

Spatial Variations of Soil Microbial Activities in Saline Groundwater-Irrigated Soil Ecosystem

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Abstract Spatial variations of soil microbial activities and its relationship with environmental factors are very important for estimating regional soil ecosystem function. Based on field samplings in a typical saline groundwaterirrigated region, spatial variations of soil microbial metabolic activities were investigated. Combined with groundwater quality analysis, the relationship between microbial activities and water salinity was also studied. The results demonstrated that moderate spatial heterogeneity of soil microbial activities presented under the total dissolved solids (TDS) of groundwater ranging from 0.23 to 12.24 g L^{-1} . Groundwater salinity and microbial activities had almost opposite distribution characteristics: slight saline water was mainly distributed in west Baqu and south Quanshan, while severe saline and briny water were dominant in east Bagu and west Hugu; however, total AWCD was higher in the east-center and southwest of Baqu and east Hugu, while it was lower in east Bagu and northwest Huqu. The results of correlation analyses demonstrated that high-salinity groundwater irrigation had significantly adverse effects on soil microbial activities. Major ions Ca²⁺, Mg²⁺, Cl⁻, and SO₄²⁻ in groundwater decisively influenced the results. Three carbon sources, carbohydrates, amines, and phenols, which had minor utilization rates in all irrigation districts, were extremely significantly affected by high-salinity groundwater irrigation. The results presented here offer an approach for diagnosing regional soil ecosystem function changes under saline water irrigation.

Keywords Spatial variations · Soil microbial activities · Saline water irrigation · Desert oasis

Introduction

Sizable arable land loss because of salinization or nutritional degradation (Mc Clung 2014) and huge fresh water shrink due to excess use, contamination or climate change impacts (Stone 2012) are currently emerging as a big challenge to agriculture development. As the world needs about 60 % more food to feed the 9 billion people in 2050 (Mc Clung 2014), sustainable management and judicious use of land and water resources appear extremely vital (Bos et al. 2005). This is particularly important in arid and semiarid regions where commonly scarcity of fresh water forces farmers to use saline water to cultivate thirsty crops (Fedoroff et al. 2010). Without proper planning and management, salt accumulation in soil profiles due to intensifying saline water irrigation typically leads to salinization, which has been the most serious threat to soil ecosystem services and functions (Ladeiro 2012).

Soil microbes are one of the most complex components of soil ecosystem (Young and Crawford 2004). They are not only the principal participants and drivers of biogeochemical cycling of elements (Song et al. 2013) but also the sensitive indicators of soil environmental change (Falkowski et al. 2008). Soil microbial activities have a direct influence on the stability and fertility of soil

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ecosystems (Hu et al. 2011). Therefore, the variations of soil microbial activities play a crucial role in diagnosing the health of soil ecosystem functions.

The topics of the effects of saline water irrigation on soil physicochemical characteristics, such as flocculation or dispersion of soil particles and imbalance of dissolved ions in soil solution have been a research priority for decades (US Salinity Laboratory Staff 1954; Shainberg and Letey 1984; Six et al. 2000; Mikutta et al. 2007; Setia et al. 2013). Various studies have also been conducted to find appropriate irrigation programs and management measures according to these researches (Wang et al. 2014). However, our understanding of the variations of soil microbial activities in saline water-irrigated regions and how they are affected by chemical compositions of irrigation water has much room for improvement (Rath and Rousk 2015). If strong reductions of soil microbial activities appear in higher saline water-irrigated regions, soil structure and function could be destructed in the long term. But if microbes manage to adapt to, by up-regulating metabolic activities, the destruction could be slowed down. Meanwhile, if the threshold of the sensitivity could be found, it would be a potentially useful supplement for saline water irrigation management.

With this review, we conducted field samplings in a typical saline groundwater-irrigated oasis, investigated the spatial variations of chemical compositions of groundwater and soil microbial metabolic activities, and finally, analyzed their relationships. The main objective of this study is to find out the effects of saline groundwater irrigation on spatial variations of soil microbial metabolic activities. We hope this research can contribute to generate the progress in the understanding of how saline water irrigation changes regional soil structure and function by influencing soil microbial process and, as a result, be useful to manage soil and saline water resources in arid and semiarid regions.

Materials and Methods

Study area Description and Sample Collection

In order to undertake the present study, Minqin oasis, which is located between the Badain Jaran desert and Tengger desert in northwest China was chosen as study area (Fig. 1). The oasis covers approximately 2864 km² ($103^{\circ}00'$ to $103^{\circ}50'E$, $38^{\circ}30'$ to $39^{\circ}05'N$) with altitude from 1309 to 1459 m. The climate is temperate continental desert. As one of the ecologically fragile regions in China, it is characterized by scanty precipitation (about 110 mm), strong evaporation (more than 2664 mm), frequent wind, and abundant sources of sand. The average annual temperature is 7.8 °C.

Agriculture accounts for about 95 % of the water use in the oasis (Chen and Feng 2013). More than 90 % of the irrigation water comes from underground reserves (You et al. 2011). The study area contains three irrigation districts: Baqu (Xuebai, Daba, Sanleizhen, Suwu, Jiahe and Dongbazhen, Fig. 1), Quanshan (Shuangcike, Datan, Quanshanzhen and Hongshaliang), and Huqu (Xiquzhen, Donghuzhen and Shoucheng).

The mean value of local groundwater salinity was reported 3.34 g L^{-1} (with electrical conductivity closely approximate to 4.33 dS m⁻¹; Chen and Feng 2013). In order to obtain the present research datasets, saline groundwater (during April to August) and soil (during August 21–28) samples were collected in 2014. Totally, 48 wells were selected for groundwater sampling (Fig. 1). GPS (global positioning system) was used to define the location. Each sample was taken after 30-min intensive pumping in order to avoid contamination. Sealed samples were then taken to the Key Lab in Cold and Arid Regions Environmental and Engineering Research Institute of Chinese Academy Sciences for chemical analysis.

Soil samples were collected from 48 farmlands which were irrigated by above sampling wells. All these farmlands planted the same variety of sunflower. Soil classified mainly as sandy loam in the upper 20 cm with average bulk density of 1.55 g cm⁻³. A total of 13 soil cores (0–20 cm depth) were collected from each farmland according to an S-shaped curve, and then completely mixed into one composite fresh soil sample. Totally, 48 soil samples with 1 kg of each were obtained by way of quartering. All soil samples were packed in insulated containers and transported to laboratory, where they were stored at 4 °C until biological analysis were conducted.

Laboratory Analysis

Anion concentrations Cl⁻ and SO₄²⁻ of groundwater were analyzed by ion chromatography (Shimadzu), and CO₃²⁻ and HCO₃⁻ were analyzed by titration (pH 4.8 alkalinity). Major cation concentrations (Ca²⁺, Mg²⁺, Na⁺, and K⁺) were analyzed by inductively coupled plasma (ICP-1,000 III C, Shimadzu).

Soil microbial metabolic activity was determined using Biolog EcoPlatesTM. The plate is composed of 96 wells, which contain a triplicate set of 31 carbon sources and three control wells with no carbon. The method is based on the capacity of microbes to utilize different carbon sources. If the microbe inoculated in the plate can utilize the carbon source, a purple color appears in the well and can be read by the equipment automatically. In this study, 5 g fresh soil was suspended in 45 ml of buffered saline solution (0.85 % NaCl) and shaken for 30 min at 25 °C. With additional NaCl solution, the suspension was subsequently diluted to

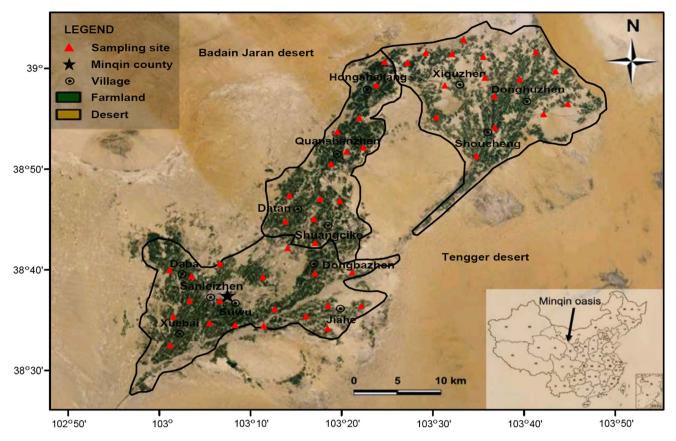


Fig. 1 Location of sampling sites in Minqin oasis

 10^{-3} and transferred to the wells. The plates were incubated for 7 days at 25 °C. The color development was followed over time by measuring the absorbance at 590 and 750 nm every 24 h. The final values used to denote activity in each well were the 590 nm values minus the 750 nm values after correcting for readings in the control well at these wavelengths. The negative values were set to zero (Classen et al. 2003). Average well color development (AWCD) was calculated both as the total sum and after different incubation periods. As the 31 carbon sources could be assigned into six categories (ten carbohydrates, seven carboxylic acid, four polymers, six amino acids, two amines, and two phenols), the AWCD of each category after 96-h incubation was also calculated (Elfstrand et al. 2007).

Statistical Analysis

Descriptive statistics were calculated using SPSS 18.0. The statistical distribution of each dataset was evaluated using skewness, kurtosis, and Kolmogorov–Smirnov (K–S). When the distribution was not normal, the data were log-transformed. The spatial patterns of all variables were produced by ordinary kriging interpolation. The maps were

obtained using ArcGIS 9.2. The correlation analyses which were conducted using SPSS 18.0 were used to examine the relationship between the variables.

Results

Descriptive Statistics

Descriptive statistics for all datasets are presented in Table 1. The mean value of groundwater TDS was 2.98 g L^{-1} with a range of 0.23–12.24 g L^{-1} , nearly 70.83 % was brackish (TDS ≥ 1.0 g L^{-1}). Among the cations, Na⁺ and Mg²⁺ had higher mean and median values. Meanwhile, the mean values of SO₄²⁻ and Cl⁻ were 0.52 and 1.31 g L^{-1} (accounted for average 62.49 and 27.54 % of total anions), respectively, demonstrating that sulfates and chlorides were the main composition of local irrigation groundwater.

Higher AWCD values indicate higher metabolic activity of microbial communities. In the first 24 h, the changes of AWCD values (minimum, maximum, mean, and median) were all slight (Table 1). However, after this lag phase, the values were increased significantly. Three

Table 1	Descriptive	statistics	of the	sample	dataset	in the	study	area
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Variable	No.	Min	Max	Mean	Median	SD	CV	Skew	Kurtosis	K–S	Distribution
Chemical compos	itions o	f saline gro	undwater sam	ples (mg L ⁻	-1)						
TDS	48	232.64	12236.95	2980.57	1334.95	3342.96	1.12	1.64	1.46	0.00	Lognormal
K^+	48	1.76	76.95	10.84	7.30	12.02	1.11	4.02	19.75	0.00	Lognormal
Na ⁺	48	14.12	3031.25	667.85	238.83	862.70	1.29	1.55	1.10	0.00	Lognormal
Ca ²⁺	48	20.00	294.00	99.35	94.00	70.08	0.71	1.16	1.00	0.25	Normal
Mg^{2+}	48	25.04	1215.81	279.34	118.82	338.31	1.21	1.68	1.35	0.00	Lognormal
CO_{3}^{2-}	36	0.00	18.99	3.75	2.53	3.30	0.88	2.98	12.54	0.12	Normal
HCO ₃ ⁻	48	48.91	244.55	90.93	79.16	38.94	0.43	1.77	3.96	0.01	Lognormal
Cl-	48	21.02	1810.22	521.72	279.17	544.93	1.04	1.42	0.60	0.00	Lognormal
SO_4^{2-}	48	84.38	6422.55	1307.73	602.04	1625.89	1.24	1.99	3.00	0.00	Lognormal
Variables of micr	obial m	etabolic act	ivities of soil	samples							
Total AWCD	48	1.17	3.93	2.84	2.84	0.66	0.23	-0.56	0.07	0.89	Normal
AWCD-24 h	48	0.00	0.05	0.01	0.01	0.01	0.83	3.33	14.04	0.00	Lognormal
AWCD-48 h	48	0.02	0.29	0.15	0.15	0.06	0.43	0.14	-0.08	0.93	Normal
AWCD-72 h	48	0.07	0.49	0.31	0.30	0.10	0.32	-0.42	0.03	0.59	Normal
AWCD-96 h	48	0.14	0.64	0.43	0.43	0.12	0.27	-0.56	0.26	0.65	Normal
AWCD-120 h	48	0.22	0.76	0.55	0.56	0.13	0.24	-0.71	0.26	0.74	Normal
AWCD-144 h	48	0.31	0.89	0.66	0.67	0.14	0.21	-0.62	-0.07	0.71	Normal
AWCD-168 h	48	0.40	0.97	0.73	0.75	0.14	0.19	-0.53	-0.45	0.84	Normal
СН	48	0.09	0.50	0.34	0.35	0.10	0.29	-0.79	0.55	0.79	Normal
AA	48	0.04	0.83	0.52	0.52	0.17	0.32	-0.58	0.21	0.96	Normal
CA	48	0.10	0.77	0.44	0.46	0.15	0.33	-0.39	0.01	0.80	Normal
AM	48	0.09	0.86	0.37	0.31	0.19	0.53	1.04	0.74	0.19	Normal
PHe	48	0.00	0.91	0.40	0.34	0.23	0.57	0.58	-0.12	0.38	Normal
PM	48	0.08	0.93	0.55	0.52	0.15	0.28	-0.52	1.52	0.79	Normal

Average absorbencies of six biochemical categories of substrates were calculated after 96-h incubation

No. number of samples, *Min and Max* minimum and maximum values, *SD* standard deviation, *CV* coefficient of variation, *K–S* Kolmogorov– Smirnov test, *TDS* total dissolved solids of groundwater, *Total AWCD* the summation of average well color development (AWCD) from 24 to 168 h, *AWCD-x h* AWCD calculated after *x* h incubation time, *CH* carbohydrates, *AA* amino acids, *CA* is carboxylic acids, *AM* amines, *PHe* phenols, *PM* polymers

categories of carbon sources (amino acids, carboxylic acids, and polymers) had higher AWCD which accounted for average 19.92, 16.90, and 21.85 % of total six categories, revealing that they were major categories utilized by soil microbes.

K–S test showed that Ca^{2+} and CO_3^{2-} in groundwater, total AWCD, AWCD after 48 up to 168 h of incubation, and AWCD of all six categories had near normal distributions. Other variables followed lognormal distributions (Table 1). The CV values of variables (Ca^{2+} , CO_3^{2-} and HCO_3^{-} in groundwater, AWCD in total, AWCD after whatever incubation period, and AWCD for all six categories) ranged from 0.19 to 0.88, indicating that these variables had moderate spatial variations. Other ions (K^+ , Na^+ , Mg^{2+} , Cl^- , and SO_4^{2-}) in groundwater and TDS characterized with CV > 1.0 suggesting that these variables had strong spatial variations.

Spatial Variations of Chemical Compositions of Groundwater

The spatial distribution of TDS and major ions of groundwater are shown in Fig. 2. The values of these variables were all low in the southern part of the Mingin oasis and gradually increased along the groundwater flow path (from southwest to northeast). According to Figs. 1 and 2, three irrigation districts had different groundwater types and distribution characteristics. In Baqu, groundwater salinity was lower in center (average TDS < 0.45 g L⁻¹), mediate in west (0.45 < average TDS < 2.25 g L^{-1}), and higher in east $(2.25 < \text{average TDS} < 3.15 \text{ g L}^{-1})$. The average TDS in this district was 1.22 g L^{-1} with fresh (TDS < 1.0 g) L^{-1}) and slight saline water $(1.0 < TDS < 3.0 \text{ g} \text{ L}^{-1})$ accounted for 38.89 and 55.56 %, respectively. In Quanshan, groundwater salinity

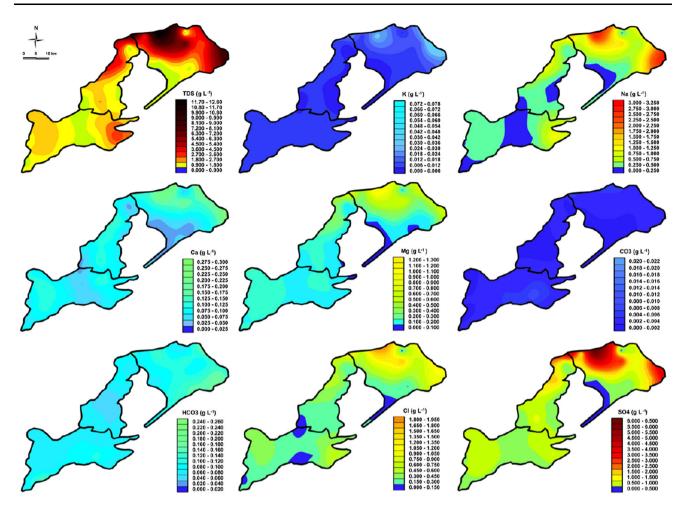


Fig. 2 Spatial variations of chemical compositions of groundwater. TDS is total dissolved solids of groundwater

increased from south to north and east to west with average TDS of 2.06 g L⁻¹. Fresh (28.57 %), slight (50 %), and severe (21.43 %; 3.0 < TDS < 10.0 g L⁻¹) saline water were all contained in this district. In Huqu, slight and severe saline water accounted for 68.75 %, while briny water (TDS > 10.0 g L⁻¹) accounted for 12.50 %. The only exception was southern part of the district, where fresh groundwater was rarely occurred with average TDS of 0.67 g L⁻¹ (accounted for 18.75 %). According to Fig. 2, the concentrations of Na⁺, Mg²⁺, Cl⁻, and SO₄²⁻ increase more prominently than others along the groundwater flow path. The groundwater-type SO₄²⁻-Cl⁻-Na⁺-Mg²⁺ was dominant in most areas of the oasis, while a little SO₄²⁻-Mg²⁺, SO₄²⁻-Cl⁻-Na⁺, and other types also existed.

Spatial Variations of Soil Microbial Activities

Total AWCD in Baqu was higher in the east of center (from 2.84 to 3.93) and southwest corner (from 2.97 to 3.86), while the minimum value was 1.78 in the east

(Fig. 3). In Quanshan, total AWCD was basically equal except a litter higher (from 2.80 to 3.31) in the northeast corner. The minimum total AWCD was 1.17 in northwest Huqu and gradually increased eastwardly up to 3.68. The spatial distribution of AWCD after 96-h incubation had the same tendency with total AWCD, although the value was lower. Compared with Fig. 2, it can be seen that the region with higher TDS of groundwater had nearly lower total AWCD, indicating that possibly negative effects of saline water irrigation on soil microbial metabolic activities.

Soil microbial communities in southwest Baqu seemed preferring to utilize amino acids and polymers, while in the east of center they mainly utilized amino acids, amines, phenols, and polymers (Fig. 3). The AWCD of polymers in east of the center was 0.77, while in the east it was 0.58, and no significant difference was observed. In Quanshan, amino acids and polymers were the dominant carbon sources utilized by soil microbes, illustrating that these two categories were very important for maintaining microbial metabolic activities in this district. Amino acids and carboxylic acids were the main carbon sources utilized by soil

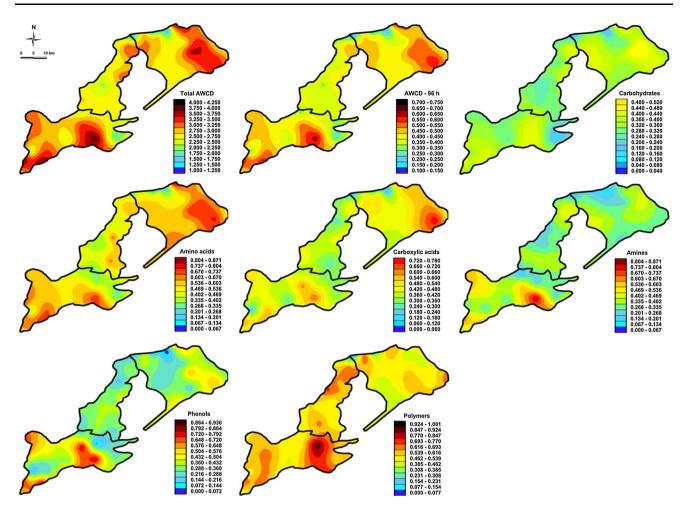


Fig. 3 Spatial variations of variables of soil microbial metabolic activities. Total AWCD is the summation of average well color development (AWCD) from 24 to 168 h; AWCD-96 h AWCD calculated after 96-h incubation, CH carbohydrates, AA amino acids,

CA carboxylic acids, *AM* amines, *PHe* phenols, *PM* polymers. Average absorbencies of six biochemical categories of substrates were calculated after 96-h incubation

microbes in east Huqu. The tendencies of spatial distribution were similar between amino acids and carboxylic acids, and between carbohydrates and amines. Among all the six categories, the AWCD of polymers was always higher in all three districts, demonstrating that soil microbes in saline groundwater-irrigated farmlands may tend to utilize this carbon source dominantly.

Relationship Between Parameters

To examine the relationships between the chemical composition of saline groundwater and the microbial metabolic activities of all soil samples, spearman's correlation coefficients were calculated (Table 2). There was a significantly negative correlation (r = -0.346, p < 0.05) between TDS of groundwater and total AWCD. This suggests a strong adverse impact of high-salinity groundwater irrigation on soil microbial activities. Among

the ions of groundwater, Ca2+, Mg2+, Cl-, and SO42were the dominant ions which affected the microbial activities. For different carbon sources, the effects of saline groundwater irrigation were significantly different. High-salinity groundwater irrigation could significantly reduce the AWCD of carbohydrates, amines and phenols. The average value reduced to 18.63, 26.04 and 20.47 %, respectively, under severe saline water irrigation, while it reduced to 32.91, 59.50 and 73.66 %, respectively, under briny water irrigation. Major chemical ions (Na⁺, Ca²⁺, Mg²⁺, Cl⁻, and SO₄²⁻) in groundwater all had significant negative correlations with the AWCD of these three carbon sources (Table 2). However, both TDS and ions in groundwater had no effect on the average AWCD of amino acids, carboxylic acids, and polymers. Based on the results of 3.1 and 3.3., it can be concluded that highsalinity groundwater irrigation mainly reduced the utilization rates of less-utilized carbon sources. Major
 Table 2
 Spearman correlation
analysis of soil microbial metabolic activity and saline groundwater composition

	Total AWCD	AWCD-96 h	СН	AA	CA	AM	РНе	PM
TDS	-0.346*	-0.331*	-0.371**	-0.204	-0.228	-0.425**	-0.397**	0.021
\mathbf{K}^+	-0.210	-0.191	-0.232	-0.096	-0.016	-0.375**	-0.162	-0.081
Na ⁺	-0.282	-0.257	-0.287*	-0.186	-0.148	-0.380**	-0.441**	0.129
Ca^{2+}	-0.304*	-0.300*	-0.358*	-0.151	-0.180	-0.369**	-0.323*	0.004
Mg^{2+}	-0.321*	-0.305*	-0.359*	-0.168	-0.186	-0.361*	-0.308*	-0.051
CO_{3}^{2-}	-0.228	-0.233	-0.129	-0.242	-0.144	-0.374*	-0.138	-0.284
HCO_3^-	-0.057	-0.007	-0.130	0.064	0.240	-0.062	0.109	-0.228
Cl-	-0.288*	-0.258	-0.301*	-0.152	-0.177	-0.343*	-0.392**	0.064
$\mathrm{SO_4}^{2-}$	-0.367*	-0.368**	-0.394**	-0.235	-0.233	-0.462^{**}	-0.392**	-0.033

Total AWCD the summation of average well color development (AWCD) from 24 to 168 h; AWCD-96 h AWCD calculated after 96-h incubation, CH carbohydrates, AA amino acids, CA carboxylic acids, AM amines, PHe phenols, PM is polymers; average absorbencies of six biochemical categories of substrates were calculated after 96-h incubation; TDS is total dissolved solids of groundwater

* Correlation is significant at the 0.05 level (2-tailed)

** Correlation is significant at the 0.01 level (2-tailed)

carbon sources utilized by soil microbial communities would not be influenced.

Discussion

In our study, soil microbial activities had moderate spatial variations in this long-term saline groundwater-irrigated oasis. Groundwater salinity was also found to have a significantly negative correlation with the variations. Although there is limited evidence for a connection between the salinity level of irrigation water and soil microbial activities, there are some indications that microbial characteristics are often affected by soil salinity (Rietz and Haynes 2003; Yuan et al. 2007). Intensive saline water irrigation is known to increase the risk of soil salinization. Salinization leads to osmolarity elevation outside the microbial cell. In order to maintain cell turgor and prevent dehydration, microbes have been shown to accumulate and produce osmolytes in their cytoplasm (Empadinhas and da Costa 2008). Under this process, some microbes die or become inactive (Placella et al. 2012) and, thereby, microbial community composition and function change (Mavi and Marschner 2012; Chowdhury et al. 2011). Our results support these analogous studies and prove that long-term saline groundwater irrigation could significantly influence the level of spatial variations of soil microbial metabolic activities in a regional scale.

From specific metabolic activities on each biochemical carbon source, we attempted to explain the spatial variations and the reasons. We found that soil microbial communities under different irrigation groundwater salinities preferred to utilize different carbon sources. The utilization rates of carbohydrates, amines, and phenols were low under

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high-salinity groundwater irrigation. Major chemical ions $(Na^+, Ca^{2+}, Mg^{2+}, Cl^-, and SO_4^{2-})$ in groundwater all had significant negative effects on the utilization rates of these categories. However, they did not reduce the utilization rates of major carbon sources (amino acids, carboxylic acids, and polymers). These findings provided some useful information about soil microbial metabolic activities research under saline groundwater-irrigated soil ecosystem.

Soil microbes were used as indicators of the soil's tolerance for saline water irrigation. Although spatial variations of water salinity are generally known by local government, directly predicting the effects on soil environment and plant growth is a challenging as they are affected by multiple interrelated factors (Wong et al. 2010; Stevens and Partington 2013). Soil microbes have been shown to be especially vulnerable to soil disturbance. Therefore, early diagnosis could be conducted through measuring their metabolic activities.

Conclusions

The TDS of groundwater in Minqin oasis was ranged from 0.23 to 12.24 g L^{-1} where moderate spatial variations of soil microbial activities were found. Slight saline water was mainly distributed in the west Bagu and south Quanshan, while severe saline and briny water were dominant in the east Baqu and west Huqu. Sulfates and chlorides were the main composition of local irrigation groundwater. On the contrary, total AWCD was higher in the east-center and southwest of Bagu and east Hugu, while it was lower in the east Bagu and northwest Hugu. The results of correlation analyses demonstrated that high-salinity groundwater irrigation had significantly adverse effects on soil microbial

metabolic activities. Major ions Ca^{2+} , Mg^{2+} , Cl_{-} , and SO_4^{2-} in groundwater decisively influenced the results. Three carbon sources, carbohydrates, amines, and phenols, which had minor utilization rates in all three irrigation districts, were significantly affected by high-salinity groundwater irrigation. Further work (e.g., molecular surveys of microbial communities) will be conducted to provide enough proofs to reveal the mechanism of the effects.

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