Abundance and distribution of ammonia-oxidizing microorganisms in the sediments of Beiyun River, China

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Abstract Ammonia oxidation, driven by ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB), plays an important role in the global nitrogen cycle. However, the population and distribution of ammonia-oxidizing microorganisms in the sediments of urban rivers with intensive anthropogenic nitrogen inputs is still unclear. In this study, we compared the diversity and abundance of AOA and AOB in Beiyun River sediments from summer to winter. AOB dominated numerically over AOA and the abundance of both the *amoA* genes were much higher in winter than in summer, while AOA communities were more phylogenetically diverse than AOB in this study area. Phylogenetic analysis revealed that *Nitrosospira* sp. was the dominant AOB in summer, with *Nitrosomonas* sp. dominant in winter, while no specific genus was found for AOA in present study. The main pollutants and microbial communities in the sediments deriving from Yangwa Watergate were different from other sites. Clone libraries showed that a considerable percentage of *amoA*

sequences matched closely with those from wastewater treatment plant (WWTP) systems. The seasonal change in nitrate content was significantly and positively related to the seasonal variation in *amoA* abundance ($P < 0.05$). Total nitrogen, total organic carbon, ammonium, and pH were the main environmental factors accounting for the difference in community composition of ammonia-oxidizing microorganisms in the sediments of Beiyun River. These findings suggest that effluent inputs, water gate operation, and seasonal changes might be key factors determining the abundance and distribution of AOB and AOA in the sediments of urban rivers.

Keywords Ammonium · Ammonia-oxidizing bacteria · Ammonia-oxidizing archaea · Sediment · Urban river

Introduction

Anthropogenic nitrogen inputs from agriculture and waste management have resulted in a significant increase in aquatic ecosystems eutrophication and emission of the greenhouse gas N_2O (Kroeze and Seitzinger 1998; Joo et al. 2013). In consequence, the load of the global nitrogen cycle has doubled since the last century (Fowler et al. 2013). Nitrification is a process in which ammonia is oxidized to nitrite and then nitrate, and this process is of vital importance for the global nitrogen cycle. The majority of anthropogenic nitrogenous pollutants can be removed through nitrification process and denitrification processes (Dang et al. 2010; Ward 2013). Ammonia oxidation, catalyzed by the ammonia monooxygenase (AMO), is the first and rate-limiting step of nitrification, and is performed mainly by ammonia-oxidizing bacteria (AOB) and archaea (AOA) (Rotthauwe et al. 1997; Francis et al. 2005, 2007). Studies based on *amoA* genes and PCR techniques have so far indicated that AOB and AOA

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were present in various environments, including soil (Leininger et al. 2006), freshwater systems (Zeng et al. 2012), wastewater treatment plants (WWTP) (Gao et al. 2013), and estuary and open sea (Cao et al. 2011). The impacts of environmental factors (e.g., pH, temperature, nutrients, and salt) on community diversity and abundance have also been studied deeply (Prosser and Nicol 2008; Erguder et al. 2009). Although AOA likely dominates ammonia oxidation in coastal and soil environments, the community characteristics of AOA and AOB, and their relative contribution to nitrification in freshwater ecosystems, especially in rivers and wetlands, should be further investigated (Francis et al. 2007; Fernández-Guerra and Casamayor 2012; Shen et al. 2014).

Sediments are the main nitrogen sink in river systems and represent one of the most important sites in the global nitrogen cycle because the strong gradient of dissolved oxygen allows oxic nitrification, anoxic denitrification, and anammox to operate in close proximity (Zaghmouri et al. 2013). Associated with microbial communities, sediments can act as biocatalytic filters for overlying water column in aquatic environments (Flood et al. 2015). Therefore, the presence of AOA and AOB in sediments can efficiently facilitate ammonia oxidation, which can further stimulate nitrogen loss from river ecosystems. Microorganisms and environmental conditions are the main factors that determine the efficient conversion and removal of excessive nitrogen in the water column and sediments of eutrophic rivers. AOA prefer a relatively low ammonia concentration. It has reported that AOA was more abundant than AOB by approximately two to four orders of magnitude in fresh water of low-ammonia concentration (Liu et al. 2011). Bai et al. (2014) also found that AOB was more abundant than AOA in the surface water of an urban river receiving treated wastewaters. However, the overall understanding of microbial characteristics of both AOA and AOB in the sediments of such urban rivers, and their relationships with environmental factors, is still far from systematic.

Beiyun River (Beijing-Tianjin-Hebei region, China) has been exposed to strong anthropogenic disturbance caused by increased population and the development of the economy. Organics and ammonium are the most important pollutants in this urban river and the water quality is better during high flow periods than in rainless periods as the WWTP effluent is the main water supply to the river all year round, especially in several main tributaries (e.g., Ba River and Liangshui River). In the meantime, numerous sediments and nutrients have precipitated to the riverbed because of the slow flow rate and a series of rubber dams along the watercourse. Hence, sediments have become one of the main sources of potential pollution of Beiyun River. In addition, previous studies revealed that AOA and AOB were ubiquitous in riparian zones of Beiyun River and the sediments of Wenyu River (the upstream section of Beiyun River) (Fan et al. 2010; Zhang

et al. 2013). In this study, we aimed to compare the abundance and phylogenetic diversity of AOA and AOB in order to understand the community pattern of ammonia-oxidizing microorganisms in the sediments of Beiyun River, which is heavily affected by anthropogenic nitrogen inputs. To achieve this objective, we carried out this study in summer (wet season) and in winter (dry season) along the Beiyun River flowing through Beijing municipality. We investigated the spatial and seasonal variation of AOA and AOB community diversity and abundance in the river sediments using clone library analysis and real-time quantitative polymerase chain reaction (qPCR).

Materials and methods

Sample collection and chemical analysis

Sediments were collected from mainstream and tributary debouchment of the Beiyun River in Beijing Section in July 2013 (summer) and February 2014 (late winter). Four sampling sites were selected from the upper reaches to the lower reaches (Fig. 1). Site 1 was located in upstream. Sites 2 and 3 were located in the debouchment of two main tributaries. Site 4 in the lower stream was behind Yangwa Watergate, which is the last channel controlling the river flowing out of Beijing. Undisturbed surface sediments at a depth of 3–5 cm from the four representative sites were sampled using a column sample device and enclosed with sterilized valve bags. Each sample was homogenized from sediments randomly grabbed from the riverbed at least five times, and then the samples were transported in a car refrigerator at 4 °C. Back in the laboratory, some of these samples were stored at –80 °C for DNA extraction and the remaining samples were dried for chemical analysis.

Sediment pH was measured using a HANNA pH211 meter (<http://hannainst.com>). Ammonium ($\text{NH}_4^+\text{-N}$), nitrate ($\text{NO}_3^-\text{-N}$) were extracted from sediments with 2 M KCl solutions by shaking for 1 h at room temperature, and determined using a flow injection analyzer (FIAstar5000, Foss, Hillerød, Denmark). Total organic carbon (TOC) was measured using a Liquid TOC II analyzer (Elementar, Hanau, Germany). Total nitrogen (TN) was determined by the Kjeldahl method. All tests and analyses were carried out in duplicate.

DNA extraction and *amoA* gene amplification

Genomic DNA was extracted from 0.3 g wet sediment sample using Ultraclean soil DNA isolation kits (MO BIO, Carlsbad, CA). The primers for bacterial *amoA* gene amplification were *amoA*-1 F (5'-GGGGTTTCTACTGGTGGT-3') and *amoA*-2R (5'-CCCCTCKGSAAAGCCTTCTTC-3') (Rotthauwe et al. 1997). Archaeal *amoA* gene were amplified using the

Fig. 1 Location of Beiyun River in Beijing section and sampling sites along the main stream

primers Arch-amoAF (5'-STAATGGTCTGGCTTAGAC-3') and Arch-amoAR (5'-GCGGCCATCCATCTGTATGT-3') (Francis et al. 2005). Approximately 10 μ L extracted DNA was used as template in 50 μ L PCR mixture, which contained 25 μ L 2 $\times$ Taq PCR Master Mix (Tiangen Biotech, China) and 2 μ L of each primer (10 μ M). The thermocycler programs for PCR were as follows: 3 min at 95 $^{\circ}$ C for initial denaturation, 35 cycles of 95 $^{\circ}$ C for 45 s, 55 $^{\circ}$ C (for AOB) or 53 $^{\circ}$ C (for AOA) for 1 min, and 72 $^{\circ}$ C for 1 min, followed by an extension cycle at 72 $^{\circ}$ C for 7 min (Li et al. 2011). Appropriate fragments (AOB and AOA were 491 bp and 653 bp, respectively) of PCR amplified products were separated by electrophoresis in 1 % agarose gels and gel purified using a gel extraction kit (OMEGA Bio-tek, Norcross, GA).

Cloning, sequencing, and phylogenetic analysis

The purified PCR products were cloned into a pEASY-T1 cloning vector and transformed into *Escherichia coli* using a pEASY-T1 cloning kit (TransGen Biotech, Beijing, China). White clones were selected randomly, and the *amoA* genes of the screened positive clones were sequenced using vector primers on Capillary sequencers ABI3730xl (Applied Biosystems, Foster City, CA). Sequences displaying >98 % identity were grouped into one operational taxonomic unit (OTU). OTU-based community diversity and richness indices were obtained using MOTHUR program (Schloss et al. 2009). NCBI BLAST (<http://www.ncbi.nlm.nih.gov/BLAST>) was used to obtain the closest *amoA* sequences in the public database. Neighbor-joining phylogenetic trees with bootstrapping were constructed with MEGA 4.0 (Tamura et al. 2007).

Real-time quantitative PCR

SYBR Green real-time quantitative PCR (qPCR) assays were carried out to determine the copy numbers of bacterial and archaeal *amoA* genes by using ABI 7500 FAST (Applied Biosystems). The primers used to amplify *amoA* genes for qPCR were the same as stated above. The reaction mixture (25 μ L) contained 12.5 μ L SYBR Green qPCR master mix (Ruian Bio Technologies, Beijing, China), 0.5 μ L of each primer (10 μ M mL⁻¹), and 2 μ L template DNA. For qPCR, the following thermocycler programs were used: 95 $^{\circ}$ C for 2 min, 40 cycles of 95 $^{\circ}$ C for 15 s, 60 $^{\circ}$ C for 1 min, and 60 $^{\circ}$ C for 1 min. Samples and standard reactions were performed in triplicate. PCR efficiencies of bacterial and archaeal *amoA* genes were 92 % and 96 %, respectively. The correlation coefficients of standard curves (r^2) were both >0.99.

Statistical analysis

Diversity indices (Shannon and Simpson) and non-parametric richness estimates (Chao1 and Ace) for each clone library were also determined using the MOTHURv.1.19.0 program. The similarity of community composition in different samples was analyzed with PRIMER 5. The correlations of ammonia-oxidizer community structure and environmental factors were evaluated by canonical correlation analysis (CCA) in Canoco for Windows 4.5. Correlation coefficient between *amoA* genes abundance and environmental parameters was calculated using SPSS16. One-way ANOVA was also performed in SPSS 16 to determine the significant differences among samples.

Nucleotide sequence accession numbers

The sequences reported in this study have been deposited with GenBank under accession numbers KJ093846–KJ093958 and KJ093999–KJ094018 for bacterial *amoA* gene, and KF857004–KF857161 for archaeal *amoA* gene in summer. The sequence accession numbers of both *amoA* genes derived from winter were KJ859227–KJ859566.

Results

Physicochemical characteristics of sediments

As one of the main pollutants in Beiyun River, NH_4^+ concentration in water was four to eight times higher than that indicated in the Chinese Environmental Quality Standards for Surface Water Grade V ($\leq 2 \text{ mg-N L}^{-1}$). High nutrient content was also detected in the whole eight sediment samples (Table 1). The content of NO_3^- in the river sediments was relatively higher in winter than in summer. Moreover, the concentrations of NH_4^+ , TN and TOC at sampling site 4 (S4 and W4) were lower than those in other sites. Further statistical tests revealed that TN was significantly and positively correlated with TOC ($P < 0.05$).

Diversity and phylogeny of *amoA* genes

Totals of 316 bacterial *amoA* clones and 315 archaeal *amoA* clones were sequenced from 16 clone libraries (Table 2). Bacterial and archaeal *amoA* sequences shared 67–100 % identity to each other, and 95–100 % identity to the closest *amoA* sequences deposited in GenBank. For each clone library, 11–23 AOA OTUs and 3–24 AOB OTUs were obtained at 2 % divergence in nucleotides. On average, the number of observed AOA OTUs was about 56 % of that predicted by the Chao1 index, whereas the number of predicted AOB OTUs was 76 %. The OTU numbers of AOB clone libraries

Table 1 Environmental data of samples in summer (S) and winter (W)

Sample	pH	NO_3^- (mg-N kg ⁻¹)	NH_4^+ (mg-N kg ⁻¹)	TN (g-N kg ⁻¹)	TOC (g-C kg ⁻¹)
S1	6.78	43.15	29.84	3.63	32.30
S2	7.77	11.39	6.59	2.28	15.20
S3	7.38	6.26	13.76	4.90	56.40
S4	7.71	21.51	7.62	1.15	7.30
W1	7.61	46.03	56.07	5.91	40.15
W2	7.39	14.11	14.03	6.24	50.68
W3	7.65	95.81	11.82	4.51	22.04
W4	7.56	41.48	11.71	4.44	22.94

Table 2 Diversity of each clone library of bacterial and archaeal *amoA* genes recovered from the sediments of Beiyun River. OTU Operational taxonomic unit, AOA ammonia-oxidizing archaea, AOB ammonia-oxidizing bacteria

<i>amoA</i> genes	Sample	No. of clones	OTUs	Chao1	Shannon	Simpson ⁻¹
AOA	S1	32	11	16	1.94	5.77
	S2	40	11	18	1.51	2.71
	S3	49	22	44	2.69	12.38
	S4	37	22	46	2.75	12.81
	W1	40	13	15	2.22	8.48
	W2	48	15	30	1.81	3.32
	W3	24	11	20	1.82	4.06
	W4	45	23	68	2.71	11.38
	ALL	315	70	130	3.21	11.50
AOB	S1	20	3	3	0.86	2.13
	S2	44	24	37	2.97	23.65
	S3	33	19	45	2.73	19.56
	S4	36	10	11	1.85	4.60
	W1	42	7	13	0.99	1.82
	W2	48	13	18	2.13	7.14
	W3	45	7	8	1.57	4.16
	W4	48	9	10	1.62	3.90
	ALL	316	63	119	3.19	13.89

showed a larger variation than those of AOA (Table 2). Indices of Shannon diversity and reciprocal of Simpson indicated that bacterial *amoA* gene diversity in summer was significantly correlated with sediment NO_3^- ($P < 0.05$).

All of the bacterial and archaeal *amoA* sequences were grouped into 70 and 63 unique OTUs, respectively. A total of 29 representative bacterial *amoA* sequences and 30 archaeal *amoA* sequences from the OTUs containing two or more members were selected for phylogenetic analysis (Figs. 2, 3). The 29 sequences of bacterial *amoA* gene, together with sequences matched closely from other study areas were grouped into two main clusters (Fig. 2). Cluster A, including 28 % sequences from the 29 OTUs and three sub-clusters (clusters A1, A2 and A3), was composed mainly of the representative samples in summer (Fig. S1). The sequences in this cluster were closely related to those from soil and sediment environments. Three OTUs in cluster A, namely OTU4, OTU10 and OTU16, were grouped with known strains in genus *Nitrosospora*, and were 96–99 % identical to

Fig. 2 Neighbor-joining phylogenetic tree of bacterial *amoA* constructed with an alignment of the sequences from 29 operational taxonomic units (OTUs) and their closest matched sequences from GenBank. Numbers in parentheses are the number of clones within this OTU. GenBank accession numbers are shown following sequences from other studies. Bootstrap values greater than 50 % of 1000 resamplings are shown near the corresponding nodes. The scale bar indicates the number of nucleotide substitutions per homologous site

Nitrosospira sp. N15 (Purkhold et al. 2003), *N. multiformis* ATCC 25196 and *Nitrosospira* sp. Np39-19 (Norton et al. 2002). In addition, all of the bacterial *amoA* sequences from S2 were in cluster A. In contrast, most of the sequences obtained in winter, and sequences from S1, were grouped into cluster B, which contained 72 % of all the test sequences, including four sub-clusters (clusters B1, B2, B3 and B4) (Fig. 2), and closely matched those retrieved from sediments and WWTP systems. Interestingly, 34 % of sequences in cluster B showed substantial overlap with those from active sludge (Sliemers et al. 2004; Figuerola and Erijman 2010; Yu et al. 2011; Zhang et al. 2011; Gao et al. 2013, 2014; Langone et al. 2014), and OTU27 (mainly including sequences from W4) was 99 % identical to *Nitrosomonas europaea* ATCC 19178. This revealed that bacterial *amoA* sequences from summer and winter were associated with genera *Nitrosospira* and *Nitrosomonas*, respectively, as *Nitrosospira* and *Nitrosomonas* are the two main AOB groups in various soil and freshwater environments, especially in aquatic environments affected heavily by anthropogenic inputs (Cao et al. 2011; Wankel et al. 2011; Luo et al. 2014).

Phylogenetic analysis indicated that the 30 representative sequences of the archaeal *amoA* genes could be grouped into eight sub-clusters. Most of these sequences were closely matched with published sequences derived from soil and sediment (Fig. 3), including agricultural soil, grassland soil, wetland soil, coastal/estuarine sediment, and river or lake sediment. According to the phylogenetic tree, we found no known AOA species similar to those derived from Beiyun River sediments. Cluster 8—the largest group in the archaeal phylogenetic tree—was highly homologous to *amoA* sequences from eutrophic lake sediments (Hou et al. 2013), and was composed of only three OTUs but more than 37 % of all the sequences from Beiyun River sediments (Fig. 3). Cluster 5 was the second largest group, including 21 % detected sequences. OTU4 and OTU16 matched closely with the *amoA* sequences from WWTP sludge (Gao et al. 2013, 2014).

Quantification analysis of *amoA* genes

The abundance of AOA was substantially lower than that of AOB in Beiyun River sediments (Fig. 4). Bacterial *amoA* gene copy numbers of sediments among samples ranged from 6.79×10^5 to 5.84×10^6 copies g^{-1} in summer, and from 2.54×10^8 to 5.59×10^8 copies g^{-1} in winter. Archaeal *amoA* gene copy numbers ranged from 1.31×10^5 to 1.67×10^6 copies g^{-1} in summer and from 5.23×10^7 to 1.21×10^8 copies g^{-1} in winter, respectively. The abundances of both archaeal and bacterial *amoA* genes were significantly different among samples ($P < 0.01$). For AOB, the sediments of site 4 (S4 and W4) had higher community abundance than other sites in both seasons, while the relative community abundance of AOA among four sediment sites changed variously. Furthermore,

Fig. 3 Neighbor-joining phylogenetic tree of archaeal *amoA* constructed with an alignment of the sequences from 30 OTUs and their closest matched sequences from GenBank. Numbers in parentheses are the number of clones within this OTU. GenBank accession numbers are shown following sequences from other studies. Bootstrap values greater than 50 % of 1000 resamplings are shown near the corresponding nodes. The scale bar indicates the number of nucleotide substitutions per homologous site

the abundance of AOA and AOB in winter was 31 to 484 times greater than that in summer.

Pearson's correlation analysis was performed to evaluate the relationships between *amoA* genes copies and sediment physicochemical parameters (Table 3). The results showed that the abundance of total *amoA* genes and AOB were significantly and negatively correlated with TOC and TN in summer ($P < 0.05$). In addition, the seasonal variability of archaeal and bacterial genes, and the abundance of both *amoA* genes was significantly and positively correlated with the variability of NO_3^- content ($P < 0.05$).

Ammonia-oxidizing community and its response to environmental factors

The community composition difference of eight samples was compared. The similarity of AOB between W1 and S1 was highest (82 %), while the highest similarity of AOA found between W2 and S3 was only 62 %. Generally, the community similarity among samples was much higher in winter than in summer.

To further clarify the characteristics of community composition and regulating factors, the correlations between *amoA* community composition patterns and environmental parameters were analyzed using CCA (Fig. 5). The result showed that the first two axes (AX1 and AX2) corresponded to 37 % and 26 % of the cumulative variance of AOA species-environment relationship, respectively. Sediment TN ($r = 0.84$), TOC ($r = 0.74$), and NH_4^+ ($r = 0.69$) were positively related to AX1, whereas pH ($r = -0.86$) was highly related to AX2 (Fig. 5). For AOB, AX1 and AX2 accounted for 40 % and 28 % of the cumulative variance of species-environment relationship, respectively. The main environmental parameters, including TN ($r = -0.77$), NH_4^+ ($r = -0.69$), and TOC ($r = -0.57$), were negatively related to AX1. In contrast, sediment pH ($r = 0.58$) was positively related to AX2. In summary, TN, NH_4^+ , TOC, and pH were the main factors determining the *amoA* genes community distribution patterns. However, these parameters elicited the opposite effect on bacterial and archaeal *amoA* groups of Beiyun River sediment. CCA ordination plots also indicated that site-specific environmental conditions might play an important role in governing the community distribution of ammonia-oxidizing microorganisms in river sediments.

Fig. 4 Relative abundance of bacterial (B) and archaeal (A) *amoA* genes copies in summer (S) and winter (W) in four sample sites (1, 2, 3, and 4)

Discussion

AOA and AOB diversity and abundance in the sediments of Beiyun River

Diversity estimates based on clone libraries of *amoA* genes in this study were within the same range of that in the sediments of estuaries (Wankel et al. 2011; Zheng et al. 2013; Chen et al. 2014) and freshwater lakes (Liu et al. 2015), while these indices were generally higher than that reported in the sediments of eutrophic lake (Zeng et al. 2012; Zhao et al. 2013, 2014). Archaeal *amoA* sequences from the eight samples showed higher Shannon diversity than bacterial *amoA*, which was in agreement with the results in previous studies (Cao et al. 2011; Zheng et al. 2013). AOA communities were more phylogenetic diverse than AOB in this study area.

In general, most of the archaeal and bacterial *amoA* sequences in Beiyun River sediments were associated with soil or sediment sources while the remaining sequences, particularly 25 % of bacterial *amoA* sequences, were closely related to those obtained from WWTP systems. These sequences probably derived from WWTP effluent (Merbt et al. 2015).

In addition, previous studies showed that typical microorganisms in WWTP systems could easily colonize the niche of native species in river sediments via effluent inputs (Mußmann et al. 2013; Cébron and Garnier 2005). As the main drainage river of Beijing, Beiyun River receives large amounts of effluents from WWTP (Jing et al. 2013). This strong influence from effluent could therefore explain the considerable proportion of WWTP-related *amoA* sequences in this study. Further study is certainly needed to clarify how WWTP effluent affects ammonia-oxidizing microorganisms in river sediments. Previous studies also showed that AOA were the main microorganisms involved in the nitrogen cycle in low-pH environments, but AOB were more likely to grow well in slightly alkaline conditions (pH 7.0–8.5) (Erguder et al. 2009; Liu et al. 2011, 2013). Cao et al. (2011) indicated that AOB were sensitive to pH changes in Pearl River estuary sediments, and community diversity was significantly correlated with pH. Therefore, compared with AOA community, AOB community distribution was likely more sensitive to the sediment environment at an average pH of 7.48 in Beiyun River.

In general, AOA greatly outnumber AOB in most marine and soil ecosystems (Leininger et al. 2006). Further studies have revealed that AOB are present in greater abundance in some freshwater environments, such as river (Sun et al. 2013), wetland (Zeng et al. 2012; Liu et al. 2014), and estuary (Magalhães et al. 2009; Wankel et al. 2011; Zheng et al. 2013). In the present study, the abundance of AOB was much higher than AOA at all sampling sites in both seasons. The high concentration of NH_4^+ may account for the numerical dominance of AOB over AOA (Magalhães et al. 2005). AOA was possibly predominant in low-nutrient environments (Erguder et al. 2009), while AOB can grow significantly in high ammonium-amended soil, and can also dominate in ammonium-fertilized agricultural (Jia and Conrad 2009) and nitrogen-rich grassland (Di et al. 2009) soils. As for

Table 3 Pearson's correlation coefficient of *amoA* genes abundance with physicochemical parameters. TOC Total organic carbon, TN total nitrogen

Parameters	Summer				Winter				Winter/summer ^c		
	AOA	AOB	Ratio ^a	Total ^b	AOA	AOB	Ratio ^a	Total ^b	AOA	AOB	Total ^b
pH	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
NO_3^-	ns	ns	ns	ns	ns	ns	ns	ns	0.98*	0.98*	0.99*
NH_4^+	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
TOC	ns	−0.94*	ns	−0.96*	ns	ns	ns	ns	ns	ns	ns
TN	ns	−0.90*	ns	−0.99**	ns	ns	ns	ns	ns	ns	ns

* $P < 0.05$, ** $P < 0.01$, ns not significant

^a Abundance ratio of AOB to AOA

^b Total *amoA* abundance (sum of AOB and AOA)

^c Ratio of *amoA* abundance in winter to *amoA* abundance in summer

Fig. 5 Canonical correlation analysis (CCA) ordination plots for the first two dimensions of the relationship between ammonia-oxidizing microorganisms assemblages and environmental factors

freshwater environments, previous studies in Dongjiang River found that AOA were far more abundant than AOB in low-ammonium water (Liu et al. 2011), while the abundance of AOB was much higher than AOA in sediments containing high levels of ammonium (Sun et al. 2013). Therefore, the high NH_4^+ and relatively high total organic matter content detected in the present study, which was in accordance with a previous study on the agriculturally impacted Elkhorn Slough Estuary, California (Wankel et al. 2011), may account for the greater abundance of AOB than AOA in the sediments of Beiyun River.

Spatial and temporal distribution of *amoA* genes in the sediments of Beiyun River

Physicochemical analysis revealed that sediment NH_4^+ , TN, and TOC in site 4 (S4 and W4) were relatively lower than that in the other sites. Site 4 was located in the lower mainstream behind Yangwa Watergate and

frequently affected by gate opening. Sluice gate and dam operation can elicit complex impacts on water quality and microbial community. The water stored behind the dam works like a reservoir, which has complex hydrology characteristics and increases water residence time (Wall et al. 2005), and this special aquatorium of the river plays an important role in nutrient reduction by sedimentation and microorganism digestion. Our previous study (published in Chinese) showed that sediment nutrients and overlying water in the downstream side of the sluice gate or dam was lower than that in other sites along the river. In the present study, we also found that the majority of bacterial *amoA* sequences of S4 and W4 were grouped into separate clades (clusters A1 and B2) and obviously different from other samples in the phylogenetic tree (Fig. 2), while the abundance of both archaeal and bacterial *amoA* genes was higher in S4. It seems that the operation of Yangwa Watergate has significantly affected the distribution of pollutants and ammonia-oxidizing microorganisms in the sediments of Beiyun River. There are many water gates and rubber dams along Beiyun River, which contributes to flood retention and sewage interception, and further studies are needed to elucidate the ecological effects of river management practices on nitrogen cycle and relevant microorganisms.

Environmental parameters were vital in shaping the spatial and seasonal distribution of ammonia-oxidizing microbes. The seasonal differences in the aquatic environment between summer and winter could cause seasonal variations in microbial composition (Kan et al. 2006). In this study, the community composition was more similar in winter than in summer samples, and *amoA* genes were more abundant in winter than in summer, while a higher pollutant concentration was also observed in winter. These results were in agreement with a previous report on a hyper-nitrified estuarine tidal flat; because of minimal competition with phytoplankton and the higher ammonium concentration in winter, the abundance of ammonia oxidizers was much higher in winter than in other seasons (Zhang et al. 2014).

Phylogenetic analysis revealed that AOB community composition was seasonally different, and AOB in summer and winter belonged mainly to *Nitrosospira* sp. and *Nitrosomonas* sp. respectively. *Nitrosomonas* sp., such as *N. mobilis*, were the dominant microorganisms in wastewaters, activated sludge, and N removal applications in WWTP (Juretschko et al. 1998; Koops and Pommerening-Röser 2001; Limpiyakorn et al. 2013; Mota et al. 2014), as well as in freshwater and river sediments impacted by wastewater effluent (Cébron et al. 2004). Beiyun River is a typical seasonal river receiving municipal sewage and WWTP effluent from downtown throughout the whole year. Wastewater effluent contains

nitrogen nutrients, organic matter and heterotrophic microorganisms (Cébron and Garnier 2005; Wakelin et al. 2008), which always causes nutrient overload and a shift in microbial community by habitat disturbance in its receiving river. As for Beiyun River, in terms of seasonal precipitation, the influence of river recharge continually from effluents was more significant in the dry season (winter) than in the wet season (summer). As a result, we found that more sequences of bacterial *amoA* in winter were affiliated with *Nitrosomonas* sp.

Conclusions

AOB dominated over AOA in community abundance, and the relative abundances of AOB and AOA communities were significantly higher in winter than in summer. Phylogenetic analysis revealed that *Nitrosospira* sp. was the dominant AOB in summer, but *Nitrosomonas* sp. in winter, while no specific genus was found for AOA in this study. A substantial proportion of *amoA* sequences matched closely with those from WWTP systems, while others were close to those from soil or sediment sources. Watergate also had a significant effect on the distribution of pollutants and microbes in the river, as the nitrogen content and communities of AOA and AOB in the sediments deriving from Yangwa Watergate (S4 and W4) were quite different from those at other sites. The input of WWTP effluent with high nitrogen load and watergate operations are prevailing problems for most urban rivers. Riverbed sediments are critical spots for nitrogen conversion and microbial processes. Therefore, further studies are needed to understand the mechanisms controlling the effects of environmental parameters (including pollutants, dissolve oxygen, light exposure and etc.) on ammonia-oxidizing microorganisms in the sediments of urban rivers, and to clarify the relative activities and contributions of AOA and AOB to the nitrogen cycle in such environments.

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