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An integrative influence of saline water irrigation and fertilization on the structure of soil bacterial communities

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Abstract

Although numerous studies have investigated the individual effects of salinity, irrigation and fertilization on soil microbial communities, relatively less attention has been paid to their combined influences, especially using molecular techniques. Based on the field of orthogonal designed test and deoxyribonucleic acid sequencing technology, the effects of saline water irrigation amount, salinity level of irrigation water and nitrogen (N) fertilizer rate on soil bacterial community structure were investigated. The results showed that the irrigation amount was the most dominant factor in determining the bacterial richness and diversity, followed by the irrigation water salinity and N fertilizer rate. The values of Chao1 estimator, abundance-based coverage estimator and Shannon indices decreased with an increase in irrigation amount while increased and then decreased with an increase in irrigation water salinity and N fertilizer rate. The highest soil bacterial richness and diversity were obtained under the least irrigation amount (25 mm), medium irrigation water salinity (4.75 dS/m) and medium N fertilizer rate (350 kg/ha). However, different bacterial phyla were found to respond distinctively to these three factors: irrigation amount significantly affected the relative abundances of Proteobacteria and Chloroflexi; irrigation water salinity mostly affected the members of Actinobacteria, Gemmatimonadetes and Acidobacteria; and N fertilizer rate mainly influenced the Bacteroidetes' abundance. The results presented here revealed that the assessment of soil microbial processes under combined irrigation and fertilization treatments needed to be more careful as more variable consequences would be established by comparing with the influences based on an individual factor, such as irrigation amount or N fertilizer rate.

Crops and Soils Research Paper

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Introduction

Saline water irrigation is one major driver of soil environmental changes associated with salinity accumulation, particles flocculation/dispersion and ionic imbalance (Marschner, 1995; Wong *et al.*, 2009). As soil microorganisms play a vital role in the biogeochemical cycling, it is well recognized that more knowledge about the effects of salinity and related environmental changes on soil microbial community is needed urgently (Rath and Rousk, 2015). Hitherto, many previous studies have focused on the variation of soil microbial processes under saline conditions.

It has been widely reported that salinity in soils from natural salinity gradients could dramatically reduce microbial respiration (Muhammad *et al.*, 2008; Setia *et al.*, 2011). Pankhurst *et al.* (2001) also found a lower fungal-to-bacteria ratio in saline soil (with an electrical conductivity value of 4.0 dS/m) when investigated the changes of the microbial community structure under increasing salinity levels. However, a positive relationship was detected between salinity in tidal wetlands ranging from fresh to oligohaline and bacterial abundance by Morrissey *et al.* (2014), and more recently, no significant irrigation water salinity (from 0.85 to 15.0 dS/m) effect was discovered on the composition of bacterial community (Ibekwe *et al.*, 2017). In any case, even using the newest molecular biological techniques available to date, the underling mechanism of the responses of microbial communities to salinity has not been revealed, and at the same time, little attention has been given to the control or the alleviation of the salinity effects on soil microorganisms.

Among the various factors that affect the soil microbial processes, irrigation water amount is perhaps one of the most relevant factors. Soil drought can limit the physiological performance of the microorganisms (Chowdhury *et al.*, 2011). Soil microbial metabolic activity would significantly decrease as the soils dry out below a certain threshold (Curiel *et al.*, 2003).

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In addition, soil drought can also strengthen the impacts of salinity on the underlying soil microorganisms. Dissolved salts in the soil solutions can become more concentrated in a drought condition and then lead to more water release from the microbial cells (Empadinhas and da Costa, 2008). Considering these, the regulation of soil water is of critical importance in maintaining the levels of microbial diversity. Generally, water conditions in agricultural soils are chiefly controlled by the amount and quality of irrigation. Therefore, understanding the influence of quality and quantity of irrigation water on soil microbial processes would be important for devising irrigation strategies for alleviating the adverse influences of saline water irrigation.

Nitrogen (N) fertilizer application is another agricultural practice that could change the structural composition of the bacterial community (Wakelin *et al.*, 2007). A long-term N application could also result in an imbalance in the soil microflora (Sun *et al.*, 2015) and a reduction in bacterial diversity (Yuan *et al.*, 2013). However, Lupwayi *et al.* (2012) suggested that N applied at agronomically recommended rates (50–80 kg/ha) may have no influence on the soil microbial biomass and diversity. In addition, the levels of bacterial diversity may not be affected by short-term treatments (Zhao *et al.*, 2014). As the nitrogen application is always used along with irrigation, their coupling influences on soil microbial community should be taken into account.

With this viewpoint, the current paper aims to investigate the influences of different irrigation water amount, salinity level of water and N fertilizer rate on the soil bacterial community structure based on a field test. Furthermore, the response of each bacterial phylum to the irrigation and fertilization factors were analysed.

Materials and method

Field test

The field test was conducted at the Minqin Experimental Station for Agricultural Water-saving and Ecological Improvement (38°42′N, 103°12′E), which is located in northwest China. The Minqin oasis, as the current study region, is an extremely arid region with average annual precipitation of 110 mm and annual evaporation of 2664 mm. Agriculture accounts for about 90% of the water use and farmlands have been continuously developed since 121 BCE (Chen and Feng, 2013). Aeolian sandy soil (Entisols in the USDA soil taxonomy system) is the dominant soil type and summer maize, sunflower, cotton, seed melon and fennel are the principal crops in this oasis. Because of a severe shortage of water resources, saline groundwater irrigation has been used for several decades. The groundwater salinity in this region ranged from 0.23 to 15.05 dS/m (Chen *et al.*, 2016) and nearly 90% of the irrigation water arises from the underground aquifers.

A detailed description of the study site and the field test had been shown in a previous study by Chen *et al.* (2017*b*). In brief, cotton was planted on 25 April 2014. Three irrigation and fertilization factors were considered for regulating the influences of saline water irrigation. One of these was irrigation water amount, which included three levels of 25, 30 and 35 mm in each irrigation application. The second was irrigation water salinity with three electrical conductivity levels of 1.09, 4.75 and 8.41 dS/m. The last one was N fertilizer rate with three levels of 50, 350 and 650 kg/ha. An orthogonal test design $L_9(3^4)$, which could uniformly distribute the coverage of the levels of all three factors by reducing experimental treatments was used (Table 1). Each treatment had three replicates. A total of 27 farm plots with each size of 5 m long and 3.4 m wide were concluded. The physicochemical properties of test soil are shown in Table 2.

Table 1. The $L_9(3^4)$ orthogonal test used in the current study

Treatment	Irrigation amount (mm)	Irrigation water salinity (dS/m)	N fertilizer rate (kg/ha)
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 levels of three factors were considered: (1) irrigation amount at the levels of 25, 30 and 35 mm for each irrigation application; (2) irrigation water salinity at levels of 1.09, 4.75 and 8.41 dS/m; (3) N fertilizer rate at the levels of 50, 350 and 650 kg/ha. L, M and H in treatment names indicate the lowest, medium and highest levels of the factor in question, respectively. For example, MHL means this treatment has the medium level of irrigation amount, the highest level of irrigation water salinity and the lowest N fertilizer rate.

Mulched drip irrigation system was used for irrigation water delivery. The water salinity was obtained by mixing groundwater from two wells with different salinity. One well was located at the experimental station (EC = 1.09 dS/m) and the other was at Huanghui Village (103°36′11.9″E, 39°02′56.4″N) in Minqin County (EC = 15.92 dS/m). The water was supplied by a pump and the exact amount was monitored by water meters. The dates of irrigation were on 26 April, 21 June, 11 July, 27 July, 10 August and 23 August.

Nitrogen fertilizer was supplied in two periods. Before the cotton sowing (on 20 April), one part (50, 200 and 350 kg/ha) was inputted to given plots according to the test design. At the flowering phase of cotton (on 26 July), another part (0, 150 and 300 kg/ha) was injected into the irrigation water through fertilizer tank, and then was transported to above given plots. On 20 April, 200 kg/ha phosphorus and 100 kg/ha potassium were applied to each plot. On 10 July and 20 August, 50 g/ha mepiquat chloride was also used to each plot.

Soil sampling

The collection of soil samples was conducted on September 26, during the boll-opening stage of cotton. In this stage, the vegetative growth of cotton was gradually stopped, the photosynthetic capacity decreased and the root absorption capacity weakened. From each plot, bulk soil was collected from four random sites to a depth of 20 cm. All soil samples were put into sterile bags, packed in ice blocks and transferred to the laboratory within 24 h, where they were immediately frozen at −80°C for further molecular analysis.

Molecular analysis of bacterial community

A Power Soil deoxyribonucleic acid (DNA) Isolation Kit (MoBio Laboratories, Inc., Carlsbad, CA, USA) was used to extract the DNA of each soil sample according to the manufacturer's instructions. A detailed illustration of this can be found in a previous

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Table 2. Physicochemical properties of test soil

Soil depth	Microture (%)	BD (g/cm^3)	pH	$EC_{1:5}$	TOC (%)	TN (%)	P_a (mg/kg)	K_a (g/kg)
0–20 cm	12.07	1.56	7.61	0.83	0.63	0.06	23.10	157.92

BD, bulk density; $EC_{1:5}$, soil electrical conductivity which was analysed in a soil suspension with the soil:water ratio of 1:5; TOC, total organic carbon; TN, total nitrogen; P_a, available phosphorus; K_a, available potassium.

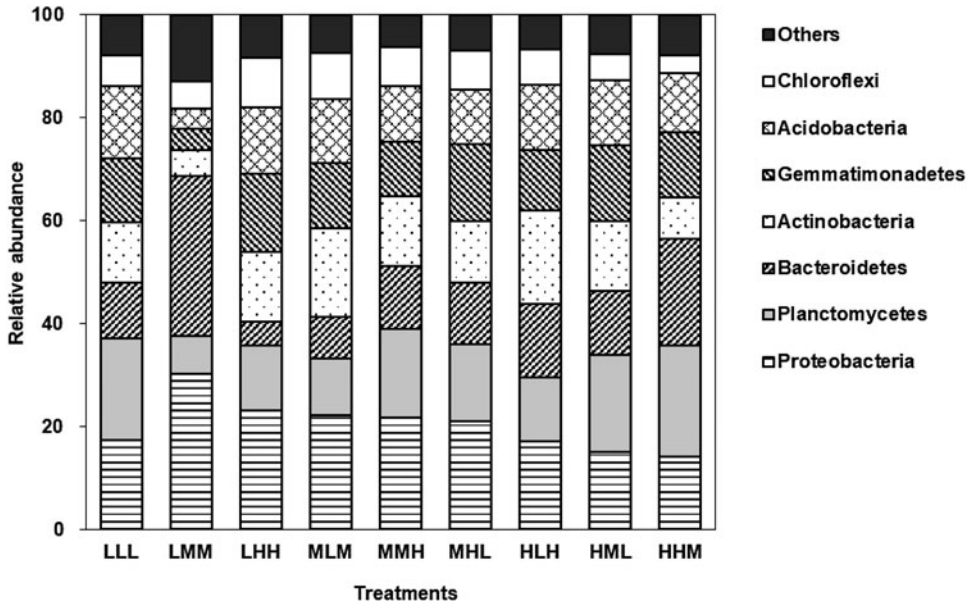

Fig. 1. Relative abundance of soil bacterial phyla under different treatments. L, M and H indicate the least, medium and highest levels, respectively, of each factor (see Table 1). The first letter of each group denotes irrigation amount, the second irrigation water salinity and the third, N fertilizer rate.

study by Chen *et al.* (2017*c*). Spectrophotometer was used for the quality and quantity assessment of the extracts and 0.8% agarose gel electrophoresis was used for integrity check. 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 using the GS-FLX Titanium system at Majorbio Bio-Pharm Technology Co., Ltd., Shanghai, China. The sequences were filtered if they had an average quality score lower than 25, contained fewer than 150 bases and had ambiguous bases. The qualified sequences were clustered into operational taxonomic units (OTUs) at 97% similarity. The taxonomic identity of each representative sequence was assigned to bacteria by using the Ribosomal Database Project classifier (Sul *et al.*, 2011) trained the Greengenes reference database.

Data analysis

The DNA sequences (OTUs) classified at 97% similarity were used for the calculation of indices of bacterial community richness and diversity (Chao1 estimator, abundance-based coverage estimator (ACE), Shannon and Simpson), which were calculated by MOTHUR. The analysis of variance (ANOVA) was conducted to evaluate the differences of the different treatments, and when the results were identified as having significant differences, multiple comparisons among means were performed. A significance level of $P = 0.05$ was chosen. All statistical analyses were performed using the SPSS18.0.

Results

Molecular summary

A total of 2 343 159 (86 572 ± 11 508 per sample) high-quality bacterial 16S rRNA gene sequences were obtained from the 27 soil samples. Based on 97% sequence similarity, 5 614 bacterial OTUs were identified, averaging 3555 ± 306 OTUs per sample. Totally, these OTUs belonged to 53 phyla, 173 classes, 318 orders, 507 families and 725 genera. *Proteobacteria*, *Planctomycetes*, *Bacteroidetes*, *Actinobacteria*, *Gemmatimonadetes*, *Acidobacteria* and *Chloroflexi* were the dominant phyla under both nine treatments (Fig. 1). In particular, *Proteobacteria* were encountered most frequently, representing an average of 20.2% of bacterial sequences across all samples. *Planctomycetes* (15.1%), *Bacteroidetes* (14.0%), *Actinobacteria* (12.6%), *Gemmatimonadetes* (12.1%) and *Acidobacteria* (11.3%) shared close frequency, together accounting for more than 65% of bacterial sequences.

Effects on bacterial richness and diversity

Irrigation amount, salinity and N fertilizer rate could all significantly influence the soil bacterial richness and diversity (Table 3). Compared with the other two factors, the irrigation amount was the most dominant factor in determining all of these indices (yielding the highest *F* values for each index). The best level of irrigation amount in obtaining the highest value of Chao1, ACE and Shannon indices was 25 mm (Fig. 2), and the values under which were all significantly higher than under the other two levels ($P < 0.01$).

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Table 3. The analysis of variance for soil bacterial richness and diversity indices

	Chao 1		ACE		Shannon		Simpson	
Factor	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Irrigation amount	26.077	<0.001	29.413	<0.001	13.652	<0.001	18.631	<0.001
Irrigation water salinity	13.078	<0.001	13.264	<0.001	7.635	0.003	3.605	0.046
N fertilizer rate	7.090	0.005	7.027	0.005	6.193	0.008	3.033	0.071

F, *F* ratio; *P*, significant level according to the LSD test.

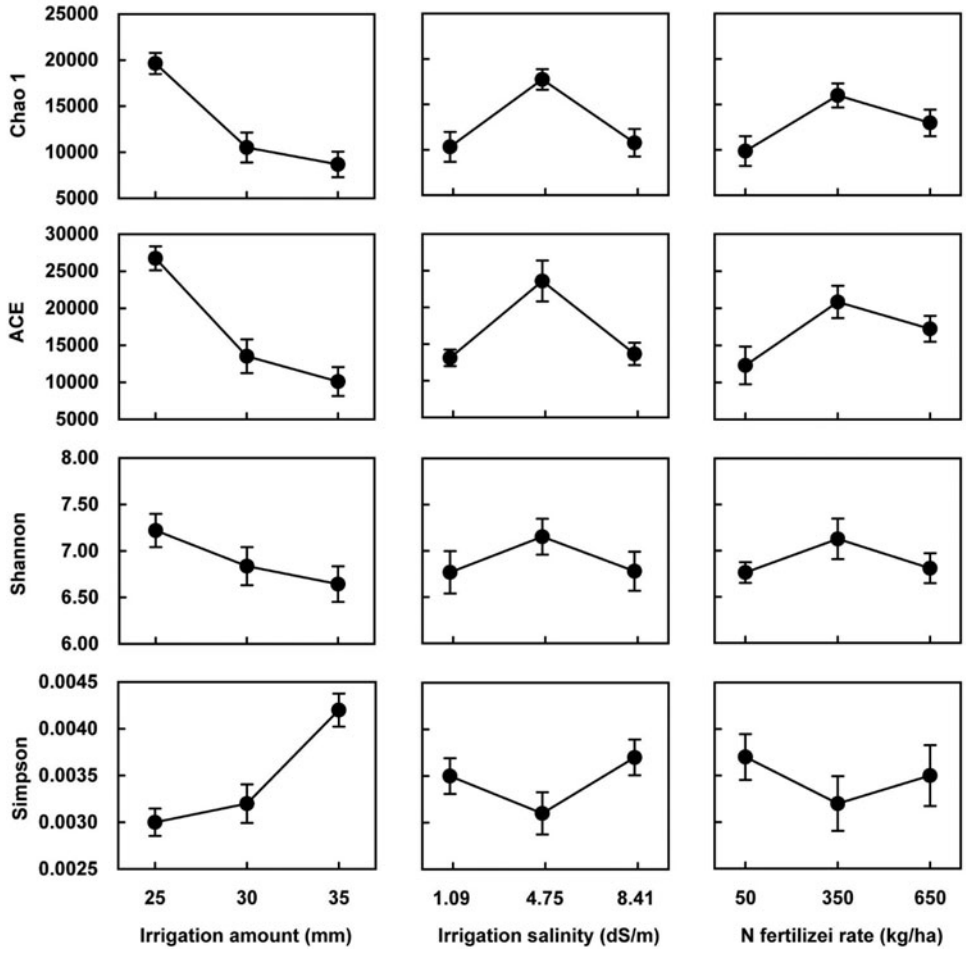

Fig. 2. The influence of the three levels of each factor on the richness and diversity indices of soil bacterial communities (Chao 1, ACE, Shannon and Simpson). The three levels of each factor were: 25, 30 and 35 mm of irrigation amount; 1.09, 4.75 and 8.41 dS/m of irrigation water salinity; 50, 350 and 650 kg/ha of N fertilizer rate.

Although there was no significant difference between the values under 25 and 30 mm irrigation amount for the Simpson index ($P = 0.430$), they were all significantly lower than under the level of 35 mm ($P < 0.001$). That meant under the least irrigation amount (25 mm), soil bacterial richness and diversity would be much higher than under the most irrigation amount (35 mm).

Similarly, the values of Chao1, ACE and Shannon indices under the irrigation water salinity of 4.75 dS/m were all significantly higher than under 1.09 and 8.41 dS/m levels (Fig. 2, $P < 0.01$). Although the value of Simpson index under 4.75 dS/m irrigation water salinity was significantly lower than under the level of 8.41 dS/m ($P = 0.018$), there was no significant difference either between 1.09 and 4.75 dS/m ($P = 0.067$) or 1.09 and 8.41 dS/m ($P = 0.526$) irrigation water salinity. This suggests that under the medium irrigation water salinity (4.75 dS/m), the highest soil bacterial richness and diversity would be obtained.

As for the influence of N fertilizer rate, each index generated a different result. Although the values of Chao1, ACE and Shannon indices under 350 kg/ha N fertilizer rate were all significantly higher than under 50 kg/ha level (Fig. 2, $P < 0.01$), neither significant difference was found between the values of Chao1 and ACE indices under 50 and 650 kg/ha or 350 and 650 kg/ha N fertilizer rate. In addition, the N fertilizer rate had no significant effect on the Simpson index (Table 3, $P = 0.071$).

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Effects on bacterial community composition

The relative abundance of each soil bacterial phylum responded differently to the irrigation amount, salinity and N fertilizer rate. Particularly, no significant effect of the three factors on the relative abundance of *Planctomycetes* was found (Table 4), which could also be confirmed from Fig. 3 by that no significant difference could be concluded under three levels of each factor.

The relative abundances of *Proteobacteria* and *Chloroflexi* were significantly affected by irrigation amount (i.e. $F = 29.6$ and 16.5, respectively), which were all followed by N fertilizer rate and irrigation water salinity (Table 4). However, they had completely different patterns of the actual influence. As for *Proteobacteria*, the relative abundance appeared to decrease with an increase in the irrigation amounts (Fig. 3), but there was no significant difference between the values under the 25 and 30 mm irrigation amount (i.e. with a P value of 0.110). In addition, *Proteobacteria* could obtain much higher frequency under the medium N fertilizer rate (350 kg/ha) and irrigation water salinity (4.75 dS/m), but no significant difference was found between 350 and 650 kg/ha levels ($P = 0.160$). Differently, the relative abundance of *Chloroflexi* was significantly increased ($P = 0.039$) and then reduced ($P < 0.001$) with an increase in the irrigation amount. Meanwhile, under a value of 650 kg/ha N fertilizer rate, the relative abundance was significantly higher than under the other two levels with a P value smaller than 0.01.

The salinity of irrigation in the current study appeared to play a vital role in the relative abundances of *Actinobacteria*, *Gemmatimonadetes* and *Acidobacteria* (Table 4), which all followed by the influence order of N fertilizer rate > irrigation amount according to the values of F-ratio, and all of the three phyla had the lowest frequency under a threshold of 4.75 dS/m irrigation water salinity (Fig. 3) with $P < 0.05$. However, *Actinobacteria* and *Gemmatimonadetes* held the significantly higher abundance under the threshold of 1.09 dS/m ($P < 0.001$) and 8.41 dS/m ($P < 0.05$) irrigation water salinity, respectively, while *Acidobacteria* obtained the highest value under both the above levels. Similarly, under 350 kg/ha N fertilizer rate, the relative abundances of these phyla were all significantly lower than under the other two levels, whereas *Actinobacteria* got the highest abundance under 650 kg/ha N fertilizer rate ($P < 0.01$) and *Gemmatimonadetes* and *Acidobacteria* obtained significantly higher value under 50 and 650 kg/ha N fertilizer rate. As for the effects of irrigation amount, the relative abundances of *Actinobacteria* and *Gemmatimonadetes* were all significantly higher under 30 and 35 mm irrigation amount. Further, no significant effect of irrigation amount on the relative abundance of *Acidobacteria* was found (Table 4 and Fig. 3).

The only phylum with the relative abundance significantly influenced by N fertilizer rate was *Bacteroidetes* ($F = 8.9$, Table 4), with the relative abundance significantly higher under 350 kg/ha N fertilizer rate compared to the other two levels ($P < 0.001$). In addition, the next important factor which was also seen to significantly influence the relative abundance of this phylum was the salinity of irrigation. Under the level of 4.75 dS/m, the relative abundance of *Bacteroidetes* was much higher than under that of 1.09 ($P = 0.006$) and 8.41 dS/m ($P = 0.021$) irrigation water salinity.

Table 4. The analysis of variance for dominant soil bacterial taxa

	Proteobacteria		*Planctomycetes*		*Bacteroidetes*		*Actinobacteria*		*Gemmatimonadetes*		*Acidobacteria*		*Chloroflexi*	
Factor	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Irrigation amount	29.578	<0.001	3.153	0.065	2.742	0.089	13.461	<0.001	3.985	0.035	2.763	0.087	16.524	<0.001
Irrigation water salinity	5.474	0.013	0.842	0.446	5.439	0.013	21.165	<0.001	12.924	<0.001	9.847	0.001	3.613	0.046
N fertilizer rate	7.915	0.003	3.371	0.055	8.896	0.002	17.169	<0.001	10.682	0.001	8.074	0.003	9.860	0.001

F, *F* ratio; *P*, significant level according to the LSD test.

Discussion

The results of the current study had shown that under the coupling influence of saline water irrigation and fertilization, the irrigation amount was the considerably dominant factor in

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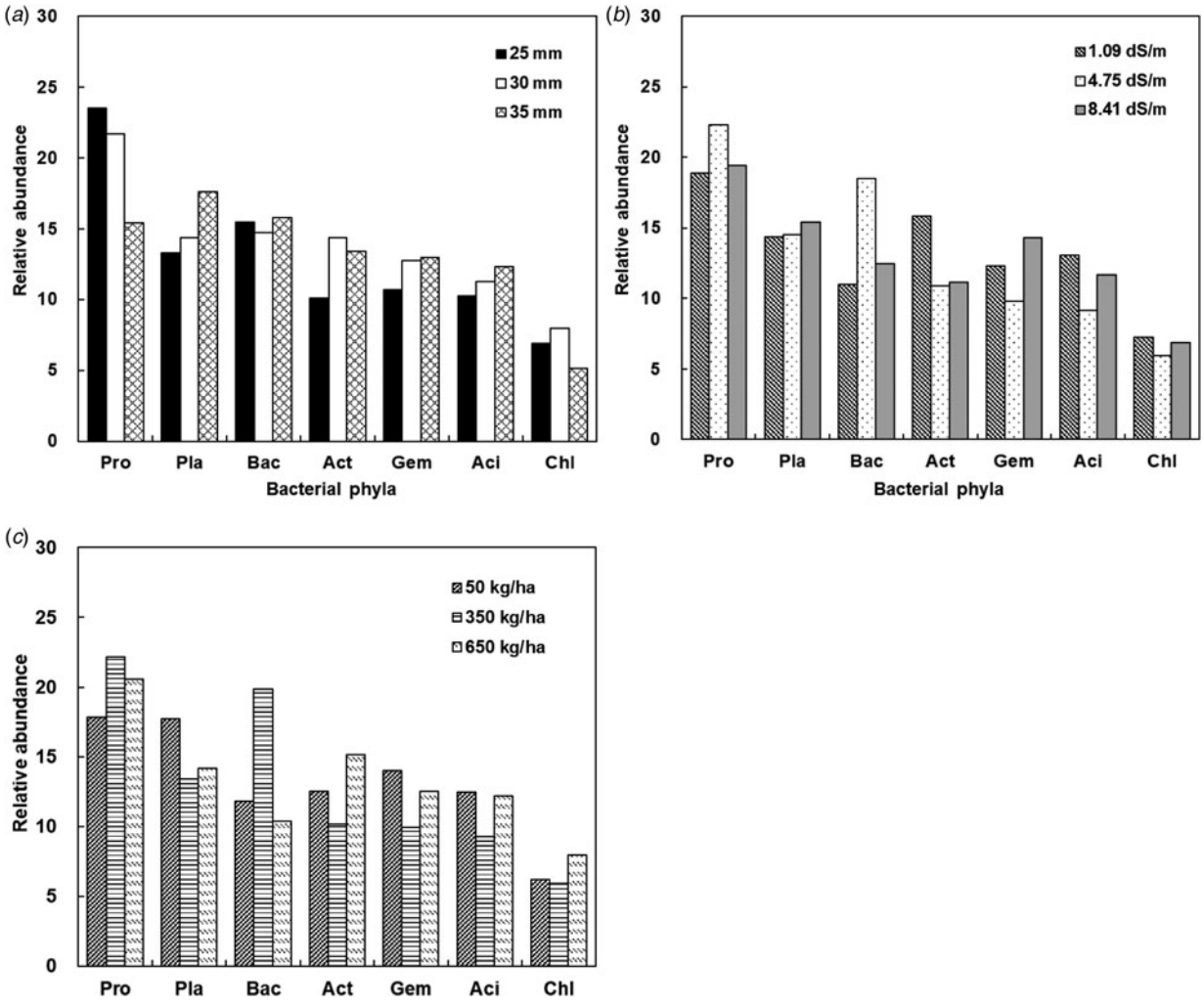

Fig. 3. The influence of the three levels of each factor on the relative abundance of the dominant soil bacterial phyla. The three levels of each factor were: 25, 30 and 35 mm of irrigation amount (*a*); 1.09, 4.75 and 8.41 dS/m of irrigation water salinity (*b*); 50, 350 and 650 kg/ha of N fertilizer rate (*c*). Seven dominant bacterial phyla were concerned: Pro (*Proteobacteria*), Pla (*Planctomycetes*), Bac (*Bacteroidetes*), Act (*Actinobacteria*), Gem (*Gemmatimonadetes*), Aci (*Acidobacteria*) and Chl (*Chloroflexi*).

determining the bacterial richness and diversity and that the lowest irrigation amount (25 mm) yielded the highest values. This was consistent with previous studies, suggesting that in dry soil conditions, increased heterogeneity of microsites and spatial isolation of soil pores may facilitate microbial diversity and species coexistence (Frey, 2007). The mechanism of soil microbial tolerance to drought appeared to involve the microbial physiology (Williams and Xia, 2009; Kakumanu *et al.*, 2013). Therefore, these results may occur due to the structural and physiological adjustment of soil microbial communities. The second influencing factor was irrigation water salinity which showed the highest soil bacterial richness and diversity under the medium irrigation water salinity. Although several previous studies had reported the negative impacts on soil microbial activities (Rietz and Haynes, 2003; Wong *et al.*, 2008; Ke *et al.*, 2013) as soil salinity increased under saline water irrigation (with water salinity generally bigger than 10 dS/m), few studies have examined bacterial richness and diversity at the OTU level in this type of ecosystem (Rath and Rousk, 2015). The current data based on molecular analysis indicated that bacteria were most diverse in medium saline water irrigation treatment (4.75 dS/m) under the coupling effects of irrigation water amount, salinity and N fertilizer. This was especially true in the compound factors' influenced background as critical factors had been changed. Under the coupling influence of irrigation water amount and salinity, the influence of N fertilizer rate on bacterial richness and diversity may be slightly weakened. The results may probably be the best explanation of these changes as the majority of previous studies demonstrated the strong effects of N fertilizer on soil microorganisms (Fierer *et al.*, 2012; Min *et al.*, 2014).

The analysis of the microbiota indicated dominance by the members of the bacterial phyla *Proteobacteria*, *Planctomycetes*, *Bacteroidetes*, *Actinobacteria*, *Gemmatimonadetes*, *Acidobacteria* and *Chloroflexi*. All of these phyla contained taxa commonly found within soils that are capable of having various effects on soil health including beneficial and pathogenic interactions (Lee *et al.*, 2008; Berendsen *et al.*, 2012; Philippot *et al.*, 2013). *Proteobacteria* is one of the most prominent phyla represented among soil bacteria around the world (Lauber *et al.*, 2009; Russo *et al.*, 2012; Tripathi *et al.*, 2012). This phylum dominated average 20.2% relative abundance of bacterial sequences across all samples in the current study, actually the highest members in

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bacteria. It was reported that fertilization can increase the abundance of *Proteobacteria* (Jangid *et al*., 2008). The current results also showed much higher relative abundances of *Proteobacteria* under the medium and high N fertilizer treatments (Fig. 3). Actually, the *Proteobacteria* are a most important bacterial community in global carbon cycling (Kersters *et al*., 2006). Our previous research also confirmed that the relative abundance of *Proteobacteria* and soil organic carbon reduced simultaneously in aquaculture wastewater irrigated soil (Chen *et al*., 2017*a*). In addition, soil salinity can also influence the relative abundance of *Proteobacteria*. For example, Wu *et al*. (2006) showed that the relative abundance of the Betaproteobacteria decreased, whereas the Alphaproteobacteria and the Gammaproteobacteria increased with increasing salinity. However, the current paper found that the effects of irrigation amount were higher than N fertilizer and irrigation water salinity, demonstrating that the *Proteobacteria* may be more sensitive to irrigation water amount than fertilization and irrigation water salinity. In future, further work is needed to reveal the mechanism of these effects.

The phylum *Planctomycetes* were previously reported in association with functioning ectomycorrhizal fungi hyphal networks (Lindahl *et al*., 2010) and higher activity of selected bacterial taxa that harboured mycorrhiza-helper bacteria such as *Burkholderia* spp., *Streptomyces* spp. or *Sphingomonas wittichii* (Churchland and Grayston, 2014). No significant effect of the three factors on the relative abundance of *Planctomycetes* was found in the current study, which in fact illustrates its higher survival capabilities. *Bacteroidetes* are typically copiotrophic and are most abundant in soils that have relatively large amounts of labile organic carbon, including rhizosphere soils (Fierer *et al*., 2007). Thus, the higher abundance of *Bacteroidetes* under 350 kg/ha N fertilizer may, in part, reflect the increased organic carbon availability. As its relative abundance was found to either increase (Wang *et al*., 2016) or decrease (Campbell and Kirchman, 2013; Canfora *et al*., 2014) along an increasing salinity gradient in previous studies, its higher abundance under 4.75 dS/m may be reasonable in the current study.

Actinobacteria held the highest abundance under a threshold of 1.09 or 8.41 dS/m irrigation water salinity, 600 kg/ha N fertilizer rate and 30 or 35 mm irrigation amount in the current study. A large number of this phylum has been found in rhizosphere which is involved in the turnover of recalcitrant plant organic matter producing a balance in the ecosystem (Priyadharsini and Dhanasekaran, 2015). The relative abundance of *Gemmatimonadetes* was also higher under 1.09 and 8.41 dS/m irrigation water salinity, as well as 50 and 650 kg/ha N fertilizer rate, which was contradict with the results of Nemergut *et al*. (2008) and Cederlund *et al*. (2014), indicating that they may be less ecologically coherent in terms of their responses to salinity and N fertilizer.

In order to analyse the effects of saline water irrigation and fertilization on soil bacterial communities, we used the orthogonal test design in this study. The design can consider complicated processes in fewer experiments and thus find the optimal levels of various factors and develop the best processing combination (Ranil *et al*., 2015). However, in this study, we only analysed the main effects of the factors. In some cases, interaction effects may also dominate the main effects. For example, manipulation of irrigation water quantity will affect the salinity situation in the dry environment in addition to the direct impact of water quality (Damgaard *et al*., 2018). Under this circumstance, although the effective information was obtained from this study, further work is still needed to thoroughly verify the effects.

Conclusions

The irrigation water amount was the most important factor in affecting soil bacterial richness and diversity and the highest values were obtained under the least irrigation amount, medium irrigation water salinity and medium N fertilizer rate. The relative abundances of *Proteobacteria* and *Chloroflexi* were more sensitive to irrigation water amount than fertilization and irrigation water salinity, which were significantly different as the results under an individual factor. Irrigation water salinity mainly played an important role in the members of *Actinobacteria*, *Gemmatimonadetes* and *Acidobacteria* while nitrogen fertilizer significantly influenced the *Bacteroidetes*' abundance.

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Conflict of interest. None.

Ethical standards. Not applicable.

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