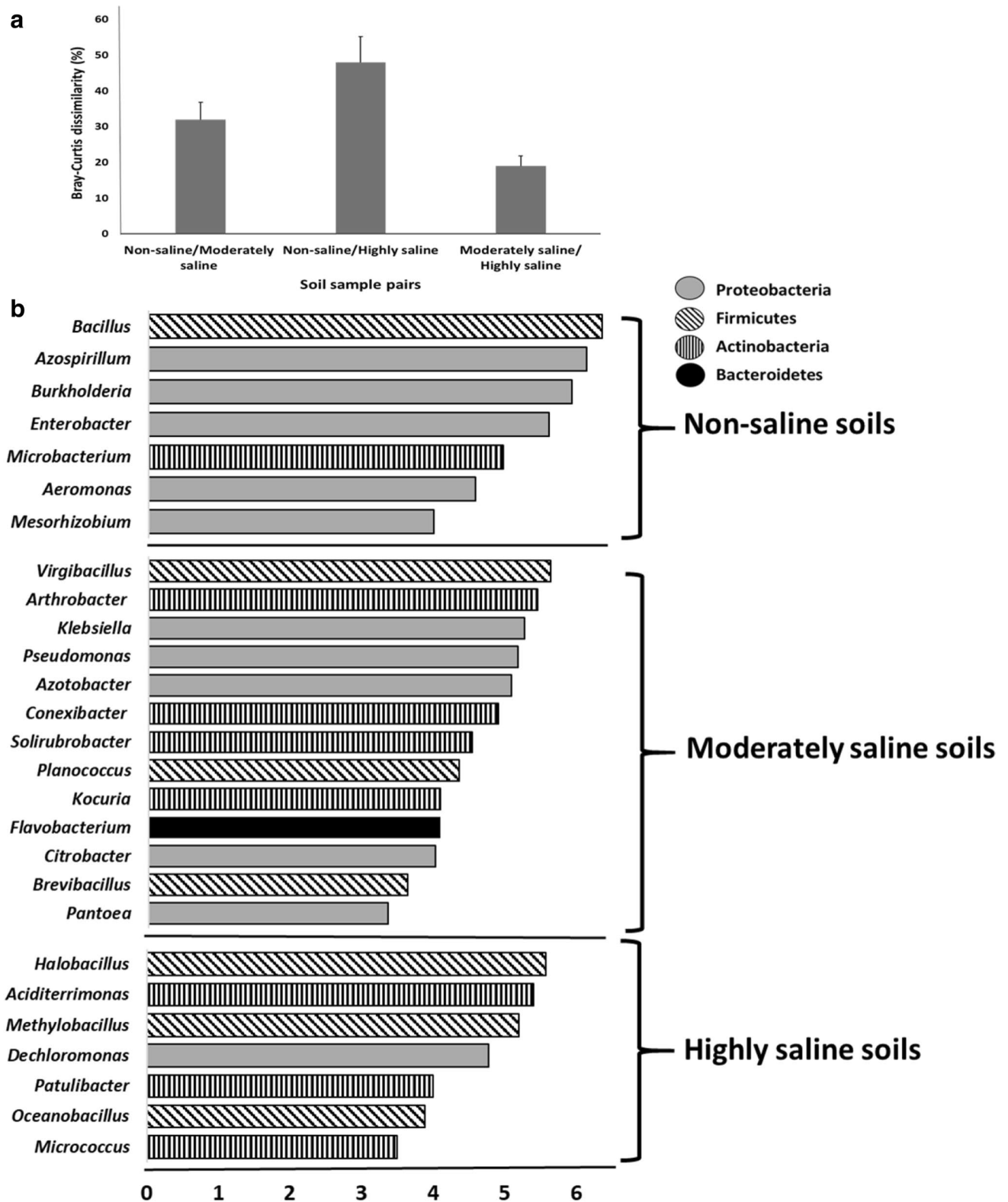


Fig. 7 Variations in the bacterial genera detected across non-saline, moderately, and highly saline environments. **a** Comparison of community structure between non-saline, moderately and highly saline soils by SIMPER analysis. Bars represent the standard error. **b** The

results of LEfSe analysis, which identified bacterial genera that were significantly abundant in one site as compared to other sites. Bacterial genera with the LDA score of more than 3 are shown

Urochloa, *Arthrobacter* (17.52%), *Virgibacillus* (12.34%), and *Pseudomonas* (9.57%) were found to be abundant bacterial genera while from *Kochia* rhizosphere, *Rubrobacter* (12.95%), *Asanoa* (10.67%), and *Planococcus* (7.85%) were abundant bacterial genera (Table 2). From highly saline environment, *Aciditerrimonas* (14.62%), *Terrimonas* (8.54%), and *Citrobacter* (6.32%) were identified from the rhizosphere of *Salsola*, *Halobacillus* (17.52%), *Staphylococcus* (11.23%), and *Rhizobium* (4.14%) were abundant in the rhizosphere of *Atriplex*, *Oceanobacillus* (14.23%), *Patulibacter* (9.89%), and *Kocuria* (7.98%)

and *Xanthomonas* (10.29%), *Streptococcus* (8.38%), and *Clostridium* (6.93%) were identified as dominant genera from lake-bank soil samples (Table 2).

Bacterial and archaeal genera with specific functions (PGPR activities) were also characterized from all saline and non-saline soil samples. N₂-fixing bacteria were found to be abundant in the rhizosphere of halophytes (*Kochia*, *Salsola*) as well as in *Triticum* (non-halophyte). P-solubilizers and IAA producers were more abundant in the rhizosphere of halophytes as compared to the non-halophyte. Bacterial genera involved in biocontrol and bioremediation of toxic

Table 2 Average relative abundance of top 5 OTUs with an abundance > 1% at family and genus level

Soil samples	Family	%	Genus	%
Non-saline soils				
<i>Triticum</i> rhizosphere	Pseudomonadaceae	19.21	<i>Pseudomonas</i>	11.98
	Enterobacteriaceae	14.65	<i>Enterobacter</i>	10.57
	Bacillaceae	13.57	<i>Bacillus</i>	9.45
	Rhodospirillaceae	10.42	<i>Azospirillum</i>	5.58
	Aeromonadaceae	9.87	<i>Aeromonas</i>	3.97
Moderately saline soils				
<i>Urochloa</i> rhizosphere	Micrococcaceae	24.25	<i>Arthrobacter</i>	17.52
	Bacillaceae	19.87	<i>Virgibacillus</i>	12.34
	Pseudomonadaceae	15.44	<i>Pseudomonas</i>	9.57
	Zoogloeaceae	13.67	<i>Azoarcus</i>	5.98
	Armatimonadaceae	10.45	<i>Armatimonas</i>	3.65
<i>Kochia</i> rhizosphere	Rubrobacteraceae	21.52	<i>Rubrobacter</i>	12.95
	Micromonosporaceae	18.54	<i>Asanoa</i>	10.67
	Bacillaceae	15.74	<i>Planococcus</i>	7.85
	Acetobacteraceae	9.85	<i>Belnapia</i>	4.23
	Flavobacteriaceae	7.77	<i>Flavobacterium</i>	1.57
Highly saline soils				
<i>Salsola</i> rhizosphere	Acidimicrobiaceae	17.41	<i>Aciditerrimonas</i>	14.62
	Chitinophagaceae	14.12	<i>Terrimonas</i>	8.54
	Enterobacteriaceae	8.58	<i>Citrobacter</i>	6.32
	Thermomonosporaceae	3.23	<i>Actinomadura</i>	1.95
	Bacillaceae	8.65	<i>Virgibacillus</i>	5.14
<i>Atriplex</i> rhizosphere	Bacillaceae	23.41	<i>Halobacillus</i>	17.52
	Staphylococcaceae	17.56	<i>Staphylococcus</i>	11.23
	Rhizobiaceae	12.41	<i>Rhizobium</i>	4.14
	Solirubrobacteraceae	9.74	<i>Solirubrobacter</i>	3.48
	Azonexaceae	5.44	<i>Dechloromonas</i>	1.89
Non-rhizospheric saline soil samples				
	Bacillaceae	17.12	<i>Oceanobacillus</i>	14.23
	Patulibacteraceae	12.54	<i>Patulibacter</i>	9.89
	Micrococcaceae	10.24	<i>Kocuria</i>	7.98
	Hyphomicrobiaceae	7.58	<i>Vasilyevaea</i>	5.12
	Bradyrhizobiaceae	5.64	<i>Bosea</i>	2.95
Lake-bank soil samples				
	Xanthomonadaceae	20.56	<i>Xanthomonas</i>	10.29
	Streptococcaceae	13.89	<i>Streptococcus</i>	8.38
	Clostridiaceae	11.57	<i>Clostridium</i>	6.93
	Moraxellaceae	8.45	<i>Moraxella</i>	5.44
	Micrococcaceae	4.23	<i>Nesterenkonia</i>	2.18

compounds were abundant in the non-rhizospheric saline soil samples. Rhizospheric and non-rhizospheric saline soil samples had high numbers of halophilic bacterial and archaeal genera (Table 3).

Strains belonging to 14 PGPR genera (*Bacillus*, *Arthrobacter*, *Ensifer*, *Burkholderia*, *Nocardioides*, *Gemmatimonas*, and *Mesorhizobium*) were identified from all soil samples (Fig. 8). Sequences from 14 PGPR genera *Aeromonas*, *Bradyrhizobium*, *Citrobacter*, *Delftia*, *Flavobacterium*, *Kocuria*, and *Skermanella* were detected only from

the saline environments (*Urochloa*, *Kochia*, *Salsola*, and *Atriplex*) while *Achromobacter*, *Azotobacter*, *Pseudomonas*, *Klebsiella*, *Lysobacter*, *Micrococcus*, and *Pantoea* were detected only from the non-saline environment (Triticum).

Ecological interpretation of overall microbial diversity patterns

A multivariate variation partitioning approach was used to study effects of soil salinity on the overall variation of

Table 3 Number of genera linked to functional categories in the rhizosphere of halophytes, non-rhizospheric soil samples and non-halophyte (*Triticum*)

Bacteria	<i>U. mutica</i>	<i>K. indica</i>	<i>S. stocksii</i>	<i>A. amnicola</i>	Non-rhizospheric saline soil samples	Lake-bank soil samples	<i>T. aestivum</i>
Nitrogen fixation	12 ± 2	14 ± 3	18 ± 3	15 ± 2	7 ± 2	5 ± 1	16 ± 2
Phosphorus solubilization	15 ± 2	11 ± 2	16 ± 2	17 ± 2	8 ± 1	6 ± 1	13 ± 1
Indole acetic acid production	19 ± 3	21 ± 2	12 ± 2	16 ± 2	12 ± 2	8 ± 1	13 ± 2
Biocontrol	5 ± 1	8 ± 1	9 ± 1	8 ± 1	18 ± 2	5 ± 1	6 ± 1
Pathogen	12 ± 2	7 ± 1	11 ± 1	5 ± 1	15 ± 2	6 ± 1	9 ± 1
Bioremediation	20 ± 2	11 ± 2	9 ± 1	6 ± 1	25 ± 3	12 ± 2	10 ± 1
Exopolysaccharides production	19 ± 2	15 ± 2	14 ± 2	11 ± 1	17 ± 2	9 ± 2	6 ± 1
Halophilic bacteria	112 ± 5	86 ± 4	102 ± 5	78 ± 4	110 ± 6	64 ± 4	3 ± 1
Haloarchaea	3 ± 1	3 ± 1	2 ± 1	2 ± 1	2 ± 1	2 ± 1	0
Unclassified function	6 ± 1	5 ± 1	8 ± 1	6 ± 1	16 ± 2	8 ± 1	4 ± 1

Each value is the mean of four biological replicates (±SE) with significant differences (P<0.05) among the bacterial communities of the analyzed soil samples

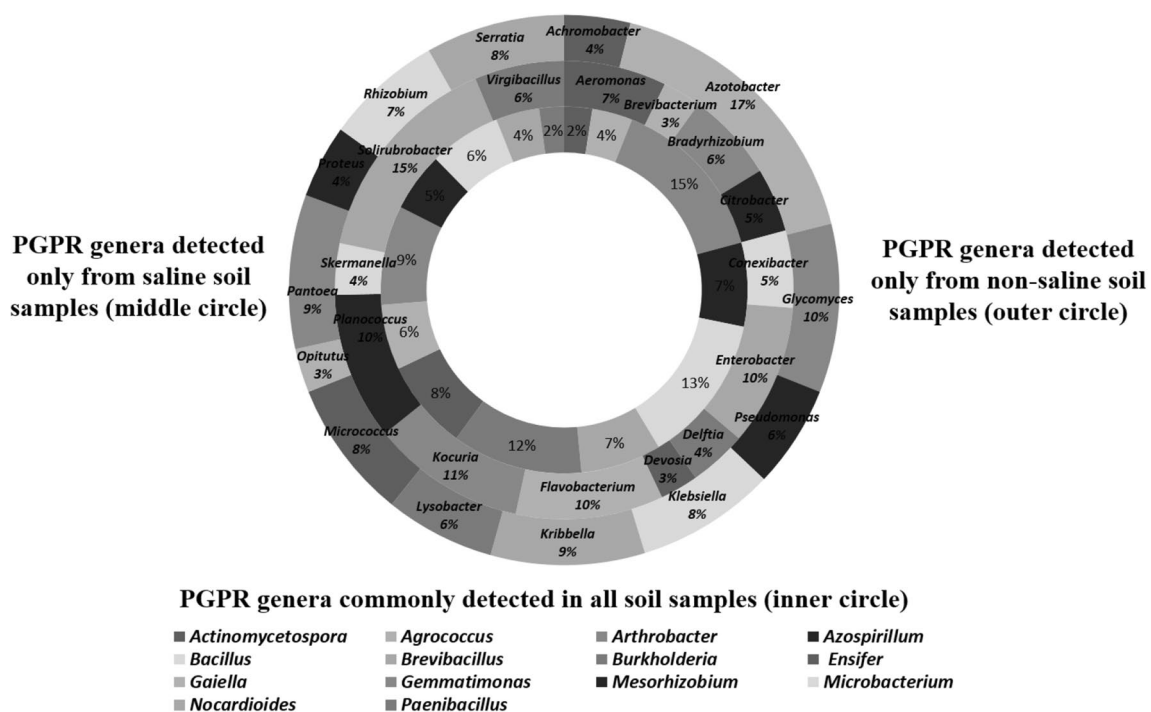


Fig. 8 PGPR genera detected by 16S rRNA gene pyrosequencing in all soil samples collected from saline and non-saline environments

rhizosphere microbiome based on the most abundant OTUs. The results showed that significant environmental variables (moderately saline, highly saline, and non-saline soil samples) included in the most parsimonious multivariate models were qualitatively different for the resident OTU, the phylum level OTU, OTU_{all} (all sequences) and SSO_{rel} (reduced sequence) (Fig. 9a). This study also included rare types, the resident single-sequence OTU relative (SSO_{rel}) data sets or sequence related to highly salt tolerant bacteria and archaea (*Halomonas*, *Halobacillus*, *Halococcus*, *Halobacterium* and *Halalkalicoccus*) to better understand the patterns of rare fraction of microbial community. A combination of environmental parameters (moderately saline, highly saline, and non-saline soil samples) could explain 59% of their overall biological variation while 41% variations were unexplained (Fig. 9b).

Discussion

In this study, we determined that the microbial community structure and the abiotic factors like salinity affect the microbial community. The rhizospheric soil samples were taken from moderately saline, highly saline, and non-saline environments. Using metagenomic analysis, we provided information about different bacterial and archaeal genera with potential functions in the rhizosphere microbiomes of halophytes and non-halophytes. Increases in salinity cause changes in soil organic matter, leaching and erosion, loss of nutrients, and changes in both quantity and composition of soil microbial communities (Langenheder et al. 2010; Yusuf et al. 2012). We determined that the differences in

microbial communities were strongly correlated with specific soil properties such as SAR and CEC, both of which can be correlated with salinity (Fig. 1). The link among plants, rhizosphere microbial communities and soil properties are often described as complex derivatives of the ecosystem and any modification in this relationship might affect the microbial structure and the ecological processes (Fierer et al. 2012; Pan et al. 2014).

In general, microbial diversity from non-saline soils (*T. rhizosphere*) was found to be less as compared to moderately and highly saline soils. The number of observed OTUs and Shannon index were maximum from the rhizosphere of *Urochloa* and *Kochia* (moderately saline soils). These plants grow under moderate saline environments (4.14 EC_{1:1} dS/m) and the physiological conditions of these plants influence the development of rhizosphere microbial communities. Some OTUs were commonly identified from all the saline soil samples and some OTUs were identified only from the rhizosphere of *Urochloa* and *Kochia* (Fig. 3). The number of observed OTUs and Shannon index for halophytes *Sal-sola* and *Atriplex* which are growing under highly saline environments (5.19 EC_{1:1} dS/m) was found to be less as compared to moderately saline soils and more as compared to non-saline soils, but the abundance of certain bacterial groups especially the *Firmicutes* were more in these soils as compared to non-saline and moderately saline soils (*Urochloa* and *Kochia* rhizosphere). Beta diversity analysis by PCoA using unweighted Unifrac distances and UPGMA tree also showed the differences in microbial diversity in three sites: non-saline, moderately and highly saline soils (Fig. 4).

Sequence analysis through the RDP classifier showed that the rhizosphere microbiomes of halophytes and the

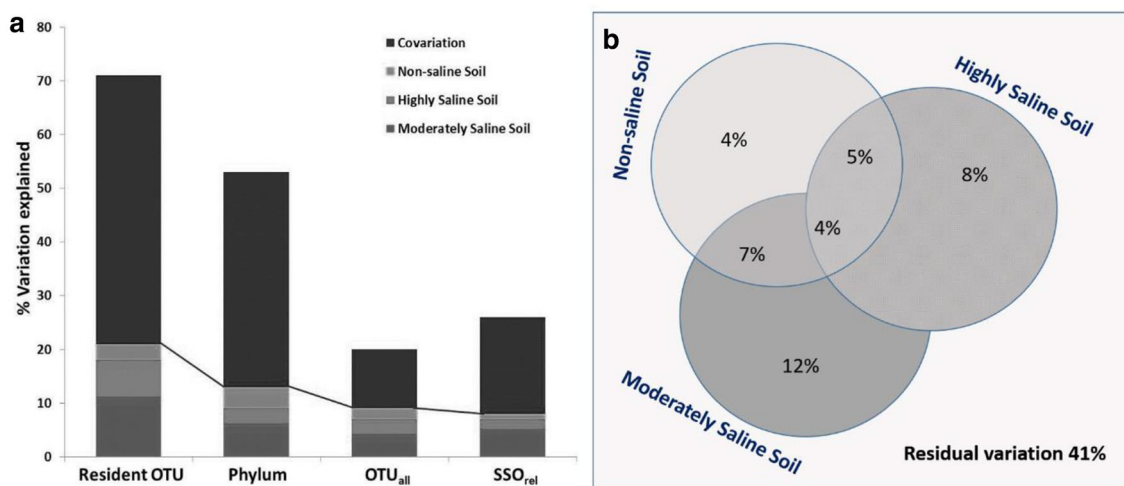


Fig. 9 Partitioning between the biological variations in the bacterial community structure based on soil salinity. Three explanatory matrices were used here, containing variables pertaining to moderately saline, highly saline and non-saline soil samples. Variation explained

on basis of **a** the resident OTU, the phylum level OTU, OTU_{all} and SSO_{rel} **b** highly salt tolerant bacteria and archaea (*Halomonas*, *Halobacillus*, *Halococcus*, *Halobacterium* and *Halalkalicoccus*)

non-halophyte *Triticum* had different abundances of bacterial and archaeal phyla. Microbial communities from moderately saline environments exhibited more diversity compared to highly saline and non-saline environments at the phylum level (Fig. S5). The decrease in microbial diversity with increased salinity might be due to decline in hydrolytic and oxidative enzyme activities and carbon cycling (Gholilarata and Raiesi 2007). Some of the bacterial and archaeal phyla, namely *Actinobacteria*, *Proteobacteria*, *Firmicutes*, *Acidobacteria*, *Planctomycetes*, *Bacteroidetes*, *Chloroflexi*, *Gemmatimonadetes*, and *Cyanobacteria* were found to be abundant in salt-affected soils as compared to non-saline soils. Sequences from *Euryarchaeota* were identified only from the rhizosphere of halophytes. Overall microbial diversity composition was significantly different among the three sites; non-saline, moderately and highly saline soils.

Our results showed that members of *Actinobacteria*, *Arthrobacter*, *Solirubrobacter*, *Patulibacter*, *Kocuria*, and *Actinomycespora* were identified from moderate and high salinity environments. These strains were found to promote plant growth and to degrade a variety of hazardous agricultural and industrial pollutants including atrazine, butane, p-nitrophenol, trinitrophenol, and vinyl chloride (Deng et al. 2015). The main difference in previous studies and our current findings was that *Actinobacteria* were more abundant in moderately saline environments especially in the rhizosphere of *Kochia* at genus level. Overall, bacterial genera *Arthrobacter* and *Kocuria* were found to be abundant in all the saline soils. These bacteria have ability to survive in hypersaline environments. They contain glutamic acid, glycine, alanine, and meso-diaminopimelic acid as cell-wall-bound amino acids (Bodenhausen et al. 2013; Niemho et al. 2013). Bacterial genera *Euzebya*, *Marmoricola*, *Aciditerrimonas*, and *Aeromicrobium*, which were detected only in highly saline environments (rhizospheres of *Salsola* and *Atriplex* and non-rhizospheric soil samples), are mostly saccharolytic bacteria (they obtain carbon and energy by carbohydrate hydrolysis) and contribute to carbon cycling in soil ecosystems (Krivushin et al. 2015). They also show the ability to degrade various hazardous compounds and can be used as biocatalysts in the production of fossil fuels and bioactive steroids (Deng et al. 2015).

Among *Proteobacteria* the *Gammaproteobacteria* were the most abundant. Bacterial genera *Devosia*, *Halomonas*, *Burkholderia*, *Rubrobacter*, *Geobacter*, *Azospirillum*, and *Aeromonas* were more abundant in the rhizospheres of halophytes as compared to non-halophyte (*T.* rhizosphere). These bacteria are involved in plant growth promotion, antibacterial and antifungal activities, and bioremediation of various toxic compounds and are also a source of naturally occurring bioactive products (Bodenhausen et al. 2013). Bacterial genera *Azoarcus*, *Kosakonia*, *Dongia*, and *Sorangium* identified only from the rhizosphere of *Urochloa* and *Kochia*

(moderate salinity) were previously reported from the rhizosphere of grasses (para grass and kallar grass) as having good plant growth-promoting abilities (Malik et al. 1997; Mukhtar et al. 2016). These results indicated that microbial communities in the rhizosphere of each plant vary to some extent even if these plants were collected from the same environments. For example, microbial diversity from the rhizosphere of *Urochloa* showed different community composition when compared to *Salsola*.

Members of the *Firmicutes* *Bacillus*, *Staphylococcus*, *Planococcus*, *Sporosarcina* were dominant in saline soils as compared to non-saline soils. *Bacillus*-like organisms are dominant in saline environments due to their ability of spore-forming and Gram-positive cell walls (Schimel et al. 2007). It is well known that *Bacillus*-like organisms play an important ecological role in biogeochemical cycles in different ecosystems such as marine waters and saline soils (Taprig et al. 2013; Mukhtar et al. 2017). Halophilic *Bacillus* strains promote plant growth, produce industrially important enzymes (proteases, amylases, cellulases and lipases) and involved in bioremediation of different toxic chemicals and pollutants from saline environments (López-López et al. 2010; Mukhtar et al. 2018). *Planococcus*, and *Planomicrobium* were dominant genera identified from moderately saline environments (rhizosphere of *Urochloa* and *Kochia*). They are involved in calcite precipitation and biological cementation (Ma and Gong 2013; Tsuda et al. 2015).

Bacterial genera related to *Bacteroidetes* (*Flavobacterium*, *Salinibacter*, *Cytophaga*, *Adhaeribacter*, and *Pontibacter*) were identified from the rhizospheres of *Urochloa*, *Salsola* and *Atriplex* and are important in the bioremediation of complex organic compounds. They have also been reported in previous studies from marine environments (Vaisman and Oren 2009; Wang et al. 2012). All members of the phylum *Thaumarchaeota* identified so far are ammonia oxidizers and have important roles in biogeochemical cycles such as carbon and nitrogen cycles (Yanagawa et al. 2014; Doxey et al. 2015). Sequences attributed to the genus *Nitrososphaera* were frequently detected in the rhizosphere of *Salsola* and *Atriplex* (highly saline soils) compared to moderately saline and non-saline soils.

Bacterial genera belonging to *Acidobacteria* (*Gp2*, *Gp4*, *Gp6*, *Gp21*, and *Gp25*) were abundant in the rhizosphere of halophytes (*Salsola* and *Atriplex*) from highly saline soils. Members of *Acidobacteria* were a dominant part of microbial communities from saline soils and marine sediments (Ghosh et al. 2010; Mukhtar et al. 2016). Bacterial genera *Sphaerobacter* and *Litorilina* (phylum *Chloroflexi*) were identified from *Salsola* and *Atriplex* plants. They have been previously reported from hypersaline wastewater (Valenzuela-Encinas et al. 2008; Rincon-Florez et al. 2013). Bacterial genera *Armatimonas*, *Fimbriimonas* and *Chthonomonas* (phylum *Armatimonadetes*) were detected from the

rhizosphere of *Urochloa*, *Salsola* and *Atriplex*. These genera have been studied from various habitats including hypersaline, marine and hot spring water, and anaerobic sludge (DeBruyn et al. 2011; Tamaki et al. 2011).

Archaeal sequences belonging to *Euryarchaeota* were abundant in the rhizospheres of the halophytes and non-rhizospheric saline soil samples. Members of *Euryarchaeota* from marine environments are reported to be involved in bioremediation, and they can break down complex lipoprotein molecules to survive in extreme conditions (Garrity and Holt 2001; Iverson et al. 2012). Sequences assigned to *Nanohaloarchaeota* were identified only in the rhizosphere of *Urochloa* and *Salsola*. Previous studies reported that they are abundant in surface waters of hypersaline lakes worldwide (Narasingarao et al. 2012; Pan et al. 2014). However, from the rhizosphere of halophytes, identification of *Nanohaloarchaeota* was reported for the first time in this study. From the rhizosphere of *Urochloa*, unclassified bacteria were more prevalent as compared to unclassified bacteria from other soil samples. Previous studies on metagenomics have also reported a higher proportion of unclassified bacteria from different environments; however, these studies need thorough excellent sequencing procedures (Chakraborty et al. 2015).

Sequences related to plant growth-promoting rhizobacteria (PGPR) such as *Arthrobacter*, *Bacillus*, *Burkholderia*, *Kocuria*, *Nocardioidea*, and *Planococcus* were relatively more abundant in the rhizospheres of the halophytes compared to that of the non-halophyte (Fig. 8). PGPR genera *Achromobacter*, *Klebsiella*, and *Lysobacter* were identified only from the rhizosphere of *Triticum* (Fig. 8). Application of these PGPR strains as a biofertilizer has resulted in improved growth and grain yield of various crops including maize, wheat, rice, and sugarcane (Gupta et al. 2015). Overall, our pyrosequencing results analysis revealed significant differences in the microbial community composition from saline and non-saline environments.

Conclusion

To our knowledge, this is the first study of moderate and high salinity soils of the Khewra salt mines of Pakistan that is based on a metagenomic analysis. The soil microbiomes of the halophytes *Urochloa* and *Kochia* from a moderately saline environment, the high saline-adapted halophytes *Salsola* and *Atriplex*, the non-rhizospheric and lake-bank samples of a high salinity environment, and a non-halophyte (*Triticum*) exhibited significant differences in microbial populations especially at the genus level. At each site, specific bacterial and archaeal species dominated. The *Salsola* and lake-bank soil samples showed great variation even within biological replicates and from site to site. In the soils studied

here, we observed significant differences in alpha and beta diversity in microbial communities from saline (both moderate and high) and non-saline soils. The rhizosphere microbiomes of the halophytes (*Urochloa* and *Kochia*) from the moderately saline soils showed more alpha diversity compared to highly saline (*Salsola* and *Atriplex*) and non-saline (*Triticum*) soils. By contrast, soil microbiomes of *Salsola* and *Atriplex* and non-rhizospheric soil samples from highly saline environments showed more beta diversity compared to the other soil samples. A novel representative of *Nanohaloarchaeota* (*Candidatus Nanosalina*) was detected only in moderately saline soils. More than 230 bacterial and archaeal genera with potential PGPR functions (N_2 fixation, P solubilization, IAA, exopolysaccharide production, and biocontrol and bioremediation activity) were identified from both moderately and highly saline soils. With deeper study, we may find that many of inhabitants of the microbial communities from naturally salt-affected soils will prove to have significant biotechnological potential for promoting crop growth in harsh environments.

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Author contributions SM: Conducted experiment and prepared manuscript; BSM: pyrosequencing and data analysis; SM: manuscript preparation; MSM: supervised research and manuscript preparation; JM: pyrosequencing and data analysis; KAM: guided in experiment plan and edited manuscript.

Compliance with ethical standards

Conflict of interest The authors declared that they have no conflict of interest in the publication.

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