



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



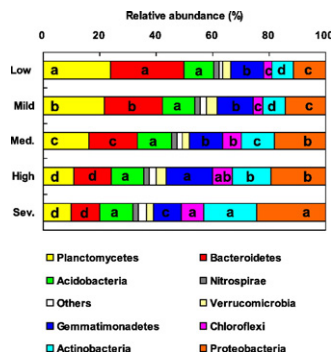
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## HIGHLIGHTS

- 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  $5.0 \text{ dS m}^{-1}$  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 ( $103^{\circ}12'3.4'' \text{ E}$ ,  $38^{\circ}42'40.2'' \text{ N}$ ) 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  $7.8^{\circ}\text{C}$ , and winter temperature minima (during December to next January) can fall to  $-27.3^{\circ}\text{C}$  whereas summer maxima (during July to August) rise to  $41.1^{\circ}\text{C}$ . The test soil is classified as sandy loam, with bulk density of  $1.56 \text{ g cm}^{-3}$  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 ( $\text{EC}_w$ ) of 1.09 (Low), 2.40 (Mild), 3.64 (Medium), 4.88 (High) and 6.12 (Severe)  $\text{dS m}^{-1}$  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  $\text{EC}_w$  of  $1.09 \text{ dS m}^{-1}$  and the other was in Huanghui Village ( $103^{\circ}36'11.9'' \text{ E}$ ,  $39^{\circ}02'56.4'' \text{ N}$ ) in Minqin County with  $\text{EC}_w$  of  $15.92 \text{ dS m}^{-1}$ . A large tank was used to store mixed water and the  $\text{EC}_w$  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  $200 \text{ kg ha}^{-1}$  P along with  $200 \text{ kg ha}^{-1}$  P and  $100 \text{ kg ha}^{-1}$  K were inputted to each plot as a base fertilizer. On July 26th, another  $150 \text{ kg ha}^{-1}$  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  $4^{\circ}\text{C}$  and others frozen at  $-80^{\circ}\text{C}$  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  $150 \mu\text{l}$  sample of the suspension was inoculated into each well. The plates were incubated at  $25^{\circ}\text{C}$ . 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).

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