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Different microbial distributions in the Yellow River delta

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ABSTRACT

The microbial rules of nitrogen removal in wetlands with high salinity were unclear, which was important in the study of saline wastewater purification. In this study, microbial quantity, diversity and physicochemical factors related to nitrogen cycling processes in a saline natural wetland were conducted. Results of real-time quantitative polymerase chain reaction (qPCR) revealed that relative abundance of anammox 16S rRNA gene (anammox for short) took the majority of the four tested nitrogen cycle genes (*amoA*, *Nitrospira* 16S rRNA gene or *nobL* for short, *nirS*). The abundances of anammox were coupled well with salinity, while the aerobic bacteria were inhibited. Deep 16S rRNA gene analysis of bacterial and archaea communities from soils showed that there were no significant differences in bacteria community structure between inland and inshore soils. However, the archaea community analysis indicated interesting results that *Euryarchaeota* was dominant in inshore soils, while *Woesearchaeota* took up over half of the inland soils. Furthermore, the subdivision *halobacteria* of *Euryarchaeota* inshore and *Woesearchaeota Incertae Sedis* AR15-18 inland were the most potential candidates to remove the nitrogen in high-salt areas. These findings provided a preliminary conception for the design of constructed wetlands in treating high saline wastewater.

Keywords: Natural wetland; Nitrogen cycle; Salinity; Anammox; Real-time qPCR; Illumina sequence

1. Introduction

Saline wastewater generated from agro-food, petroleum and leather industries has become a hot issue recently. Biological reactor [1] and physicochemical means [2] have been applied in saline effluent purification with much high cost. In contrast to those technologies, constructed wetlands are low consumption in energy, more environmental friendly [3] and efficient in pollutant removal [4]. Although it was reported that the plants in constructed wetlands treatment did not grow well with a saline concentration above 24‰ [5], many haloduric plants grow well in the Yellow River delta area, and scientists have found with the low concentration of nitrogen pollutants [6]. Therefore, it is necessary to further study the natural wetlands in Yellow River delta area and find ways to raise the removal efficiency of saline wastewater in constructed wetlands [7].

Plants and microbes are two major players in the nitrogen removal process in wetlands. It was reported that the plants are able to absorb nitrogenous compounds, provide carbon sources for nitrate removal and provide surface for microbial growth [8]. Moreover, some microorganisms, such as the ammonia-oxidizing bacteria (AOB), the nitrite-oxidizing bacteria (NOB), denitrifying bacteria and also anoxic ammonium-oxidizing bacteria (anammox) [9], play the main role

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in nitrogen removal. These processes mediated by the above microbes are limited by salinity [10]. Although scientists have separated and identified some salt-tolerant bacteria in nitrogen removal [11], there was little studies on the spatial distribution (soil, water and plant rhizosphere) of nitrogen cycle related microbes in coastal wetlands [12], because different environmental parameters could show a various system of nitrogen cycle microorganisms [13].

Furthermore, in long-term natural wetlands, such as the Yellow River delta area, haloduric microbial communities in soils have been stable and responsible for the relatively high pollution removal rates [14]. Other research suggested that a diverse and unique microbial community inhabited in the constructed wetlands treating saline wastewater [15]. Therefore, it is of vital importance to figure out the quantity and diversity of different nitrogen removal microbes in natural saline wetland soils.

The object of this paper was to figure out which kinds of microbes, including anammox, NOB, AOB, and denitrifying bacteria, were dominant in hyperhaline environments. The study site is located in the Yellow River delta area (Fig. 1). The real-time qPCR was performed to illustrate the absolute abundance of anammox 16S rRNA genes (anammox), *Nitrospira* 16S rRNA genes (*nobL*) (other nitrite-oxidizing bacteria was not detected in our samples), and two functional genes *amoA* and *nirS* in water, soil and rhizosphere microbes, respectively. We also investigated the effect of temperature, dissolved oxygen (DO), pH and salinity on the process of nitrogen cycle in a high salinity wetland. Analysis of the bacterial and archaea community structure of soils was conducted by Illumina high-throughput 16S rRNA gene amplicon sequencing. Based on the results, these dominant microbes would be enriched in order to improve the effect of saline wastewater treatment in the future.

2. Materials and methods

2.1. Survey region

The Yellow River delta is located in the northeast of Shandong Province, China. The delta was formed as the Yellow River re-entered the Bohai Sea in 1855. Until now, it has stretched to an area of 6,010 km^2. The Yellow River delta expands every year for the accumulation of sediment from the upstream and midstream. The warm temperature and semi-humid climate make the annual mean temperature range from 11.7°C to 12.6°C, while the annual mean precipitation is 530–630 mm. The total area of wetlands is 4,167 km^2. The study site was chosen in a typical estuary wetland with the dominant species of *Phragmites australis* (*P. australis*) and *Suaeda salsa* (*S. salsa*) (119°10′08″E, 37°46′01″N to 119°10′17″E, 37°45′23″N).

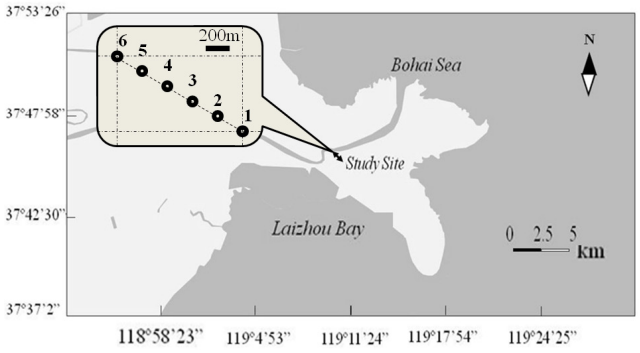

Fig. 1. Sampling sites in the Yellow River delta area, China (Coordinates: 119°10′08″E, 37°46′01″N to 119°10′17″E, 37°45′23″N (linear)).

2.2. Samples collection and analysis

Six sampling plots were selected every 200 m along the downstream tidal ditch with three paralleled points for each plot, of which water, soil, and plant samples were all collected. Each substrate core was 20 cm diameter and 15 cm depth, which was then put into iceboxes before depositing to the lab. Water samples (200 mL) were homogenized and collected in polyethylene plastic bottles, while temperature (*T*), pH, DO, and salinity (‰) of water and soil solution were measured on the spot. Temperature was measured by mercury thermometers. DO was tested with an HQ30d 53LEDTM DO analyzer (HACH, USA). Soil salinity was measured by a HI98331 soil electrical conductivity meter (HANNA, Shanghai), while water salinity was measured by DDBJ-350 portable electrical conductivity meter (Leici, Shanghai).

All water, soil, and plant samples were placed in polyethylene bottles or bags and brought to the laboratory at once and stored at 4°C for further analysis. Before soil character analysis, the soil samples were freeze-dried and sieved through a 2-mm nylon sieve to remove coarse debris and stones and then ground with a pestle and mortar until all particles passed a 0.149-mm nylon sieve. Rhizosphere soil samples were taken from two kinds of the plants with the largest numbers (*P. australis* and *S. salsa*). The soil in rhizosphere was shaken off, while 15-cm roots were cut down and immerged into the NaCl solution (0.85%).

2.3. DNA extraction and quantification

Total DNA of the soils were extracted using soil DNA rapid Isolation kit (Bioteke Ltd., China). The rhizosphere microbial samples were isolated by adding 0.85% NaCl solution to the roots and shaking for 20 min. DNA extraction kits (Omega, USA) were used to extract and purify the total genomic DNA from water samples and rhizosphere microbial samples treated above. Extracted genomic DNA was detected by 1% agarose gel electrophoresis and stored at –20°C until analyzed. Real-time qPCR was conducted on the target fragments of the following functional genes or 16S rRNA genes: *amoA* (ammonia monooxygenase), *nobL* (*Nitrospira* 16S rRNA), *nirS* (cd1-containing nitrite reductase), and anammox (anammox bacterial 16S rRNA). Of these genes, *amoA* and *nirS* are the functional genes in ammonia oxidation and denitrification, while anammox and *nobL* represent the total bacteria in anaerobic ammonia oxidation and nitrite-oxidation stage, respectively. Specially, in all samples, we didn't detect other NOB 16S rRNA genes expect *Nitrospira* (*nobL*). That was why we represented NOB by *nobL* abundance. The primers information, protocols, and parameters of target genes used in qPCR analysis are listed in Tables 1 and 2. All primers were synthesized by TAKARA Biotechnology Company (Dalian, China), and diluted to 20 μmol·L^{-1}.

Table 1
Primers of target genes used in qPCR analysis[a]

Target gene	Primer	Primer sequence (5′–3′)	Amplification size (bp)	Reference
Bacterial 16S rRNA	338F	ACTCCTACGGGAGGCAGCAG	180	[16]
	518R	ATTACCGCGGCTGCTGG		
anammox 16S rRNA	AMX809F	GCCGTAAACGATGGGCACT	257	[17]
	AMX1066R	AACGTCTCACGACACGAGCTG		
amoA	amo598f	GAATATGTTCGCCTGATTG	120	[18]
	amo718r	CAAAGTACCACCATACGCAG		
nirS	nirScd3aF	GT(C/G)AACGT(C/G)AAGGA(A/G)AC(C/G)GG	425	[19]
	nirSR3cd	GA(C/G)TTCGG(A/G)TG(C/G)GTCTTGA		
Nitrospira 16S rRNA	NSR1113f	CCTGCTTTCAGTTGCTACCG	119	[18]
	NSR1264r	GTTTGCAGCGCTTTGTACCG		

[a]This table was made by taking our previous work [20] as a reference.

Table 2
Protocols and parameters of target genes used in qPCR analysis[a]

Target gene	Primer (uL)	Programs
Bacterial 16S rRNA	0.4	Pre-heating at 50°C for 2 min, pre-denaturation at 95°C for 10 min, denaturation at 95°C for 15 s, annealing at 60°C for 1 min, and extension at 72°C for 1 min
anammox	0.4	Pre-heating at 50°C for 2 min, pre-denaturation at 95°C for 10 min, denaturation at 95°C for 15 s, annealing at 56°C for 45 s, and extension at 72°C for 30 s
amoA	1.6	Pre-heating at 50°C for 2 min, pre-denaturation at 95°C for 10 min, denaturation at 95°C for 15 s, annealing at 56°C for 45 s, and extension at 72°C for 30 s
nirS	0.4	Pre-heating at 50°C for 2 min, pre-denaturation at 95°C for 10 min, denaturation at 95°C for 15 s, annealing at 57°C for 30 s, and extension at 72°C for 30 s
nobL	0.4	Pre-heating at 50°C for 2 min, pre-denaturation at 95°C for 10 min, denaturation at 95°C for 15 s, annealing at 58°C for 45 s, and extension at 72°C for 30 s

[a]This table was made by taking our previous work [20] as a reference.

The plasmids containing *amoA*, *nobL*, *nirS*, and anammox were prepared as standard samples based on the standard-curve quantification method by Light Cycler 480II/96 (Roche Diagnostics Ltd., Switzerland) user's manual. The standard samples were diluted to yield a series of 10-fold concentrations and used for qPCR standard curves. The R^2 value for each standard curve exceeded 0.99, indicating linear relationships over the concentration ranges used in this study. The qPCR procedures are presented in Table 2. Each qPCR amplification was conducted in 40 cycles and followed by a melting curve analysis. The qPCR efficiencies were from 80.66% to 98.52% [20]. Since there were large differences of total bacteria abundance among water, soil, and rhizosphere samples, we applied relative abundance to analyze the microbial abundances. The relative abundances of *amoA*, *nobL*, *nirS* and anammox were equal to the absolute amount of these genes divided by that of the bacterial 16S rRNA gene.

2.4. High-throughput 16S rRNA gene amplicon sequencing-Illumina

In order to describe the data, we divided all six sampling sites into two groups. The sites that are relatively far from the sea were defined as inland, in which the salinity was exceeded 1‰. Those near the sea were called inshore with salinity under 1‰. The PCR reactions and Illumina MiSeq sequencing was carried out in Shanghai Majorbio Bio-pharm Technology Co., Ltd. (Shanghai, China). After sequencing, BIPES pipeline was used to process the raw sequences. Then we used a two-stage clustering (TSC) algorithm to cluster tags into operational taxonomy units (OTUs) at sequence divergences of 3% [21]. We assigned the taxonomy of all tags using the global alignment for sequence taxonomy method [22]. The coverage percentage was calculated by Good's method [23]. Rarefaction curves, Shannon weaver, Simpson diversity indices, abundance-based coverage estimators (ACE), Chao1, and Good's coverage were calculated by Mothur analysis (http://www.mothur.org). Illumina sequencing data was deposited in the national center for biotechnology information Sequence Read Archive under BioProject Number PRJNA304204 (A1), PRJNA304207 (B1), PRJNA304209 (A2), and PRJNA304214 (B2).

2.5. Quality control and data analysis

Differences of spatial distribution of four nitrogen cycle genes were analyzed by histograms using origin 8.0 (Origin

Lab, US). Effect of different physical conditions on the absolute abundances of nitrogen cycle genes was carried out by principal components analysis (PCA) by means of IBM SPSS Statistics 20.0 and Canoco for windows 4.5. Statistical significant of difference between different samples was determined with a one-way analysis of variance (ANOVA) by using SPSS 13.0.

3. Results and discussion

3.1. Spatial distribution of the genes in different nitrogen transformation processes

The relative abundance of the nitrogen cycle related genes of *amoA*, *nobL*, *nirS* and anammox were shown in Fig. 2. Furthermore, the absolute abundances of these genes and total bacteria were listed in Table 3. In Fig. 2(A), the relative abundance of *amoA* in inland water and plant rhizosphere samples were much larger than inshore ($P < 0.05$), with the aquatic plants rhizosphere inland yield oxygen into the water [24]. However, in soils, the two groups shared a similar *amoA* abundance ($P > 0.05$) for the relative low DO. In addition, Fig. 2(A) shows that for the samples inland, there were more *amoA* genes around rhizosphere of *S. salsa* than that in the soil, water, and *P. austrilis* rhizosphere ($P < 0.05$). The different salt-tolerant strategies might responsible for the result with the Na^+ ions accumulation in the rhizosphere of *P. australis* [25], and Na^+ ions decreasing with the ion uptake into the vacuole of *S. salsa* [26]. For samples inshore, the relative abundance of *amoA* in water and soil was similar ($P > 0.05$), which was much larger than that of *P. austrilis* and *S. salsa* rhizosphere ($P < 0.05$). It is reported that the organic pollution inshore was very serious [27], which may be correlated with the large amount of *amoA* gene [28].

As shown in Fig. 2(B), the relative abundance of soil, water, and *S. salsa* rhizosphere *nobL* had no significant differences in inland and inshore samples ($P > 0.05$). However, the *nobL* amounts of inland *P. austrilis* were far more than those of inshore ones ($P < 0.05$). For both inland and inshore, the relative abundance of *nobL* in soil was the largest, far more than that in water and plant rhizosphere ($P < 0.05$) [29]. Figs. 2(A) and (B) together showed that the *amoA* gene relative abundance was always lower than that of the *nobL* gene. This is quite different to the other studies [30], in which the AOB is generally more than NOB. In this investigation conditions, it could not be ignored that the impact of salinity on AOB was stronger than on NOB, and led to the big differences between gene copies AOB and NOB [31]. In addition, the expression amount of the 16S rRNA of *Nitrospira* is generally larger than the functional genes of AOB.

From Fig. 2(C), the relative abundance of *nirS* gene of soil, water, and plant rhizosphere had no significant difference between inland and inshore ($P > 0.05$). This was probably due to the similar salinity in all study sites (Table 1), for which among all the environmental factors,

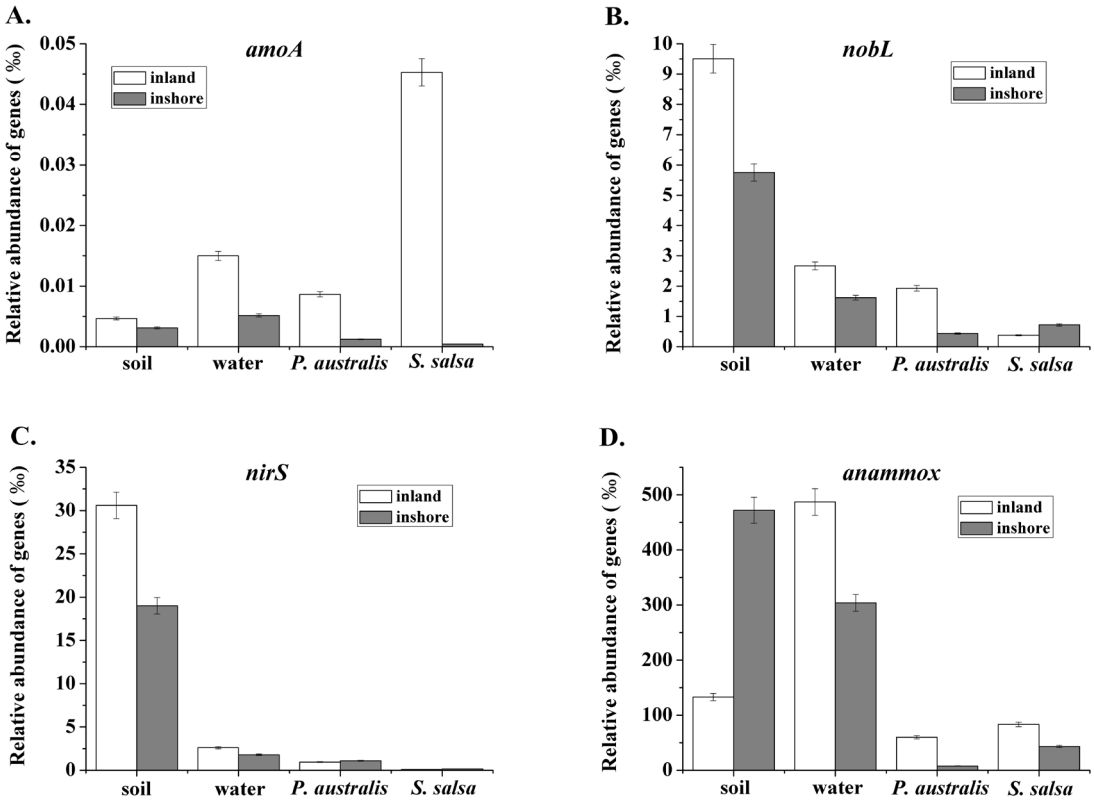

Fig. 2. Relative abundance of four genes in different samples, ($n = 3$) (Figure A–D represents the relative abundance of *amoA*, *nobL*, *nirS* and *anammox*, respectively).

Table 3
Real-time qPCR results of each sample

Genes	Sample number	Soil	Water	*P. austrilis*	*S. salsa*
Bacteria 16s rRNA	1	1.76×10^8	2.56×10^8	4.33×10^{10}	1.39×10^{11}
	2	2.61×10^9	6.17×10^7	1.49×10^{10}	5.79×10^{10}
	3	2.84×10^8	7.93×10^7	1.12×10^{10}	1.17×10^9
	4	2.01×10^{10}	1.30×10^8	5.15×10^{10}	4.96×10^{10}
	5	1.62×10^{10}	1.08×10^9	1.80×10^{10}	2.58×10^{10}
	6	6.58×10^8	2.11×10^8	1.41×10^{10}	1.06×10^{11}
amoA	1	2.14×10^3	1.99×10^3	1.18×10^5	3.68×10^6
	2	6.80×10^3	2.11×10^3	2.85×10^5	3.30×10^6
	3	5.38×10^3	1.86×10^3	1.97×10^5	1.99×10^6
	4	3.50×10^4	1.99×10^3	5.88×10^4	2.88×10^4
	5	4.93×10^4	2.10×10^3	2.17×10^4	1.84×10^4
	6	3.07×10^4	3.25×10^3	2.14×10^4	3.28×10^4
nobL	1	7.56×10^6	6.03×10^5	5.71×10^7	1.33×10^7
	2	1.78×10^7	2.75×10^5	5.56×10^7	1.69×10^7
	3	3.85×10^6	1.81×10^5	2.14×10^7	4.46×10^7
	4	9.78×10^7	6.49×10^5	1.83×10^7	3.60×10^7
	5	6.70×10^7	1.08×10^6	8.57×10^6	3.45×10^7
	6	4.78×10^7	5.76×10^5	9.64×10^6	6.05×10^7
nirS	1	4.64×10^7	4.28×10^5	3.70×10^7	1.09×10^7
	2	1.96×10^7	4.91×10^5	2.16×10^7	7.49×10^6
	3	2.78×10^7	1.13×10^5	7.08×10^6	1.65×10^6
	4	3.33×10^8	2.76×10^5	5.95×10^7	2.67×10^6
	5	2.09×10^8	1.84×10^6	1.93×10^7	5.92×10^6
	6	1.60×10^8	4.21×10^5	1.30×10^7	1.98×10^7
anammox	1	1.34×10^8	1.27×10^8	1.25×10^9	6.53×10^9
	2	1.40×10^8	3.20×10^7	1.99×10^9	5.83×10^9
	3	1.34×10^8	3.42×10^7	9.10×10^8	4.11×10^9
	4	7.00×10^9	8.23×10^7	2.26×10^8	2.01×10^9
	5	6.12×10^9	2.48×10^8	3.03×10^8	1.66×10^9
	6	4.34×10^9	1.01×10^8	1.20×10^8	4.19×10^9

the *nirS* gene abundance was only significantly related to the change of salinity [32]. Similar to *nobL*, for both inland and inshore, the *nirS* relative amount in soil was the largest, which was far larger than in other parts. The soil chemical parameters were much fitter for these microbes [33]. As is shown in Fig. 2(D), the relative abundance of anammox of water inland and soil inshore were the largest, probably because of their high total organic carbon content [34].

In Fig. 2, the anammox 16S rDNA copies were always the most in the four tested nitrogen cycle genes, which was similar to Bae et al. reported [35]. Obviously, soil provided more nutrient and larger solid surface than flow water, in which more microbes stayed and worked in their appropriate position [36]. Rhizospheric microorganisms of *S. salsa* and *P. australis* differed from the non-rhizospheric microorganisms in the soil because of the different living environment, such as pH, enzymes activities, and so on [37]. In a word, both salt concentration and rhizosphere parameters affected the microbial activity and quantity of nitrogen cycle microbes [38].

3.2. Effect of physicochemical parameters on the absolute abundance of functional genes

The characteristics of water and soil were listed in Table 4. The correlations among various characters of water and soil and relative abundance of nitrogen cycle genes were studied (Fig. 3). The first and second PCA axis explained 74.41% and 24.52% of variation in gene types, respectively. The angles between the arrows indicated the correlations between different soil characters and nitrogen cycle genes. The correlation value was deduced by perpendicular projection of one arrow tip onto the line overlaying the other arrow.

It seemed that temperature was a negative factor for *amoA*, *nobL*, and *nirS* in traditional nitrogen cycle. For example, *nirS*-type denitrifiers tend to be less sensitive to warming [39]. The pH has a larger positive effect on *nirS* than on *amoA* and *nobL*, which is probably due to the higher pH tolerance of nitrification bacteria [40]. It has already been found that the community structures of AOB are influenced by soil pH [41,42]. Moreover, the sequences representative of *Nitrosospira* cluster 2 were found in greater relative

Table 4
Characteristics of the water and soil from the study sites

Coordinates	1	2	3	4	5	6
	119°10′08″E, 37°46′01″N to 119°10′17″E, 37°45′23″N (linear)					
Water						
DO (mg·L^{-1})	1.48	4.34	3.87	1.7	1.27	2.59
Temperature (°C)	23.4	25.3	24.7	22.8	23.5	23.7
pH values	8.10	8.21	8.12	8.12	8.04	8.11
Salinity (‰)	14.19	14.66	15.42	14.90	14.88	12.67
Soil						
DO (mg·L^{-1})	1.67	2.24	3.26	1.07	1.85	5.24
Temperature (°C)	18.3	18.1	19.6	18.4	17.5	18.7
pH values	8.08	8.07	8.17	8.01	8.04	8.40
Salinity (‰)	17.32	15.84	14.31	6.85	6.77	6.92

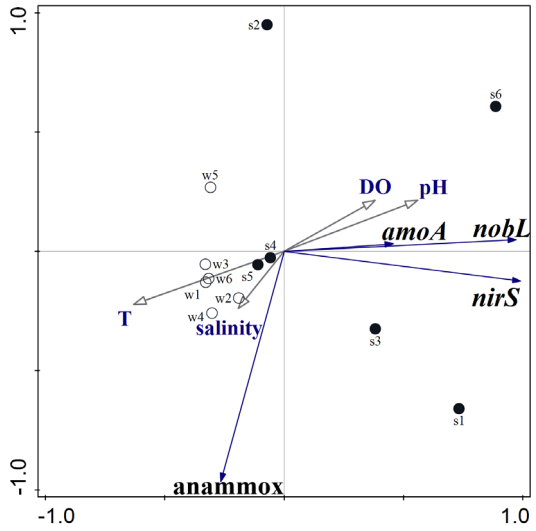

Fig. 3. Correlation between abundance of nitrogen pathway genes and impacting factor by principal components analysis (PCA), (n = 3).

abundance in acidic agricultural soils, which suggesting that cluster 2 may be adapted to growth at low pH, even pH 2.9 [1,43]. DO had a negative effect on the abundance of anammox for its anaerobic characters [44]. Salinity was correlated with anammox positively, confirming that both low DO and high salinity stimulated the anaerobic microbes. In other words, although DO concentration is limited, the anaerobic microbes can slowly adapt to increasing salinity after a long period of time [45].

3.3. Bacterial and archaea communities of soils

The pyrosequencing-based analysis of the 16S rRNA gene was used to assess the soil bacterial and archaea community compositions. In order to better understand the difference between the samples, A1 and B1 represent the archaea and bacterial communities of inland, respectively, while A2 and B2 represent the archaea and bacterial communities of inshore, respectively.

The comparison of phylotype coverage, diversity, and richness estimators at a phylogenetic distance of 3% between bacteria and archaea are shown in Table 5. For archaea, a total of 31,442 reads were obtained from the two samples through pyrosequencing analysis with an average read length of 370–376 bp. For bacteria, a total of 56,265 reads were obtained with an average read length of 420–423 bp, which was larger than that of archaea. The numbers of sequences per sample were 15,331 in A1, 16,111 in A2, 31,237 in B1, and 25,028 in B2. Good's coverage estimates were 99.26%, 98.37%, 95.52%, and 90.36%, which indicated that the number of sequences was sufficient to characterize the microbial communities (Table 5).

The community diversity was analyzed by calculating the Shannon and Simpson diversity index (Table 5). The Chao estimator indicated that the richness of B2 (9,186.67) was larger than that of B1 (5,752.38), followed by A2 (957.60) and A1 (706.37). These results were most correlated with the different salinity. Brandon et al. also found that archaeal and bacterial communities in anoxic sediments of a California hypersaline lake responded differently to environmental gradients of salinity and carbon [46]. Besides, the Shannon index of B1 and B2 were similar (6.47 and 6.40, respectively), which were larger than that of A1 (5.32) and A2 (3.59). These results suggest that B2 had the highest community richness, and the similar bacterial community diversity as B1. Community richness of A2 was larger than A1. However, A2 had smaller archaea community diversity than A1, for the relatively low salinity in soils [46].

Beside the bacterial and archaea community richness and diversity, community composition is also important to understand the difference between soil inshore and inland. The composition of bacterial and archaea communities at the phylum level in the soils is shown in Fig. 4. As shown in Fig. 4(a), there are no significant differences among the ten bacterial phyla, including AOB, NOB, and anammox bacteria (in accordance with Fig. 2). However, in Fig. 4(b), the relative abundance of

Table 5
Comparison of phylotype coverage, diversity and richness estimators at a phylogenetic distance of 3%

Sample	OTUs (97%)	ACE	Chao	Shannon	Simpson (×10^{-3})	Good's coverage (%)
A1	472	778.92	706.37	5.32	7.89	99.26
A2	579	1,345.37	957.60	3.59	84.5	98.37
B1	3062	7,136.09	5,752.38	6.47	6.98	95.52
B2	4108	1,3094.27	9,186.67	6.40	8.26	90.36

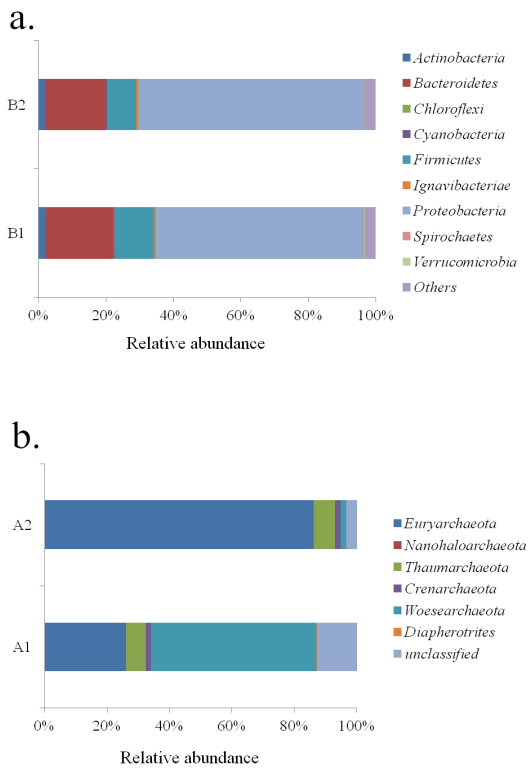

Fig. 4. Composition of different archea (A1 and A2) and bacteria (B1 and B2) communities at phylum level. A1 and B1 represent the archea and bacteria communities of inland soils, while A2 and B2 represent archea and bacteria communities of inshore soils.

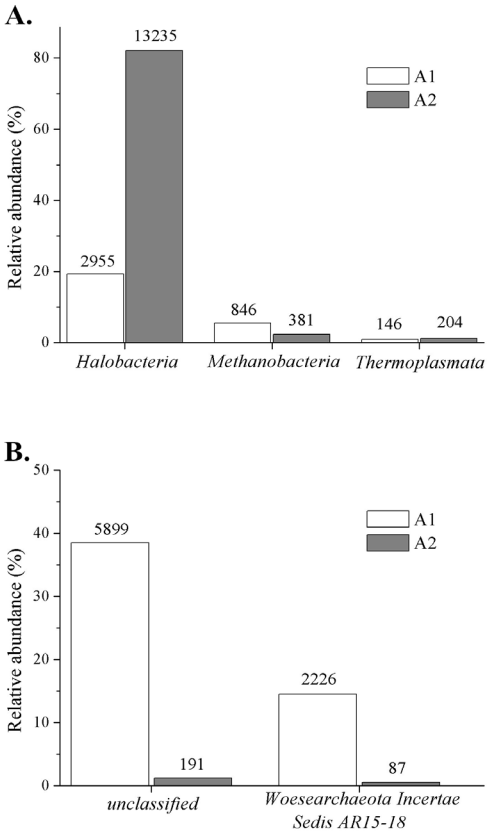

Fig. 5. Relative abundance of *Euryarchaeota* (A) and *Woesearchaeota* (B) subdivisions at class level (The number of reads was marked over the column).

different archaea phyla of the two samples was quite different from each other. The major six phyla observed in the two samples were *Euryarchaeota*, *Nanohaloarchaeota*, *Thaumarchaeota*, *Crenarchaeota*, *Woesearchaeota*, and *Diapherotrites*. Of the six phyla, *Euryarchaeota* was dominant in A2 with a relative abundance of 86.16% (325) OTUs and (13,881) reads, while *Woesearchaeota* took up 53.00% (378) OUTs and (8,125) reads in A1. Sequences that could not be classified into any known group were assigned as *unclassified*, with a proportion of 12.81% and 3.42% in A1 and A2, respectively.

Since the archaea community composition of A1 and A2 was quite different, the subdivisions relative abundance of the two different phyla *Euryarchaeota* and *Woesearchaeota* were shown in Fig. 5. We supposed that the most abundant and different phyla might be the potential candidate for nitrogen removal under high salinity. In previous investigations about salinity effects on the microbial community composition, halophilic *Halobacteriales* of the *Euryarchaeota* was the most important group in the hypersaline sediments [47]. Therefore, in natural saline wetlands, halophilic microorganisms might be the main force in nitrogen cycle and pollutant removal. Interestingly, in this study, the most abundant *Woesearchaeota* inland was AR15-18. Besides, it was reported that AR10-13 took up the majority in the DNA samples of complex sediment and planktonic consortia from an aquifer adjacent to the Colorado River (USA) [48], which was different from our findings. In other words, our findings provided another source for the newly expanded archaea – *Woesearchaeota* [49]. The relationship between these new archaea and the nitrogen removal still needs further study.

4. Conclusion

The results revealed that the microbial distribution in the Yellow River delta were quite different as the changes of distance away from the estuary. The relative abundance of four nitrogen cycle genes showed that anammox was the dominant microbes and might be the candidate to remove nitrogen pollutants in hypersaline areas. In addition, more environmental parameters could affect the microbial nitrogen removal process. In the archaea community study, we dramatically detected a couple of the domain archaea and found different dominant domains. This study, together with the recently published work on expanded genetic diversity within archaea, improves the knowledge of the archaea community and diversity.

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Compliance with ethical standards

This article does not contain any studies with human participants performed by any of the authors. All authors declare that they have no conflict of interest.

References

[1] O. Lefebvre, R. Moletta, Treatment of organic pollution in industrial saline wastewater: a literature review, Water Res., 40 (2006) 3671–3682.
[2] E. Ellouze, R.B. Amar, S. Boufi, A.B. Salah, Coagulation-flocculation performances for cuttlefish effluents treatment, Environ. Technol., 24 (2003) 1357–1366.
[3] J.T. Verhoeven, B. Arheimer, C. Yin, M.M. Hefting, Regional and global concerns over wetlands and water quality, Trends Ecol. Evol., 21 (2006) 96–103.
[4] A.K. Kivaisi, The potential for constructed wetlands for wastewater treatment and reuse in developing countries: a review, Ecol. Eng., 16 (2001) 545–560.
[5] J.M. Jesus, C.S. Calheiros, P.M. Castro, M.T. Borges, Feasibility of Typha latifolia for high salinity effluent treatment in constructed wetlands for integration in resource management systems, Int. J. Phytorem., 16 (2014) 334–346.
[6] Z. Yang, L. Wang, J. Niu, J. Wang, Z. Shen, Pollution assessment and source identifications of polycyclic aromatic hydrocarbons in sediments of the Yellow River delta, a newly born wetland in China, Environ. Monit. Assess., 158 (2009) 561–571.
[7] N. Bassi, M.D. Kumar, A. Sharma, P. Pardha-Saradhi, Status of wetlands in India: a review of extent, ecosystem benefits, threats and management strategies, J. Hydrol.: Region. Studies, 2 (2014) 1–19.
[8] H. Brix, Functions of macrophytes in constructed wetlands, Water Sci. Technol., 29 (1994) 71–78.
[9] A. Mulder, A.A. Van de Graaf, L. Robertson, J. Kuenen, Anaerobic ammonium oxidation discovered in a denitrifying fluidized-bed reactor, FEMS Microbiol. Ecol., 16 (1995) 177–183.
[10] C.E. Bower, J.P. Bidwell, Ionization of ammonia in seawater: effects of temperature, pH, and salinity, J. Fish. Res. Board Can., 35 (1978) 1012–1016.
[11] W. Tanthanuch, P. Tittabutr, S. Mohammed, R. Matthiesen, M. Yamabhai, M. Manassila, O.N. Jensen, N. Boonkerd, N. Teaumroong, Identification of salt-tolerant Sinorhizobium sp. strain BL3 membrane proteins based on proteomics, Microbes Environ., 25 (2010) 275–280.
[12] H.-P. Koops, A. Pommerening-Röser, Distribution and ecophysiology of the nitrifying bacteria emphasizing cultured species, FEMS Microbiol. Ecol., 37 (2001) 1–9.
[13] T.H. Erguder, N. Boon, L. Wittebolle, M. Marzorati, W. Verstraete, Environmental factors shaping the ecological niches of ammonia-oxidizing archaea, FEMS Microbiol. Rev., 33 (2009) 855–869.
[14] M.S. Moussa, D.U. Sumanasekera, S.H. Ibrahim, H.J. Lubberding, C.M. Hooijmans, H.J. Gijzen, M.C. van Loosdrecht, Long term effects of salt on activity, population structure and floc characteristics in enriched bacterial cultures of nitrifiers, Water Res., 40 (2006) 1377–1388.
[15] C.S. Calheiros, A. Teixeira, C. Pires, A.R. Franco, A.F. Duque, L.F. Crispim, S.C. Moura, P.M. Castro, Bacterial community dynamics in horizontal flow constructed wetlands with different plants for high salinity industrial wastewater polishing, Water Res., 44 (2010) 5032–5038.
[16] G. Muyzer, E.C. de Waal, A.G. Uitterlinden, Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA, Appl. Environ. Microbiol., 59 (1993) 695–700.
[17] I. Tsushima, T. Kindaichi, S. Okabe, Quantification of anaerobic ammonium-oxidizing bacteria in enrichment cultures by real-time PCR, Water Res., 41 (2007) 785–794.
[18] H.M. Dionisi, A.C. Layton, G. Harms, I.R. Gregory, K.G. Robinson, G.S. Sayler, Quantification of *Nitrosomonas oligotropha*-like ammonia-oxidizing bacteria and *Nitrospira* spp. from full-scale wastewater treatment plants by competitive PCR, Appl. Environ. Microbiol., 68 (2002) 245–253.
[19] I.N. Throback, K. Enwall, A. Jarvis, S. Hallin, Reassessing PCR primers targeting nirS, nirK and nosZ genes for community surveys of denitrifying bacteria with DGGE, FEMS Microbiol. Ecol., 49 (2004) 401–417.
[20] J. Liu, Z. Yang, W. Yu, J. Hu, H. Xie, S. Wang, J. Zhang, Relationship between nitrogen transformation and its related genes: comparison among riparian, marsh, and full-scale constructed wetlands, Desal. Wat. Treat., (2015) 1–11.
[21] X.T. Jiang, H. Zhang, H.F. Sheng, Y. Wang, Y. He, F. Zou, H.W. Zhou, Two-stage clustering (TSC): a pipeline for selecting operational taxonomic units for the high-throughput sequencing of PCR amplicons, PLoS One, 7 (2012) e30230.
[22] S.M. Huse, L. Dethlefsen, J.A. Huber, D.M. Welch, D.A. Relman, M.L. Sogin, Exploring microbial diversity and taxonomy using SSU rRNA hypervariable tag sequencing, PLoS Genet., 4 (2008) e1000255.
[23] I.J. Good, The population frequencies of species and the estimation of population parameters, Biometrika, 40 (1953) 237–264.
[24] Q. Wang, H. Xie, J. Zhang, S. Liang, H.H. Ngo, W. Guo, C. Liu, C. Zhao, H. Li, Effect of plant harvesting on the performance of constructed wetlands during winter: radial oxygen loss and microbial characteristics, Environ. Sci. Pollut. Res. Int., 22 (2015) 7476–7484.
[25] S.S. Al-Garni, Increasing NaCl-salt tolerance of a halophytic plant *Phragmites australis* by mycorrhizal symbiosis, Am.-Eurasian J. Agric. Environ. Sci., 1 (2006) 119–126.
[26] L. Zheng, C. Chen, F.Y. Zhang, Development of water quality model in the Satilla River Estuary, Georgia, Ecol. Modell., 178 (2004) 457–482.
[27] H. Wu, Y. Huo, M. Hu, Z. Wei, P. He, Eutrophication assessment and bioremediation strategy using seaweeds co-cultured with aquatic animals in an enclosed bay in China, Mar. Pollut. Bull., 95 (2015) 342–349.
[28] X. Sun, A. Wang, L. Yang, L. Guo, Q. Chen, Z. Hu, L. Jiang, L. Xiao, Spatial distribution of ammonia-oxidizing archaea and bacteria across eight freshwater lakes in sediments from Jiangsu of China, J. Limnol., 73 (2014) 312–324.
[29] L.M. Pitombo, J.B. Carmo, M. Hollander, R. Rossetto, M.V. López, H. Cantarella, E.E. Kuramae, Exploring soil microbial 16S rRNA sequence data to increase carbon yield and nitrogen efficiency of a bioenergy crop, GCB Bioenergy, 8 (2015) 867–879.

[30] D.W. Graham, C.W. Knapp, E.S. Van Vleck, K. Bloor, T.B. Lane, C.E. Graham, Experimental demonstration of chaotic instability in biological nitrification, ISME J., 1 (2007) 385–393.
[31] A. Malovanyy, E. Plaza, J. Trela, M. Malovanyy, Ammonium removal by partial nitritation and Anammox processes from wastewater with increased salinity, Environ. Technol., 36 (2015) 595–604.
[32] Y. Zheng, L. Hou, M. Liu, J. Gao, G. Yin, X. Li, F. Deng, X. Lin, X. Jiang, F. Chen, H. Zong, J. Zhou, Diversity, abundance, and distribution of nirS-harboring denitrifiers in intertidal sediments of the Yangtze Estuary, Microb. Ecol., 70 (2015) 30–40.
[33] T. Ligi, M. Truu, J. Truu, H. Nõlvak, A. Kaasik, W.J. Mitsch, Ü. Mander, Effects of soil chemical characteristics and water regime on denitrification genes (nirS, nirK, and nosZ) abundances in a created riverine wetland complex, Ecol. Eng., 72 (2014) 47–55.
[34] N.J. Bale, L. Villanueva, H. Fan, L.J. Stal, E.C. Hopmans, S. Schouten, J.S. Sinninghe Damste, Occurrence and activity of anammox bacteria in surface sediments of the southern North Sea, FEMS Microbiol. Ecol., 89 (2014) 99–110.
[35] H. Bae, K.-S. Park, Y.-C. Chung, J.-Y. Jung, Distribution of anammox bacteria in domestic WWTPs and their enrichments evaluated by real-time quantitative PCR, Process Biochem., 45 (2010) 323–334.
[36] B. Schippers, A.W. Bakker, P.A. Bakker, Interactions of deleterious and beneficial rhizosphere microorganisms and the effect of cropping practices, Annu. Rev. Phytopathol., 25 (1987) 339–358.
[37] D.A. Frank, P.M. Groffman, Plant rhizospheric N processes: what we don't know and why we should care, Ecology, 90 (2009) 1512–1519.
[38] L. Borruso, G. Bacci, A. Mengoni, R. De Philippis, L. Brusetti, Rhizosphere effect and salinity competing to shape microbial communities in Phragmites australis (Cav.) Trin. ex-Steud, FEMS Microbiol. Lett., 359 (2014) 193–200.
[39] J. Jung, J. Yeom, J. Kim, J. Han, H.S. Lim, H. Park, S. Hyun, W. Park, Change in gene abundance in the nitrogen biogeochemical cycle with temperature and nitrogen addition in Antarctic soils, Res. Microbiol., 162 (2011) 1018–1026.
[40] W. Bae, S. Baek, J. Chung, Y. Lee, Optimal operational factors for nitrite accumulation in batch reactors, Biodegradation, 12 (2001) 359–366.
[41] S. Avrahami, W. Liesack, R. Conrad, Effects of temperature and fertilizer on activity and community structure of soil ammonia oxidizers, Environ. Microbiol., 5 (2003) 691–705.
[42] G.A. Kowalchuk, J.R. Stephen, Ammonia-oxidizing bacteria: a model for molecular microbial ecology, Annu. Rev. Microbiol., 55 (2001) 485–529.
[43] R.A. Nugroho, W.F. Roling, A.M. Laverman, H.R. Zoomer, H.A. Verhoef, Presence of Nitrosospira cluster 2 bacteria corresponds to N transformation rates in nine acid Scots pine forest soils, FEMS Microbiol. Ecol., 53 (2005) 473–481.
[44] M.M. Kuypers, A.O. Sliekers, G. Lavik, M. Schmid, B.B. Jorgensen, J.G. Kuenen, J.S. Sinninghe Damste, M. Strous, M.S. Jetten, Anaerobic ammonium oxidation by anammox bacteria in the Black Sea, Nature, 422 (2003) 608–611.
[45] K. Windey, I. De Bo, W. Verstraete, Oxygen-limited autotrophic nitrification-denitrification (OLAND) in a rotating biological contactor treating high-salinity wastewater, Water Res., 39 (2005) 4512–4520.
[46] B.K. Swan, C.J. Ehrhardt, K.M. Reifel, L.I. Moreno, D.L. Valentine, Archaeal and bacterial communities respond differently to environmental gradients in anoxic sediments of a California hypersaline lake, the Salton Sea, Appl. Environ. Microbiol., 76 (2010) 757–768.
[47] H. Jiang, H. Dong, B. Yu, X. Liu, Y. Li, S. Ji, C.L. Zhang, Microbial response to salinity change in Lake Chaka, a hypersaline lake on Tibetan plateau, Environ. Microbiol., 9 (2007) 2603–2621.
[48] C.J. Castelle, K.C. Wrighton, B.C. Thomas, L.A. Hug, C.T. Brown, M.J. Wilkins, K.R. Frischkorn, S.G. Tringe, A. Singh, L.M. Markillie, R.C. Taylor, K.H. Williams, J.F. Banfield, Genomic expansion of domain archaea highlights roles for organisms from new phyla in anaerobic carbon cycling, Curr. biol., 25 (2015) 690–701.
[49] L. Eme, W.F. Doolittle, Microbial diversity: a bonanza of phyla, Curr. biol., 25 (2015) R227–R230.