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Original Research

The Abundance and Community Composition of Ammonia-Oxidizing Prokaryotes in Small-Reservoir Sediments in China's Huashan Watershed

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Abstract

We investigated the effects of nutrient levels on the abundance and diversity of ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB), seven surface sediment samples from small reservoirs at different nutrient levels were collected from the eastern, central, and western parts of Huashan watershed in Chuzhou, Anhui Province to determine the abundance and community composition of AOA and AOB. The results showed that the abundance of bacterial *amoA* gene (1.85×10^7 to 2.86×10^8 g/dry sediment) was higher than that of archaeal *amoA* gene (1.25×10^5 to 1.23×10^6 g/dry sediment) in all sediment samples. The abundance of the archaeal *amoA* gene exhibited significant positive correlations with total nitrogen concentrations, whereas the abundance of bacterial *amoA* gene showed significantly negative correlation with pH. Archaeal *amoA* gene sequences included *Nitrososphaera* and *Nitrosopumilus* clusters and the majority of *Nitrosospira* and *Nitrosomonas oligotropha* lineages.

Keywords: nitrogen cycling, ammonia-oxidizing prokaryotes, community composition, nutrient

Introduction

Nitrogen (N) is an essential nutrient and limits biological productivity in most freshwater and marine ecosystems [1]. Nitrification, mediated by the microbial process, plays a central and critical role in global nitrogen cycling in both soils and sediments [2-3]. Bacteria were thought to be responsible for catalyzing the first and rate-limiting step of this process [3]. Recent studies have found that, in addition to ammonia-oxidizing bacteria (AOB), ammonia-oxidizing archaea (AOA) also could catalyze the process [4], and that AOA exists widely in various ecosystems [5-11].

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Previous studies have investigated the distribution of AOA and AOB in lake sediments [9-13], the rhizosphere of freshwater macrophytes [14], estuary sediments [15], mangrove sediments [16], and marine sediments [1, 17]. It has been found that AOA dominated in marine sediments [1, 17], indicated by its better adaptation to anaerobic, low pH, and low ammonia habitats than AOB [18-19], whereas higher nutrients in the freshwater sediments promoted AOB dominance, such as river sediments [20], mangrove sediments [16, 21], and shore side wetlands [22]. Zhao et al. [13] found that the abundance of archaeal *amoA* in lake sediment was higher than bacterial *amoA*, whereas Moiser and Francis [23] found that bacterial *amoA* copy numbers were greater than archaeal *amoA* in most of the estuary. This contradiction could be attributed to the different adaptations of the two ammonia-oxidizing groups to nutrients in different nutrient level sediments. It is important to figure out the relationships between the abundance and community composition of AOA and AOB and the sediment properties, including pH- and nitrogen-associated factors.

Previous studies have indicated that environmental factors such as pH [24], temperature [25], ammonium concentration [26], nitrogen fertilization [27-28], and organic carbon [28-29] could affect the abundance and community composition of AOA and AOB. pH significantly affected the AOA community in river sediments [20]. Elevated temperature increased AOA abundance, whereas it decreased AOB abundance [30]. Strong correlations were found between ammonium concentrations and the abundance of both AOA and AOB in mangrove [21] and freshwater sediments [9, 11]. Other nutrient elements such as available phosphorus could affect the community composition of AOA [9, 12]. Moreover, the concentration of organic carbon was found to significantly correlate with the community composition of AOA and AOB in the intertidal sediments of the Yangtze Estuary [15]. Moreover, the effects of nutrient levels on the abundance, diversity, and community compositions of AOA and AOB have been investigated. Wang et al. [31] investigated the effects of nitrogen fertilizer in rice soil and found that three major AOB groups (*Nitrosomonas communis* cluster, *Nitrosospira* clusters 3a and 3b) were clearly affected by N fertilizer, whereas the AOA community remained unchanged. Evidence revealed that AOB and AOA preferred different N levels for growth, with AOB growing significantly only at high ammonia nitrogen levels and AOA growing substantially at low ammonia nitrogen levels [32]. However, most of these results have been obtained in the soil ecosystem, which left gaps in the freshwater sediments.

Huashan watershed has been exploited by agriculture for years. The small reservoirs all over the watershed have been influenced by agricultural runoff. The reservoirs have been confirmed to be severely eutrophic with nitrogen input [33]. pH- and nitrogen-associated factors have been found to have significant effects on the community composition of AOA and AOB in soils [24, 27-28, 31]. Therefore, it is necessary to find out the differences of abundance and community composition of AOA and AOB at different nutrient levels in these reservoir sediments. In the present study, seven sediment samples from seven freshwater reservoirs with different nutrient levels were collected and subjected to chemical and molecular biological analysis to elucidate the effects of nutrient levels on the abundance and diversity of AOA and AOB.

Materials and Methods

Sample Collection

Our study was conducted in Huashan watershed in the eastern part of Chuzhou City in Anhui Province, China. Seven reservoirs were selected for sampling within the eastern, central, and western parts of the watershed on 27 November 2013, namely ZC (Zhuchong Reservoir: 32.2959N, 118.188898E), WY (Wangying Reservoir: (32.26846N, 118.156268E), SSK (Shishankou Dam: 32.28577N, 186.18611E), LQ (Longquan Reservoir: 32.24426N, 118.17009E), YF (Youfang Dam: 32.25742 N, 186.199439E), DSW (Dashanwa Reservoir: 32.22676N, 118.189137E), and HHQ (Honghuaqiao Reservoir: 32.27961N, 118.233967E). These seven small reservoirs are located at the branches of the main stream in this watershed. Restricted to agricultural nitrogen fertilizer, these sampling reservoirs were under the impact of the nitrogenous process. Three sampling sites were randomly selected as replicates at each target reservoir. Surface sediment columns were collected at each site with the columnar sediment sampler (11 cm diameter; HYDRO-BIOS, Germany). All samples were transferred to sterile tubes immediately after sampling, refrigerated with ice, and then transported to a laboratory for further analysis. Surface sections (0-1 cm) of each site were separated. Water samples were collected at 0.5 m depth in each reservoir and the concentrations of COD_{Mn} were measured using titration. The air temperature was 7ºC on sampling day.

Physicochemical Analysis of Sediment Samples

All seven sediment samples were dried with a freeze dryer (ALPHA1-2, CHRIST, Germany). Total nitrogen (TN) was measured using an elemental analyzer (EA3000, Euro Vector, Italy). Sediment ammonia and nitrate concentrations were extracted with 2 M KCl and measured by a continuous flow analyzer (San++, SKALER, Netherlands). Sediment samples were measured for pH after being treated with 2 M KCl. The physicochemical analysis included three replicates for each sample.

DNA Extraction

DNA was extracted from 0.25 g sediment sample using a PowerSoil DNA Isolation Kit (MoBio Laboratory, Solana Beach, CA). DNA was extracted from the three

replicates and combined. The extracted DNA was checked on 0.8% agarose gel and the concentration of the extracted DNA was determined using a BioPhotometer (Eppendorf, Hamburg, Germany).

Real-Time Quantitative PCR

Real-time quantitative PCR was performed using SYBR Green I on an IQ5 Thermocycler (RG65HD, Corbett, Australia). The primers for archaeal *amoA* PCR amplification were Arch-amoAF/Arch-amoAR [1], and the primers for bacterial *amoA* PCR amplification were amoA-1F/amoA-2R [34]. The standard curve was constructed by 10-fold serial dilutions of plasmid DNA of a known concentration covering 3.40×10^2 to 3.40×10^8 of archaeal *amoA* copies per µl, and 1.59×10^2 to 1.59×10^8 of bacterial *amoA* copies per µl.

The volumes of reaction mixtures for archaeal and bacterial *amoA* PCR amplification were 20 µl, including 5 ng of DNA template, 1 × SYBR Premix Ex Taq buffer (Takara, Japan), and 0.2 µM each of archaeal or bacterial *amoA* primers. For the thermal cycles, the protocol for archaeal *amoA* PCR amplification was as follows: 3 min at 95ºC, 45 cycles of 30 s at 95ºC, 1 min at 53ºC, 20 s at 72ºC, and 7 min at 72ºC. The protocol for bacterial *amoA* PCR amplification was as follows: 3 min at 95ºC, 45 cycles of 30 s at 95ºC, 1 min at 55ºC, 20 s at 72ºC, and 7 min at 72ºC.

Melting curve and 2% agarose gel electrophoresis were employed to check the specificity of the PCR products. Data analysis was carried out with the Rotor-Gene 6000 software package. The PCR amplification efficiencies were 0.97-1.02 for archaeal *amoA* and 0.95-0.98 for bacterial *amoA*. The obtained data were calculated with the mass of the sediment samples to determine the abundance of archaeal and bacterial *amoA* copies per gram sediment (dry weight).

Clone Library and Phylogenetic Analysis

Clone libraries were constructed with the DNA extracted from the seven sediment samples. PCR reactions were carried out with Ex Taq DNA polymerase (Takara, Otsu, Japan) in all *amoA* PCR amplifications. Triplicate PCR products were pooled, gel-purified using the Axygen PCR cleanup purification kit, and cloned by the pGEM-T vector (Promega, Madison, WI, USA). The resulting ligation products were used to transform to *Escherichia coli*-competent cells (DH5α, Takara, Japan). Picked transformants were grown overnight on LB agar plates containing 100 µg/mL ampicillin, 40 µg/mL X-Gal, and 24 µg/mL IPTG. Clones were checked by PCR amplification using vector primers (T7 and SP6) and conveyed to Shanghai MajorBio Biotechnology Co., Ltd. for DNA sequencing.

The sequences acquired in accordance with the above procedure were translated into conceptual protein sequences. All *amoA* sequences were compared with GenBank database sequences using BLAST. Operational taxonomic units (OTUs) were defined as sequence groups in which sequences differed by ≤ 5% nucleotide differences. Amino acid sequence alignments were generated using ClustalX [35]. Indices of diversity and nonparametric richness estimations were performed using DOTUR software [36]. The estimated coverage of the constructed *amoA* gene sequences was calculated as $C = [1- (n_1/N)] \times100\%$, where n_1 is the number of singletons sequences and N is the total number of sequences. Neighbor-joining phylogenetic trees (based on Jukes-Cantor distances) were constructed based on alignments of amino acid sequences using MEGA4.0 [37].

Statistical Analysis

Significant differences of sediment properties and the abundance of archaeal or bacterial *amoA* were assessed by one-way ANOVA and post hoc comparisons with the SPSS 13.0 package (SPSS, Chicago, IL). Correlation analysis (two-tailed Pearson correlation coefficients) between *amoA* abundance and environmental factors were also performed. P-values <0.05 were considered statistically significant.

Nucleotide Sequence Accession Numbers

The *amoA* sequences obtained in this study have been deposited in the GenBank database under accession numbers KJ583248-KJ583307, KJ583338-KJ583457, and KJ583488-KJ583517 for archaeal *amoA* sequences, and KJ583548-KJ583643, KJ583691-KJ583875, and KJ583924-KJ583973 for bacterial *amoA* sequences.

Results

Physicochemical Properties of Sediment Samples

The pH of sediments collected in the present study was generally neutral (Table 1). The lower pH was found in the sediments of Zhuchong (pH = 6.66) and Longquan (pH = 6.98) reservoirs. The pH values of sediment samples in Wangying and Honghuaqiao reservoirs were highest (7.36 and 7.34). The concentration of COD_{Mn} in Wangying Reservoir was remarkably lower (2.42 mg/L) than those of other samples, whereas higher concentrations of COD_{Mn} were found in Zhuchong (7.88 mg/L), Longquan (7.32 mg/L) and Dashanwa (7.48 mg/L) reservoirs. The nitrate concentration of the sediment sample collected from Shishankou Dam (0.86 mg/kg) was significantly higher than that of the others, whereas the nitrate concentrations in sediment samples of Youfang Dam (0.46 mg/kg), Dashanwa Reservoir (0.36 mg/kg), and Wangying Reservoir (0.25 mg/kg) were significantly lower. The highest ammonia nitrogen concentration was found in the sediment sample of Longquan Reservoir (47.87 mg/kg). Lower concentrations of ammonia were found at Dashanwa (15.31 mg/kg) and Honghuaqiao

Table 1. The physicochemical parameters of different sediment samples collected from Huashan Watershed. Data are presented as means ± standard deviation (n = 3). Different letters indicate significant differences between samples.

Sample sites	pH	COD_{Mn} (mg/L)	NO_3^--N (mg/kg)	NH_4^+-N (mg/kg)	TN (mg/kg)
ZC	6.66	7.88	0.65±0.11^{c}	19.65±2.15^{a}	590.73±27.62^{a}
WY	7.36	2.42	0.25±0.02^{a}	39.26±3.39^{c}	1315.68±36.54^{d}
SSK	7.26	5.08	0.86±0.07^{d}	31.27±3.89^{b}	922.59±37.81^{b}
LQ	6.98	7.32	0.69±0.04^{c}	47.87±5.95^{d}	1097.21±83.99^{c}
YF	7.01	5.50	0.46±0.05^{b}	28.58±3.85^{b}	1053.79±124.38^{c}
DSW	7.22	7.48	0.36±0.04^{ab}	15.31±1.91^{a}	880.22±63.68^{b}
HHQ	7.34	5.60	0.76±0.10^{cd}	16.02±1.37^{a}	580.12±21.51^{a}

COD_{Mn}: chemical oxygen demand; NO_3^--N: nitrate nitrogen, NH_4^+-N: ammonia nitrogen, TN: total nitrogen, ZC: Zhuchong Reservoir, WY: Wangying Reservoir, SSK: Shishankou Dam, LQ: Longquan Reservoir, YF: Youfang Dam, DSW: Dashanwa Reservoir, HHQ: Honghuaqiao Reservoir.

(16.02 mg/kg) reservoirs. Significant differences were observed in the TN concentrations from all the sediment samples. The sediment sample from Wangying Reservoir contained high levels of TN (1315.68 mg/kg), whereas lower levels of TN appeared in Zhuchong (590.73 mg/kg) and Honghuaqiao (580.12 mg/kg) reservoirs.

Abundance of Archaeal and Bacterial *amoA* Genes

The abundance of archaeal *amoA* ranged from 1.25×10^5 copies per gram of dry sediment to 1.23×10^6 copies per gram of dry sediment, whereas the abundance of the bacterial *amoA* ranged from 1.42×10^7 copies per gram of dry sediment to 2.86×10^8 copies per gram of dry sediment (Fig. 1). Bacterial *amoA* abundances were higher than those of the archaeal *amoA* abundance in all sediment samples. The highest archaeal *amoA* abundance was found in the sample from Wangying Reservoir and the lowest from Zhuchong Reservoir. No significant changes in archaeal *amoA* abundance were observed among all the other five sediment samples with all values between 10^5 and 10^6 copies per gram of dry sediment. In contrast, samples collected from Zhuchong Reservoir were found to be the highest bacterial *amoA* abundance of 2.86×10^8 copies per gram of dry sediment, followed by the sediment samples from Youfang Dam (1.31×10^8 copies per gram of dry sediment) and Honghuaqiao Reservoir (6.85×10^7 copies per gram of dry sediment), whereas the Dashanwa Dam sediment sample was the lowest – even lower than that of Zhuchong Reservoir by nearly one order of magnitude. Other samples demonstrated similar abundance in bacterial *amoA* with all values slightly above 10^7 copies per gram of dry sediment.

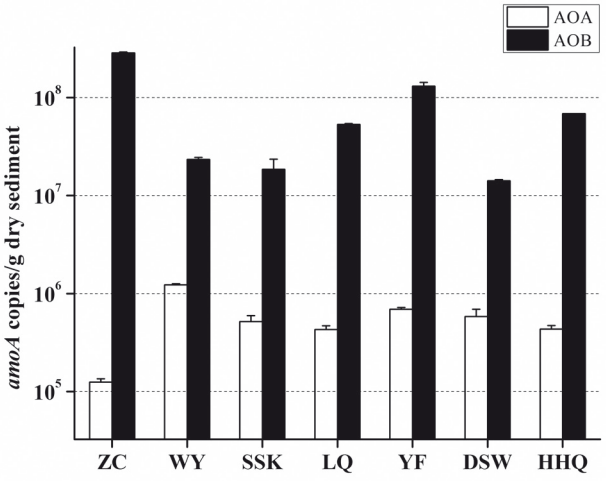

Fig. 1 The copy numbers of the archaeal (white columns) and bacterial (black columns) *amoA* genes in different sediment samples. Data are presented as means ± standard deviation (n = 3). ZC: Zhuchong Reservoir; WY: Wangying Reservoir; SSK: Shishankou Dam; LQ: Longquan Reservoir; YF: Youfang Dam; DSW: Dashanwa Reservoir; HHQ: Honghuaqiao Reservoir.

Diversity Analysis of Archaeal and Bacterial *amoA* Genes

In total, 210 archaeal *amoA* sequences and 331 bacterial *amoA* sequences were recognized, respectively (Table 2). Operational taxonomic units (OTUs) were calculated using the DOTUR software package [37]. Consequently, 68 OTUs were generated for the archaeal *amoA* clone library, whereas the number of OTUs in the bacterial *amoA* clone library was 52 in total. Among individual clone libraries, the number of OTUs ranged from 5 to 14 for both archaeal *amoA* and bacterial *amoA*. For archaeal *amoA*, Longquan Reservoir sediment contained 14 OTUs, which was nearly three times as high as Dashanwa Reservoir. For bacterial *amoA*, Longquan Reservoir and Youfang Dam sediment samples both contained 14 OTUs, whereas the lowest number was observed in the sediment of Zhuchong Reservoir (5 OTUs). The clone library coverage varied from 76.6% (Longquan Reservoir) to 96.7% (Dashanwa Reservoir and Honghuaqiao Reservoir) for archaeal

Table 2. Diversity of the archaeal and bacterial amoA gene sequences from different sediment samples collected from Huashan Watershed.

Samples	Number of clones	Number of OTUs	C%	S_{chaol}	*H'*
AOA					
ZC	30	9	80	16.5	1.55
WY	30	7	90	8.5	1.52
SSK	30	11	80	14.8	1.80
LQ	30	14	76.7	21	2.42
YF	30	12	80	17	2.18
DSW	30	10	96.7	10	2.15
HHQ	30	5	96.7	5	1.38
AOB					
ZC	50	5	96	5.5	0.84
WY	46	11	89.1	16	1.93
SSK	47	9	93.6	10	1.68
LQ	43	14	83.7	18.7	1.73
YF	50	14	84	42	2.18
DSW	45	8	88.9	13	1.21
HHQ	50	11	90	16	1.90

C%: coverage of each clone library, S_{chaol}: the Chao 1 index, *H'*: the Shannon-Wiener index, ZC: Zhuchong Reservoir, WY: Wangying Reservoir, SSK: Shishankou Dam, LQ: Longquan Reservoir, YF: Youfang Dam, DSW: Dashanwa Reservoir, HHQ: Honghuaqiao Reservoir.

amoA, and from 83.7% (Longquan Reservoir) to 96% (Zhuchong Reservoir) for bacterial *amoA*. Diversity indices of Shannon-Wiener (*H'*) and S_{Chao1} demonstrated that the Longquan Reservoir sediment sample (2.42 and 21, respectively) had the highest diversity for archaeal *amoA* and the Youfang Dam sediment sample (2.18 and 42, respectively) maintained the highest diversity for bacterial *amoA*, whereas the Honghuaqiao Reservoir sediment sample (1.38 and 5, respectively) had the lowest diversity for archaeal *amoA* and the Zhuchong Reservoir sediment sample (0.84 and 5.5, respectively) had the lowest diversity for bacterial *amoA*.

Relationships between Environmental Factors and Abundance, Diversity of Archaeal and Bacterial *amoA* Genes

Pearson's correlation coefficients were calculated to investigate the relationships between environmental factors and the abundance and diversity of archaeal and bacterial *amoA*s (Table S1). The abundance of the bacterial *amoA* gene was negatively correlated to pH ($P<0.05$), whereas there was no significant correlation found between archaeal *amoA* abundance and pH (Table S1). TN concentrations exhibited positive relationships with the abundance of the archaeal *amoA* gene ($P<0.05$). However, TN concentrations showed no significant correlations with the abundance of the bacterial *amoA* gene. The concentration of COD_{Mn} was found to significantly negatively correlate with the abundance of the archaeal *amoA* gene ($P<0.05$). No other environmental factors showed significant correlations with the numbers of both archaeal and bacterial *amoA* OTUs.

Phylogenetic Analysis of Archaeal and Bacterial *amoA* Genes

In the present study, all the obtained archaeal *amoA* sequences are affiliated with two major clusters: 69.05% of all the sequences fell into the *Nitrososphaera* cluster, and the others fell into *Nitrosopumilus* cluster (Fig. S1a). Archaeal *amoA* sequences isolated from Zhuchong Reservoir contained only the *Nitrososphaera* cluster, suggesting that this sample was low in the diversity in the community composition of archaeal *amoA* sequences, whereas all of the other samples contained both two clusters in archaeal *amoA* sequences with different percentages (Fig. 2a). The *Nitrososphaera* cluster was predominant with a high percentage ≥90% in all

Table S1. Pearson correlation coefficients of environmental variables and abundance as well as diversity of AOA and AOB communities.

Environmental variables	Pearson correlation coefficients								
	AOA					AOB			
	amoA abundance	OTU numbers	S_{chao1}	*H'*		*amoA* abundance	OTU numbers	S_{chao1}	*H'*
pH	0.653	-0.437	-0.718	-0.230		-0.870*	0.290	0.026	0.554
COD_{Mn}	-0.863*	0.406	0.449	0.419		0.449	-0.323	-0.204	-0.663
NO_3^--N	-0.707	0.121	0.246	-0.100		0.148	-0.085	-0.289	-0.087
NH_4^+-N	0.388	0.531	0.534	0.408		-0.292	0.602	0.199	0.427
TN	0.815*	0.372	0.207	0.409		-0.521	0.578	0.398	0.532

*$P<0.05$. S_{chaol}: the Chao 1 index, *H'*: the Shannon-Wiener index, COD_{Mn}: chemical oxygen demand, NO_3^--N: nitrate nitrogen, NH_4^+-N: ammonia nitrogen, TN: total nitrogen.

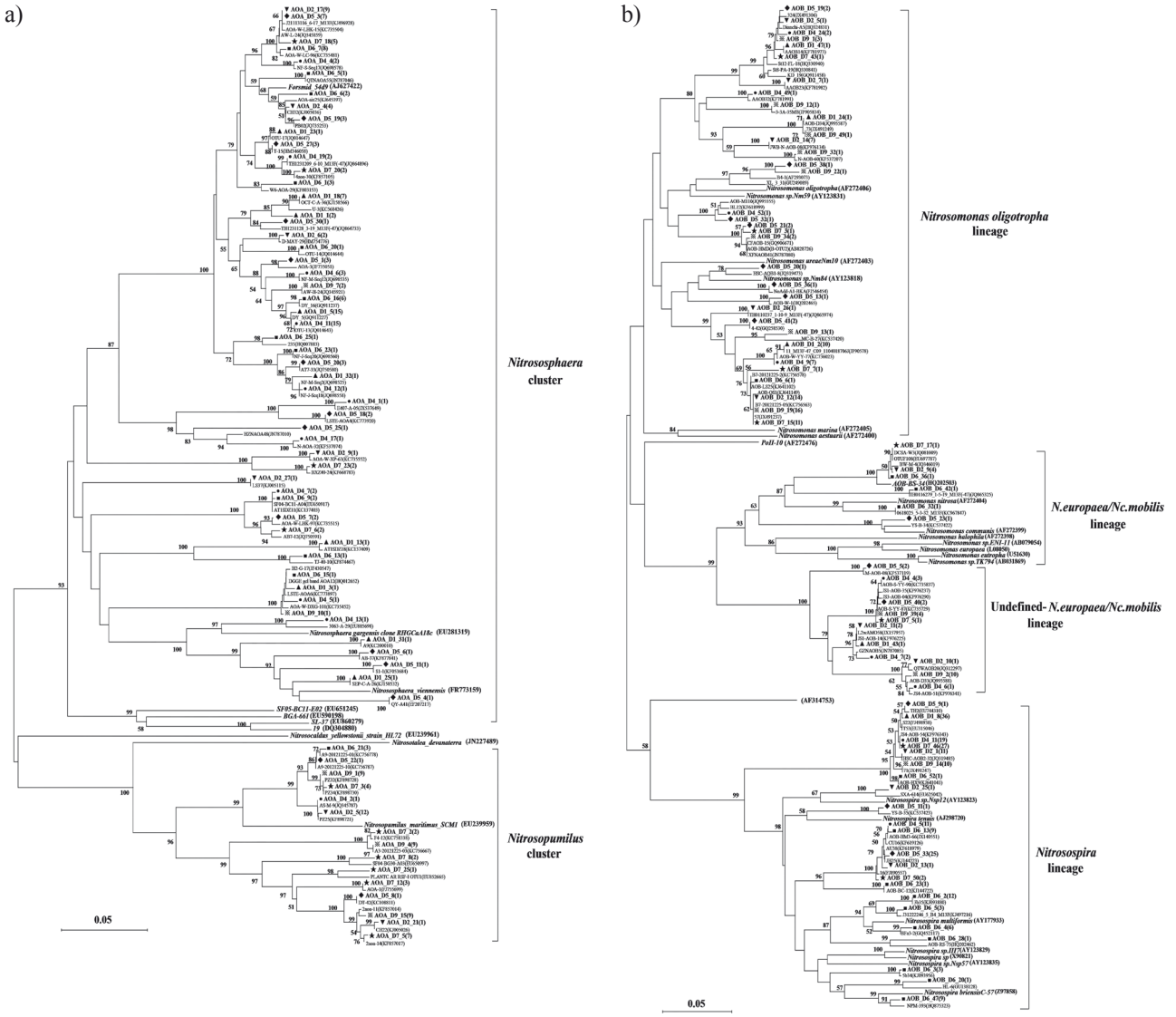

Fig. S1 Phylogenetic trees of archaeal a) and bacterial b) *amoA* gene sequences obtained in this study (▲: Zhuchong Reservoir; ▼: Wangying Reservoir; ●: Shishankou Dam; ♦: Longquan Reservoir; ■: Youfang Dam; ★: Dashanwa Reservoir; ⊡: Honghuaqiao Reservoir). Only the representative sequences from each OTU were presented and the numbers in parentheses indicated the number of sequences affiliated to the same OTU. Only bootstrap values greater than 50% are shown near nodes.

archaeal *amoA* sequences of Shishankou Dam (97%), Longquan Reservoir (93%), and Youfang Dam (90%) (Fig. 2a). The sediment sample from Wangying Reservoir contained *Nitrososphaera* and *Nitrosopumilus* clusters with similar percentages (57% and 43%, respectively). The *Nitrosopumilus* cluster was predominant in the sediment samples collected from Dashanwa (63%) and Honghuaqiao (90%) reservoirs (Fig. 2a).

Bacterial *amoA* sequences obtained in this study were divided into *Nitrosomonas oligotropha*, *Nitrosospira*, *N.europaea*/*Nc.mobilis,* and undefined- *N.europaea*/*Nc.mobilis* lineages (Fig. S1b). All the sediment samples contained *Nitrosomonas oligotropha* and *Nitrosospira* lineages with distinct proportions. The *Nitrosomonasoligotropha* lineage was predominant in sediments collected from Wangying (52%) and Honghuaqiao (54%) reservoirs, whereas other samples were all predominant in the *Nitrosospira* lineage (Fig. 2b). The highest percentage of *Nitrosospira* lineage appeared in sediment from Youfang Dam (92%), followed by sediments from Zhuchong Reservoir (73%), Dashanwa Reservoir (65%), Shishankou Dam (64%), and Longquan Reservoir (63%) (Fig. 2b). Except for *Nitrosomonas oligotropha* and *Nitrosospira* lineages, the undefined *N.europaea*/*Nc.mobilis* lineage was the only cluster found in the sediments of Zhuchong Reservoir, Shishankou Dam, and Honghuaqiao Reservoir (2%, 13%, and 28%, respectively). The *N.europaea*/*Nc.mobilis* lineage was the only cluster collected from Youfang Dam with a relative percentage of 6% (except for *Nitrosomonas oligotropha* and *Nitrosospira* lineages). Wangying, Longquan, and Dashanwa sediment samples contained all four lineages, suggesting that they maintained high bacterial *amoA* sequence diversity (Fig. 2b).

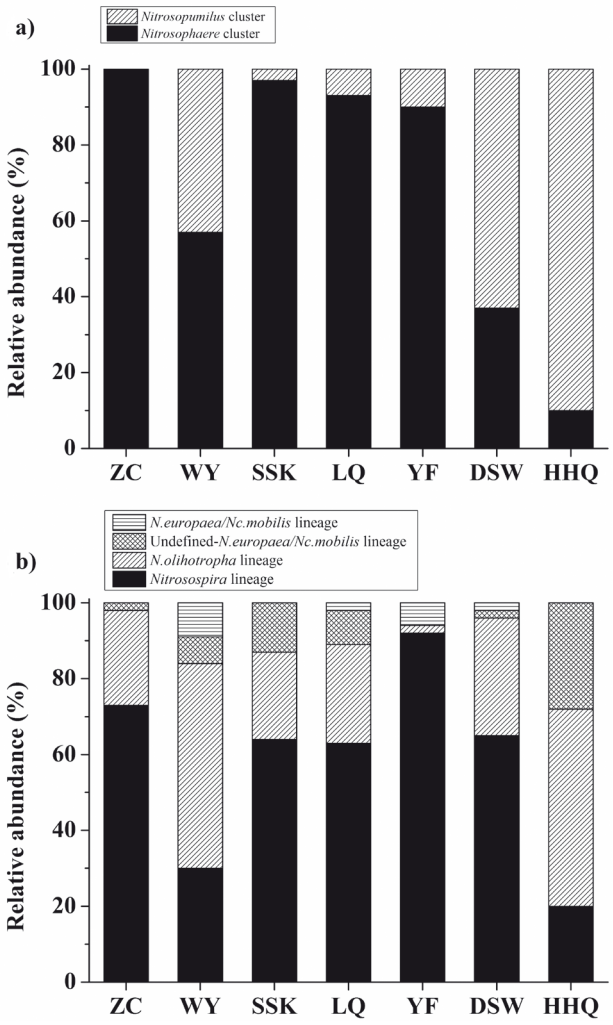

Fig. 2 Relative proportions of archaeal a) and bacterial b) amoA gene representing different groups from different sediment samples. ZC: Zhuchong Reservoir; WY: Wangying Reservoir; SSK: Shishankou Dam; LQ: Longquan Reservoir; YF: Youfang Dam; DSW: Dashanwa Reservoir; HHQ: Honghuaqiao Reservoir.

Discussion

Ammonia oxidation is the first and rate-limiting step of nitrification and plays an important role in the nitrogen cycling of various ecosystems. A recent study has found that the trophic status can affect the abundance and diversity of AOA and AOB [38]. In this study, seven sediment samples with distinct trophic status were collected to elucidate the relationships between nutrient levels and the abundance and diversity of AOA and AOB.

In the present study, bacterial *amoA* abundance was higher than archaeal *amoA* abundance in all sediment samples. Previous research showed that the abundance of AOA and AOB changed in various sediments [9, 12-13, 39]. Limpiyakorn et al. [40] found that the abundance of AOB was more than four orders of magnitude greater than AOA, which was similar to the present study. In contrary, Zhao et al. [13] found that abundance of AOA was higher than AOB in unvegetated sediment of Lake Taihu. Another study demonstrated that AOA far outnumbered AOB in sediments of Lake Taihu [39]. These discrepancies may be caused by different sediment environments. A previous report had shown that the sediment type and living substrates were suggested to affect abundances of ammonia oxidizers [41].

In the present study, pH was only found to negatively correlate with AOB abundance. Previous studies have demonstrated that pH is the key factor determining the abundance and community composition of AOA and AOB. Nicol et al. [24] found that AOA abundance decreased with elevated soil pH, whereas AOB abundance increased with elevated pH. Another study showed that soil pH was significantly positively correlated with the abundance of AOB [27], which was inconsistent with the present study. These differences may be attributed to the narrower pH range (6.66-7.36) in the present study compared to the previous studies (pH ranging 4.5-7.5).

The results of Pearson correlation analysis demonstrated that TN was positively correlated with AOA abundance. However, ammonia concentration was found to be of no significant correlation with AOA abundance. AOA was known for its preference to live in low ammonia nitrogen environments [42]. It was not found in the present study. Samples of LQ and DSW were found to be the highest and lowest ammonia concentrations, respectively, while the abundances of AOA in these two samples were generally moderate.

AOA sequences obtained from different sediment samples contained two major clusters, *Nitrososphaera* and *Nitrosopumilus*. The results of correlation analysis indicated that the relative percentage of *Nitrosopumilus* was positively related to pH, whereas nutrient levels showed no obvious correlation with any clusters of AOA. The result was in accordance with previous research that AOA community composition remained unchanged with three levels of N (urea) fertilizer in rice soil [31]. Limited information was available about the effect of trophic status on the community composition of AOA. Thus, more studies need to be undertaken to elucidate the mechanism of ammonia oxidation.

AOB sequences obtained in this study were divided into four lineages: *Nitrosomonas oligotropha*, *Nitrosospira*, *N.europaea/Nc.mobilis,* and undefined-*N.europaea/Nc.mobilis* lineages. The relative percentages of the *N.europaea/Nc.mobilis* lineage was found to be positively correlated with TN ($R = 0.824$, $P<0.05$) and negatively correlated with nitrate nitrogen ($R = -0.817$, $P<0.05$). In the sediments samples of ZC, DSW, and HHQ with low ammonium nitrogen concentrations, the *N.europaea/Nc.mobilis* lineage was found to barely exist, which was consistent with the study of Sui et al. [43], who found that the diversity of the AOB community declined with the decreased ammonium nitrogen concentrations and *N.europaea* gradually disappeared. Wang et al. [31] conducted a microcosm experiment to determine the effects of nitrogen fertilizer on the composition of AOB and AOA communities, and results showed that *Nitrosospira* decreased with the increasing application of N fertilizer

– in contrast to our results. In the sediment samples of SSK, LQ, and YF with higher nitrogen input, the *Nitrosospira* cluster demonstrated correspondingly higher abundance. Another analysis of bacterial *amoA* sequences revealed that the *N.europaea* cluster is commonly found with high ammonium levels and the *N. oligotropha* is the dominant cluster with low ammonium levels [40]. The findings in this study were not remarkable.

Conclusion

In the present study, seven surface sediment samples from small reservoirs were collected and investigated for the abundance and community composition of AOA and AOB. The abundance of the bacterial *amoA* gene was higher than that of the archaeal *amoA* gene in all samples. The highest diversity of archaeal *amoA* gene was found in the LQ reservoir and the highest diversity of bacterial *amoA* gene was found in the YF reservoir. Significant positive correlation was found between TN and the abundance of archaeal *amoA* gene, and significant negative correlation was found between pH and the abundance of the bacterial *amoA* gene. *Nitrososphaera* and *Nitrosopumilus* clusters were the main clusters of archaeal *amoA* sequences. Bacterial *amoA* sequences mainly consisted of *Nitrosospira* and *Nitrosomonas oligotropha* lineages. The distinctive nutrient levels in the seven reservoirs remarkably affected the abundance and community composition of AOA and AOB. Further studies need to be undertaken to elucidate the mechanism underlying how trophic status changes the abundance and community composition of AOA and AOB.

Abbreviations

Abbreviation	Full name
AOA	ammonia-oxidizing archaea
AOB	ammonia-oxidizing bacteria
amoA	ammonia monooxygenase α-subunit
ZC	Zhuchong Reservoir
WY	Wangying Reservoir
SSK	Shishankou Dam
LQ	Longquan Reservoir
YF	Youfang Dam
DSW	Dashanwa Reservoir
HHQ	Honghuaqiao Reservoir
N	nitrogen
TN	total nitrogen
COD_{Mn}	chemical oxygen demand
NO_3^--N	nitrate nitrogen
NH_4^+-N	ammonia nitrogen

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