The responses and adaptations of microbial communities to salinity in farmland soils: A molecular ecological network analysis

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ABSTRACT

Soil salinity is an increasing problem deteriorating soil fertility in degraded farmland soils. However, the responses and adaptations of microbial communities and interactions to salinity in farmland are not yet fully understood. In this study, we used 16S rRNA Miseq-sequencing technique to investigate the phylogenetic compositions, diversity and structure of soil microbial communities under different salinity conditions. The results indicated that prokaryotic diversity decreased with salinity. The change in prokaryotic community was primarily driven by salinity levels. The relative abundances of *Proteobacteria*, *Bacteroidetes* and *Firmicutes* were higher, and those of *Acidobacteria* and *Thaumarchaeota* were lower under high salinity than in medium and low salinity soils. Further, microbial network interactions changed along the salinity gradient revealed by a phylogenetic molecular ecological networks (pMENs) analysis. Salinity enhanced the interactions between microorganisms, evidenced by more links, higher average degree and average clustering coefficients within the pMENs in high salinity soils. Furthermore, we constructed the sub-networks of *Flavobacterium* and *Acidobacteria Gp4* to explore the changes of interactions among different microbial groups under salinity. We found that salinity shifted the interactions among different microbial taxa, and such changes vary among different microbial populations. This study provides solid evidences that microbial communities adapt to salinity through the adjustments of microbial compositions and interactions.

1. Introduction

Soil salinity is an increasingly serious problem in agricultural soils all over the world (Qadir et al., 2000). Many studies observe the influences of salinity on soil microbial communities (Pankhurst et al., 2001; Muhammad et al., 2006; Ghollarata et al., 2007; Valenzuela-Encinas et al., 2009; Johannes et al., 2011; Mavi et al., 2012; Campbell and Kirchman, 2013; Sun et al., 2015a,b). The effect of salt on soil microbes is stronger than that of heavy metals (Sardinha et al., 2003). However, it is still not fully conclusive on the salt effects on microbial community. The dominant parameters to characterize microbial responses to salt exposure were microbial biomass, respiration, microbial activity, composition, diversity and structure. For example, Muhammad et al. (2006) observe that the microbial biomass C decreased from approximately $190 \mu\text{g g}^{-1}$ to $80 \mu\text{g g}^{-1}$ in response to salinity stress ranged from 2.1 to 6.0 mg g^{-1} . In contrast, Mavi and Marschner (2012) observed the microbial biomass C slightly increased from $93 \mu\text{g g}^{-1}$ to

$148 \mu\text{g g}^{-1}$ in response to salinity stress ranged from EC1.0 to EC2.5. Microbiological activities including soil respiration and enzyme activities are also depressed by salinity (Ghollarata and Raiesi, 2007). However, Rousk et al. (2011) reveal that soil salinity is not a decisive factor for bacterial growth.

The effects of salinity on microbial compositions and structure are widely studied (Pankhurst et al., 2001; Campbell and Kirchman, 2013). Sun et al. (2015a,b) report that high alkali-saline level could reduce soil microbial quantity, but not materially alter soil microbial community composition. The medium alkaline-saline soil had the highest diversity indices at the order and species level, in comparison with the high and low ones (Valenzuela-Encinas et al., 2009). Killham (1994) describes two main adaptation strategies of microorganisms to osmotic stress (e.g. salinity, drought or freezing), including ion accumulation in the cell to exclude salt solute, and production of organic acids to antagonize salt gradient. However, these mechanisms are known from single microorganisms, but seldom focus on microbial interactions among

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various populations. We are still not fully understood how a community adapts to salinity through community-level adjustments of the compositions and interactions.

Microbial interactions may provide system-level adaptations of prokaryotic communities to soil salinity. Co-occurrence of prokaryotic populations in a community reflects their similar niche adaptations of the co-occurring species, or interspecies interactions, either by competition or by cooperation (Rui et al., 2015). It is not clear how the salinity drives the changes of microbial interactions. Many network methods have been developed, including equation-based network methods (Yeung et al., 2002; Gardner et al., 2003), Bayesian network methods (Gerstung et al., 2009) and relevance/co-expression network methods (Zhang and Horvath, 2005; Horvath et al., 2006; Oldham et al., 2006). However, most studies use arbitrary thresholds, and thus the constructed networks are subjective rather than objective (Barabasi and Oltvai, 2004). The phylogenetic molecular ecological networks (pMENs) have been proposed based on a novel conceptual framework using a random matrix theory (RMT)-based approach (Deng et al., 2012). It provides good solutions to some common problems concerning high-throughput metagenomic data (Deng et al., 2012). The pMENs have been applied to characterize network interactions of microbial communities in response to elevated CO₂ (Zhou et al., 2011), heavy metal pollutions (Yin et al., 2015), ocean acidification (Wang et al., 2015) and livestock grazing (Sun et al., 2015a,b).

Different soil salinity gradients have been formed along the Bohai Bay in Tianjin, China, due to interlace of fluvial outwash and marine deposit, shallow groundwater level and unreasonable exploration of groundwater resources (Wang et al., 2011). High soil salinity seriously influences the crop production and sustainable development of agriculture in Tianjin. In this study, the pMENs was applied to investigate the responses of soil microbial communities and microbial interactions to salinity changes in Tianjin farmland soils in May and November. We hypothesized that (1) the phylogenetic diversity and structure of microbial community would shift under different salinity conditions; (2) soil salinity would affect microbial network interactions among different salinity gradients.

2. Material and methods

2.1. Study sites and sampling

Field sites located in the Bohai Bay in Tianjin municipality. The research area is affected by the warm temperate semi-humid continental monsoon climate with an average annual temperature of 11.4–12.9 °C. The annual precipitation is 520–660 mm, with 75% of the total precipitation occurring from June to August (Yue et al., 2010). Due to regional differences in topography, precipitation, evaporation, groundwater depth and soil properties, soil salinity gradients are formed from north to south in Tianjin. Six farmland sites were selected along the salinity gradients, including low salinity region (L group) with braunerde soil (L1: 40°04′14.87″N, 117°20′01.90″E; L2: 40°05′25.91″N, 117°38′10.21″E); medium salinity region (M group) with fluvo-aquic soil (M1: 39°36′54.49″N, 116°58′04.20″E; M2: 39°32′09.31″N, 116°59′32.31″E), and high salinity region (H group) with coastal saline soil (H1: 38°43′09.78″N, 117°26′44.00″E; H2: 38°49′08.84″N, 117°03′38.57″E). The physico-chemical properties in 6 sampling sites were shown in Table S1. The salt levels in six sampling sites at different layers were shown in Table S2. The hierarchical cluster of soil sampling sites based on soil salinity was exhibited in Fig. S1.

Samplings were conducted twice in May and November 2013. The unsaturated zone cores were collected with a 5.5 cm diameter hollow-stem hand auger. Samples were taken continuously in total 10 layers from the ground surface to a depth of 3 m in a profile, including: 0–0.2 m, 0.2–0.4 m, 0.4–0.6 m, 0.6–0.8 m, 0.8–1.0 m, 1.0–1.3 m, 1.3–1.6 m, 1.6–2.0 m, 2.0–2.5 m and 2.5–3.0 m. Samples were stored in

polyethylene bags. Moisture content was determined by drying a minimum of 50 g of soil sample at 108 °C for 24 h. Soil water extracts were obtained by adding 200 ml Mili Q water to 100 g soil sample, shaking 45 min at room temperature, centrifuging at 4000 rpm, and filtering supernatant. Soil water extracts were used to measure pH and salinity by a portable analyzer (Orion Star A329, Thermo, USA), SO₄²⁻ and Cl⁻ by an ion chromatography (ICS-2100, Dionex, USA), K⁺, Na⁺, Ca²⁺, Mg²⁺ by an Inductively coupled plasma atomic emission spectrometry (Optima 8300, PE, USA), TOC and TN by a total organic carbon analyzer (vario, Elementar, Germany); and NO₃⁻ and NH₄⁺ by a continuous flow analyzer (Auto Analyzer 3, Seal, Germany). Part of soil samples were freeze dried and stored at –20 °C for genomic DNA extraction.

2.2. DNA extraction, miseq sequencing and data analysis

DNA extraction was extracted using Ezup genomic DNA extraction kit for soil (Sangon Biotech, China, Cat# SK8264). DNA concentration and quality were checked using a NanoDrop Spectrophotometer. Extracted DNA was diluted to 10 ng μL⁻¹.

Universal primer 515F (5′-GTGCCAGCMGCCGCGTAA-3′) and 806R (5′-GGACTACHVGGGTWTCTAAT-3′) was used to amplify the V4 hypervariable region of 16S rRNA gene for pyrosequencing using Miseq sequencer (Lin et al., 2016). The PCR mixture (25 μl) contained 1 x PCR buffer, 1.5 mM MgCl₂, each deoxynucleoside triphosphate at 0.4 mM, each primer at 1.0 μM and 0.5 U of Ex Taq (TaKaRa, Dalian) and 10 ng soil genomic DNA. The details about PCR amplification program were described before (Li et al., 2014). In total, 115 samples were sequenced using Reagent Kit v2 2 × 250 bp by the Illumina Miseq platform at Environmental Genome Platform of Chengdu Institute of Biology.

The sequence data were processed using QIIME Pipeline-Version 1.7.0 (<http://qiime.org/>). All sequence reads were trimmed based on their unique barcodes. The sequences with high quality (length > 300 bp, without ambiguous base 'N', and average base quality score > 20) were used for downstream analysis. Chimera sequences were removed using the UCHIME algorithm (Edgar et al., 2011). Sequences were clustered by the complete-linkage clustering method incorporated in the RDP pyro pipeline. Operational taxonomic units (OTUs) were classified using a 97% nucleotide sequence similarity cutoff. Taxonomy was assigned using the Ribosomal Database Project classifier (Wang et al., 2007). All the samples were randomly-resampled to 2590 reads. The indices of alpha-diversity were calculated, including chao1 estimator of richness, observed species and Shannon's diversity. The original sequence data were deposited at the European Nucleotide Archive by accession PRJEB21751 (<http://www.ebi.ac.uk/ena/data/view/PRJEB21751>).

2.3. Statistical analysis

The hierarchical cluster analysis of sampling sites based on soil salinity was performed by IBM SPSS 21. The average rarefaction curves among H, M and L groups were generated from the observed species. The univariate ANOVA was used to identify the factors making the differences of microbial community diversity by IBM SPSS 21. Then, the one-way ANOVA based on Chao1 richness and Shannon diversity index was applied to compare microbial community diversity by IBM SPSS 21. The principal coordinate analysis (PCoA) (Wang et al., 2015) was used to compare the microbial community structure of six sampling sites by CANOCO 5.0 based on Bray-Curtis distance using the relative abundance data of OTU. The ANOSIM of microbial composition was applied to test the differences among three groups by Primer 7. The Mantel test (Yin et al., 2015) was applied to evaluate the correlations between microbial communities with environmental variables using PCORD 5.0. The principal coordinate analysis was performed with averaging values from the ten layers of each point, and all other analyses were performed with raw data.

2.4. pMENs construction and network analysis

Phylogenetic molecular ecological networks (pMENs) analysis was performed based on the relative abundances of all samples through the pMENs analysis pipeline (<http://ieg2.ou.edu/MENA>; Zhou et al., 2010, 2011; Deng et al., 2012). The whole process and details are given in a previous MENA study elsewhere (Deng et al., 2012). Briefly, OTUs in at least 9 out of each group were used to construct network. Various network properties such as average degree, average path distance, average clustering coefficient and modularity index were characterized based on similarity matrices. The network modules were generated by fast greedy modularity optimization (Newman, 2006). Identification of key module members was based on within-module connectivity (Zi) and among-module connectivity (Pi) of each node (Olesen et al., 2007). Besides, based on singular value decomposition (SVD), eigengene network analysis was performed to summarize the gene abundance data from each module in pMENs. Finally, mantel tests were used to measure the correlations between pMENs and environmental properties. The Cytoscape 3.1.1 was used to visualize network graphs. Since we were primarily interested in the impact of seasonal salinity on network interactions, the pMENs were constructed under H, M and L groups for May and November, respectively.

3. Results

3.1. Phylogenetic compositions and structure of soil microbial communities

A total of 1199572 high quality 16S rRNA gene sequences were obtained for all 115 samples (Table S3). They were resampled to 2590 sequences per sample, which were clustered into 7403 OTUs. The rarefaction curves showed that our sequencing efforts were enough to distinguish the differences between different salinity levels (Fig. S2). The univariate ANOVA results showed that the effects of soil depth on prokaryotic community diversity were not significant, but those of salinity were significant. Therefore, the key factor causing the differences of community diversity was not soil depth, but salinity (Table S4). Both the Shannon and Chao1 indices demonstrated significant difference between the H group and L group ($P < 0.05$) in May, while only the Shannon index showed a significant difference between the H and L groups ($P < 0.05$) in November (Table 1).

Furthermore, the PCoA results further revealed that the microbial community structures were changed by salinity in both samplings (Fig. 1). Similar community compositions at phylum level were observed in May and November samples at the same salinity level (Fig. 2). The ANOSIM showed that microbial compositions were significantly different among H, M and L group at both samplings (Table 2). The Mantel tests indicated that the shifts in microbial communities were significantly correlated with salinity, K^+ , Na^+ , Mg^{2+} , NO_3^- , Cl^- and SO_4^{2-} ($P < 0.05$) both samplings (Table 3). Ca^{2+} , TN, moisture and pH were significantly correlated with microbial communities either in May or November.

The top seven phyla were *Proteobacteria*, *Thaumarchaeota*, *Acidobacteria*, *Bacteroidetes*, *Actinobacteria*, *Chloroflexi* and *Firmicutes* among H, M and L groups in both samplings. These phyla accounted for

more than 70% of the total sequences at all sites (Fig. 2). However, the relative abundances of *Proteobacteria*, *Bacteroidetes* and *Firmicutes* were apparently higher in H group than those in M and L groups both samplings. In contrast, the relative abundances of *Thaumarchaeota* and *Acidobacteria* were relatively lower in H group compared with those in M and L groups. In May, the most abundant genera were *Nitrososphaera* (6.46%) and *Lactobacillus* (3.31%) in H group, *Nitrososphaera* (16.43%) and *Nitrosopumilus* (13.09%) in M group, and *Nitrososphaera* (18.22%) and *Gp4* (6.41%) in L group. In November, the most abundant genera were *Bacteroides* (6.21%) and *Lactobacillus* (3.60%) in H group, *Nitrososphaera* (9.62%) and *Bacteroides* (5.05%) in M group, and *Nitrososphaera* (12.91%) and *Gp4* (6.83%) in L group (Table S5).

3.2. Microbial community interactions

To understand the interactions of microbial populations in H, M and L microbial communities, we used OTU data of 16S rRNA sequences to construct pMENs for H, M and L groups by RMT-based network approach. Major topological properties of six empirical MENs (H-MEN, M-MEN, and L-MEN in May; H-MEN, M-MEN, and L-MEN in November) of microbial communities showed that salinity did influence the connectivity of micro-organisms with similar threshold (0.710 for H, 0.730 for M and 0.730 for L in May, and 0.710 for H, 0.720 for M and 0.710 for L in November, respectively) in two sampling times (Table 4). Compared with M- and L-MENs, the H-MENs demonstrated less total nodes (259 and 262), less modules (17 and 15), but more links (912 and 1259), higher average degree (7.042 and 9.611) and average clustering coefficients (0.560 and 0.587) both in May and November. This indicated that salinity may enhance the interaction between micro-organisms, and the network became denser with high salinity in May and November, respectively. The higher modularity suggested that the microbial communities were highly complex (Olesen et al., 2007). It was interesting to see that the modularity demonstrated highest value (0.862) in May while the lowest value (0.696) in November for H-MENs. The values of R square of power-law were above 0.7, and this indicated ecological networks based on RMT should be scale-free (Zhou et al., 2010). The average path distance (GD) means the shortest path between two nodes (Wang et al., 2015). It demonstrated irregular variation based on salinity gradients.

Strong positive correlations were observed in all six pMENs, while negative correlations were rare (Fig. S3). It implied that microbes might cooperate more to adapt to high salinity or similar niches. Further, we constructed the sub-networks of *Flavobacterium* and *Acidobacteria_Gp4* to explore the possible interactions between *Flavobacterium*/*Acidobacteria_Gp4* and other microbes. The top three *Flavobacterium* OTUs in H group had more complex interactions than their corresponding OTUs in M and L groups, evidenced by more nodes and links (Fig. 3), while *Acidobacteria_Gp4* had much less complex interactions in H group (Fig. 4). For example, in November samplings, OTU383 was connected with ten nodes in L-MEN and three nodes in M-MEN, but only one in H-MEN. It might implicate that the interactions of *Acidobacteria_Gp4* with other microbes were weakened at high salinity environments, evidenced by less nodes and links.

Furthermore, eigengene analysis (Langfelder and Horvath, 2007;

Table 1
Shannon and Chao1 indices in May and November samples.

Group	May		November	
	Shannon	Chao1	Shannon	Chao1
H	7.63 ± 0.65 ^a	958.22 ± 195.69 ^a	7.13 ± 1.07 ^a	951.99 ± 166.42 ^a
M	7.89 ± 0.46 ^{ab}	1232.50 ± 120.85 ^b	7.78 ± 0.52 ^{ab}	1055.01 ± 99.30 ^a
L	8.10 ± 0.34 ^b	1245.13 ± 113.05 ^b	7.85 ± 0.37 ^b	1003.14 ± 122.22 ^a

The values in the table were the means ± standard deviation (SD) of 20 (2 sites × 10 layers) replicates. The values in the same column with different letters indicated significant differences (at $P < 0.05$).

Fig. 1. Principle coordination analysis of microbial communities from six sampling sites based on the OTU relative abundances in May (a) and November (b).

Horvath and Dong, 2008) was performed to reveal the higher-order organization of the constructed pMENs. In this analysis, each module is represented by its singular value decomposition (SVD) of abundance profile, which is referred to as the module eigengene. The results showed that module eigengene explained 43–83% of the variances in relative OTU abundances across different samples in H group, 34–66% in M group and 38–75% in L group in May, respectively. In November, module eigengene explained 41–94% of the variances in H group, 38–70% in M group and 36–90% in L group, respectively. For example, in May, module E10 in H group had 31 OTUs derived from *Bacteroidetes*, *Chloroflexi*, *Proteobacteria*, *Actinobacteria*, *Euryarchaeota* and *Deinococcus-Thermus*. Its eigengene could explain 48% of all variations (Fig. S4). In addition, the relationships between microbial network modules and environmental properties were analyzed with Mantel tests. It was found that Na^+ , Mg^{2+} , Cl^- , SO_4^{2-} and salinity were significantly correlated with module E10 in H group.

Connectivity analysis among or within the modules showed that different OTUs (nodes) played distinct roles in the pMENs (Fig. 5). From an ecological perspective, the peripherals may represent specialists, whereas module hubs and connectors may be more generalists and network hubs may be super-generalists (Olesen et al., 2007; Deng et al., 2012). Two connectors (OTU2269 and OTU968) were observed in H group network, which were derived from *Firmicutes* (May) and *Bacteroidetes* (November). One module hub (OTU70) was detected in the M group network, which was derived from *Actinobacteria* (November). One connector and four module hubs were present in the L group network, which included *Acidobacteria* OTU59 (November), *Thaumarchaeota* OTU157 (May), *Proteobacteria* OTU350 (May), *Actinobacteria* OTU79 (November) and *Unclassified* OTU578 (May). No network hubs were detected in any pMENs. The above results suggested that salinity markedly altered the network structure and topological roles of individual OTUs and key microbial populations.

Table 2

ANOSIM of microbial compositions between three groups in May and November.

Groups compared	May		November	
	R	P	R	P
H-M-L group	0.490	0.001	0.329	0.001

R > 0 indicated that differences between three groups were greater than within-groups.

Table 3

The correlations between environmental variables and microbial community composition in May and November by Mantel test.

Environmental Variable	May	November
K ⁺	0.1873**	0.1808**
Ca ²⁺	0.1174	0.2143**
Na ⁺	0.3363**	0.2569**
Mg ²⁺	0.2247**	0.1538*
NH ₄ ⁺	0.0249	−0.0588
NO ₂ [−]	−0.0547	−0.0209
NO ₃ [−]	0.1799**	0.3520**
Cl [−]	0.3521**	0.2270**
SO ₄ ^{2−}	0.3277**	0.2949**
TOC	0.0149	0.0097
TN	0.0117	0.3405**
moisture	0.1223*	0.0902
pH	0.0444	0.1571*
salinity	0.4429**	0.3574**

Significant:

* P < 0.05.

** P < 0.01.

Fig. 2. Relative abundances of the top seven phyla in H, M and L groups in May (a) and November (b). The different letters above the columns indicated significant differences (at P < 0.05) among three groups. The error bar indicated standard deviation (SD) of 20 (2 sites × 10 layers) replicates.

Table 4
Topological properties of molecular ecological networks under different salinities.

Community		No. of original OTUs	Similarity threshold	Total nodes	Total links	R square of power-law	Average degree (avgKK)	Average clustering coefficient (avgCC)	Average path distance (GD)	Module	Modularity
May	H group	2713	0.710	259	912	0.777	7.042	0.560	5.327	17	0.862
	M group	2912	0.730	275	725	0.840	5.273	0.369	3.048	25	0.691
	L group	2690	0.730	349	880	0.696	5.043	0.434	7.799	25	0.860
November	H group	2379	0.710	262	1259	0.604	9.611	0.587	4.750	15	0.696
	M group	2252	0.720	281	929	0.803	6.612	0.410	5.748	18	0.782
	L group	2243	0.710	267	657	0.815	4.921	0.395	4.021	22	0.834

In addition, we explored the correlations between environmental variables and pMENs microbial compositions (Table S6). *Thaumarchaeota*, *Bacteroidetes* and *Proteobacteria* were significantly correlated with environmental properties under H group in May and November; *Bacteroidetes* and *Actinobacteria* were significant under M group in May, and *Actinobacteria* were significant under L group in November. The results suggested that the network interactions among different phylogenetic groups/populations were dramatically shifted by salinity, and such impacts in network interactions were significantly correlated to soil environmental properties.

4. Discussions

Understanding the shift of microbial composition and structure under different soil salinity gradients is critical to reveal the adaptation mechanisms of microbial community to salinity. In this study, significant differences in the microbial community compositions and microbial interactions networks were observed along salinity gradients. Salinity is identified to be the key factor driving the variations of prokaryotic community diversity, structure and microbial interactions. Our study provided deep insights into the responses and adaptations of soil prokaryotic community to salinity at community level based on microbial interactions analysis.

More *Proteobacteria*, *Firmicutes* and *Bacteroidetes*, but less *Thaumarchaeota* and *Acidobacteria* were observed in H group compared to M and L groups. Thus, the phyla *Proteobacteria*, *Firmicutes* and *Bacteroidetes* are likely more resistant to high salinity, while *Acidobacteria* and *Thaumarchaeota* are less resistant or susceptible to high salinity. In a previous study, Sand et al. (2014) demonstrate that *Acinetobacter baylyi*ADP1 (*Proteobacteria*) can cope with high salinity by uptake and accumulation of the well-known compatible solute glycine betaine. Another study (Samaei et al., 2013) shows that some

Proteobacteria such as *Acinetobacter radioresistens* and *Pseudomonas aeruginosa* (strain 1) are halotolerant, and another *Pseudomonas aeruginosa* strain 2 may be halophile. Similarly, Egamberdieva et al. (2008) also report that two *Acinetobacter* strains and *Pseudomonas aeruginosa* are salt tolerant. Above results and our data supported that *Proteobacteria* is likely resistant to high salinity.

A recent study (Hidri et al., 2016) in saline soil demonstrates that *Bacillus* sp. (*Firmicutes*) has a great surviving capacity under salt conditions. *Halobacillus salinus* (*Firmicutes*) and *Bacillus simplex* (*Firmicutes*) are the bacteria suitable for bioremediation in hypersaline conditions (Nicholson and Fathepure, 2005). These results supported that *Firmicutes* possess the high salinity resistance. Some studies also show that *Bacteroidetes* is a phylum resistant to salt (Valenzuela-Encinas et al., 2009; Keshri et al., 2013), and it can be one of dominant populations in alkaline saline soil (Valenzuela-Encinas et al., 2009). A similar study (Keshri et al., 2013) shows that the dominant phylum in the haloalkaline soil is *Bacteroidetes* followed by *Proteobacteria*.

On the contrary, microbes with low resistance to high salinity would decrease their abundances in H group samples, such as *Acidobacteria* and *Thaumarchaeota*. *Acidobacteria* was reported to be abundant in medium-saline soils (Valenzuela-Encinas et al., 2009), saline sediments from Qinghai Lake (Dong et al., 2006) and mangrove sediments of Sundarban (Ghosh et al., 2010). However, higher percentages of *Acidobacteria* are also observed in low salinity soil than that in high salinity soil (Yang et al., 2016), which is consistent with our study. Archaeal phylum *Thaumarchaeota* are chemolithoautotrophic ammonia-oxidizers including genus *Nitrososphaera*. Our data showed that the *Nitrososphaera* genus was abundant in most samples and decreased in H salinity, indicating their sensitivities to salinity. These *Thaumarchaeota* microbes play important roles in carbon and nitrogen cycles. Thus, changes in salinity may significantly alter biogeochemical cycles in farmland soil.

Fig. 3. Effects of salinity on the network interactions of *Flavobacterium* in H (a), M (b) and L (c) group in May and in H (d), M (e) and L (f) group in November. A blue line indicates a positive interaction between two individual nodes, while a red line indicates a negative interaction. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 4. Effects of salinity on the network interactions of *Acidobacteria_Gp4* in H (a), M (b) and L (c) group in May and in H (d), M (e) and L (f) group in November. A blue line indicates a positive interaction between two individual nodes, while a red line indicates a negative interaction. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Microorganisms in naturally saline habitats are supposed to share some common strategies to resist high salinity (Ma and Gong, 2013). Multiple adaptations must be developed for maintaining resistant populations to cope with salt stress. Our pMENs analysis showed that salinity significantly altered microbial interactions. Thus, it is likely that the adjustment of microbial interactions at community level is a strategy to cope with high salinity stress. In the network, positive links could be attributed to niche overlap and cross-feeding, while negative relationships could be attributed to competition and amensalism (Faust and Raes, 2012). Microbial communities owned more positive links among OTUs in the H group, indicating that they may cooperate or adapt to similar ecological niches. Adversely, some microorganisms who did not own the ability to compete with others would be filtered

out (Pointing et al., 2009). Specifically, the genus *Flavobacterium* (*Bacteroidetes*) is considered as moderately halophilic bacteria (Ren and Zhou, 2003). However, the relative abundance of *Flavobacterium* was highest in H group. *Acidobacteria_Gp4* was most abundant genus of *Acidobacteria* phylum in M and L groups. The sub-networks of *Flavobacterium*/*Acidobacteria_Gp4* indicated that the top three *Flavobacterium* OTUs in H group had more complex interactions than their corresponding OTUs in M and L groups, while *Acidobacteria_Gp4* had much less complex interactions in H group. It appeared that salinity selected for *Flavobacterium* but against *Gp4*. This suggested that the interactions among different microbial taxa were substantially changed by salinity, and such impacts were different among various populations.

A previous study reports that the positive correlations percentage

Fig. 5. pMEN submodules under H (a), M (b) and L (c) group in May and under H (d), M (e) and L (f) group in November. Each dot represents an OTU from six networks. The topological roles of each OTU is defined by two parameters, within-module connectivity (Z_i) and among-module connectivity (P_i). Z_i indicates the connectivity of nodes inside the module, P_i indicates the connectivity of nodes with other modules. According to values of Z_i (2.5) and P_i (0.62), the roles of nodes were classified into four categories as below.

ranges from 57.2% to 63.4% in four C and N cycling gene networks (Sun et al., 2015a,b). It was beyond our expectation that the positive link percentage were above 98% in all the six networks. It is likely that high salt habitat forces microbes to intensify their cooperations against salt stress. Alternatively, it may be attributed to the mutualisms among microbes in long-term co-evolution processes (Zhang et al., 2014) under high salinity.

5. Conclusions

This study revealed the changes in the diversity, structure and interactions of soil prokaryotic communities driven by salinity. High salinity decreased prokaryote community diversity, but enriched some taxa, e.g. *Proteobacteria*, *Bacteroidetes* and *Firmicutes*. Additionally, high salinity enhanced microbial interactions. It implies that microbes in naturally saline habitats could develop multiple adaptations to survive under high salt concentrations, including changing the interactions among microbes. Our results provide solid evidences that microbial communities adapt to salinity through the adjustments of microbial compositions and interactions. This study is an important step towards an integrated understanding of the mechanisms that microorganisms may apply to adapt to salinity.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.apsoil.2017.08.019>.

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