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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 μg g^{-1} to 80 μg 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 μg g^{-1} to 148 μg 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 has been applied to characterize network interactions of microbial communities in response to elevated CO_2 (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), SO_4^{2-} and Cl^- by an ion chromatography (ICS-2100, Dionex, USA), K^+, Na^+, Ca^{2+}, Mg^{2+} by a Inductively coupled plasma atomic emission spectrometry (Optima 8300, PE, USA), TOC and TN by a total organic carbon analyzer (vario, Elementar, Germany); and NO_3^- and NH_4^+ 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′-GTGCCAGCMGCCGCGGTAA-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 $MgCl_2$, 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.