

# Effects of saline water irrigation and fertilization regimes on soil microbial metabolic activity

Li -Juan Chen<sup>1,2</sup> · Qi Feng<sup>1</sup> · Yong-Ping Wei<sup>2</sup> · Chang-Sheng Li<sup>3</sup> · Yan Zhao<sup>2</sup> · Hui-Ya Li<sup>1</sup> · Bao-Gui Zhang<sup>1</sup>

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## Abstract

**Purpose** Irrigation and fertilization can change soil environment, which thereby influence soil microbial metabolic activity (MMA). How to alleviate the adverse effects by taking judicious saline water irrigation and fertilization regimes is mainly concerned in this research.

**Materials and methods** Here, we conducted a field orthogonal designed test under different saline water irrigation amount, water salinity, and nitrogen fertilizer application. The metabolic profiles of soil microbial communities were analyzed by using the Biolog method.

**Results and discussion** The results demonstrated that irrigation amount and fertilizer application could significantly change MMA while irrigation water salinity had no significant effect on it. Medium irrigation amount (30 mm), least (50 kg ha<sup>-1</sup>) or medium (350 kg ha<sup>-1</sup>) N fertilizer application, and whatever irrigation water salinity could obtain the optimal MMA. Different utilization rates of *carbohydrates*, *amino acids*, *carboxylic acids*, and *polymers* by soil microbial

communities caused the differences of the effects, and *D-galactonic acid  $\gamma$ -lactone*, *L-arginine*, *L-asparagine*, *D-glucosaminic acid*, *Tween 80*, *L-threonine*, and *D-galacturonic acid* were the indicator for distinguishing the effects.

**Conclusions** The results presented here demonstrated that by regulating irrigation water amount and fertilizer application, the effects of irrigation salinity on MMA could be alleviated, which offered an efficient approach for guiding saline water irrigation.

**Keywords** Biolog · Fertilization · Saline water irrigation · Soil microbial activity

## 1 Introduction

Agriculture accounts for about 70 % of the world's water use, over 90 % applied for irrigation (Bourzac 2013). As freshwater shrink due to excess use, contamination, or climate change impacts (Stone 2012) is currently becoming a big challenge worldwide, saline water, which is in plentiful supply in the world (Mantell et al. 1985), will play an increasingly important role on agricultural irrigation (Pereira et al. 2002). However, farming soils are highly susceptible to saline water irrigation. Soil water, pH, organic carbon, and other physico-chemical characteristics would be influenced by potential soil salinity increase (Wong et al. 2010; Mavi et al. 2012). Microbial communities, which directly participate in some important ecological processes in soil, such as decomposition of organic matter, formation of soil aggregation, and nutrient cycling, would thereby be affected due to these soil environment changes (Strickland and Rousk 2010; Amini et al. 2016). It has been reported that soil salinity detrimentally influences microbiological processes due to the restricted availability of

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✉ Li -Juan Chen  
ljchen@lzb.ac.cn

<sup>1</sup> Key Laboratory of Ecohydrology of Inland River Basin, Northwest Institute of Eco-Environment and Resources, Chinese Academy of Sciences, Lanzhou 730000, China

<sup>2</sup> School of Geography, Planning and Environmental Management, The University of Queensland, Brisbane 4072, Australia

<sup>3</sup> Plant Protection and Quarantine Station of Gansu Province, Lanzhou 730020, China

water or the influence on cellular physiology and metabolic processes (Sardinha et al. 2003; Oren 2008). It also has been shown that soil salinity over a critical level has a strong negative impact on microbial respiration (Setia et al. 2011). As soil microbial activities have a direct influence on the stability and function of soil ecosystems (Manzoni et al. 2012), how to alleviate these adverse effects by taking judicious irrigation programs and sustainable management measures, therefore, appears extremely vital for saline water utilization.

Besides irrigation, fertilization is another important agricultural practice. Previous studies have shown that long-term N application reduces the microbial functional diversity (Lovell et al. 1995; Sarathchandra et al. 2001), but some recent studies have suggested that a certain amount of N had no effect on soil microbial biomass and functional diversity (Ogilvie et al. 2008; Lupwayi et al. 2012). However, little research concerned the coupling effects of saline water irrigation and fertilization on soil microbial metabolic activities under field conditions. By regulating saline water irrigation and fertilization regimes, can adverse effects be alleviated? If so, it will be much more useful for guiding saline water irrigation.

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 (>83 % of papers by using Biolog method published prior to June 2001; 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, this method is based on microbial growth, through which the active microbes are mainly concerned. 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). Currently, it is more commonly used in a variety of different aspects (Lyons et al. 2010; Sala et al. 2010; Gilbert et al. 2012; Mouchet et al. 2012).

With this review, we conducted a field test to investigate the effects of different irrigation water amount, salinity, and N fertilizer application on soil microbial metabolic activity (MMA) by using Biolog method. We explored under which irrigation and fertilization combination that the metabolic activity of soil microbial communities would be optimal. Furthermore, we also analyzed the sensitive biochemical categories of substrates and its sole carbon source for distinguishing carbon utilization by soil microbial communities under different saline water irrigation and fertilization regimes. We hope that this research can contribute to understand how saline water irrigation and fertilization regimes influence soil microbial process and, as a result, be useful to manage saline water irrigation.

## 2 Materials and methods

### 2.1 Field test

The field test 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. The station is located at the boundary of Tengger Desert, where average annual evaporation is 2664 mm and annual precipitation is 110 mm. The average annual temperature is 7.8 °C, and winter temperature minima can fall to -27.3 °C, whereas summer maxima rise to 41.1 °C. The test soil is classified as sandy loam, with bulk density of 1.56 g cm<sup>-3</sup> in the 0–20-cm depth.

The test was conducted in 2014 with cotton (*Gossypium hirsutum* Xinluzao 7) planted on April 25 and harvested on November 7. Three irrigation and fertilization factors, which chiefly needed to be controlled under saline water irrigation, were considered: irrigation water amount (with three levels of 25, 30, and 35 mm for each irrigation application and six applications during the cotton growth period), irrigation water salinity (with three levels of 1.09, 4.75, and 8.41 dS m<sup>-1</sup>), and N fertilizer rate (with three levels of 50, 350, and 650 kg ha<sup>-1</sup>). An orthogonal design was used (Table 1), which could scatter the test samples uniformly over the feasible space with less computational cost and allow the statistical testing to be conducted over only a few combinations of factors rather than all the possible combinations (Yang and Ma 2016). Each treatment had three replicates. Totally, 27 plots were used with each size of 5 m long and 3.4 m wide. A randomized block design was used for the plot distribution.

A drip irrigation system mulched with plastic film was used to deliver irrigation water. Different salinity irrigation water was obtained by mixing water from two groundwater wells in specified proportions. One well was located at the experimental station (freshwater (FW), EC = 1.09 dS m<sup>-1</sup>), and the other was in Huanghui Village (103° 36' 11.9" E, 39° 02' 56.4" N) in Minqin County (saline water (SW), EC = 15.92 dS m<sup>-1</sup>). The ion concentrations of the groundwater are presented in Table 2. According to local irrigation practice, the source water of the first irrigation event was surface water which was supplied from Hongyashan Reservoir by channels (EC = 0.52 dS m<sup>-1</sup>, see Table 2). The irrigation water was supplied by a pump, with the exact amounts of water supplied monitored by water meters. Further details of the irrigation system can be found in Chen et al. (2014).

Fertilization was carried out in two periods. Before cotton sowing (on April 20), one part of N fertilizer (50, 200, and 350 kg ha<sup>-1</sup>) was inputted to given plots (according to the test

**Table 1** Orthogonal test design of different saline water irrigation and fertilization regimes

Treatment	Irrigation amount (mm)	Irrigation salinity (dS m <sup>-1</sup> )	N fertilizer rate (kg ha <sup>-1</sup> )
LLL	25	1.09	50
LMM	25	4.75	350
LHH	25	8.41	650
MLM	30	1.09	350
MMH	30	4.75	650
MHL	30	8.41	50
HLH	35	1.09	650
HML	35	4.75	50
HHM	35	8.41	350

Three factors were concerned. Each factor had three levels: irrigation amount (levels indicate 25, 30, and 35 mm for each irrigation application), irrigation salinity (levels indicate 1.09, 4.75, and 8.41 dS m<sup>-1</sup>), and N fertilizer rate (levels indicate 50, 350, and 650 kg ha<sup>-1</sup>). The “L” in treatment name means the least level of a certain factor, while “M” and “H” indicate the medium and highest levels, respectively

design) as a base fertilizer, along with 200 kg P ha<sup>-1</sup> and 100 kg K ha<sup>-1</sup> to each plot. At flowering phase of cotton (on July 26), another part of N fertilizer (0, 150, and 300 kg ha<sup>-1</sup>) was injected into the irrigation water through fertilizer tank and then was transported to above given plots. According to local practices and recommendations, the dates of irrigation was April 26, June 21, July 11, July 27, August 10, and August 23, while about 50 g ha<sup>-1</sup> mepiquat chloride was used on July 10 and August 20.

## 2.2 Soil sampling

Soil samples were collected during the most important phase of cotton (boll opening phase) on September 26, 2014. Four non-rhizosphere soil samples were collected from each plot to a depth of 20 cm by using a standard soil corer (5-cm diameter) and then completely mixed into one composite fresh soil sample. After sieving out plant roots and stones, 1 kg of soil sample was obtained and put into sterile bags. All soil samples were packed in ice blocks and transported to laboratory within 24 h for further measurements. In the laboratory,

**Table 2** Chemical composition of source water used in the experiment

Water	HCO <sub>3</sub> <sup>-</sup> (mg L <sup>-1</sup> )	Cl <sup>-</sup> (mg L <sup>-1</sup> )	SO <sub>4</sub> <sup>2-</sup> (mg L <sup>-1</sup> )	Ca <sup>2+</sup> (mg L <sup>-1</sup> )	Mg <sup>2+</sup> (mg L <sup>-1</sup> )	Na <sup>+</sup> (mg L <sup>-1</sup> )	K <sup>+</sup> (mg L <sup>-1</sup> )	TDS (mg L <sup>-1</sup> )	EC (dS m <sup>-1</sup> )
FW	267	93	307	97	40	109	7.0	921	1.09
SW	689	2906	6334	438	1043	2655	33.6	14,099	15.92
SuW	150	29	126	41	13	64	2.0	425	0.52

FW is the freshwater from the well located at the experimental station; SW is the saline water from the well located at Huanghui Village; SuW is the surface water from Hongyashan Reservoir; TDS is the total dissolved solid; EC is the electrical conductivity

the soil samples were immediately refrigerated at 4 °C for microbial analysis.

## 2.3 Microbial analysis

Soil microbial metabolic activity was measured by using Biolog Ecoplates<sup>TM</sup>. The plates have 96 wells, and each plate consists 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 by 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 595 and 750 nm at 24-h intervals for 168 h.

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 average well color development (AWCD), 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 Statistical analyses

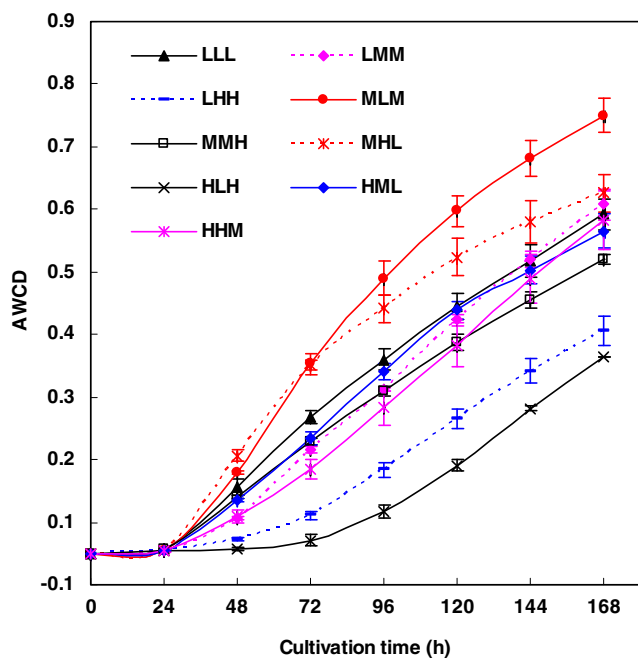
The results of different treatments were evaluated by using variance analysis (ANOVA) and pairwise multiple comparisons. Principal coordinate analysis (PCoA) based on Bray-Curtis distances was analyzed by using Canoco 5.0. All statistical analyses were conducted by using SPSS18.0 software.

### 3 Results

#### 3.1 Effects on microbial metabolic activity

The changes of the AWCD under different treatments are shown in Fig. 1. In the first 48 h, the changes in AWCD of all treatments were insignificant, but after this lag phase, the values were increased significantly, demonstrating that all microbial communities obtained the capacity to metabolize the organic substrates in Biolog Ecoplates. The increase rates, however, under different treatments were different. For example, while the AWCD of MHL increased gently after 72 h, the value for HHM increased faster and faster across the cultivation time. Among all nine treatments, the accumulated AWCD value of MLM was the highest. However, it is not the best choice of saline groundwater irrigation and fertilization design according to the characteristic of orthogonal test.

From the ANOVA results (Table 3), we could see that irrigation amount and N fertilizer rate could significantly change MMA. The AWCD for medium irrigation amount (30 mm) was significantly higher than the least (25 mm) and the most (35 mm) irrigation amount (Table 4,  $P < 0.001$ ). However, there was no significant difference between 25 and 35 mm irrigation water amount at the 0.01 level ( $P = 0.038$ ). The AWCD under the highest N fertilizer rate (650 kg ha<sup>-1</sup>) was significantly lower than 50 and 350 kg ha<sup>-1</sup> N fertilization ( $P < 0.001$ ). However, there was also no significant difference between 50 and 350 kg ha<sup>-1</sup> N fertilization ( $P = 0.279$ ). From Table 3, we can see that there was no significant effect of irrigation water salinity on MMA. The mean values showed



**Fig. 1** Changes of average well color development (AWCD) under different treatments across the incubation time

that AWCD decreased with increasing irrigation water salinity, but no significant difference was found (Table 4). The optimal irrigation and fertilization combination which had the highest soil microbial metabolic activity was medium irrigation amount (30 mm), 50 or 350 kg ha<sup>-1</sup> N, and whatever irrigation water salinity.

#### 3.2 Effects on different biochemical categories of substrates

In this research, the 31 substrates in the Biolog Ecoplates were assigned into six categories. The average absorbance of each category at 96 h is presented in Fig. 2. Different treatments showed diverse performances on different categories of substrates. For example, two categories of substrates (*amines and amino acids*) were hardly utilized by soil microbial communities under HLH treatment, while microbes under LHH and HHM treatments disliked utilizing *phenols*, revealing distinctly different microbial community structures under these treatments (Fig. 2). The ANOVA results showed that irrigation water amount and N fertilizer rate had significant effects on utilization of *carbohydrates, amino acids, carboxylic acids, and polymers* (Table 3). The highest utilization rates of above categories by soil microbes all occurred under 30 mm irrigation amount and 50/350 kg ha<sup>-1</sup> N fertilization (Table 4). Furthermore, irrigation water salinity had significant effect on *carboxylic acid* uptake ( $P = 0.027$ ). The highest utilization rate was found under 4.75 or 1.09 dS m<sup>-1</sup> irrigation salinity. However, irrigation water salinity had no significant effects on utilization rates of *carbohydrates, amino acids, and polymers*. In addition, irrigation amount and water salinity had no significant effects on utilization rates of *amines and phenols* either.

#### 3.3 Effects on sole carbon source utilization

In order to reveal the different effects on sole carbon source utilization, PCoA analysis was used. The first and second axes explained 56.5 % of the total variation (Fig. 3a). Soil samples under LHH, HLH, and HHM treatments were clearly separated along the two axes of the PCoA, presenting the obviously different utilization patterns on 31 carbon sources from others (Fig. 3). From Fig. 3b, we could see that the main drivers for PC1 were one *carbohydrate* (D-galactonic acid  $\gamma$ -lactone, -0.745), two *amino acids* (L-arginine, -0.605; L-asparagine, -0.730), one *carboxylic acid* (D-glucosaminic acid, -0.655), and one *polymer* (Tween 80, -0.696). The PC2 axis was mainly determined by one amino acid (L-threonine, -0.616) and one *carboxylic acid* (D-galacturonic acid, 0.566). It means that the soil microbial metabolic activity under different saline water irrigation amount, salinity, and N fertilizer rate was mainly related to the consumption of D-galactonic acid  $\gamma$ -lactone, L-arginine, L-asparagine, D-glucosaminic acid,

**Table 3** Analysis of variance (ANOVA) of the average well color development (AWCD) and six biochemical categories of substrates

Factor	AWCD		Carbohydrates		Amino acids		Carboxylic acids		Amines		Phenols		Polymers	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Irrigation water amount	50.828	<0.001**	9.455	0.001**	11.868	<0.001**	16.788	<0.001**	0.954	0.402	3.431	0.052	4.128	0.032*
Irrigation water salinity	0.839	0.447	2.025	0.158	0.555	0.583	4.341	0.027*	0.379	0.689	0.279	0.759	0.471	0.631
N fertilizer rate	63.051	<0.001**	9.781	0.001**	17.995	<0.001**	14.673	<0.001**	3.525	0.049*	3.592	0.046*	7.157	0.005**

*F* is the *F* ratio; *P* is significance level

\*Significant at the 0.05 level; \*\*significant at the 0.01 level

Tween 80, L-threonine, and D-galacturonic acid substrates. These carbon sources can be used to distinguish utilization rate of categories by soil microbial communities under saline water irrigation and fertilization regimes.

#### 4 Discussion

Saline water irrigation can influence soil microbial metabolic activity by changing soil environment. Previous studies have shown that soil microbial biomass, respiration, and enzymatic activity were all highly susceptible to saline water irrigation (Chander et al. 1994; Chowdhury et al. 2011; Ke et al. 2013). We found that medium irrigation amount (30 mm) obtained the highest AWCD, while the least irrigation amount (25 mm) had no significant difference with the most (35 mm). Li et al. (2014) reported similar findings and explained that mild drought could improve soil aerating conditions, which thereby increased general microbial metabolic activity. The mechanism of soil microbial tolerance to drought appeared involving the microbial physiology (Williams and Xia 2009; Kakumanu et al. 2013). Therefore, we inferred that the result may occur

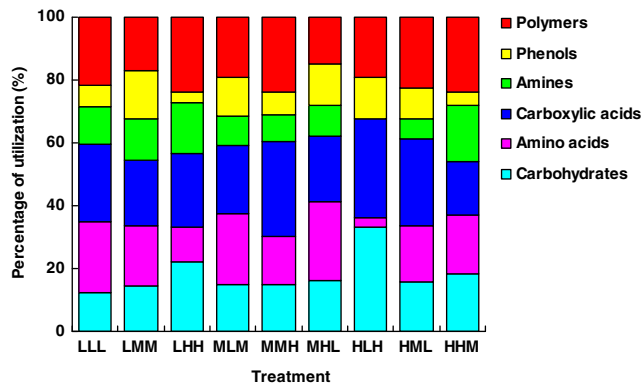
due to the structural and physiological adjustment of soil microbial communities.

Fertilization has been shown to increase (Tyree et al. 2006) or decrease (Maier and Kress 2000) or have no effect on soil microbial activity (Pangle and Seiler 2002). We found that the most fertilizer N (650 kg ha<sup>-1</sup>) had a significant negative impact on MMA. However, there was no significant difference between the least (50 kg ha<sup>-1</sup>) and medium (350 kg ha<sup>-1</sup>) N fertilization. Min et al. (2014) found that medium N fertilization could stimulate microbial breeding. Schimel and Weintraub (2003) proposed that adding N may alleviate N limitation, which allows microbes to divert carbon from overflow metabolism to microbial cell biomass. Nitrogen fertilization may influence MMA through a variety of mechanisms including changes in microbial biomass, microbial diversity, mycorrhizal infection, aboveground and belowground substrate quantity and quality, labile carbon inputs, and production of extracellular enzymes by soil microbes (Phillips et al. 2015; Toshiko et al. 2016). However, different soil environment, original nitrogen content, types of plant species, and other factors could all change the results (Rath and Rousk 2015).

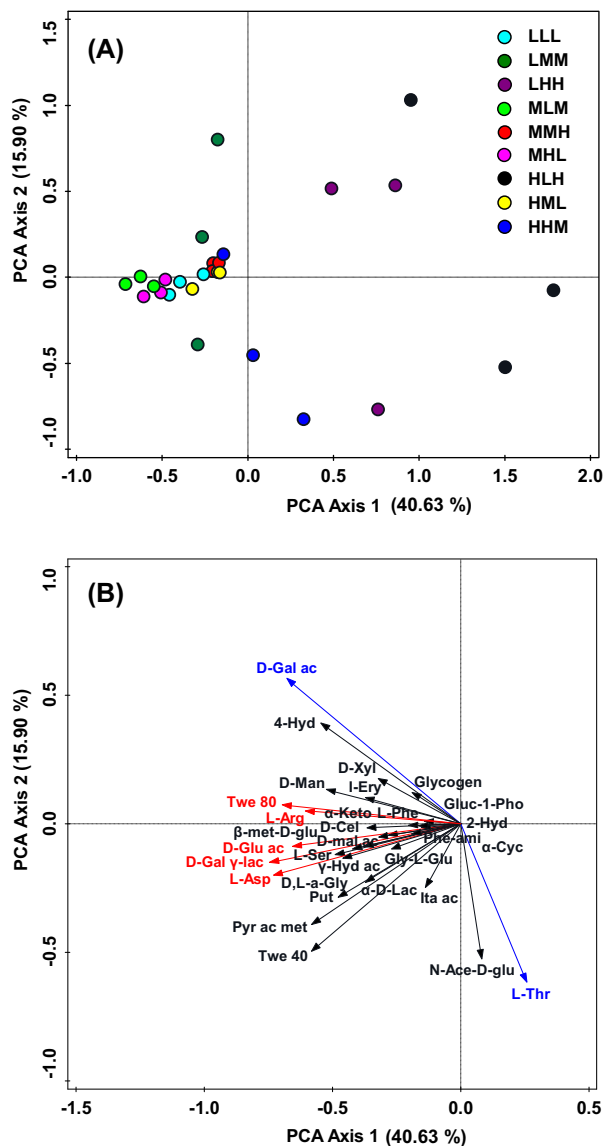
**Table 4** Pairwise multiple comparisons indicate the effects of the three levels of each factor on the average well color development (AWCD) and six biochemical categories of substrates

Factor	Level	AWCD	Carbohydrates	Amino acids	Carboxylic acids	Amines	Phenols	Polymers
Irrigation water amount	25	0.235bB	0.157bB	0.253bB	0.306bB	0.174aA	0.126abA	0.268bA
	30	0.364aA	0.255aA	0.435aA	0.466aA	0.182aA	0.231aA	0.375aA
	35	0.197cB	0.177bB	0.175bB	0.249bB	0.111aA	0.083bA	0.239bA
Irrigation water salinity	1.09	0.272aA	0.224aA	0.317aA	0.351abAB	0.144aA	0.155aA	0.303aA
	4.75	0.271aA	0.179aA	0.26aA	0.391aA	0.139aA	0.163aA	0.313aA
	8.41	0.252aA	0.185aA	0.286aA	0.278bB	0.184aA	0.122aA	0.267aA
N fertilizer rate	50	0.331aA	0.218aA	0.403aA	0.437aA	0.170abA	0.186aA	0.349aA
	350	0.312aA	0.234aA	0.361aA	0.356bA	0.222aA	0.197aA	0.349aA
	650	0.153bB	0.136bB	0.100bB	0.228cB	0.075bA	0.057bA	0.185bB

Mean value with the same letter means no significant difference between levels, and different letter means significant difference. Small letter is significant at the 0.05 level and capital letter is at the 0.01 level



**Fig. 2** Utilization percentages of six biochemical categories of carbon substrates under different treatments



**Fig. 3** Principal coordinate analysis (PCoA) of sole carbon source utilization based on Bray-Curtis distances. The resulting plots of 27 samples (a) and 31 sole carbon sources (b) are shown. Abbreviations of carbon sources are listed in Table S1 (Electronic Supplementary Material)

We expected by regulating irrigation water amount and N fertilizer rate that the effects of irrigation water salinity on MMA would be alleviated. The result was consistent with our expectation. Compared with irrigation water amount and N fertilization, irrigation salinity had no significant effect on MMA. Many previous studies concerned soil salt effects on the soil microbial physiology (Kakumanu and Williams 2014), community structure (Campbell and Kirchman 2013), biomass (Egamberdieva et al. 2010), respiration (Chowdhury et al. 2011), and C use efficiencies (Malik and Gleixner 2013). However, there is a shortage of assessments of soil microbial metabolic activity responses to saline water irrigation. Generally, the amount of salts introduced in soil during saline water irrigation is completely decided by irrigation amount. Salt transport processes in soil are also controlled by soil water flow (Ramos et al. 2011). It has been reported that mulched drip irrigation allows application of water at a low rate and high frequency over extended periods of time; the soil salt introduced during the early stages of saline water irrigation can be leached effectively by subsequent applications (Kang 1998). Furthermore, this irrigation system is able to maintain high soil matric potential in the root zone, which, therefore, avoiding soil salt accumulation exceeds the tolerance limits of soil microbial communities (Burt and Isbell 2005). Therefore, drip irrigation might alleviate the effects of irrigation water salinity on MMA.

The average absorbance of the Biolog Ecoplates could be used to compare the metabolic activities of soil microbial communities on all the substrates preliminarily but could not present the specific metabolic property on each biochemical category of substrates (Zhang et al. 2014). Some soil microbes may prefer to utilize the carbohydrates, and others may tend to metabolize carboxylic acids. In this research, we found that different utilization rate of carbohydrates, amino acids, carboxylic acids, and polymers by soil microbial communities decisively influenced the results of the effects, demonstrating that these four categories can be regarded as sensitive carbon sources for distinguishing carbon utilization of soil microbial communities under different saline groundwater irrigation and fertilization regimes. Meanwhile, the significant correlations between above categories and experimental factors were highly consistent with the AWCD and the factors. From specific metabolic activity on each sole carbon source, we explained the effects further. We concluded that D-galactonic acid  $\gamma$ -lactone, L-arginine, L-asparagine, D-glucosaminic acid, Tween 80, L-threonine, and D-galacturonic acid were the indicated sole carbon sources for distinguishing utilization rate of categories by soil microbial communities. However, these findings could not provide enough proofs to reveal the mechanism of the effects. After all, the Biolog technique could only analyze the activities of the cultivable microbial community. Therefore, molecular method use may be urgent in the future, which will enable us to obtain more clear evidence.

In this study, we used the orthogonal test design to obtain the optimal saline water irrigation and fertilization combination for the highest soil microbial metabolic activity and analyze the effects of all factors. This type of design is particularly advantageous in rapid characterization of complicated processes in fewer experiments and therefore has been widely used in industrial and agricultural production (e.g., Dingjian et al. 2011; Ranil et al. 2015; Pengguo et al. 2016) to find the optimal levels of various factors and develop the best processing combination. However, as a restriction of this design, we only analyzed the main effects of the factors. In some cases, interaction effects may also dominate the main effects. For example, irrigation water amount and irrigation water salinity may produce an interactive effect on the available water and jointly may affect the metabolic microbial activity. Under this circumstance, although effective information of the effects of saline water irrigation and fertilization regimes on soil microbial metabolic activity are obtained from this study, further work is still needed to verify that the effects are indeed true.

## 5 Conclusions

Different irrigation amount and nitrogen fertilizer application could significantly change MMA. Based on systematic analysis, we found that the major influencing factors on MMA in this study were irrigation water amount and N fertilizer application, while irrigation salinity had no significant effect. Medium irrigation amount (30 mm) obtained the highest AWCD, while no significant difference was found between the least (25 mm) and most (35 mm) irrigation amount. Although the most N fertilizer (650 kg ha<sup>-1</sup>) had a significant negative impact on MMA, there was no significant difference between 50 and 350 kg ha<sup>-1</sup> N fertilization either. The optimal combination which had the highest MMA was 30 mm irrigation amount, 50 or 350 kg ha<sup>-1</sup> N fertilization, and whatever irrigation water salinity.

Different utilization rate of carbohydrates, amino acids, carboxylic acids, and polymers by soil microbial communities decisively influenced the carbon utilization under different saline groundwater irrigation and fertilization regimes. D-galactonic acid  $\gamma$ -lactone, L-arginine, L-asparagine, D-glucosaminic acid, Tween 80, L-threonine, and D-galacturonic acid were the indicated sole carbon sources for distinguishing utilization rate of categories by soil microbial communities. Further work will be conducted to provide enough proofs to reveal the mechanism of the effects.

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