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Community structures of ammonia-oxidizing and their role in the nitrogen circle in surface sediments of shallow lakes with different pollutant sources

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ABSTRACT

The purpose of this work was to investigate the effects of fenced aquaculture, livestock and poultry rising, agricultural non-point source pollution, and submerged plant enrichment on the diversity and community composition of ammonia-oxidizing microorganisms in the sediments of shallow lakes. Ammonia-oxidizing archaea (AOA) has been found in surface sediments of shallow lakes in the Lixiahe region, Jiangsu Province, China, which provided an example of the global extent and geographical distribution of AOA habitats. The distribution of AOA in these shallow lakes was examined in this study. Rich organic matter promoted the growth of AOA. There was a small difference of destination sequences between Group 1.1b and Group 1.1a at the sediments of Lake Dazong sample point in Lake Dazong, in which the AOA came mainly from terrigenous environment and lake water and sediments of Lake Dazong. The factories and fisheries around the shores of Lake Desheng and the livestock-raising area in the upper reaches of Lake Jiulongkou influence the community features of surface sediments. The long-term fenced breeding has increased the injection of artificial pollution source, reduced the ratio of nitrogen isotope, and resulted in the reduction of AOB abundance and diversity at this sampling point. The relatively high diversity and abundance of AOA or AOB accelerate the transformation of ammonia nitrogen to nitrate nitrogen, reduce the ammonia nitrogen in surface sediments and increase the nitrate nitrogen in the sediments and overlying water.

Keywords: Surface sediments; Ammonia-oxidizing archaea; Ammonia-oxidizing bacteria; Community structure; Pollutant sources

1. Introduction

Lake sediments are vertically structured ecosystems in which ammonia-oxidizing microbial activity is predominantly influenced by the availability of nitrogen element and organic matters. The close interchange of chemical, physical and biological processes along vertical gradient also provides niches for metabolically ammonia-oxidizing microorganisms. Ammonia-oxidizing microorganisms are important drivers for the nitrogen cycle of natural systems [1,2] as ammonia oxidation can be carried out by ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) in soil or sediments. Studies have already been conducted on the role and function of AOA and AOB in the nitrogen cycle [3]. Recent studies have shown that AOA with the

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ammoxidation ability contains the *amoA* gene [4,5]. Auguet and Casamayor [6] and Herrmann et al. [7] discovered AOA in the rhizospheres of aquatic plants in alpine and freshwater lakes. Following this discovery, the extensive research was conducted for examining AOA in lake ecological systems. AOA-related nitration reaction processes are more prevalent in cases of low nitrogen [8,9]. AOA have been classified into three types [10,11]: the nitrated archaea communities of marine environments in oceans, most water bodies and sediments (Group 1.1a); the nitrated archaea communities from soil environments and other terrigenous environments (Group 1.1b); and the nitrated archaea communities of thermophilic environments (Group 1.1c). Pester et al. [12] classified AOA sequences into five groups: *Nitrosocaldus cluster*, *Nitrosopumilus cluster*, *Nitrosotalea cluster*, *Nitrososphaera sister cluster* and *Nitrososphaera cluster*. Of these, the *Nitrososphaera cluster* includes Group 1.1b, and the *Nitrosopumilus cluster* includes Group 1.1a. Llirós et al. [13] found that in environments with a high content of dissolved oxygen (DO), most AOA belonged to Group 1.1b and Group 1.1a. In their study of Lake Taihu sediments, Wu et al. [14] found that AOA mostly belonged to Group 1.1b and rarely belonged to Group 1.1a.

Some previous studies have shown that the abundance and community structure of ammonia-oxidizing microorganisms changed following different external environmental factors [8,15,16], and understanding their diversity was important to provide an insight into mitigation eutrophication problems [17,18]. Changes in environmental factors, e.g., soil type [19], submerged macrophytes [20], fertilization practices [16,21,22], and ammonium concentrations [23–25], may lead to change in AOA and AOB richness and distribution, and consequently result in change in soil nitrogen migration and transformation processes. The differences among habitats affect the biochemical reaction processes in which microbes participate, and their ability to carry out ecological functions [25]. AOA and AOB respond to changes in the ambient environment, and their richness and diversity can be used as important indicators to reflect environmental conditions, and to assess and predict the health of ecological systems [2].

Depending on the environment, the $^{15}N/^{14}N$ ratio of sediment nitrate can provide information on dinitrogen fixation, uptake of fixed nitrogen by phytoplankton, nitrification, and denitrification [26]. The denitrified method was used to measure the nitrogen isotopic composition of nitrate in seawater and freshwater [27,28]. In addition, few studies have reported the effect of multiple pollutant sources in a limited area on the relative richness and community distribution of AOA and AOB in sediments. In current research, a bacterial method was used for measuring the isotopic composition of sediment nitrate, which is based on the analysis of nitrous oxide gas (N_2O) that is produced quantitatively from nitrate by denitrifying bacteria. Therefore, the aim of this study was to investigate the influence of fenced aquaculture, livestock and poultry rising, agricultural non-point source pollution, and submerged plant enrichment on ammonia-oxidizing microorganism diversity and community distribution in sediments of shallow lakes, and to investigate the impact of sediments AOA and AOB community structures on inorganic nitrogen sources (ammonia and nitrate).

2. Materials and methods

2.1. Site description and sample collection

In September 2013, surface sediment samples (0–20 cm) were collected by grab bucket from four sample points, which were Lake Desheng (DS), Lake Jiulongkou (JLK), lake entry in Lake Dazong (DZ) and lake center in Lake Dazong (DZ5) in the Lixiahe region in the lower reach of Huaihe River (Fig. 1), and the sample locations were surveyed by GPS. Each of these sampling points was subject to intensive human activities. The densities of submerged plants, degree of fenced aquaculture, and discharges of industrial wastewater and non-point source pollutants at each of these sites had varying influences on the lake environment. DS was at the center of DS, and the water was about 3.5 m deep. DS is a river channel-type lake, and the riverbank environments exert a strong influence on the water quality and sediment environment. There were chemical fertilizer factories, sandpits, and other industries close to the riverbanks, and the lake surface was mostly fenced off and isolated by aquaculture. Site JLK was in the southern inlet of JLK. It had just been dredged, and the water depth was 1.5 m. There was very little fenced aquaculture in this area, and the water flowed freely. There are livestock farms in this area, and fish and livestock are bred in its upstream area. DZ was at the outlet of the Mangshe River, in the northern area of Lake Dazong. The water was about 2.1 m deep, and the fenced aquaculture at this site was extensive. DZ5 was in the central area of Lake Dazong; the water was about 1 m deep, and the water was clear. This region has a dense cover of water plants, such as duckweed, *P. prispus*, water caltrop, and hornwort. The surface sediment is black, and there is a thick black layer of decomposing organic material.

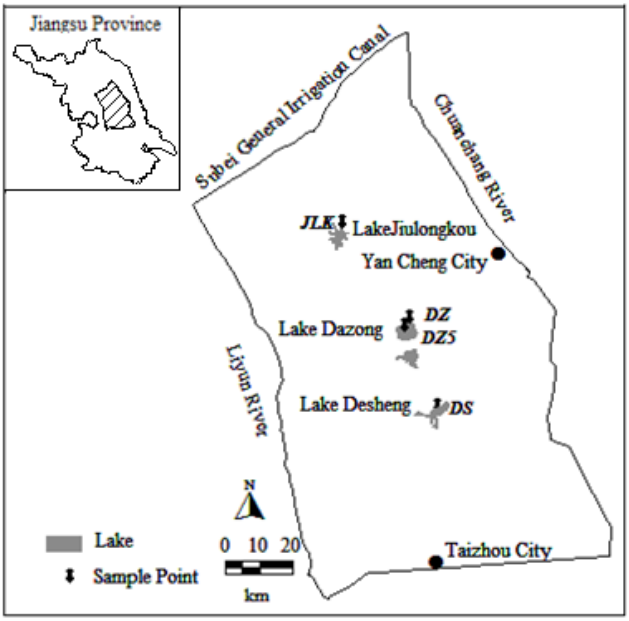

Fig. 1. Sketch map of the lake sampling points in the research region.

2.2. Chemical analysis method

After sampling, the sediment samples were taken to the laboratory in sealed plastic bags, stored in iceboxes (–80°C) (DW-HL388, Meiling, China), and were then freeze-dried (ALPHA 1-2, CHRIST, Germany). All of the samples were ground and sieved with a standard 150-lm sieve. Water samples were kept in a cooler at 4°C until analysis. Total nitrogen (TN), ammonia nitrogen (NH_4^+), nitrite nitrogen (NO_2^-), and nitrate nitrogen (NO_3^-) extracted with 2 M KCl were measured using flow injection analyzer (Skalar SAN^{++}, SKALAR, Netherlands). The total organic carbon (TOC) was measured by elemental analyzer (EA 4000, Jena, Germany). All analytical operations were conducted using of strict quality control guidelines and analysis of replicates. All samples were measured in triplicate, and the results were expressed as the mean.

2.3. DNA extraction and PCR amplification

DNA was extracted from a 0.5-g freeze-dried and sieved sediment sample using an extraction and purification agent (FastDNA® Spin Kit for Soil, MP Biomedical, and Santa Ana, CA, USA). The samples were washed twice with precooled 70% alcohol and were resuspended in a sterilized Tris-EDTA (TE) buffer solution. The final volume of the sample was 50 μL. DNA was analyzed by agarose electrophoresis, and the samples were stored below –20°C. The DNA was extracted three times from each sample before consolidation to ensure uniformity of microbes. The consolidated DNA was diluted tenfold and was then used as a template. The archaeal *amoA* gene was amplified using the primers *amoA-AF* (5′-STAATGGTCTGGCTTAGACG-3′) and *amoA-AR* (5′-GCGGCCATCCATCTGTATGT-3′) [5], and AOB gene copies were quantified using primers *amoA-1F* (5′-GGGGTTTCTACTGGTGGT-3′) and *amoA-2R* (5′-CCCCTCKGSAAAGCCTTCTTC-3′) [29]. These primers were used to extract purified DNA in a 25-μl amplification system. The PCR product was analyzed by gel electrophoresis with a 1.0% agarose gel. The sample was treated with fluorescent dye (0.5 μg/ml) before a photo was taken with a gel imager (Bio-Rad, Chemi Doc XRS TOTAL, CA, USA).

2.4. Clone library and phylogenetic analysis

To construct the clone library, the PCR amplification product was purified using a Promega Agarose Gel DNA purification kit (Takara, Japan). The PCR recovery product was connected to the pGEM-T vector (Promega, Madison, WI, USA) before being shifted to DH5α-competent cells (Takara, Japan). This was followed by blue-white sieving. Forty white clones were selected for both AOA and AOB. Positive clones were identified by PCR amplification using the universal primers T7 and SP6, and typical bacterial strains were selected for sequencing (Shanghai Major Bio-Pharm Technology Co., Ltd.). The nucleotide sequence accession number obtained in this study was KM516351-KM516472 for AOA and KM516473-KM516706 for AOB. After amplification of AOA and AOB, 40 clones of AOA and 60 clones of AOB from DS, DZ, DZ5, and JLK were selected out for further sequencing.

DOTUR software [30] was used to independently calculate the operational taxonomic units (OTUs) for each library. The most typical sequence in each OTU was selected for Basic Local Alignment Search Tool (BLAST) similarity comparison on GenBank (http://www.ncbi.nlm.nih.gov/BLAST/). The sequence with the highest similarity was selected using as the reference sequence. ClustalX 1.8 (the National Center for Biotechnology Information, MD, USA) was used for multiple sequence alignment, and the Jukes-Cantor parameter model was applied to calculate evolutionary distances [31]. The Kimura two-parameter calibration model in MEGA 5.0 software [32] was used to verify different evolutionary distances, and then a two-by-two comparison was carried out. A phylogenetic tree was constructed using the neighbor-joining method, and bootstrapping was used to assess the reliability of the tree, with the value of bootstrap set at 1,000 times. DOTUR software was used to calculate a range of indexes, including Shannon–Wiener and Chao1.

The coverage of the clone library (also called the capacity of clone library) was calculated with the following formula: $C = [1- (n1/N)] \times 100\%$. In this formula, *n1* is the number of singleton OTUs, and *N* is the total number of clones in the corresponding clone library [33].

2.5. Denitrified method for measuring nitrogen isotopic composition of nitrate

P. aureofaciens (ATCC 13985) was used for nitrogen isotopic measurements. The bacterial strains and culture conditions accord to Casciotti's research [27]. 2 mL aliquots were poured into 20 mL headspace vials, and purged for 3 h with N_2. 1 g sediment was added to centrifuge tube filled with 5 mL pure water, and soaked for 1 h at 80°C, then 2 ml of supernatant were took to the headspace vials. Finally, $\delta^{15}N$ in N_2O was analyzed by using MAT-253 isotope mass spectrometer.

3. Results and discussion

3.1. Diversity analysis and community structures of the microorganism

The DNA extraction of microbes in the surface sediments of shallow lakes in the study area was relatively complete, which favored the PCR amplification. Gel recovery, purification, and clone sequencing analysis show that the sequence length is 629 and 491 bp. The BLAST comparison showed that the homology between the PCR product sequence and other *amoA* genes through which AOA and AOB could not be cultivated reached 99%, which indicates that the PCR product matched the design and amplification of the experiment well, and that the primers and amplification conditions were suitable for this study. It also indicates that the AOA and AOB sequence has been extracted from the sediments, and therefore, AOA and AOB are present in sediments of DS, JLK, and Lake Dazong. The destination sequence clone quantities were described as Table 1. The Chao1 and Shannon–Wiener (H) indexes of each OTU were calculated using DOTUR software (Table 1). The plasmids of surface sediments from JLK were complicated, so the quantity of destination sequences (16) obtained at the time of clone sequencing was considered small, and the diversity index of JLK could not be calculated with DOTUR software. Analysis by the clone library showed that the coverage rates were all higher than 65%, which indicates that the library included most of the AOA and AOB *amoA* gene types.

Table 1
Comparison of microbial diversity by coverage rate, Shannon and Chao1 from clones in sampling sites in the sediments of lakes in the Lixiahe region

Ammonia-oxidizing microorganisms	Sampling stations	No. of clones	No. of OTUs	C %	H	Chao1
AOA	DS	27	8	70.37	1.9259	8
	DZ	40	9	77.50	1.8285	9
	DZ5	39	14	64.10	2.3307	19
AOB	DS	59	15	74.58	2.1855	51
	DZ	58	4	93.10	0.6778	5
	DZ5	60	8	86.67	1.3332	14
	JLK	58	9	84.48	1.3541	19

The Shannon–Wiener diversity index of AOA was the greatest in DZ5 and was slightly lower in DS and DZ. Values of the Shannon–Wiener diversity index was the lowest for DZ. We compared the differences in the AOA community structure complexities at DS, DZ, and DZ5. Diversity and richness were the highest at DZ5, located at the center of Lake Dazong, but were relatively low at DZ and DS. To investigate the diversity and community composition of ammonia-oxidizing populations, four sediment samples were selected for construction of the clone libraries of archaeal and bacterial. Abundances of bacterial and archaeal *amoA* gene in sediment samples collected from three lakes are shown on Table 2.

Table 2
Abundances of AOA and AOB in sediments of Lake Dazong, Lake Desheng and Lake Jiulongkou

Sample size	AOA (copies/g dw)	AOB (copies/g dw)
DS	3.27×10^5	1.28×10^7
JLK	None	4.13×10^6
DZ	1.16×10^6	7.44×10^5
DZ5	1.38×10^7	1.04×10^6

Based on the 99% homology, the AOA of DS, DZ, DZ5, and JLK were categorized into 16 OTUs, and AOB were categorized into 20 OTUs. A representative sequence was selected out from each of these OTUs, and the reference sequence with a similar source was searched through BLAST. After the given sequence was added, the phylogenetic tree was set up with Mega 5 software, as shown in Fig. 2. In this study, there were 122 AOA sequences in Group 1.1b and Group 1.1a. Out of the 122 destination sequences, 87 sequences belonged to Group 1.1b, accounting for 71.3%. Among these 87 sequences, 25 were derived from DS, 4 from JLK, 21 from DZ, and 37 from DZ5, respectively. This accounted for 92.6%, 52.5%, 94.9%, and 25% of the destination sequences of DS, DZ, DZ5, and JLK, respectively. For other 35 sequences belonged to Group 1.1a, there were 2 (7.4%), 19 (47.5%), 2 (5.1%), and 12 (75%) sequences from DS, DZ, DZ5, and JLK, respectively. Therefore, the AOA were mainly in Group 1.1b at DS and DZ5, but in Group 1.1a at JLK. The percentages of AOA in Group 1.1a and Group 1.1b at DZ were similar.

There were 235 AOB sequences in groups of *Nitrosomonas* and *Nitrosospira*. The AOB were mainly in *Nitrosomonas*, with 217 out of the 235 destination sequences belonged to *Nitrosomonas*. For the 87 sequences in Group 1.1b, there were 13 and 72 sequences were clustered into the sub-clusters of 111clone5D 11 GenBank (EU671952) and 222cloneCF-SAOA-24 GenBank (GQ906649), respectively. For the 13 sequences in 111clone5D 11 GenBank, 5 were from DS, 2 from DZ, and 6 from DZ5. For the 72 sequences in 222cloneCF-SAOA-24 GenBank, 20 were from DS, 19 from DZ, 30 from DZ5, and 3 from JLK. Out of the 16 OTUs, only OTU1, which belongs to the Group 1.1b sub-cluster, exists in all the samples from DS, DZ, DZ5, and JLK, accounting for 21.3%. The OTU with the largest quantity is OTU1 and is very similar to the archaea sequence discovered in sediment samples from the Yangtze River estuary [34]. The 87 sequences of Group 1.1b are clustered into 11 OTUs. The reference sequences of these 11 OTUs include KJ496929 from wastewater treatment systems; KC108808 from the sediments of Dongting Lake; KF857068 from river sediment; KC735552, KC735379, and KC735383 from the sediment of Yangtze River estuary; KF537016 from the sediment of the Beijing-Hangzhou Grand Canal; JQ698526.1 from the sediment at the outlet of Chaohu Lake; and KF169744 from mangrove forest soil. The 35 sequences from the Group 1.1 sub-cluster are divided into five OTUs; their reference sequences included FJ601570 separated from the sediment of tropical estuary; KF898586 and KC735494 from alpine lakes, and KJ005026 and KF413247 from the Yangtze River estuary. The homologies of the gene segments of the representative sequences of the 16 OTUs and their respective reference sequences at the nucleotide level are all 99% and have a very high degree of similarity.

The 235 sequences of AOB were clustered into four clusters. For the 68 sequences (28.9%) in cluster 1, 21 of them were from DS, 14 from JLK, 2 from DZ, and 31 from DZ5. The reference sequences of cluster 1 include KC735739, KC758470, KC735650, JX157932, KF803116, and KJ497222 from the Yangtze River estuary, abandoned mining ponds, sediments in Sandy river, and wastewater treatment systems. There were 147 AOB sequences (62.6%) in cluster 2, with 32 of them from DS, 39 from JLK, 50 from DZ, and 26 from DZ5. The reference sequences of cluster 2 were from sediments of eutrophic lake (JF905885), urban lake (FJ498948), Yangtze River estuary (KC736034), and wastewater treatment systems (JQ277664 and JQ277611). There were only 2 sequences (0.9%) from DS belong to cluster 3, which were similar with wastewater treatment systems (JQ277652 and KJ497262). Sequences of cluster 1, cluster 2, and cluster 3 were mainly in

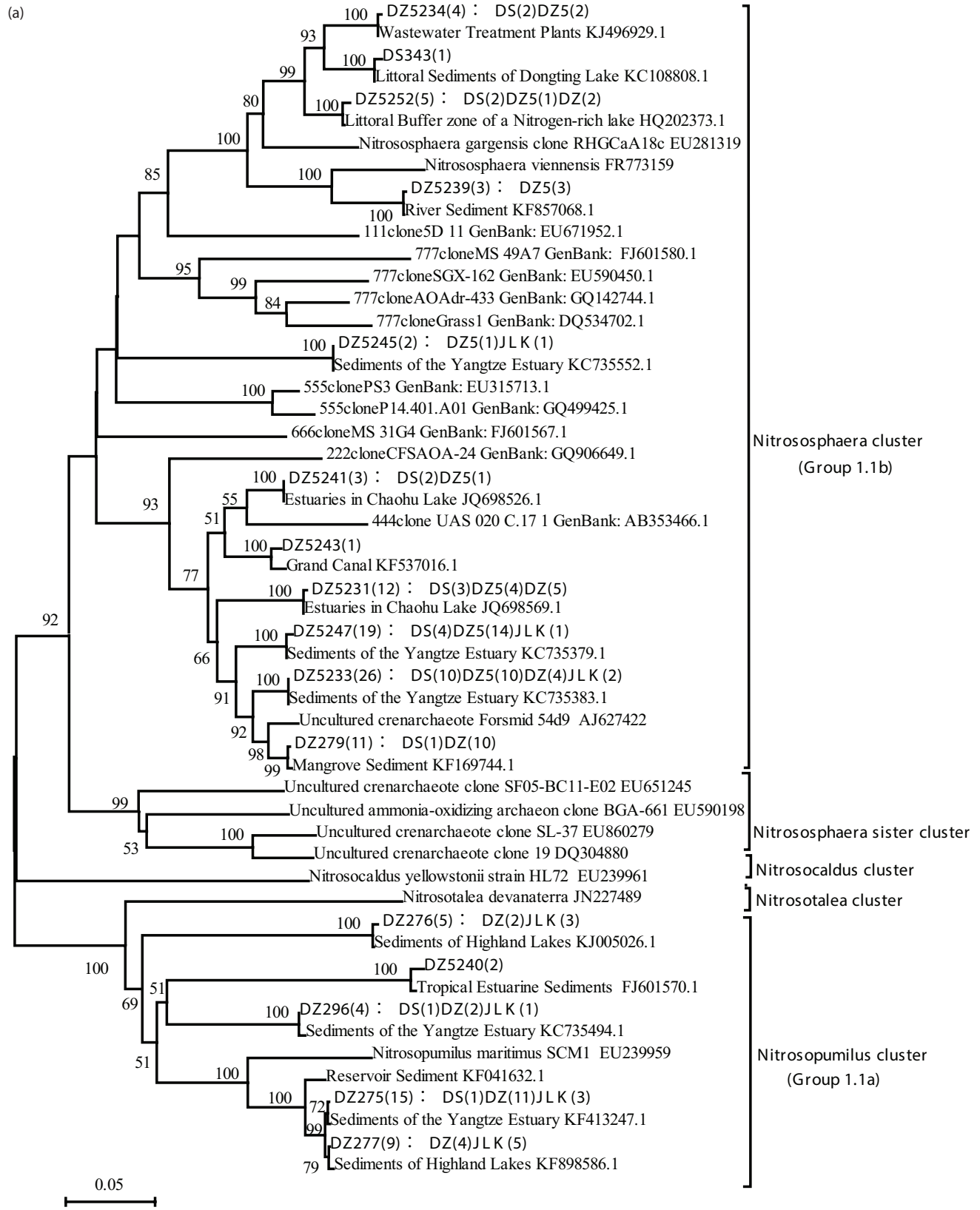

Fig. 2. Neighbor-joining phylogenetic tree of archaeal *amoA* gene sequences from the sediments of lakes in the lower reach of the Lixiahe region: (a) AOB.

Note: Numbers at nodes represent the percentages bootstraps resampling based on 1,000 replicates; only the values higher than 50 are presented to indicate at branch points. Branch lengths correspond to sequence differences as indicated by the scale bar.

(b)

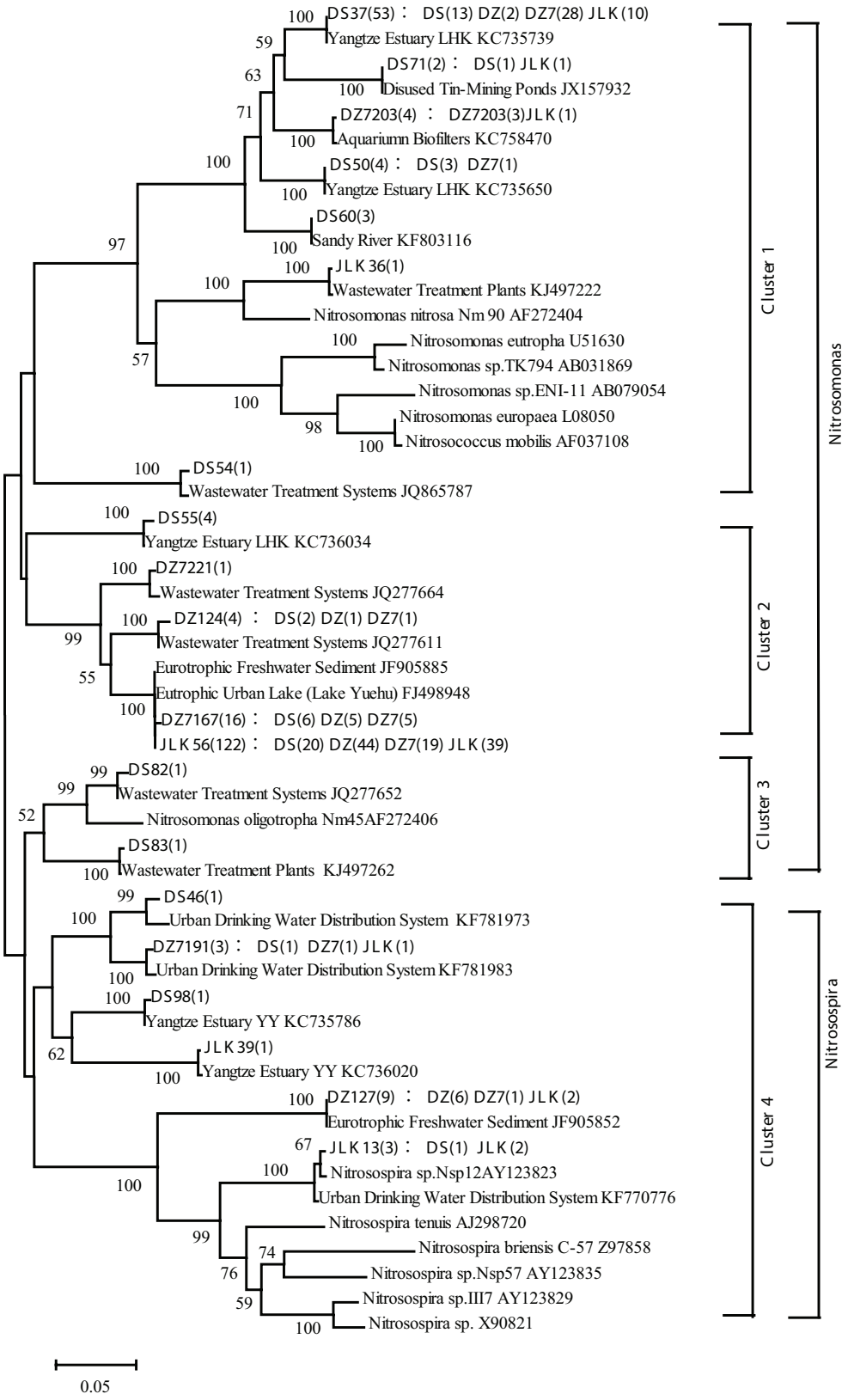

Fig. 2. (Continued) Neighbor-joining phylogenetic tree of archaeal *amoA* gene sequences from the sediments of lakes in the lower reach of the Lixiahe region: (b) AOA.

Note: Numbers at nodes represent the percentages bootstraps resampling based on 1,000 replicates; only the values higher than 50 are presented to indicate at branch points. Branch lengths correspond to sequence differences as indicated by the scale bar.

Nitrosomonas. Other 18 AOB sequences were in cluster 4, with 4 of them from DS, 6 from JLK, 6 from DZ, and 2 from DZ5. Sequences of cluster 4 were mainly in *Nitrosospira*. The reference sequences of cluster 4 include Urban drinking water distribution system (KF781983 and KF770776), Yangtze River estuary (KC735786 and KC736020), and sediments in eutrophic freshwater system (JF905852).

The *amoA* gene sequence of AOA in the lake sediments in Lixiahe region has a similarity of more than 97% with *Nitrososphaeraviennensis* (FR773159) and uncultured crenarchaeote Forsmid 54d9 (AJ627422), which comes from soil. Its similarity to the *Nitrososphaeragargensis* clone RHGCaA18c (EU281319), which is concentrated in thermal springs, reaches 93% [12]. However, its similarity to *Nitrosopumilusmaritimus* SCM1, a marine AOA bacterial strain that was successfully separated from an oceanarium in Seattle, WA, USA, is as low as 71% [35].

Out of the 122 destination sequences, 71.3% belong to Group 1.1b, and 28.7% belong to Group 1.1a. As pointed out by Schleper et al. [11], the AOA of oceans, most water bodies and sediment sources are clustered in Group 1.1a, and the AOA of soil and other terrigenous environment sources are clustered in Group 1.1b. Therefore, AOA in lake sediments in the Lixiahe area are mainly from soil and other terrigenous environment sources, which is similar to the results found in sediments in Lake Taihu by Wu et al. [14]. Based on cluster analysis on more than 100 archaea *amoA* gene sequences, Francis et al. [5] found that the distribution of AOA types was different among habitats and sampling points. Among the four sampling points selected for current research, 92.6% of the AOA from DS are mainly from Group 1.1b. The DS is a river channel-type lake. Subject to the influence of industrial wastewater and sewage from the surroundings, the AOA from DS are mainly from soil and other terrigenous environment sources.

In contrast, AOA from the center of Lake Dazong are also mainly from Group 1.1b, accounting for 94.9%. Because this site located at the center of the lake, there is much less AOA from terrigenous environments. The high richness of the Group 1.1b sub-cluster is possibly attributable to the rich submerged plants at this site. Phylogenetic analysis shows that the AOA in DZ5 have the highest diversity and richness, while the AOA in DZ and DS have the lowest diversity and richness. Submerged plants can influence the microbial community composition of rhizospheres by releasing DO, organic matter, and nutrient elements, thus affecting the nitrogen cycling process of shallow lakes at the sediment–water interface [36]. Herrmann et al. [37] indicate that plant rhizosphere can increase the richness of AOA in sediments by releasing oxygen and organic carbon in oligotrophic, mesotrophic, or eutrophic lake ecosystems. There are many submerged plants such as *Hornwort* and *P. prispus* at DZ5, which, once they die, were settle onto the bottom of the lake. As a result, the TN content of sediments is very high, and more than twice the TN content of sediment from DS, DZ, and JLK. The submerged plants around DZ5 were the reason for higher richness of AOA in Group 1.1b; it also resulted in the higher AOA diversity and richness at DZ5 than at DS and DZ. Therefore, the high organic content in surface sediments is conducive to the growth of AOA. However, several previous studies indicated that the high organic content in sediments may have an inhibiting effect upon AOA [35]. For the sampling site of DZ, the sequences belong to Group 1.1b and Group 1.1a are 21 and 19. The AOA from both groups are almost balance. It indicates that the main sources of AOA at this sampling point are the erogenous environment and the Lake Dazong water body and sediments.

In the clone library construction process, 40 typical bacterial strains from DS and JLK were selected for sequencing after white clone cultivation. After running a BLAST comparison, it was found that only 27 and 16 strains from DS and JLK, respectively, were AOA sequences. The other sequences are plasmid sequences, which have primer binding sites. The environments of DS and JLK were quite different from those of DZ and DZ5. DS was located in a river channel-type lake; the features of AOA community structures were strongly influenced by riverbank environments of DS. Field investigations indicated that there are factories, sandpits and other industries around the riverbanks of DS. Additionally, the surface of DS is mostly fenced for fish and crab breeding. Site JLK was at the southern inlet of JLK and the sediments in this part of the lake had been dredged just before sampling. Fish and livestock breeding are mainly found in the area upstream of JLK. All of these factors may have significantly influenced the distribution of AOA community structures in surface sediments. Therefore, we had to identify and add a new specific primer pair for pre-cloning PCR amplification to ensure that the clone library had a sufficient coverage rate, and that it more accurately reflected the community structure features of AOA in the sedimentary environment. In contrast, DZ was located at the outlet of Lake Dazong, while DZ5 was located at the center of the lake. The community structures of AOA in the surface sediments at both sites were somewhat similar to those of ordinary shallow lakes or estuaries. We can bring a desirable coverage rate to the clone sequence by using a universal specific primer pair. The research results of Jin et al. [38] also indicated that two or more pairs of primers may be needed for a more accurate result when new environmental samples are studied. In this research, three pairs of primers were compared to set up the AOA clone library. The results show that individual taxonomic units can possibly be obtained through specific primers. Therefore, to study the AOA in DS and JLK more accurately and thoroughly we need to identify suitable specific primers that meet the requirements of setting up a clone library.

3.2. Distribution of NH_4^+ and NO_3^- in the water-sediment surface and response mechanism for the ammonia-oxidizing prokaryotes

Table 3 shows the features of N concentrations in water samples of Lake Dazong, DS and JLK. The average concentration of NH_4^+ was 0.58 mg/L, with the range between 0.29 and 0.99 mg/L; the average concentration of NO_3^- was 0.54 mg/L, ranging between 0.27 and 0.83 mg/L. The difference between TN concentrations and inorganic nitrogen (NH_4^+, NO_3^-) concentrations could be considered as the organic nitrogen. The organic nitrogen of overlying water is the main part of TN in Lake Dazong, DS and JLK. The concentrations of NH_4^+ and NO_3^- in interstitial water at the depth of 5 cm are shown in Fig. 3. The concentrations of NO_3^- in interstitial water at DS, DZ and DZ5 generally are lower than the concentrations of NH_4^+, while the contrast trend appears in the JLK sample.

Table 3
TN, NO_3^- and NH_4^+ concentrations in water samples of Lake Dazong, Lake Desheng and Lake Jiulongkou (data are presented as mean ± standard deviation, n = 3)

Sample size	TN (mg/L)	NO_3^- (mg/L)	NH_4^+ (mg/L)
DS	5.41 ± 0.23	0.83 ± 0.04	0.67 ± 0.03
JLK	2.98 ± 0.15	0.56 ± 0.02	0.99 ± 0.05
DZ	2.93 ± 0.11	0.27 ± 0.01	0.32 ± 0.02
DZ5	3.05 ± 0.09	0.48 ± 0.02	0.29 ± 0.01

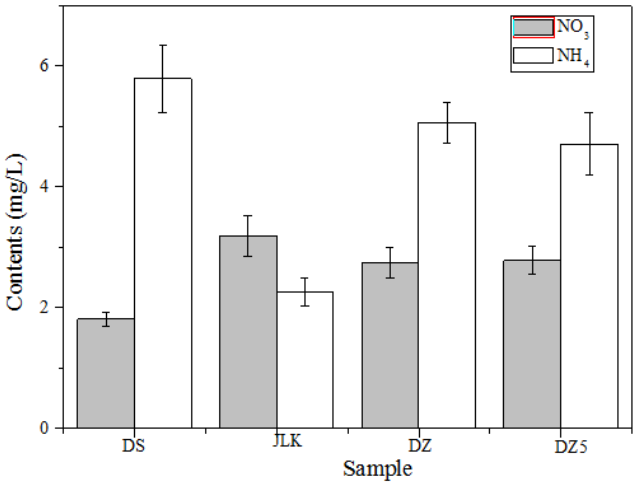

Fig. 3. Distribution of NO_3^- and NH_4^+ in interstitial water of lakes in the lower reach of the Lixiahe region.

The concentrations of NH_4^+ and NO_3^- in sediments in DZ, DZ5, DS and JLK are shown in Fig. 4. The maximum values of NH_4^+ were found in DZ sediment core. In general, the maximum NO_3^- concentrations were found in the surface sediment of DS.

The 235 destination sequences of AOB are all β-Proteobacteria in the Lixiahe region, Out of the which, 92.3% belong to *Nitrosomonas*, and 28.7% belong to *Nitrosospira*. From Figs. 3 and 4, it is seen that the mean concentrations of NH_4^+ were rich in the surface sediments (>20 mg/kg), and the *Nitrosomonas* in four sample sites were rich too. The results are consistent with Schramm's [39] study that *Nitrosomonas* have a competitive advantage when they were in the environment rich in ammonia, while the *Nitrosospira* have a competitive advantage in the environment poor in ammonia. Due to the effect of long-term fertilization treatment on the composition of AOB community structure [40], the non-point source pollution brought by the long-term farmland fertilization around DS and the livestock and poultry breeding in the upstream of JLK has increased the abundance and diversity of AOB. A higher diversity and relative abundance has promoted the transformation of NH_4^+ in the sediments to NO_3^-, thus resulting in the highest NO_3^- concentration on the DS sample (followed by JLK), and resulting in the NH_4^+ concentration of the surface sediments being JLK < DS < DZ < DZ5 (Fig. 4). Due to the existence of concentration gradient, the NO_3^- in the interstitial water enters the overlying water through the diffusion effect, thus adding the nitrate nitrogen

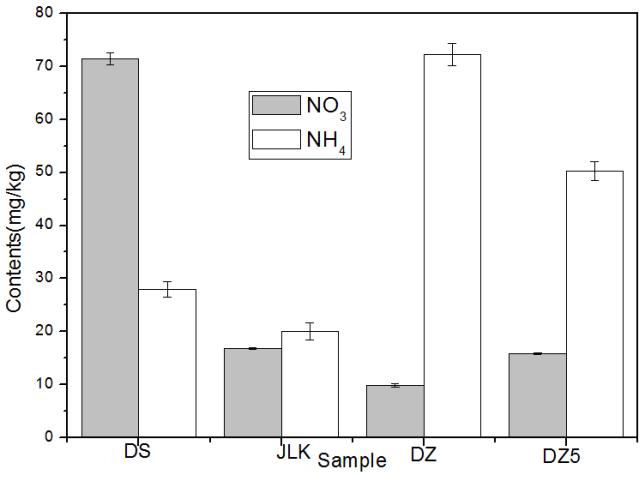

Fig. 4. Distribution of NO_3^- and NH_4^+ in sediments of lakes in the lower reaches of the Lixiahe region.

in the overlying water, and promoting the nitrate nitrogen concentration in the DS overlying water (Table 3). The diversity and relative abundance of AOB in the JLK surface sediments are lower than those of DS, which is related to the fact that JLK has just been dredged. The NH_4^+ concentration of DZ is the highest, and NO_3^- is the lowest, which is maybe because long-term fenced breeding has reduced the diversity and relative abundance of AOA and AOB, thus reducing the quantity of transformation from NH_4^+ to NO_3^-.

The NH_4^+ content and the TN content in the surface sediments at the four sampling points are approximate in order and are contrary to the size order of the Shannon–Wiener index and Chao1 index of AOB. The interstitial NO_3^- content is consistent with the size order of diversity index and abundance index. A higher diversity and abundance gives the AOB community in surface sediments stronger feedback ability to environment, ensures the greater buffer ability of AOB community structure, promotes the progressing of nitration reaction, and increases the generation of NO_3^- at the place of DS and JLK, which is released into the overlying water. The TN and ammonia nitrogen content in DZ surface sediments is the highest, while the nitrate nitrogen content is the lowest, which is maybe because there is a lower diversity and abundance of AOB at this sampling point and there is little nitrate nitrogen transformed from ammonia nitrogen. The AOB diversity and abundance at DZ5 are relatively low, with a relatively weak nitration reaction and a relatively low NO_3^- content. In addition, the abundant submerged plant at this sampling point directly absorbs and assimilates nitrate nitrogen as its own organic substance in the process of growth, which may also be a reason for why the surface nitrate nitrogen content at this sampling point is relatively low.

The NO_3^- migration and transformation process in the interstitial water is not only subject to the conditions of sediments but also subject to the penetration effect of overlying water. Moreover, the difference of DO and bioactivity of sediments-water interface will also affect the vertical distribution of interstitial NO_3^-. The interstitial NO_3^- content at the 0.5 cm height of DS and JLK is higher than that at the height

of 1 cm, which is maybe because the DO is high in the surface sediments of above 0.5 cm, and the AOB diversity and abundance indexes are relatively high. Under the condition of DO sufficiency, the nitrate nitrogen content would increase as the nitration speed increases. In contrast, the NO_3^- content in DZ and DZ5 overlying water is relatively low and the NO_3^- in the interstitial water enters the overlying water. In addition, the growth of *Potamogetoncrispus* and hornwort at DZ5 will also absorb NO_3^- from the interstitial water of surface, thus reducing the NO_3^- content at 0.5 cm of the surface at these two points. The NO_3^- in the interstitial water of DS and JLK will increase as the depth increases between 1 and 8 cm, while the change of DZ5 is moderate and DZ shows a decreasing trend. The NO_3^- contents at the 4 sampling points meets the DS > JLK > DZ5 > DZ and accords with the order of AOB diversity and abundance. This demonstrates that the AOB diversity and abundance play an important role to the NO_3^- migration and transformation of sediments-water interfaces in the shallow water lakes in the region. DS has the highest AOB diversity and abundance in its surface sediments. The relatively high diversity and abundance of DS accelerate the transformation of ammonia nitrogen to nitrate nitrogen, reduce the ammonia nitrogen in surface sediments and increase the nitrate nitrogen in the sediments and overlying water.

3.3. Response mechanism of AOA and AOB abundance with different nitrogen pollutant sources

The *Nitrosospira* community of surface sediment AOB at the DZ5 sampling point accounts for 3.34% of the overall clone sequence of this sampling point. The nitrogen isotope at this point measured with the anti-nitration method is 10.0‰. In connection with the denseness of submerged plants at this point, we can learn that the nitrate in the sediments at this point mainly comes from decomposition of plants. In contrast, the *Nitrosospira* community at the other three sampling points accounts for a percentage in the overall clone sequence, which is over twice of the DZ5 percentage. Therein, *Nitrosospira* at the DS sampling point accounts for 6.8% of the overall clone sequence. Both the *Nitrosospira* communities of surface sediment AOB at the sampling point of DZ and JLK account for 10.4% of the overall clone sequence at this sampling point. The nitrogen isotope ratios at the point of DS, DZ and JLK are positive, being 8.29‰, 5.69‰ and 8.84‰, respectively. In connection with the site investigation, these three points not only have the afflux of breeding pollution but also have agricultural non-point source pollution, thus resulting in the higher *Nitrosospira* community at the three points than that of DZ5, which has no afflux of agricultural non-point source pollution. This has common characteristics with researches of Ibekwe et al. [41]. He finds out that AOB focuses on *Nitrosospira* in the processing system of farming and animal husbandry wastewater wetlands, while AOB focuses on *Nitrosomonas* in the livestock feces and flushing water.

DS has the highest AOB diversity and abundance in its surface sediments, which is maybe related to its complicated AOB source. The nitrate source-determining result of DS is mixed pollution (8.29‰). Therefore, the complexity of surroundings can also affect the diversified distribution and relative abundance of AOB in surface sediments. The AOB diversity and nitrogen isotope ratio in DZ surface sediments is lower than those of DS, DZ5 and JLK, which is related to the fact that the surface sediments at this sampling point are highly subject to the direct influence of fenced breeding. The long-term fenced breeding has increased the injection of artificial pollution source, reduced the ratio of nitrogen isotope, and resulted in the reduction of diversity and relative abundance at this sampling point. Therefore, the difference of pollutant source has resulted in the difference of nitrate nitrogen $\delta^{15}N$ in the lake surface sediments with different pollutant sources in the same region. The long-term fenced breeding (DZ) will result in the reduction of permillage (5.7‰) value of δ15N and the reduction of AOB abundance and diversity. The long-term excess accumulation of decayed plants (DZ5) will result in the increase of permillage (10.0‰) value of $\delta^{15}N$ and the reduction of AOB abundance and diversity.

4. Conclusion

The AOB diversity and abundance play an important role to the NO_3^- migration and transformation of sediments-water interfaces in the shallow water lakes in the region. Desheng Lake has the highest AOB diversity and abundance in its surface sediments. The relatively high diversity and abundance of DS accelerate the transformation of ammonia nitrogen to nitrate nitrogen, reduce the ammonia nitrogen in surface sediments and increase the nitrate nitrogen in the sediments and overlying water. The results of nitrogen isotope shows that: the sediments, which not only have the afflux of breeding pollution, but also have agricultural non-point source pollution, have the higher *Nitrosospira* community than that of sediment, which has no afflux of agricultural non-point source pollution. The long-term fenced breeding will result in the reduction of the ratio of nitrogen isotope ($\delta^{15}N$) and the reduction of AOB abundance and diversity. The long-term excess accumulation of decayed plants will result in the increase of the ratio of nitrogen isotope ($\delta^{15}N$) and the reduction of AOB abundance and diversity.

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