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Shifts in soil microbial metabolic activities and community structures along a salinity gradient of irrigation water in a typical arid region of China

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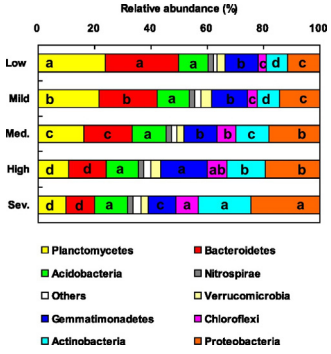
HIGHLIGHTS

- Soil microbial activities were greatly restrained in saline water irrigated soils.
- Significant differences in sole carbon source utilization were detected.
- Soil bacterial richness and diversity increased with irrigation salinity.
- The number of bacterial phyla decreased with irrigation salinity.

GRAPHICAL ABSTRACT



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ABSTRACT

Saline water irrigation can change soil environment, which thereby influence soil microbial process. Based on a field experiment, the shifts in soil microbial metabolic activities and community structures under five irrigation salinities were studied using Biolog and metagenomic methods in this study. The results demonstrated that microbial metabolic activities were greatly restrained in saline water irrigated soils, as average well color development (AWCD) reduced under all saline water irrigation treatments. Although no significant difference in carbon substrate utilization of all six categories was observed among Mild, Medium, High and Severe treatments, the consumption of sole carbon source was significantly varied. Especially, asparagine, galacturonic, putrescine and 4-benzoic acid played a decisive role in dominating the differences. Soil bacterial richness and diversity increased with irrigation salinity while the number of bacterial phyla decreased. Three significantly increased (Proteobacteria, Actinobacteria and Chloroflexi), two decreased (Planctomycetes, Bacteroidetes) and two irresponsive (Gemmatimonadetes and Acidobacteria) phyla were observed as the dominant groups in saline water irrigated soils. The results presented here could improve the understanding of the soil biological process under saline circumstance.

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1. Introduction

The scarcity of fresh water has forced farmers to use saline water to cultivate thirsty crops in arid and semiarid regions (Fedoroff et al., 2010). Continuous irrigation with saline water, however, may result in soil salinization, land degradation and yield decrease (Wong et al., 2010; Setia et al., 2011). Although efforts are being made to prevent or limit salt accumulation in the root zone (Pereira et al., 2002), saline water irrigation can inevitably alter soil physicochemical and biological properties.

The influence of salinity as a major stress to soil microorganisms has been assessed in several studies. Although microbial biomass has been found often to be low in salt-affected environments (Batra and Manna, 1997; Pankhurst et al., 2001; Rietz and Haynes, 2003; Yuan et al., 2007), Rath and Rousk (2015) explained that it may not only be the result of a direct negative effect of salinity but also could be caused by a reduced input of organic matter due to sparse plant growth in saline soils. Differently, the effects on microbial respiration have been detected clearly negative (Sardinha et al., 2003; Yuan et al., 2007; Muhammad et al., 2008). Setia et al. (2010) reported that saline soil with electrical conductivity bigger than 5.0 dS m^{-1} reduced microbial respiration even by >50%, demonstrating significant metabolic function impaired. Besides, the effects of salinity on soil microbial metabolic activity have also been widely evaluated, as described by Jin et al. (2014) who found AWCD values decreased significantly with increasing irrigation salinity. However, our understanding of the effects of saline water irrigation on the metabolic activities and structures of the soil microbial community is still fragmented and incomplete as we have noted an alarming lack of assessments of how saline water irrigation changes simultaneously the soil microbial metabolic activities and community structures, especially using up-to-date microbial analysis methods.

The Biolog microplate technique, a method based on the capacity of microorganisms to utilize different carbon substrates, is initially widely used for soil microbial community functional diversity analysis (Pierce et al., 2014). Compared with other microbial research methods, it has a problem in presenting a complete picture of the microbial community (Weber and Legge, 2010). However, because most biogeochemical processes are driven by active microbes (Blagodatskaya and Kuzyakov, 2013), the Biolog method is still useful in comparing the functional ability of the entire soil microbial community in contrasting environmental samples (Ros et al., 2008).

In recent years, the metagenomic methods have opened new doors for mapping soil microbial phylogeny (Verastegui et al., 2014). Metagenomics is a culture-independent approach that seeks to access the biosynthetic capacity of microbial species (Charlop-Powers et al., 2014). By directly capturing the total soil microbial DNAs, metagenomics has the potential to provide a complete toolkit for facilitating the characterization of soil microbial community structures (Fierer et al., 2012). Recent developments in the use of rDNA homology and conserved features of biosynthetic pathways have made metagenomic approach more easy and reliable (Bhattacharyya et al., 2014). Most of the microbial species are now assigned to 'RNA similarity groups' (Claesson et al., 2009) for better understanding of the diversity and community dynamics of whole soil microbiota.

In this study, we conducted a field experiment to investigate the effects of different irrigation salinity on soil microbial metabolic activities and bacterial community structures using Biolog Ecoplates™ and metagenomic methods. We hope this research can contribute to improve the understanding of how saline water irrigation changes soil structure and function by influencing soil microbial processes and as a result, be useful to manage soil and saline water resources in arid and semiarid regions.

2. Materials and methods

2.1. Field experiment

The field experiment was conducted at the Minqin Experimental Station for Agricultural Water-saving and Ecological Improvement (103°12′3.4″ E, 38°42′40.2″ N) in Minqin County, Gansu Province, China (Fig. 1). The station is located at the boundary of Tengger Desert, where average annual evaporation is 2664 mm and precipitation is 110 mm. The average annual temperature is 7.8 °C, and winter temperature minima (during December to next January) can fall to −27.3 °C whereas summer maxima (during July to August) rise to 41.1 °C. The test soil is classified as sandy loam, with bulk density of 1.56 g cm^{-3} in the 0–20 cm depth (Table 1).

A cotton field was used to conduct the experiment during April 25th to November 7th, 2014. Five irrigation water salinity levels with electrical conductivity (EC_w) of 1.09 (Low), 2.40 (Mild), 3.64 (Medium), 4.88 (High) and 6.12 (Severe) dS m^{-1} were involved. Each treatment had three replicates. Totally 15 plots were used with each size of 15 m long and 3.4 m wide. A randomized block design was used for the plots distribution. Different salinity was obtained by mixing water from two groundwater wells in specified proportions. One well was located at the experimental station with EC_w of 1.09 dS m^{-1} and the other was in Huanghui Village (103°36′11.9″ E, 39°02′56.4″ N) in Minqin County with EC_w of 15.92 dS m^{-1}. A large tank was used to store mixed water and the EC_w of desired salinity was calibrated using a conductivity meter.

A drip irrigation system mulched with plastic film was used to deliver irrigation water. Water was supplied by a pump and the amount was controlled by a water meter. Each plot had the same irrigation amount, totally 210 mm (7 applications of 30 mm each) during cotton growth period. The irrigation interval was determined based on soil moisture and cotton growth requirements. Totally 200 kg ha^{-1} N along with 200 kg ha^{-1} P and 100 kg ha^{-1} K were inputted to each plot as a base fertilizer. On July 26th, another 150 kg ha^{-1} N was injected into the irrigation water and then transported to each plot. All plots had same mepiquat chloride use and other necessary agronomic operations.

2.2. Soil sampling

Soil samples were collected on September 26th, during the most important growth phase of cotton (Boll Opening Phase). Four non-rhizosphere soil samples were collected from each plot to a depth of 20 cm using a standard soil corer (5 cm diameter) and then completely mixed as one fresh soil sample. After sieving out plant roots and stones, 1 kg of soil sample was obtained and put into a sterile bag. All soil samples were packed in ice blocks and transported to laboratory within 24 h. In the laboratory, each soil sample was divided into two parts: one was refrigerated at 4 °C and others frozen at −80 °C for microbial analysis.

2.3. Microbial functional diversity analysis

Soil microbial metabolic activity was measured using Biolog Ecoplates™. The plates have 96 wells and each plate consisting of three replicates (comprising 31 sole carbon sources and one water blank). In this study, 5 g of each soil sample was suspended in 45 ml of sterile saline solution (0.85% NaCl) and shaken 30 min on an orbital shaker. Then 1 ml of soil suspension was transferred into a microcentrifuge tube and centrifuged at 10,000 rpm for 20 min. The supernatant was removed. The pellets were washed twice to remove water soluble carbon using the sterile saline solution and resuspended in 20 ml of the same solution. A 150 μl sample of the suspension was inoculated into each well. The plates were incubated at 25 °C. Color development in each well was recorded as optical density at 590 nm (color development + turbidity) and 750 nm (turbidity only) at 24 h intervals for 168 h (Classen et al., 2003).

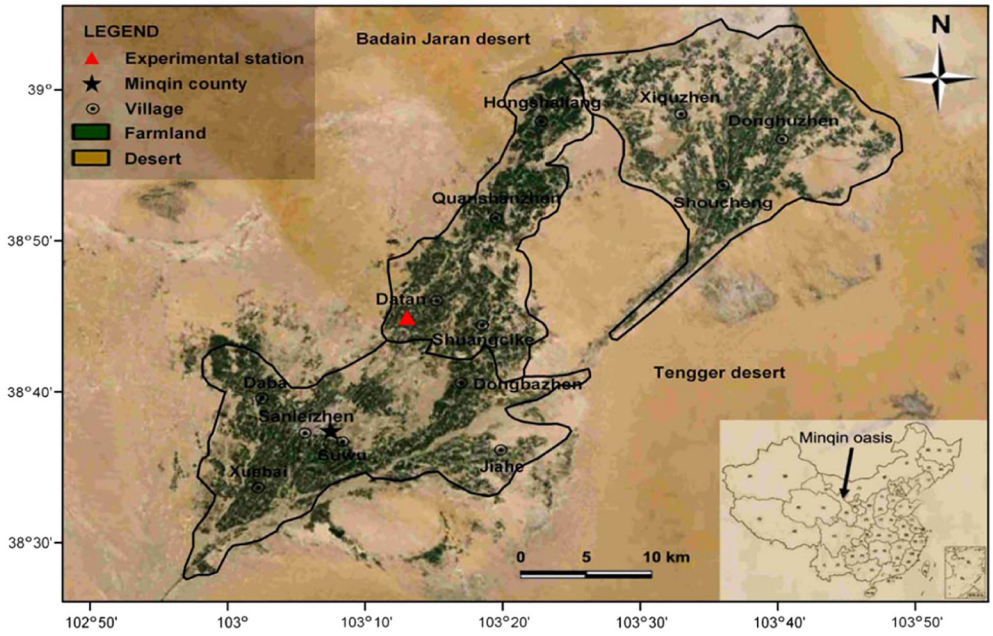

Fig. 1. Location of the study site.

The well absorbance values were adjusted by subtracting the absorbance of the control well. The final values in each well were the 590 nm values minus the 750 nm values. Negative readings were set to zero (Classen et al., 2003). Microbial activity in each microplate, expressed as AWCD value was determined according to Garland and Mills (1991). The 96 h optical density value (chosen according to exponential phase of growth curves of all plates) for each sample in triplicate, divided by their AWCD to normalize the values, was used to calculate the utilization of carbon sources (Zak et al., 1994).

2.4. Genetic analysis of bacterial community

The DNA was extracted from 0.25 g of soil sample using a Power Soil DNA Isolation Kit (MoBio Laboratories, Inc.) according to the manufacturer's instructions. The extracts were assessed for quality and quantity using a spectrophotometer and then checked for integrity by 0.8% agarose gel electrophoresis. The V4 regions of the bacterial 16S rRNA genes were amplified using primers 520F (5-AYT GGG YDT AAA GNG-3) and 802R (5-TAC NVG GGT ATC TAA TCC-3). The amplicons were sequenced with the GS-FLX Titanium system (Majorbio Bio-Pharm Technology Co., Ltd., Shanghai, China). The sequences were quality-filtered using the Quantitative Insights Into Microbial Ecology (QIIME) 1.7.0 (Caporaso et al., 2010), with which the sequences <150 bp and reads containing ambiguous bases or any unresolved nucleotides were removed. Chimeras were checked by the MOTHUR program with the UCHIME algorithm (Schloss et al., 2009; Edgar et al., 2011). The qualified sequences were clustered into operational taxonomic units (OTUs) at 97% identity threshold. Indices of bacterial community richness (Chao 1, ACE) and diversity (Shannon and Simpson) were calculated using MOTHUR (Schloss et al., 2009). The community composition of bacteria and principal coordinates analysis (PCoA) based on weighted UniFrac distances was analyzed using QIIME.

2.5. Data analysis

One way analysis of variance (ANOVA) was performed to test the significant effects of the different irrigation salinity treatments on microbial properties using the PASW Statistics 18.0. Heatmap was generated by R packages.

3. Results

3.1. Microbial metabolic activities

Completely different effect patterns in soil microbial metabolic activities were detected under saline water irrigation. Although significant decreases in AWCD values were found under Mild, Medium, High and Severe irrigation salinity treatments versus Low treatment (Fig. 2A), there was no significant difference between each pair of them. Under Low salinity treatment, soil microbes consumed all six categories of substrates (Fig. 2B). In contrast, five categories, i.e. carbohydrates, amino acids, carboxylic acids, amines and polymers, the absorbance values of which accounted of 18.4, 18.5, 16.9, 18.1 and 23.8% of the total amount, were dominantly consumed by microbes in Mild saline water irrigated soils. Under the other three treatments, in addition, four main categories, carbohydrates (20.9%, average absorbance value of the three treatments), amino acids (21.8%), carboxylic acids (26.0%) and polymers (20.1%) were dominantly utilized.

Although there was no significant difference in carbohydrates utilization among treatments (Fig. 2B), the sole carbon source uptake under different treatments were completely varied (Fig. 3). Compared with Low salinity treatment, significant decreases in mannitol utilization were observed under all the other four treatments ($P < 0.001$). However, we simultaneously detected significant increases in cellobiose utilization under Medium and High treatments, in erythritol utilization under Mild,

Table 1
Physicochemical properties of experimental soil.

Soil depth	Texture	Microture (%)	BD (g cm^{-3})	TOC (%)	TN (%)	P_a (mg kg^{-1})	K_a (g kg^{-1})	pH
0–20 cm	Sandy loam	12.07	1.56	0.63	0.06	23.10	157.92	7.61

BD: bulk density; TOC: total organic carbon; TN: total nitrogen; P_a: available phosphorus; K_a: available potassium.

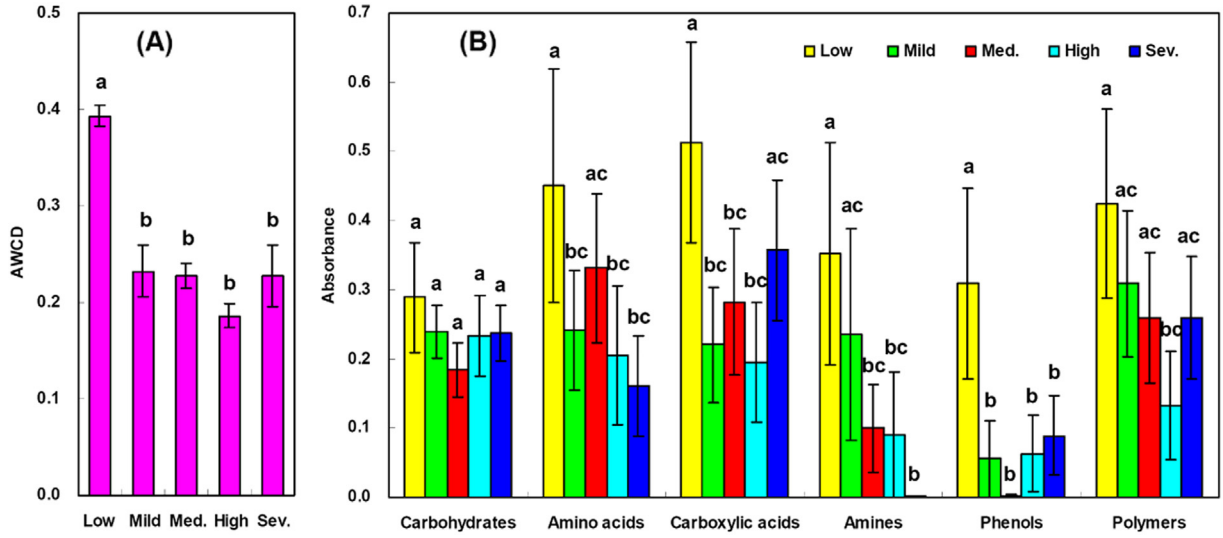

Fig. 2. Effects of saline water irrigation on microbial metabolic activities (A) and six functional categories of carbon substrates (B). Values with different letters indicated significant difference at $P < 0.05$ according to the LSD test.

High and Severe treatments and still in glucose phosphate utilization under Severe treatment ($P < 0.001$). Similarly, as for amino acids utilization, significant decreases were found under Mild, High and Severe treatments ($P < 0.05$, Fig. 2B), which were mainly caused by asparagine utilization reduction ($P < 0.001$, Fig. 3) although a significant increase in serine uptake ($P < 0.001$) was also detected. Still, a significant decrease in galacturonic utilization under Mild treatment ($P < 0.001$, Fig. 3), which mainly accounted for the carboxylic acids utilization reduction ($P = 0.004$, Fig. 2B), was accompanied by a significant increase in itaconic acid uptake ($P < 0.001$, Fig. 3). However, this trend under Medium and High treatments were less remarkable although the reduced galacturonic utilization also resulted in significant decreases in carboxylic acids utilization ($P = 0.016$ and 0.003, respectively). Significant decreases, which were mainly caused by reduced putrescine and 4-benzoic acid utilization (Fig. 3), were discovered in amines utilization under Medium ($P = 0.036$), High ($P = 0.031$) and Severe ($P = 0.007$) treatments and in phenols utilization under all treatments except Low salinity. Particularly, the utilization of polymers was significantly reduced under High salinity treatment ($P = 0.007$). From Fig. 3 we could find that the significant decreases in Tween 40 and Tween 80 uptake were the main reasons.

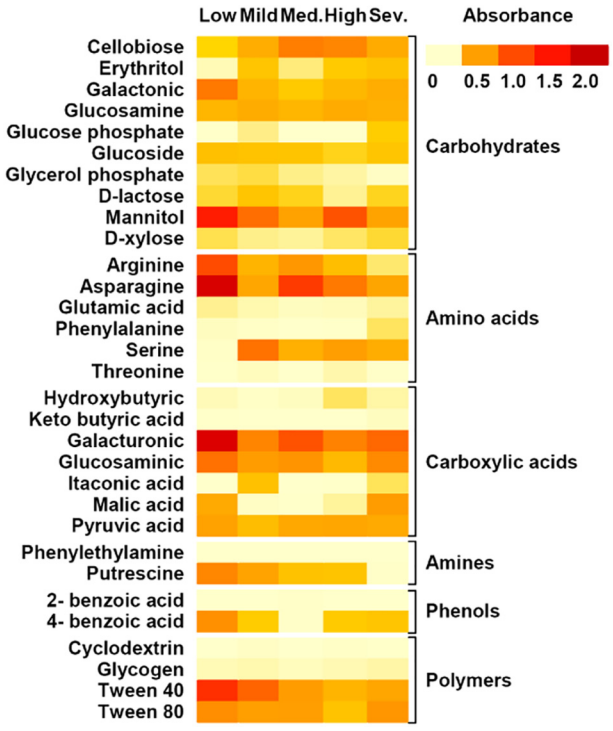

Fig. 3. Heatmap of 31 carbon substrates utilization under five saline water irrigation treatments.

3.2. Composition of the bacterial communities

Saline water irrigation could significantly influence soil bacterial community richness and diversity (Table 2). The values of Chao 1 and ACE indices increased with irrigation salinity except the High treatment, the values of which were significantly lower than that of the Medium treatment ($P < 0.001$). In addition, the value of Shannon index also significantly increased with irrigation salinity ($P < 0.001$), indicating that the higher salinity in irrigation water, the higher diversity of bacterial community structures was detected.

Remarkable changes were discovered for bacterial communities (Fig. 4). Principal coordinates analysis showed that the three samples from the same treatment almost grouped together. A pronounced shift of bacterial communities from Low to Severe treatments was exhibited along PC1. Especially, samples of the Low, Mild and Medium treatments were well separated from samples of the Severe treatment. In addition, a weak association was also demonstrated along PC2, revealing a clear adaptation and selectivity of bacteria to irrigation water salinity.

There was a significant influence of saline water irrigation on the composition of the bacterial communities across the irrigation salinity gradient. Totally 45 phyla were found under Low treatment, while 44, 40, 40, 35 phyla were detected for Mild, Medium, High and Severe

Table 2
Means and standard deviation of bacterial community richness and diversity indices across irrigation water salinity gradient.

Treatment	Chao 1	ACE	Shannon
Low	6821 (258)a	6904 (211)a	6.455 (0.0123)a
Mild	7209 (245)a	7355 (207)a	6.615 (0.0135)b
Med.	12,947 (649)c	17,633 (502)c	6.786 (0.0128)c
High	10,005 (426)b	12,391 (342)b	6.825 (0.0149)d
Sev.	14,899 (769)d	20,754 (578)d	6.931 (0.0128)e

Values with different letters (i.e. a, b, c, d and e) indicated significant difference at $P < 0.05$ according to the LSD test.

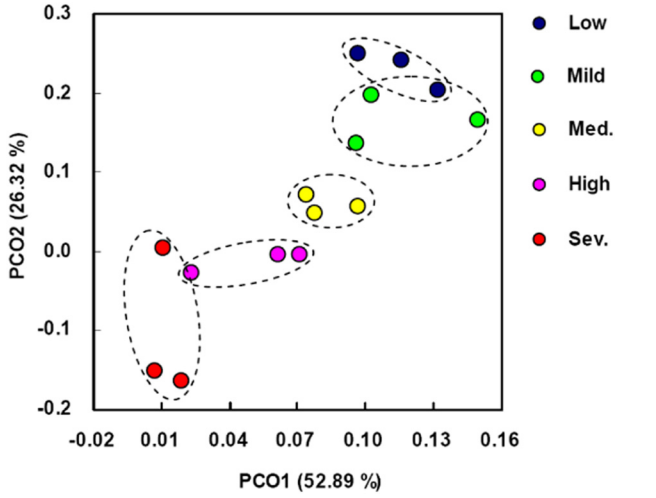

Fig. 4. Principal coordinate analysis (PCoA) based on weighted UniFrac distances of bacterial communities.

treatments, respectively. Phyla of Planctomycetes, Bacteroidetes and Proteobacteria were the dominant groups in all saline water irrigated soils (Fig. 5A), the total relative abundances of which were 61.4, 56.4, 51.4, 43.5 and 44.2% along the irrigation salinity gradient. All these phyla were extremely responsive. The Proteobacteria increased strongly with irrigation salinity (from 11.6 to 24.3% in relative abundance), whereas the Planctomycetes and Bacteroidetes decreased significantly (from 23.7 to 9.8% and 26.1 to 10.1%, respectively; $P < 0.05$). Besides, Actinobacteria and Chloroflexi phyla, which were also abundant across all treatments, followed the Proteobacteria pattern while the other two main phyla (Gemmatimonadetes and Acidobacteria) seemed not very responsive to irrigation salinity. At the class level (Fig. 5B), Phycisphaerae and Cytophagia were the most abundant classes and followed the patterns similar to that of the Planctomycetes and Bacteroidetes while the dominant other responsive classes (Alphaproteobacteria and the other classes of Actinobacteria) followed the Proteobacteria pattern. The other classes of Gemmatimonadetes and Acidobacteria-6 classes followed the similar patterns of Gemmatimonadetes and Actinobacteria and the Planctomycetia and Saprospirae classes followed that of Planctomycetes and Bacteroidetes.

4. Discussion

Decreases in AWCD, which were found under Mild, Medium, High and Severe irrigation salinity treatments versus under Low treatment in this study, had revealed that saline water irrigation could greatly restrain soil microbial metabolic activities. Majority previous studies had also confirmed the negative impacts of salinity increase on soil microbial activities (Ghollarata and Raiesi, 2007; Setia et al., 2010; Wong et al., 2009). Especially, Rietz and Haynes (2003) found a significant negative exponential relationship between irrigation-induced salinity and indices of microbial activity (arginine ammonification and fluorescein diacetate hydrolysis rates) and concluded that increasing salinity resulted in a smaller, more stressed microbial community which was less efficient in using carbon resources. This was also confirmed by Yuan et al. (2007) who analyzed that substrate utilization efficiency was a significant factor influencing microbial activities in salt affected soils. Nevertheless, with increasing irrigation salinity, the conditions were not becoming increasingly detrimental in this study as no significant difference of AWCD values was found among Mild, Medium, High and Severe treatments. A possible reason may be the drip irrigation system used in this study. It has been reported that mulched drip irrigation is able to maintain high soil matric potential in the root zone and avoid soil salt accumulation exceed the tolerance limits of soil microbial communities (Burt and Isbell, 2005). In addition, the type of salt changed (e.g. sodic Na-salts vs. Ca-salts) may also resulted in higher availability of organic substrates at increasing salinity. Formerly, Saviozzi et al. (2011) incubated originally non-saline soil at different concentrations of NaCl and even observed an increase in respiration compared to the control. However, from the analysis of the carbon substrate utilization, we found that although no significant difference exhibited for dominant substrates (carbohydrates, amino acids, carboxylic acids and polymers) among Mild, Medium, High and Severe treatments, the consumption of sole carbon source was significantly changed. Dominantly, asparagine, galacturonic putrescine, 4-benzoic *aci* and Tween 40 accounted for these changes and affected the total metabolic activities of soil microbes. It meant that microbes in higher salinity water irrigated soils may reduce to use one certain carbon source, but they may simultaneously use others, which consequently accounted for remaining the total metabolic activities.

Analysis of the effects of saline water irrigation on soil bacterial richness as measured by both Chao1 and ACE indicated that the values increased with irrigation salinity except the High treatment. However,

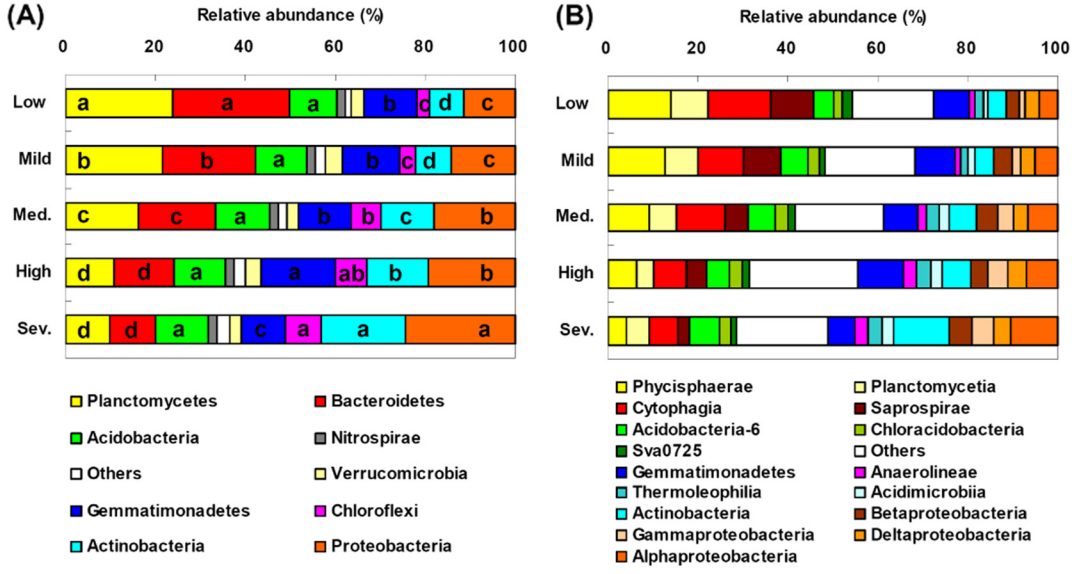

Fig. 5. Relative abundance of soil bacterial phyla (A) and classes (B) under different treatments. Values with different letters indicated significant difference at $P < 0.05$ according to the LSD test.

the number of bacterial phyla decreased with irrigation water salinity. The dominant groups in all saline water irrigated soils were Planctomycetes, Bacteroidetes and Proteobacteria, followed by Actinobacteria, Chloroflexi, Gemmatimonadetes and Acidobacteria. Some of the above bacterial phyla were similarly found in the salt-affected soils (Ma and Gong, 2013), which were reported that 90% of the bacterial sequences were belonged to six phyla (Proteobacteria, Actinobacteria, Firmicutes, Acidobacteria, Bacteroidetes and Chloroflexi). We noticed that the relative abundance of Proteobacteria increased significantly with irrigation salinity. Actually, this phylum was the highest member in bacteria which dominated 24.3% relative abundance in the Severe saline water irrigated soils. It is no surprising as this phylum to be found one of the most common bacterial taxon in saline soils (Valenzuela-Encinas et al., 2009; Canfora et al., 2014). Wu et al. (2006) showed that with increasing salinity, the relative abundance of the Betaproteobacteria decreased, whereas that of the Alphaproteobacteria and the Gammaproteobacteria increased. However, Valenzuela-Encinas et al. (2009) found that the dominant class of Proteobacteria in both high and low saline soils was Gammaproteobacteria, whereas in medium saline soils was Alphaproteobacteria. Different soil characteristics may contribute to these differences. In our study, Alphaproteobacteria was the dominant class in Proteobacteria and dominantly derived the relative abundance increase. Differently, the relative abundances of Planctomycetes and Bacteroidetes reduced significantly with irrigation salinity. Planctomycetes have been recovered from aquatic environments that varied greatly in salinity status (Schlesner, 1994), thus, the declines in their relative abundance under salinity was not easily explained. It may be that other groups (such as Proteobacteria) became more competitive. In contrast, Bacteroidetes was found ether increase (Wang et al., 2016) or decrease (Campbell and Kirchman, 2013; Canfora et al., 2014) along an increasing salinity gradient in previous studies, no certain regulation has found.

Based on the above results, we found that although the soils irrigated with Low salinity water exhibited the highest microbial metabolic activities (Fig. 2A), they showed the lowest bacterial community richness and diversity (Table 2). Likewise, soils under Mild, Medium, High and Severe salinity treatments formed similar trends. In addition, the variation of bacterial community structures was more sensitive than microbial metabolic activities. This is no surprising since even if the abundance of a community varied remarkably, the microbial function may not change so much if the community contained a high degree of functional redundancy (Allison and Martiny, 2008). Smalla et al. (1998) discovered, by comparing the temperature gradient gel electrophoresis (TGGE) profiles of the original inoculum with those in the BIOLOG wells following incubation, that fast-growing bacteria which adapted to high substrate concentrations were numerically dominant in the BIOLOG wells. Therefore, the comparatively high diversities of sequences may more accurately reflect the functional community. In the present study, however, the patterns of substrate utilization cannot be related to species or population compositions since they only indicate functional aspects of the culturable fraction of the community inoculated to the wells, future work would be needed for more accurate assessment based on functional groups analysis.

5. Conclusions

In the present study, we found that soil microbial metabolic activities were greatly restrained in saline water irrigated soils. Although no significant difference in carbon substrate utilization of all six categories was observed among Mild, Medium, High and Severe treatments, the consumption of sole carbon source was significantly different. Four sole carbon resources, i.e. Asparagine, Galacturonic, Putrescine and 4-benzoic acid, played a decisive role in dominating the differences. Soil bacterial richness and diversity increased with irrigation salinity while the number of bacterial phyla decreased. A clear adaptation and selectivity of bacteria to irrigation salinity were detected. Three significantly increased (Proteobacteria, Actinobacteria and Chloroflexi) and two decreased (Planctomycetes, Bacteroidetes), as well as two irresponsive (Gemmatimonadetes and Acidobacteria) phyla were observed as the dominant groups in all saline water irrigated soils.

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