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RESEARCH ARTICLE

Ammonia-oxidizing archaea and bacteria in water columns and sediments of a highly eutrophic plateau freshwater lake

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Abstract Both ammonia-oxidizing archaea (AOA) and bacteria (AOB) can play important roles in the microbial oxidation of ammonia nitrogen in freshwater lake, but information on spatiotemporal variation in water column and sediment community structure is still limited. Additionally, the drivers of the differences between sediment and water assemblages are still unclear. The present study investigated the variation of AOA and AOB communities in both water columns and sediments of eutrophic freshwater Dianchi Lake. The abundance, diversity, and structure of both planktonic and sediment ammonia-oxidizing microorganisms in Dianchi Lake showed the evident changes with sampling site and time. In both water columns and sediments, AOB *amoA* gene generally outnumbered AOA, and the AOB/AOA ratio was much higher in summer than in autumn. The total AOA *amoA* abundance was relatively great in autumn, while sediment AOB was relatively abundant in summer. Sediment AOA *amoA* abundance was likely correlated with ammonia nitrogen (rs = 0.963). The AOB/AOA ratio in lake sediment was positively correlated with total phosphorus (rs = 0.835), while pH, dissolved organic carbon, and ammonia nitrogen might be the key driving forces for the AOB/AOA ratio in lake water. Sediment AOA and AOB diversity was correlated with nitrate nitrogen (rs = -0.786) and total organic carbon (rs = 0.769), respectively, while planktonic AOB diversity was correlated with ammonia nitrogen (rs = 0.854). Surface water and sediment in the same location had a distinctively different microbial community structure. In addition, sediment AOB community structure was influenced by total phosphorus, while total phosphorus might be a key determinant of planktonic AOB community structure.

Keywords Ammonia-oxidizing archaea · Ammonia-oxidizing bacteria · Freshwater · Water column · Sediment

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Introduction

Microbial ammonia oxidation plays a crucial role in nitrogen cycling in aquatic ecosystems. Ammonia conversion can be achieved by both ammonia-oxidizing archaea (AOA, affiliated with archaeal phylum *Thaumarchaeota*) and bacteria (AOB, within classes *Beta-* and *Gamma-proteobacteria*) (Hayden and Beman 2014). Both use ammonia monoxygenase (AMO) to catalyze the oxidation of ammonia nitrogen. The *amoA* gene has become a well-established functional biomarker to investigate the distribution of ammonia-oxidizing archaeal and bacterial populations in marine and estuarine ecosystems. Salinity has been known as a key factor shaping the abundance of ammonia-oxidizing microbial community (Zhang et al. 2015a). AOA are typically more abundant than AOB in marine environment (Bouskill et al. 2012;

Lipsewers et al. 2014; Nakagawa et al. 2007; Newell et al. 2013), suggesting that AOA might play a more important role in ammonia oxidation. However, the numerical advantage of AOA versus AOB in estuarine ecosystem remains ambiguous (Magalhaes et al. 2009; Mosier and Francis 2008; Puthiya Veettil et al. 2015; Santoro et al. 2008; Veettil et al. 2015; Zhang et al. 2015a,b).

The distribution of sediment ammonia-oxidizing archaeal and bacterial populations has been investigated in a variety of freshwater ecosystems, such as river (Reis et al. 2015; Sonthiphand et al. 2013; Sun et al. 2013; Xie et al. 2014), reservoir (Wang et al. 2014), lake (Bollmann et al. 2014; Hou et al. 2013; Liu et al. 2015; Mukherjee et al. 2016; Zhao et al. 2013, 2014), wetland (Liu et al. 2014a; Wang et al. 2013; Yang et al. 2014), and pond (Lu et al. 2015), yet the relative importance of AOA and AOB to nitrification process in freshwater sediments remains under debate. Several previous studies indicated that AOA *amoA* gene abundance was usually greater than AOB in lake sediments (Herrmann et al. 2009; Hou et al. 2013; Zhao et al. 2014), while the numerical dominance of AOB over AOA *amoA* gene abundance was observed in sediments of many freshwater lakes on the Yunnan Plateau (Liu et al. 2015). AOA and AOB can have comparable contribution to nitrification in lake sediment (Liu et al. 2014b). So far, the links between lake sediment ammonia-oxidizing microbial populations and environmental factors still remain largely unclear. A number of factors (etc., pH, nitrogen compounds, phosphorus, and organic matter) might mutually govern the abundance, diversity and structure of lake sediment AOA and AOB (Liu et al. 2014b, 2015; Yang et al. 2016). Moreover, although the spatial variation of lake sediment AOA and AOB communities has been well-documented, information on their temporal change is still very limited. In addition, plateau lakes may harbor ammonia-oxidizing assemblages of different evolutionary origin from those in other lakes worldwide (Yang et al. 2013). However, little is known about the distribution of sediment AOA and AOB in plateau freshwater lake (Liu et al. 2015; Yang et al. 2016). Several studies have shown that planktonic AOA and AOB communities in oligotrophic high-altitude lakes can vary with sampling site and time (Auguet et al. 2011; Auguet and Casamayor 2013; Hayden and Beman 2014; Hu et al. 2010; Vissers et al. 2013; Yang et al. 2013). To date, the difference of the diversity and structure of ammonia-oxidizing microorganisms between in water column and in sediment has not been addressed, although Auguet et al. (2012) implied that AOA assemblages in water column were distantly related to their sediment counterparts.

Dianchi Lake (309 km^2), located on the Yunnan Plateau, is the sixth largest freshwater lake in China, with average water depth and altitude of 4.4 and 1886 m, respectively (Yang et al. 2016). Dianchi Lake is regarded as one of the three most eutrophic lakes in China. Because it provides the substrate for nitrogen removal through denitrification and/or anammox, ammonia oxidation can be of great importance to diminish the external nitrogen pollutants (Hou et al. 2013). Therefore, detailed knowledge of AOA and AOB communities in Dianchi Lake can aid in the possible solutions for its eutrophication. Therefore, the main objective of the present study was to investigate the variation of AOA and AOB communities in both the water columns and sediments of highly eutrophic Dianchi Lake.

Materials and methods

Study sites and sampling

Four sampling locations were located in the northern part of Dianchi Lake (Fig. S1). Triplicate water and sediment samples were collected in July (summer) and October (autumn) in 2014. Water samples (1-m depth below water surface) were collected using plexiglass water sampler, while sediment samples (0–5 cm) were collected using core sampler. In this study, samples SW1, SW2, SW3, and SW4, respectively, represented the water samples collected from the sampling sites 1–4 in summer, while samples AW1, AW2, AW3, and AW4 correspondingly represented the water samples from these four sites in autumn. Moreover, samples SS1, SS2, SS3, and SS4, respectively, were referred to the sediment samples from the sampling sites 1–4 in summer, while samples AS1, AS2, AS3, and AS4 correspondingly were referred to the four sediment samples in autumn. After collection, these water and sediment samples were immediately transported to the laboratory. For water samples, pH, dissolved oxygen (DO), ammonium nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), total nitrogen (TN), and total phosphorus (TP) were determined according to the standard protocols (China Environmental Protection Agency 2002). The level of dissolved organic carbon (DOC) in water was measured using a Shimadzu 5000A TOC analyzer. The levels of sediment total organic carbon (TOC), NH_4^+-N, NO_3^--N, TN, and TP were determined according to the literatures (Song et al. 2012; Yang et al. 2016). The properties of water and sediment samples are listed in Table S1 and Table S2, respectively.

Molecular analyses

Microbial cells in each water sample (300 mL) were retained using 0.22-μm pore-size membrane (diameter 50 mm; Millipore), and the total genomic DNA was extracted independently from each replicate using E.Z.N.A. Water DNA kit (Omega, USA). Sediment DNA was extracted independently from each replicate using Powersoil DNA extraction kit (Mobio Laboratories, USA). DNA quality was checked by 1.0 % agarose gel electrophoresis and DNA concentration

was quantified using a biophotometer (Eppendorf, Hamburg, Germany). Each replicate lake water or sediment DNA sample was individually subjected to real-time quantitative PCR (q-PCR) assays. The number of archaeal and bacterial *amoA* genes were quantified using the primer sets Arch-amoAF (5′-STAATGGTCTGGCTTAGACG-3′)/Arch-amoAR (5′-GCGGCCATCCATCTGTATGT-3′), and AmoA-1 F (5′-GGGGTTTCTACTGGTGGT-3′)/AmoA-2R (5′-CCCCTCKGSAAAGCCTTCTTC-3′), respectively, following the same PCR reactions as previously described (Francis et al. 2005; Rotthauwe et al. 1997; Wang et al. 2014). SYBR Green q-PCR assays was performed using an ABI 7500 FAST (Applied Biosystems). Standard curves ranging from 10^3 to 10^8 gene copies/mL were generated using serial dilutions of linearized plasmids (pGEM-T, Promega) containing cloned *amoA* gene amplified from environmental DNA. The amplification efficiency and coefficient (r^2) for the amplification of AOA and AOB *amoA* genes were 93 and 99 %, 0.994 and 0.998, respectively. One-way analysis of variance (ANOVA) followed by the Student–Newman–Keuls test were applied to assess the significant differences ($P<0.05$) in the copy number of *amoA* gene among different samples.

The primer sets Arch-amoAF/Arch-amoAR and AmoA-1 F/AmoA-2R were also adopted for construction of AOA and AOB clone libraries, following the same conditions as previously described in the literature (Wang et al. 2014). The PCR products from triplicate samples were mixed in equal amounts and cloned into pMD19-T vector (Takara Corp, Japan). The clones containing correct size were sequenced at Beijing Genomics Research Center Co., Ltd. The obtained valid *amoA* gene sequences were assigned into the same operational taxonomic units (OTUs) with a maximum distance of 3 % using the MOTHUR program (Schloss et al. 2009), and OTU-based Shannon diversity index was further calculated. Phylogenetic analysis of the obtained *amoA* gene sequences was performed using the MEGA 6.0 software (Tamura et al. 2013). Moreover, the links between ammonia-oxidizing assemblages and water or sediment properties were identified using Pearson's correlation analysis with the SPSS 20.0 software. Redundancy analysis (RDA) using the CANOCO 4.5 software was also applied to discriminate the links between microbial community composition and environmental factors. The fraction of *amoA* gene sequence in each OTU was assigned as species input and the measured water/sediment physicochemical properties as input for the environmental variables. The significance test of Monte Carlo permutations was performed to choose a suitable model of the microorganism-environment relationships. The obtained sequences in this study were deposited in the GenBank database under accession number KT317786–KT318082 and KT274817–KT275131 for AOA and KT323361–KT323795, KP902850–KP902954, and KP903025–KP903057 for AOB, respectively.

Results

Abundance of archaeal and bacterial amoA genes in summer and autumn

In this study, AOB *amoA* abundance was significantly greater than AOA in water samples ($P<0.05$) except for sample AW3 (Fig. 1). The number of archaeal *amoA* gene ranged from 3.72 to 1.16×10^5 copies per mL water in the water samples from Dianchi Lake. Sample AW3 (1.16×10^5 copies per mL water) and sample AW1 (6.86×10^4 copies per mL water) had much greater AOA *amoA* gene abundance than other water samples (less than 7×10^3 copies per mL water) ($P<0.05$). At each sampling site, the archaeal *amoA* gene was more abundant in the water column in autumn than in summer ($P<0.05$). Moreover, the significant difference of the bacterial *amoA* gene abundance was observed in the water columns in either summer or autumn ($P<0.05$), ranging from 8.02×10^2 to 1.42×10^5 bacterial *amoA* gene copies per mL water. Sample AW1 had the greatest AOB *amoA* abundance followed by sample SW1. These two water samples (more than 6.6×10^4 bacterial *amoA* gene copies per mL water) had significantly greater AOB *amoA* abundance than the rest (less than 3.5×10^4 bacterial *amoA* gene copies per mL water) ($P<0.05$). At both sampling sites 1 and 4, AOB *amoA* was more abundant in autumn than summer, but the reverse was observed at sites 2 and 3. In addition, the ratio of AOB to AOA *amoA* genes ranged between 533 and 905.6 in summer water samples, much greater than that in autumn water samples (0.04–2.07).

For each sediment sample, AOB showed greater *amoA* gene abundance than AOA ($P<0.05$) (Fig. 2). The number of archaeal *amoA* gene varied from 2.54×10^3 to 3.7×10^4 copies per gram dry sediment in summer and autumn sediments in Dianchi Lake. Samples AS1, AS3, and AS4 (more than 1.3×10^4 gene copies per gram dry sediment) had greater AOA *amoA* abundance than other sediment samples (less than 7.3×10^3 gene copies per gram dry sediment) ($P<0.05$). At each sampling site, the archaeal *amoA* gene in autumn sediment sample numerically outnumbered the corresponding summer one ($P<0.05$). Moreover, sediment samples from Dianchi Lake showed a significant difference in AOB *amoA* gene abundance ($P<0.05$), varying from 4.75×10^4 to 3.84×10^5 bacterial *amoA* gene copies per gram dry sediment. Sample AS2 had much lower AOB *amoA* gene abundance than other sediment samples ($P<0.05$). At each sampling site, the summer sediment sample showed greater AOB *amoA* gene abundance than the corresponding autumn one ($P<0.05$). In addition, the ratio of AOB to AOA in summer sediment samples (71.6–151) was much greater than that in autumn sediment samples (5.7–2.07).

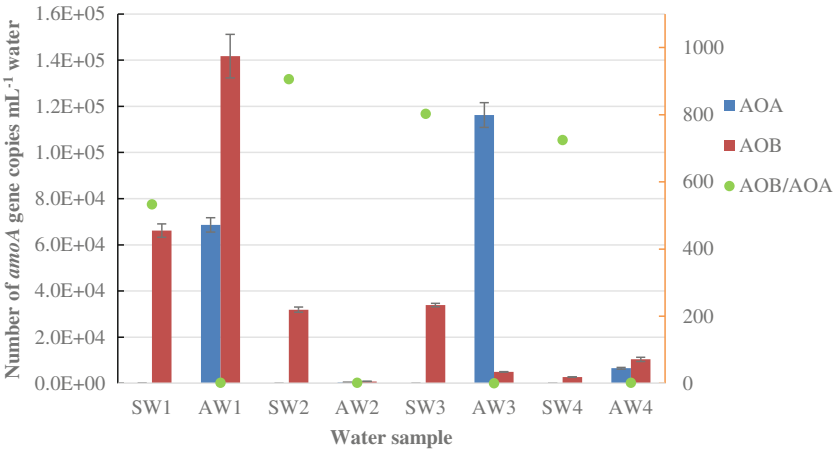

Fig. 1 Abundances of archaeal and bacterial amoA genes in lake water samples

Diversity of archaeal and bacterial amoA genes in summer and autumn

In the present study, a total of 612 archaeal and 573 bacterial *amoA* gene sequences were retrieved from water and sediment samples from Dianchi Lake. AOA libraries were composed of 5–15 archaeal OTUs (Table 1). A wide range of AOA community diversity was found in both sediment samples (with Shannon index of 0.68–2.1) and water samples (with Shannon index of 1.14–1.96). At sampling sites 1, 2, and 4, summer water samples had lower AOA community diversity than the corresponding autumn ones.

AOB libraries consisted of 1–15 bacterial OTUs (Table 2). A wide range of AOB community diversity also occurred in either sediment samples (with Shannon index of 0–2.08) or water samples (with Shannon index of 0.64–2.28). Summer sediment samples had greater AOB community diversity than the corresponding autumn ones at three sampling sites (sites 1, 3, and 4). However, at each sampling site, summer water samples showed greater AOB diversity than the corresponding autumn ones. Moreover, in both summer and autumn seasons, sediment samples showed lower AOB diversity than the corresponding surface water samples in most of the sampling sites (3 out of 4). In addition, autumn water and sediment samples always showed lower AOB diversity than AOA diversity. In contrast, summer water and sediment samples usually had relatively high AOB diversity.

Phylogeny of AOA and AOB communities in summer and autumn

In the present study, the representative sequences used for further phylogenetic analysis were selected only from the major OTUs (containing at least two *amoA* gene sequences). The archaeal *amoA* gene sequences from the major AOA OTUs could be assigned into six distinctive AOA groups (clusters A–F) (Fig. 3). Both sediment samples and water samples from Dianchi Lake differed greatly in the proportion of each AOA *amoA* cluster (Fig. S2a). In the same sampling location, a distinctive structure difference of AOA communities between in surface water and in sediment was also observed. Cluster-A was the second largest AOA group in both waters and

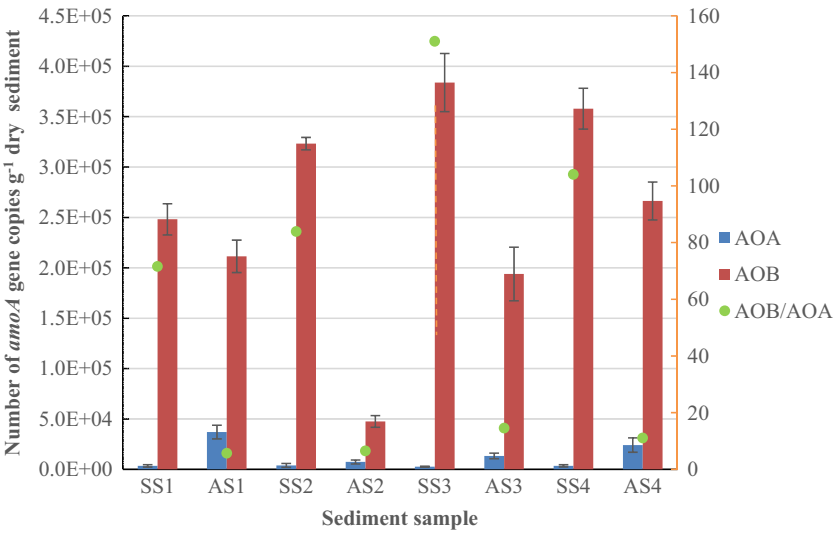

Fig. 2 Abundances of archaeal and bacterial amoA genes in lake sediment samples

Table 1 Diversity indices of each AOA clone library

Sample	Sequence number	OTUs	Shannon index
SS1	43	15	2.1
SS2	40	5	0.99
SS3	37	5	0.68
SS4	33	8	1.68
AS1	36	9	1.49
AS2	39	7	1.32
AS3	39	7	1.62
AS4	39	6	0.87
SW1	38	8	1.38
SW2	44	7	1.23
SW3	38	9	1.65
SW4	42	6	1.14
AW1	36	9	1.7
AW2	35	8	1.96
AW3	36	6	1.54
AW4	37	6	1.5

sediments. The proportion of AOA sequences affiliated with cluster-A in water samples AW1, SW3, SW4, AW2, AW3, and AW4 (25–45.5 %) was higher than SW1 (11.4 %) and SW2 (19.5 %). The AOA sequences affiliated with cluster-A comprised a greater proportion in sediment samples SS1, SS4, AS2, and AS3 (30.6–41.9 %) than in other sediment samples (0–19.4 %). The AOA sequences in this cluster were related to ≥97 % similarity to several cultured ones from wastewater treatment plant, flooding paddy soil, lake sediment. Moreover, cluster-C was the largest AOA group in both waters and sediments. They comprised a greater fraction of the total sequence library in summer water samples (SW1 65.7 %, SW2 65.6 %, SW3 50 %, and SW4 64.1 %) than autumn ones (AW1 36.4 %, AW2 30.8 %, AW3 42.4 %, and AW4 41.7 %). In addition, AOA sequences affiliated with cluster-C consisted of a greater proportion in sediment samples SS2, SS3, AS1, and AS4 (64.5–87.2 %) than other sediments (38.7–54.3 %). The sequences in cluster-C were related with ≥95 % similarity to several cultured ones from a variety of soil and sediment ecosystems, including river side, wetland, and grassland soils, and wetland, river estuary, and lake estuary sediments. Cluster-F was a minor AOA group. The sequences in this group could be related to ≥89 % similarity to the AOA *amoA* gene from two cultivated AOA strains (*Nitrososphaera* JG1 and EN76) (Kim et al. 2012; Tourna et al. 2011). They were only observed in water samples SW1 (8.6 %), AW1 (6.1 %), and AW4 (11.1 %) and sediment samples SS3 (7.7 %), AS1 (6.5 %), and AS3 (20.6 %).

Table 2 Diversity indices of each AOB clone library

Sample	Sequence number	OTUs	Shannon index
SS1	37	14	2.08
SS2	34	7	1.02
SS3	33	8	1.29
SS4	34	11	1.76
AS1	38	2	0.28
AS2	36	4	1.14
AS3	37	4	0.79
AS4	36	1	0.00
SW1	37	14	2.28
SW2	35	15	2.45
SW3	41	14	2.05
SW4	36	4	0.90
AW1	36	6	0.98
AW2	33	4	1.20
AW3	35	2	0.64
AW4	35	4	0.74

The sequences from the major AOB *amoA* OTUs could be grouped into five distinctive AOB clades (clusters I–VI) (Fig. 4). Both sediments and waters showed an evident difference in the proportion of each AOB *amoA* gene cluster (Fig. S2b). Surface water and sediment samples from the same site also showed distinct community structures. AOB sequences affiliated with cluster I were mainly observed in summer waters SW1, SW2 and SW3 (31–67.6 %) and summer sediments SS1, SS3 and SS4 (55.6–85.7 %). AOB sequences affiliated with cluster I were not detected in all the autumn waters and most of the autumn sediments (3 out of 4). These sequences could be related with ≥90 % similarity to the uncultured ones from aquaculture pond sediment, rice soil, plateau soil and riparian sediment. Cluster IV was the second largest AOB group. AOB sequences affiliated with cluster IV were mainly found in autumn waters AW1, AW2, AW3 and AW4 (57.6–79.4 %) and autumn sediments AS2 (51.4 %) and AS3 (80 %). Most of AOB sequences in cluster IV showed ≥93 % similarity to the uncultured ones from lake sediment. In addition, cluster V was the largest AOB *amoA* group and the members in this group could be distantly related (less than 82 % similarity) to the *amoA* gene from one cultivated *Nitrosomonas* strain. AOB sequences affiliated with cluster V predominated in water sample SW4 (71.4 %) and sediment samples SS2, AS1 and AS4 (89.3–100 %), and also comprised a considerable proportion in water samples AW1, AW2, AW3, AW4 and SW2 (13.8–42.4 %) and sediment samples AS2 (48.6 %) and AS3 (20 %). However, AOB sequences affiliated with cluster V were not detected in other water or sediment samples.

Influential factors regulating AOA and AOB communities

Pearson's correlation analysis indicated that the AOB/AOA ratio in lake water was positively correlated with the levels of pH, DOC, and NH_4^+-N ($P < 0.05$), while no significant

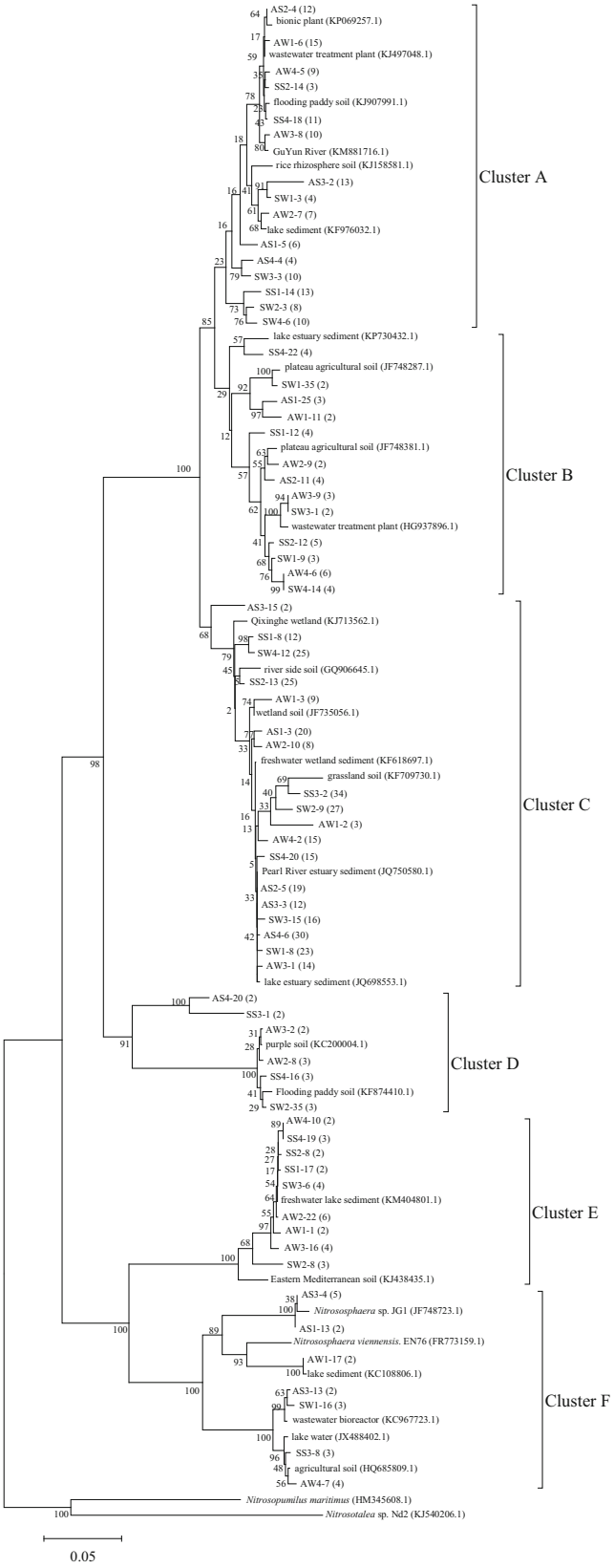

Fig. 3 Phylogenetic tree of representative archaeal amoA sequences and reference sequences from Genbank. The obtained archaeal sequences beginning with "SW1"–"SW4," "AW1"–"AW4," "SS1"–"SS4," and "AS1"–"AS4" were referred to the sequences retrieved from samples SW1–SW4, AW1–AW4, SS1–SS4, and AS1–AS4, respectively. The number in parentheses represents the numbers of the sequences in the same OTU in a given clone library. Numbers at the nodes indicate the levels of bootstrap support based on neighbor-joining analysis of 1000 resampled datasets. The bar represents 5 % sequence divergence

links were found between the determined water parameters and AOA or AOB *amoA* gene abundance ($P > 0.05$) (Table 3). Planktonic AOB diversity was found to show a positive correlation with NH_4^+-N ($P < 0.05$). Moreover, sediment AOA *amoA* gene abundance was positively correlated with sediment NH_4^+-N ($P < 0.05$), while the sediment AOB/AOA ratio showed a positive correlation with TP ($P < 0.05$) (Table 4). In addition, sediment AOA diversity was negatively correlated with NO_3^--N ($P < 0.05$), while sediment AOB diversity showed a positive correlation with TOC ($P < 0.05$).

Water environmental factors accounted for 74.9 % (the first two RDA axes, respectively, 30.5 % and 21.6 %) of the total variance for planktonic AOA OTU composition (Fig. 5a). In this study, no water parameter was found to significantly contribute to the planktonic AOA community–environment relationship. For planktonic AOB OTU composition, the water environmental factors accounted for 90.7 % (the first two RDA axes, respectively, 32 % and 27.7 %) of the total variance (Fig. 5b). TP ($P = 0.0340$, $F = 2.821$, 999 Monte Carlo permutations) significantly contributed to the planktonic AOB assemblage–environment relationship. In addition, sediment environmental factors explained 88.7 % (the first two RDA axes, respectively, 35.9 % and 25.3 %) of the total variance for sediment AOA OTU composition (Fig. 6a). No sediment parameter significantly contributed to the sediment AOA–environment relationship. The sediment environmental factors accounted for 88.2 % (the first two RDA axes, respectively, 41.5 % and 20.2 %) of the total variance for lake sediment AOB OTU composition (Fig. 6b). Only TP ($P = 0.012$, $F = 4.259$, 999 Monte Carlo permutations) showed significant contribution to the sediment AOB–environment relationship.

Discussion

AOA and AOB amoA gene abundance in freshwater lake

It remains unclear whether AOA or AOB *amoA* gene is dominant in lake sediment ecosystem. Previous studies indicated that AOA *amoA* gene outnumbered AOB in the sediments of freshwater lakes at different trophic states, such as eutrophic freshwater lakes (Chaohu Lake and Taihu Lake) (Hou et al. 2013; Zhao et al. 2014), mesotrophic Erhai Lake (Yang et al. 2016), and oligotrophic Lake Superior (Bollmann et al. 2014). AOA *amoA* gene also showed numerical dominance over AOB in the sediments of eight freshwater lakes in Jiangsu

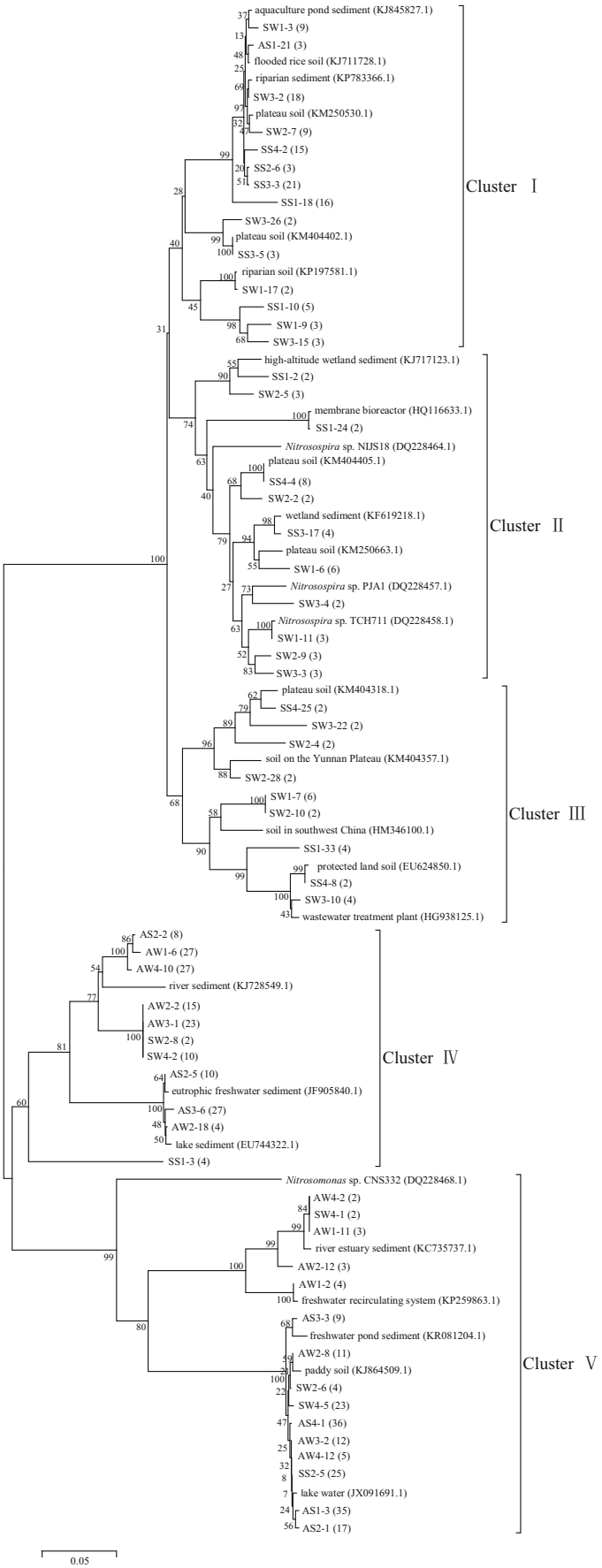

Fig. 4 Phylogenetic tree of representative bacterial amoA sequences and reference sequences from Genbank. The obtained bacterial sequences beginning with "SW1"– "SW4", "AW1"– "AW4", "SS1" –"SS4" and "AS1" –"AS4" were referred to the sequences retrieved from samples SW1– SW4, AW1– AW4, SS1 –SS4 and AS1 –AS4, respectively. The number in parentheses represents the numbers of the sequences in the same OTU in a given clone library. Numbers at the nodes indicate the levels of bootstrap support based on neighbor-joining analysis of 1000 resampled datasets. The bar represents 5 % sequence divergence

(China) (Sun et al. 2014). However, bacterial *amoA* gene was more abundant than archaeal *amoA* gene in the sediments of several small freshwater lakes on the Yunnan Plateau (Liu et al. 2015) and eutrophic freshwater Lake Erie (Bollmann et al. 2014). AOA *amoA* gene was also found to be less abundant than AOB in the sediments of saline Qinghai Lake (Jiang et al. 2009). In this study, sediment AOB always displayed greater *amoA* gene abundance than AOA, suggesting that AOB might have a greater contribution to nitrification in lake sediment of eutrophic freshwater Dianchi Lake. This was in agreement with the result reported in our recent study (Yang et al. 2016).

So far, although the spatial variation of AOA and AOB *amoA* gene abundance in freshwater lake sediment has been well-documented, there is a paucity of information on the temporal change. The present study showed the time-related change of both AOA and AOB *amoA* gene abundance in lake sediment. At a given sampling site, sediment AOA abundance was greater in autumn than summer, while sediment AOB *amoA* gene abundance was greater in summer. In addition, the sediment AOB/AOA ratio was also subject to temporal change, and it was much greater in summer than in autumn.

It remains in debate whether AOA or AOB *amoA* gene is more abundant in lake water column. AOA *amoA* gene were more abundant than AOB in the waters of saline Qinghai Lake (Jiang et al. 2009). Vissers et al. (2013) indicated that AOA *amoA* gene generally outnumbered AOB in the water column of oligotrophic freshwater Lake Lucerne, while Hayden and Beman (2014) showed that AOB *amoA* gene was more abundant than AOA in all the water samples from nine oligotrophic high-altitude freshwater lakes in California. Mukherjee et al. (2016) revealed that AOA *amoA* gene dominated compared to AOB in oligotrophic freshwater Lake Superior, whereas AOB *amoA* gene were more abundant than AOA in eutrophic freshwater Lake Erie. In the present study, both AOA and AOB *amoA* genes in water columns of eutrophic freshwater Dianchi Lake showed the evident time- and site-related changes. This was consistent with the results reported in oligotrophic freshwater lake (Vissers et al. 2013). In Dianchi Lake, similar to sediment AOA amoA gene, at a given sampling site, planktonic AOA amoA gene was also found to be more abundant in autumn than in summer. In addition, AOB amoA gene generally showed greater abundance than AOA in lake water, suggesting that AOB might play a more important role in

Table 3 Pearson's correlation analysis of water AOA and AOB communities with environmental factors

	DO	pH	DOC	TN	TP	NH_4^+-N	NO_3^--N
AOA abundance	−0.052	−0.432	−0.531	0.141	−0.142	−0.473	0.404
AOB abundance	−0.492	−0.539	−0.68	0.678	0.661	0.095	0.654
AOB/AOA	−0.132	0.845[a]	0.75[a]	0.336	0.304	0.894[a]	−0.499
AOA OTU number	−0.1	−0.175	−0.214	0.329	0.539	0.124	−0.061
AOB OTU number	−0.312	0.414	0.428	0.637	0.53	0.849	−0.317
AOA diversity	0.24	−0.522	−0.413	−0.357	−0.18	−0.62	−0.091
AOB diversity	−0.336	0.437	0.497	0.599	0.521	0.854[a]	−0.391

[a] Correlation is significant at the 0.05 level

nitrification in lake water. The temporal shift in the AOB/AOA ratio in water columns was also observed, and the AOB/AOA ratio was much greater in summer than in autumn.

AOA and AOB community diversity in freshwater lake

Several previous studies indicated that AOA generally had higher community diversity than AOB in sediments of Taihu Lake (Zhao et al. 2014) and small freshwater lakes on the Yunnan Plateau (Liu et al. 2015), while other studies showed higher AOB diversity than AOA in sediments of Jinshan Lake (Liu et al. 2014b) and Dianchi Lake and Erhai Lake (Yang et al. 2016). In the present study, both AOA and AOB diversity in sediments of Dianchi Lake illustrated a temporal shift. In addition, autumn sediment samples always showed lower AOB diversity than AOA, while summer sediment samples usually had relatively high AOB diversity. This suggested that the diversity difference between lake sediment AOA and AOB could vary with time.

Auguet et al. (2011) reported the seasonal changes of AOA diversity in waters in oligotrophic alpine lakes. Hu et al. (2010) revealed higher planktonic AOA diversity than AOB in two high-altitude oligotrophic freshwater lakes. In this study, both AOA and AOB diversity in water columns of eutrophic Dianchi Lake illustrated the evident time- and site-related changes. At a given sampling site, planktonic AOA generally had lower diversity in summer than in autumn, while planktonic AOB tended to have relatively high diversity in summer. In addition, planktonic AOB diversity was generally higher than planktonic AOA in summer, but lower in autumn, which also suggested the temporal change of diversity difference between lake planktonic AOA and AOB. Moreover, at a given sampling site, sediment usually tended to have lower AOB diversity than surface water.

AOA and AOB community structure in freshwater lake

The spatial heterogeneity of the structure of either sediment AOA or AOB community has been found in a single freshwater lake or across different freshwater lakes (Bollmann et al. 2014; Hou et al. 2013; Liu et al. 2014b; Sun et al. 2014; Yang et al. 2016). In this study, the results of phylogenetic analysis indicated the site-related difference in AOA or AOB community structure in sediments of Dianchi Lake. Moreover, the present study also provided the evidence that lake sediment AOA or AOB community structure could be subject to temporal change. In addition, the waters from eutrophic Dianchi Lake showed the evident time- and site-related variation in either AOA or AOB community structure. This was consistent with the results in other previous studies (Auguet et al. 2011; Auguet and Casamayor 2013; Hu et al. 2010). So far, to the authors' knowledge, there has been no report available on comparing the structures of lake ammonia-oxidizing microbial populations between in sediment and in water column. In the current study, surface water and sediment in the same sampling location showed a difference in both AOA and AOB community structure.

Table 4 Pearson's correlation analysis of sediment AOA and AOB communities with environmental factors

	TOC	TN	NO_3^--N	NH_4^+-N	TP	C/N
AOA abundance	−0.564	−0.284	0.194	0.963[a]	−0.687	−0.23
AOB abundance	0.051	0.105	0.082	−0.49	0.687	−0.004
AOB/AOA	0.356	0.032	−0.116	−0.799	0.835[a]	0.263
AOA OTU number	0.131	−0.632	−0.52	−0.049	0.316	0.703
AOB OTU number	0.620	0.167	0.096	−0.524	0.677	0.425
AOA diversity	−0.142	−0.446	−0.786[a]	−0.066	0.237	0.341
AOB diversity	0.769[a]	0.300	0.110	−0.528	0.579	0.407

[a] Correlation is significant at the 0.05 level

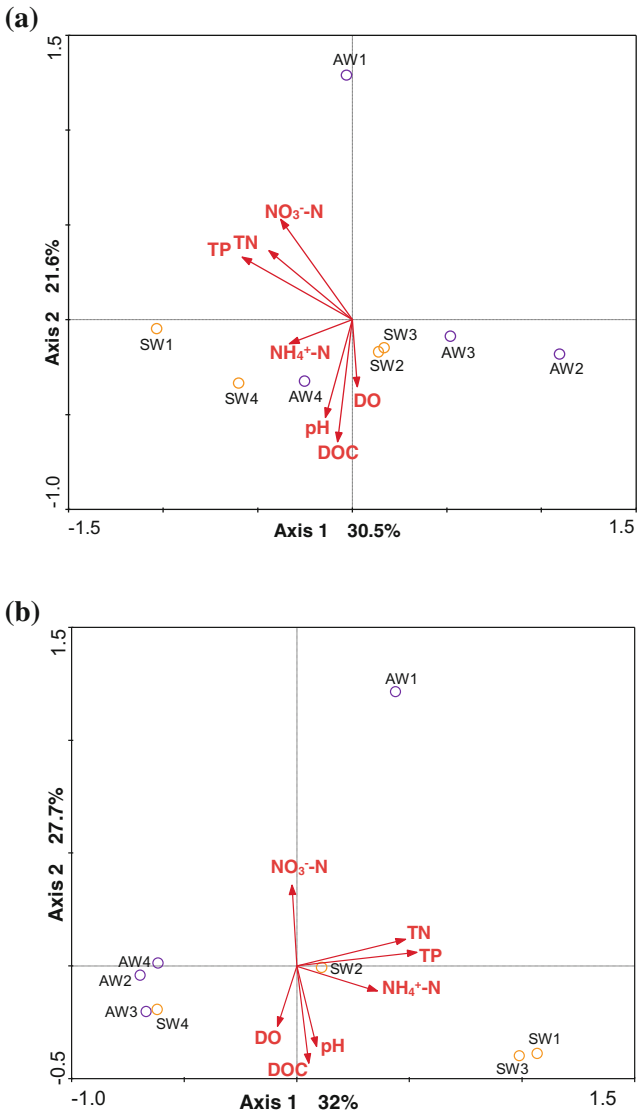

Fig. 5 RDA ordination plot for the first two principal dimensions of the relationship between water AOA OTU composition (**a**) or AOB OTU composition (**b**) and the water parameters

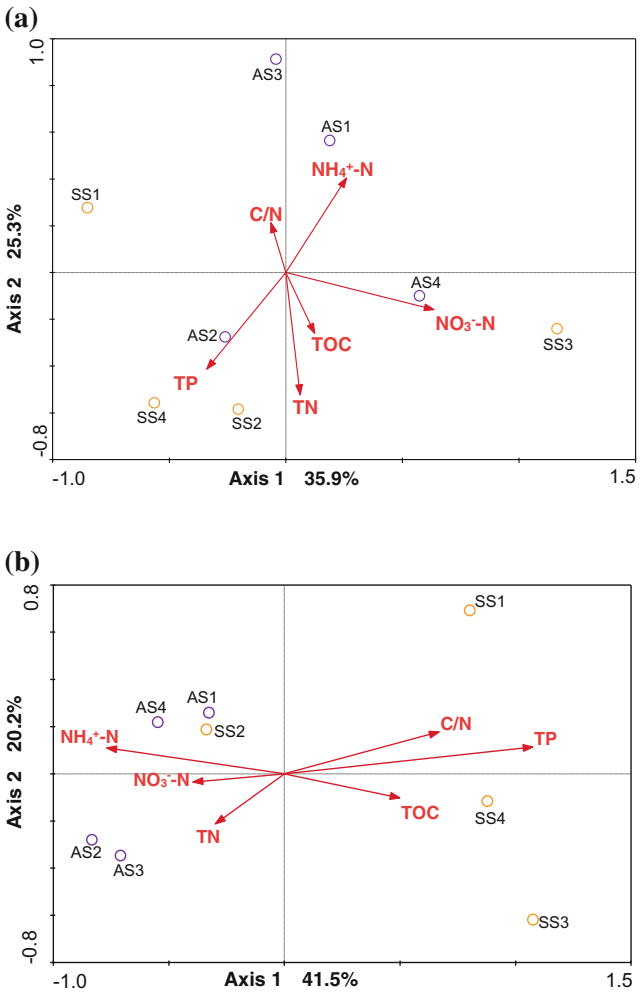

Fig. 6 RDA ordination plot for the first two principal dimensions of the relationship between sediment AOA OTU composition (**a**) or AOB OTU composition (**b**) and the sediment parameters

In this study, only a small proportion of archaeal amoA gene sequences from Dianchi Lake could be related (with ≥89 % similarity) to the AOA amoA gene from two cultivated AOA strains (Nitrososphaera JG1 and EN76) (Kim et al. 2012; Tourna et al. 2011). Most of the obtained archaeal amoA gene sequences from Dianchi Lake were distributed in AOA cluster C, and they could be grouped together with the uncultured ones from a variety of soil and sediment ecosystems. Moreover, only a small proportion of AOB sequences from Dianchi Lake could be affiliated with several cultivated Nitrosospira strains (NIJS18, PJA1, and TCH711). A majority of the obtained AOB sequences from Dianchi Lake showed no close relation to those from any known isolated AOB sequences. They were affiliated with the uncultured bacterial sequences from various soil and sediment ecosystems and wastewater bioreactor.

Environmental factors influencing AOA and AOB community

A number of environmental factors can regulate AOA and AOB amoA gene abundance in freshwater lake sediment (Yang et al. 2016). In this study, sediment AOA amoA gene abundance in Dianchi Lake was found to be likely shaped by the level of sediment ammonia nitrogen, which was consistent with the results reported in other freshwater lakes (Liu et al. 2014b; Zhao et al. 2014). Little is known about the environmental factors governing the AOB/AOA ratio in freshwater lake sediment. Our previous study indicated that the AOB/AOA ratio in freshwater lakes could be influenced by sediment NO_3^--N (Yang et al. 2016). In contrast, the current study revealed that TP might positively influence the AOB/AOA ratio in lake sediment. TP can be a key determinant of AOA or AOB amoA gene abundance in freshwater lake sediment (Liu et al. 2014b; Yang et al. 2016). The present study further suggested the potential role of TP in determining the AOB/

AOA ratio in lake sediment. In addition, previous studies indicated that lake sediment AOA diversity was likely influenced by lake trophic status (Bollmann et al. 2014; Herrmann et al. 2009; Yang et al. 2016), TN and TP (Yang et al. 2016), and temperature (Zeng et al. 2014), while AOB diversity by lake trophic status (Hou et al. 2013; Yang et al. 2016), pH (Yang et al. 2016), NO_3^--N and TN (Liu et al. 2014b), and temperature (Zeng et al. 2014). However, in this study, lake sediment AOA and AOB diversity was found to be likely affected by NO_3^--N and TOC, respectively, which was not in agreement with the results reported in these previous studies. Moreover, it has been widely accepted that lake trophic status is a key determinant of AOA and AOB community structure (Bollmann et al. 2014; Herrmann et al. 2009; Yang et al. 2016). The result of RDA in the current study also showed that TP might play an important role in shaping sediment AOB community structure in Dianchi Lake, while no obvious links were found between sediment properties and AOA community structure.

Previous work has reported that planktonic AOA amoA gene abundance in freshwater lake could be influenced by lake elevation (Hayden and Beman 2014), NH_4^+-N and nitrite nitrogen (NO_2^--N) (Auguet et al. 2011, 2012), and temperature and conductivity (Vissers et al. 2013), while planktonic AOB amoA gene abundance by phosphate (Hayden and Beman 2014). In this study, the links between environmental factors and planktonic AOA or AOB amoA gene abundance was not clear. However, the AOB/AOA ratio in waters of Dianchi Lake was found to be likely influenced by pH, DOC, and NH_4^+-N. Moreover, the present study showed that AOB diversity in waters of Dianchi Lake might be positively affected by the level of NH_4^+-N. In addition, several previous studies suggested that planktonic AOA community structure in freshwater lake was shaped by pH (Auguet and Casamayor 2013), temperature (Auguet et al. 2011), and water depth (Auguet et al. 2012). Hu et al. (2010) revealed the salinity-related differentiation of AOA community composition in high-altitude lakes on the Tibetan Plateau. To date, the report on the driving force for planktonic AOB community structure has been yet unavailable. In the current study, the result of RDA suggested that TP was likely a key determinant of planktonic AOB community structure in Dianchi Lake, while the driving force for planktonic AOA community structure was not identified.

Conclusions

The abundance, diversity and structure of planktonic and sediment AOA and AOB communities in eutrophic Dianchi Lake were subject to the evident time- and site-related changes. AOB amoA gene was generally more abundant than AOA in both water columns and sediments. At a giving sampling location, surface water and sediment showed a difference in either AOA or AOB community structure. TP might play an important role in shaping sediment AOB community structure, while planktonic AOB community structure was likely determined by TP as well as TN.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethical statement The work has not been published previously and not under consideration for publication elsewhere.

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