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ORIGINAL ARTICLE

Abundance and diversity of ammonia-oxidizing archaea in a biological aerated filter process

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Abstract Ammonia-oxidizing archaea (AOA) represent an important group of ammonia-oxidizing microorganisms that are able to convert ammonia to nitrite, a function which is crucial for the removal of nitrogen from wastewater. In this study, we investigated the abundance and diversity of AOA in a full-scale wastewater treatment plant (WWTP) which used a biological aerated filter (BAF) as the main processing mode. According to the quantitative PCR results, AOA clearly outnumbered ammonia-oxidizing bacteria (AOB) during the whole process. The abundance of AOA *amoA* genes in the filter layer of BAF was highest with the value varied from 6.32×10^3 to 3.8×10^4 copies/ng DNA. The highest abundance of AOB *amoA* genes was 1.32×10^2 copies/ng DNA, recorded in the effluent of the ACTIFLO® settling tank. The ratios of AOA/AOB in the WWTP were maintained at two or three orders of magnitude. Most AOA obtained from the WWTP fell within the *Nitrosopumilus* cluster. The abundance of AOA and AOB was significantly correlated with ammonium nitrogen concentrations and pH value. The community structure of AOA was significantly influenced by dissolved oxygen concentrations, pH value and chemical oxygen demand.

Keywords Ammonia-oxidizing archaea · Ammonia-oxidizing bacteria · Biological aerated filter · Environmental factors

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Introduction

Nitrification and denitrification are key steps in the removal of biological nitrogen from wastewater (Ferrera and Sánchez 2016). The oxidation of ammonia to nitrite is the first and rate-limiting step in nitrification (Kowalchuk and Stephen 2001). Two groups of microorganisms, ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA), are believed to be involved in this process. Both AOB and AOA contain *amoA* genes that encode ammonia monooxygenase, the key enzyme that catalyzes the oxidation of ammonia to hydroxylamine (Li et al. 2016). AOA have been found in a broad variety of natural environments, such as terrestrial systems (Chen et al. 2013), seawater (Santoro and Casciotti 2011; Hou et al. 2013; Lv 2013), estuarine and ocean sediments (Sakami 2012), suggesting the important role of AOA in global nitrogen cycle. However, compared with AOB, little research has been conducted on AOA, especially on the role of AOA in wastewater treatment processes.

Environmental factors, such as ammonium nitrogen (NH_4^+-N) concentrations, temperature, salinity, pH and dissolved oxygen (DO) levels can significantly affect the abundance and diversity of AOA (Caffrey et al. 2007). Among these factors, DO level and NH_4^+-N concentration are considered to be the most important (Yan et al. 2016). Ye and Zhang (2011) reported that DO level had a large effect on the types and quantities of different AOA and AOB. Park et al. (2006) suggested that low DO levels might be the most appropriate for the growth of AOA, but other studies have shown different results; for example, Kayee et al. (2011) identified a large number of AOA *amoA* genes were found in wastewater

treatment plants (WWTPs) at a DO concentration of 3.25 mg O_2/L. The substrate affinity (K_s) values of AOA for NH_4^+-N are one to four logs lower than those values of AOB in WWTPs (Limpiyakorn et al. 2013), suggesting that AOA may be more suitable to grow under low NH_4^+-N concentrations. These authors also reported that the expression of AOA *amoA* genes could be repressed under high NH_4^+-N concentrations.

The biological aerated filter (BAF) is a submerged wastewater treatment technology which consists of porosity filters with a large surface area for the growth of biofilm (Bao et al. 2016). Compared with conventional activated sludge processes (Wang et al. 2017), the BAF has many advantages, including higher organic loadings, higher shock resistance and less sludge production. In recent years the BAF has been widely used in WWTPs worldwide, especially for enhancing the removal of nitrogen from wastewater (Yu et al. 2016).

The presence of AOA in WWTPs has been reported by a number of research groups (Park et al. 2006; Sonthiphand and Limpiyakorn 2011; Tong et al. 2011; Yapsakli et al. 2011; Ye and Zhang 2011; Gao et al. 2013; Chen et al. 2017), but the role AOA play in WWTP systems is still open to discussion. In this study, we selected a full-scale municipal WWTP with a BAF as the main unit (Shenzhen, China) as the object of study. The abundance and diversity of AOA and AOB in different treatment stages of the WWTP were studied, with a focus on the AOA in the filter layer of the BAF. We also evaluated the relationships between environmental factors and AOA communities.

Material and methods

Description of the WWTP

Samples of wastewater and filters were taken from a full-scale municipal WWTP with a treatment capacity of 5.0×10^4 m^3/day in Shenzhen, China. The treatment processes of the WWTP are a combination of BIOSTYR® BAF and ACTIFLO® setting tank (Veolia Water Technologies, Veolia Environnement, Paris, France). As shown in Fig. 1, the wastewater is treated successively by passage through a bar screen, aerated grit chamber and primary settling tank to remove suspended solids (SS). The effluent from the primary settling tank, the backwash wastewater following treatment in the ACTIDYN® settling tank (Veolia Water Technologies) and the effluent from the anaerobic filter are introduced into the BAF for the removal of pollutants, including chemical oxygen demand (COD), NH_4^+-N, total nitrogen (TN) and total phosphorus (TP). Lime is added to the BAF to adjust the alkalinity. Of the effluent introduced into the BAF, 48% passes into an anaerobic filter, which is used as denitrification tank for nitrogen removal. Methyl alcohol is added into the anaerobic filter as the carbon source, which is necessary for denitrification. The effluent from the BAF is finally treated by ACTIFLO® settling tank for removal of the detached biofilm and SS. After UV disinfection, the effluent is discharged into the river. The sludge from the primary, ACTIFLO® and ACTIDYN® settling tanks is introduced into the sludge treatment tank for treatment and compressed to sludge cake. The exhaust gas generated during the treatment process is collected and deodorized.

Sampling sites and physicochemical analysis

Five wastewater samples were collected from the WWTP, including raw wastewater (RW), the influent of the BAF, the effluent of BAF (EB), the effluent of the anaerobic filter (EA) and the effluent of the ACTIFLO® settling tank (EAS). Two filter samples (FB and FA) were taken from the filter layers of the BAF and anaerobic filter tank, respectively. The total height of the BAF was 7 m, and the filter layer, located in the middle of the BAF, was 3.5 m high. The inlet flow and influent were located at the bottom of the BAF. In order to study the distribution of AOA communities in the BAF, we collected seven samples from the filter layer in the BAF at a height of 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 m, respectively. The concentrations ofCOD, SS, NH_4^+-N, nitrite nitrogen (NO_2^--N), nitrate nitrogen (NO_3^--N) and TN were analyzed according to standard methods (NEPA Chinese 2002). Dissolved oxygen (DO) concentrations and pH values were measured by using a DO meter (model YSI-550A; YSI Corp., Yellow Springs, OH) and pH meter (model FE20; Mettler-Toledo, Greifensee, Switzerland), respectively.

DNA extraction

To achieve high concentrations of DNA, we filtered 1 L of each water sample through a cellulose nitrate membrane (pore size 0.22 μm). The filter was then cut into pieces, and these pieces and glass beads were placed together into a centrifuge tube and 10 mL of sterile water added. The mixture was gently mixed by vortex to ensure that all residues were separated from the membrane and then centrifuged at 4874 *g* for 5 min. For the filter samples, 10 mL of the filter suspension was put into a centrifuge tube together with 10 mL of sterile water. The mixture was then gently mixed by vortex to ensure that all biofilms had fallen offthe filters, followed by centrifugation at 4874 *g* for 5 min. DNA was extracted from the centrifuged deposit (0.5 g) by the Fast DNA® SPIN Kit For Soil (MP Biomedicals, Santa Ana, CA) following the manufacturer's instructions. After being confirmed by conventional electrophoresis on a 1% agarose gel electrophoresis, the concentration of DNA was measured on a NanoDrop UV-Vis spectrophotometer (model ND-2000; Thermo Fisher Scientific, Waltham, MA).

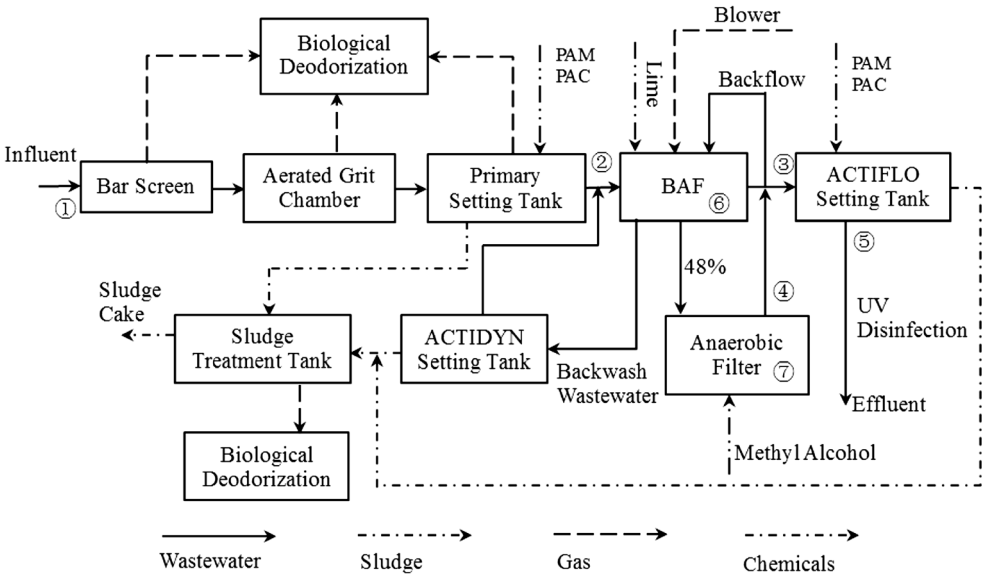

Fig. 1 The flow chart of the targeted full-scale municipal wastewater treatment plant (WWTP) showing the main wastewater treatment processes and sampling sites. Water sampling sites (①– ⑤ and filter sampling sites (⑥, ⑦) are shown. ① Raw wastewater, ② influent of the biological aerated filter (*BAF*), ③ the effluent of BAF, ④ the effluent of the anaerobic filter tank, ⑤ the effluent of the ACTIFLO® settling tank, ⑥ filter layers of the BAF, ⑦ filter layers of the anaerobic filter tank. *PAM* Polyacrylamide, *PAC* polyaluminium chloride

PCR amplification, sequencing and construction of clone libraries

Primers Arch-*amoA* 26F (5′-GACT ACATMTTCTAYACWGAYTGGGC-3′) and Arch-*amoA* 417R (5′-GGKGTCATRTATGGWGGYAAYGTTGG-3′) (Park et al. 2008) were selected to amplify the segment of AOA *amoA* gene fragments. The PCR cycling program consisted of an initial denaturation at 98 °C for 2 min, followed by 35 cycles at 98 °C for 30 s, annealing at 55 °C for 30 s and 72 °C for 1 min, with a final extension at 72 °C for 5 min. Following purification with the MiniBest Agarose Gel DNA Extraction kit Ver.3.0 (TaKaRa, Tokyo, Japan), the PCR products were ligated into the pMD 19-T vector and transformed into *Escherichia coli* DH5α competent cells following the manufacturer's instructions.

The obtained sequences sharing at least 97% identity were grouped into one operational taxonomic unit (OTU) using Mothur. OTU-based parameters, including library coverage (*C*), the Shannon–Weiner diversity index (*H*), Pielou's evenness index (*J*), and the Chao 1 richness estimate (*S*), were calculated following the methods described in the previous studies (Good 1953; Chao 1987; Hill et al. 2003). The representative sequences were aligned to the database of the NCBI using the BLAST tool. Neighbor-joining phylogenetic trees were constructed with the obtained similar sequences using MEGA 5.1 (bootstrap value was set at 1000 replicates).

Quantification of AOA and AOB *amoA* genes

Quantitative PCR was employed to measure the abundance of AOA and AOB *amoA* gene copies in the samples. The primer pair Arch-*amoA* 26F/Arch-*amoA* 417R was used for the AOA *amoA* genes, and the primer pair *amoA*-1F (5′-GGGGTTTCTACTGGTGGT-3′)/*amoA*-2R (5′-CCCC TCKGSAAAGCCTTCTTC-3′) (Rotthauwe et al. 1997) was selected for the AOB *amoA* genes. The quantification reactions were performed in a qPCR system (LightCycler 1.5; Roche Applied Science, Penzburg, Germany). The reaction mixture (20 μL) for each qPCR amplification consisted of 10 μL SYBR Premix ExTaq™ II, 0.4 μL of each primer, and 2 μL of template DNA. The purified PCR products were ligated into pMD®19-T Vector and transformed into *E. coli* DH5α competent cells. The specificity of each PCR product for the target genes was checked using gel electrophoresis, and positive colonies were sent to The Beijing Genomics Institute for sequencing. After the target genes had been confirmed, the plasmids DNA were extracted from the positive colonies with the E.Z.N.A.™ plasmid Mini Kit (Omega Bio-Tek Inc., Norcross, GA) and used as a standard sample. The mass concentration of the plasmid DNA was determined on a NanoDrop UV-Vis spectrophotometer and converted into molecular units (the copy number of the plasmids in a unit sample). The standard sample was diluted for eight different ten-fold dilutions and the threshold cycle (C_t) value was determined; the standard curve between the C_t value and the logarithm of the sample concentration was then established. The qPCR conditions were set at 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s, 55 °C for 30 s and 72 °C for 1 min; the fluorescence value was read during each cycle at 72 °C. The dissolution curve was measured immediately after the PCR reaction at 65–95 °C. The results were accurate when the melting temperature value was >80 °C, and the dissolution curve was a single peak. The correlation coefficients (R^2) were 0.9988 for AOA *amoA* and 0.9996 for AOB *amoA*.

Statistical analysis

The relationships between environmental factors and AOA abundance were evaluated by Pearson correlation coefficients using SPSS version 17.0 (IBM Corp., Armonk, NY). The links between AOA communities in the BAF and environmental factors were assessed by redundancy analysis (RDA) using CANOCO for Windows 4.5 (Plant Research International, Wageningen, The Netherlands).

Results

Characteristics of the samples

The water quality indexes of the collected samples are shown in Table 1. The concentrations of COD, NH_4^+-N and SS sharply decreased following treatment in the primary settling tank and BAF. The temperature variations among the different units in the treatment system were negligible, with the temperature ranging between 27.6 and 28.3 °C. However, the DO concentrations of the five samples fluctuated greatly, with the highest and lowest DO concentration recorded as 4.57 mg O_2/L in the EB sample and 0.67 mg O_2/L in the EA sample, respectively. The pH value ranged between 6.57 and 7.51.

Abundance of AOA and AOB in the collected samples

Figure 2 shows the qPCR results of AOA and AOB *amoA* genes of the collected samples. In general, the qPCR results indicated that AOB *amoA* genes were ubiquitous in the treatment plants, with the abundance of AOA *amoA* genes being higher than that of AOB *amoA* genes in both the BAF and ACTIFLO® settling tank. AOA *amoA* genes were not detectable in the EA sample, possibly due to the low DO concentration in the anaerobic filter. The abundance of AOA *amoA* genes was highest in the FB sample (1.53×10^4 copies/ng DNA). Among the wastewater samples, the EB sample contained the highest amount of AOA *amoA* genes (1.02×10^2 copies/ng DNA). The abundance of AOB *amoA* genes ranged between 0.54 copies/ng DNA and 1.32×10^2 copies/ng DNA, with the highest amount of AOB *amoA* genes found in the EAS sample. However, the abundance of AOA *amoA* genes in the two filter samples (FB and FA) was distinctly higher than that of AOB *amoA* genes. The AOA/AOB ratio in the FB and FA samples was 213.63 and 27.45, respectively.

Since the abundance of AOA *amoA* genes in the FB sample was the highest, we investigated further the distribution of AOA in the filter layer of the BAF. The characteristics of wastewater samples collected at different heights (0.5-m intervals) of the filter layer were measured. As shown in Fig. 3, the recorded temperatures at the different heights were in the range of 28.4 to 29.1 °C. The highest pH value was 7.48, which was recorded at a height of 2.0 m. The pH value decreased to pH 6.66 at a height of 4.0 m, but there was no significant change in pH value from 4 to 5 m in height. However, the concentration of DO increased from 2.12 to 5.78 mg O_2/L along the direction of filtration. As shown in Fig. 4a, the NH_4^+-N concentration also significantly decreased from 11.98 mg N/L at a height of 2.0 to 2.57 mg N/L at 5 m. In comparison, the NO_3^--N concentration increased at different

Table 1 Water quality indexes of the five water samples collected from the wastewater treatment plant

Physiochemical properties[b]	Water samples[a]					*F* value	*P*-value
	RW	IB	EB	EA	EAS		
COD (mg O_2/L)	171.25 ± 8.92^a	48.05 ± 3.54^b	18.07 ± 3.20^c	18.75 ± 1.34^c	18.90 ± 4.37^c	533.2	0.0000
TN (mgN/L)	21.01 ± 2.71^a	15.50 ± 3.23^{ab}	13.24 ± 2.68^{bc}	4.96 ± 0.96^{de}	9.06 ± 1.24^{ce}	20.6	0.0001
NO_3^--N (mg N/L)	0.71 ± 0.23^a	2.73 ± 0.70^a	8.96 ± 2.27^b	2.30 ± 0.67^a	3.56 ± 1.21^a	19.5	0.0001
NO_2^--N (mg N/L)	0.30 ± 0.05^a	0.50 ± 0.26^a	0.14 ± 0.06^a	3.42 ± 0.96^b	0.72 ± 0.42^a	23.6	0.0000
NH_4^+-N (mg N/L)	16.21 ± 2.82^a	10.61 ± 2.43^b	3.29 ± 0.68^c	3.86 ± 0.92^c	3.13 ± 1.14^c	30.9	0.0000
SS (mg/L)	90 ± 17.35^a	44 ± 13.53^b	10 ± 2.65^c	7 ± 3.00^c	4 ± 3.46^c	38.3	0.0000
pH	6.99 ± 0.12^a	7.51 ± 0.05^b	6.57 ± 0.03^c	6.73 ± 0.01^c	6.60 ± 0.03^c	126.5	0.0000
DO (mg/L)	1.42 ± 0.22^{ab}	0.94 ± 0.36^a	4.57 ± 0.89^c	0.67 ± 0.03^a	3.37 ± 1.36^{bc}	14.8	0.0003
Temperature (°C)	28.2 ± 0.10^a	27.6 ± 0.17^a	28.0 ± 0.06^a	27.9 ± 0.10^a	27.8 ± 0.82^a	1.1	0.4203

Values in the same row followed by the same lowcase letter are not significantly different at $P \leq 0.05$ according to one-way analysis of variance followed by the post hoc Tukey tes)

[a] RW, Raw wastewater; IB, influent of the biological aeration filter; EB, effluent of the biological aerated filter (BAF); EA, effluent of the anaerobic filter; EAS, effluent of ACTIFLO® setting tank

[b] COD, Chemical oxygen demand; TN, total nitrogen; NO_3^--N, nitrate nitrogen; NO_2^--N, nitrite nitrogen; NH_4^+-N, ammonium nitrogen; SS, soluble solids; DO, dissolved oxygen

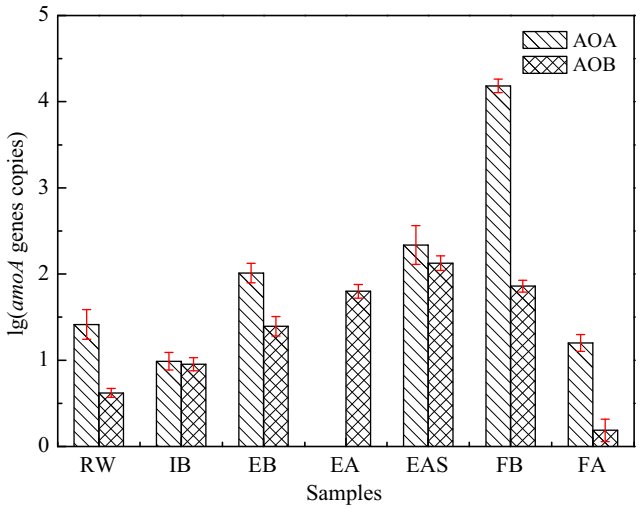

Fig. 2 Quantitative analysis of ammonia-oxidizing archaea (*AOA*) and ammonia-oxidizing bacteria (*AOB*) *amoA* genes (encoding ammonia monooxygenase) in the collected samples. *RW* Raw wastewater, *IB* influent of the BAF, *EB* effluent of the BAF, *EA* effluent of anaerobic filter, *EAS* effluent of ACTIFLO® settling tank, *FB* samples taken from the filter layers of the BAF, *FA* samples taken from the anaerobic filter

heights, with minimum and maximum concentrations of 3.64 and 12.07 mg N/L at heights of 2.0 and 5.0 m, respectively. Based on the low variations in TN and NO_2^--N concentrations at different heights, we deduced that NH_4^+-N was converted to NO_3^--N and that the nitrification reactions mainly occurred in the filter layers. The occurrence of nitrification reactions in the filter layer could also provide a possible explanation for the decrease in pH value. The COD concentration decreased sharply from 161.02 to 46.85 mg/L with increasing height of the BAF filter layers to 5 m (Fig. 4b).

The abundances of AOA and AOB in the filter samples at different heights of the BAF are shown in Fig. 5. The abundance of AOA *amoA* genes varied between 6.32×10^3 and 3.8×10^4 copies/ng DNA, while maximum value was attained

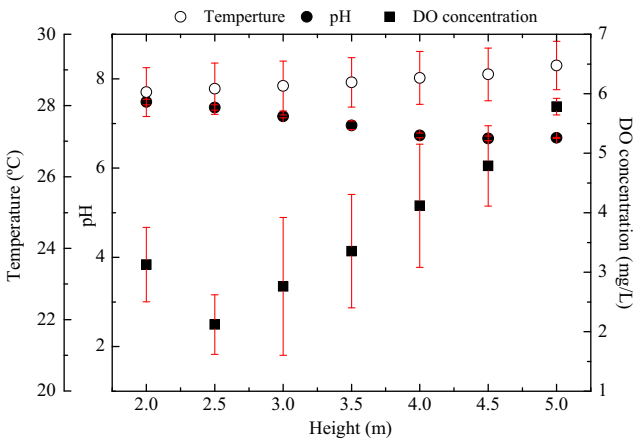

Fig. 3 Changes in the temperature, pH and dissolved oxygen (*DO*) concentration of the effluent of filter samples collected at different heights of the BAF

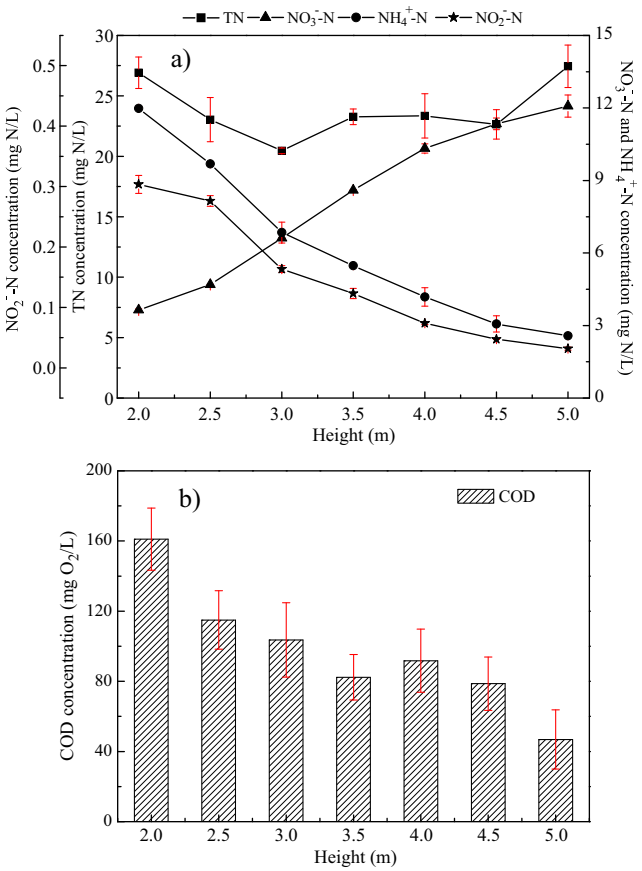

Fig. 4 Water quality indexes of the effluent of filter samples collected at different heights of the BAF. **a** Concentration of total nitrogen (*TN*), ammonium nitrogen (NH_4^+-*N*), nitrite nitrogen (NO_2^--*N*) and nitrate nitrogen (NO_3^--*N*) **b** Concentration of chemical oxygen demand (*COD*)

at a height of 5.0 m of the BAF. In comparison, the amount of AOB *amoA* genes found in the BAF varied between 20.64 and 1.05×10^2 copies/ng DNA, with the maximum values recorded at a height of 4.0 m of the BAF. The ratios of AOA/AOB ranged between 845.37 and 2784.91, which indicated that AOA were dominant over AOB in the BAF. The water quality clearly improved with increasing height along the BAF.

Diversity of AOA communities

The distribution and relative abundance of AOA *amoA* genes in the WWTP was investigated. Based on AOA *amoA* gene sequences, we constructed and evaluated five clone libraries and 27 OTUs. The distribution of the relative abundances of OTUs in the collected samples are shown in Fig 6. OTU4 and OTU8 were dominant in the RW and EAS samples, suggesting that the AOA communities in the influent and effluent of the WWTP changed little. OTU3 was dominant in FB and FA, which implied that the BAF and anaerobic filter shared the same dominant OTU type. OTU3 was also the most widely distributed OTU type, being present in all five samples tested. The results suggested that OTU3 had a strong adaptability to

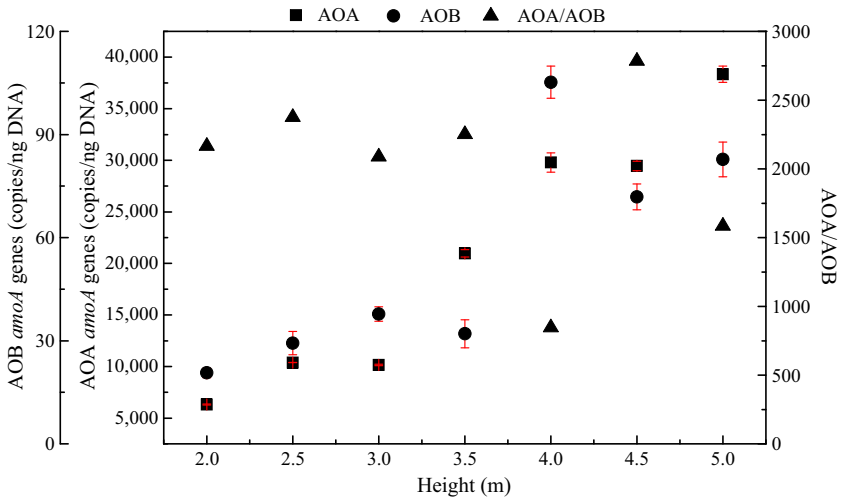

Fig. 5 The abundance of AOA and AOB *amoA* genes in filter samples collected at different heights of the BAF

the environment. Although OTU4 dominated in the RW sample, it was not present in the FB sample, suggesting that the environmental conditions in the BAF might be not suitable for the growth of OTU4. The *C*, *J*, *H* and *S* values of the AOA *amoA* gene clone libraries are presented in Table 2. The results showed that RW and EAS contained 12 and 10 OTU types, respectively. In contrast, only five OTU types were detected in the FA sample. The FA sample had the minimum *H* value (0.54) among the five samples measured, suggesting that the diversity of AOA *amoA* genes in the filter layer of the anaerobic filter was lower than in the other samples. The *C* value of the FB and FA samples was 90.0 and 90.6%, respectively, which indicated that clone libraries covered the AOA communities in FA and FB samples and that the majority of species could be detected and identified. For the samples taken from wastewater (RW, EB and EAS), the *H* value and *S* value were all higher than the samples from the filers (FB and FA), indicating that the AOA from wastewater had a richer species diversity. The highest *H* and *S* values were 2.47 and 34.5, respectively, detected from the EAS sample (Table 2).

The distribution and relative abundance of AOA *amoA* genes in the filter layer of the BAF was further investigated (Fig. 7). Six clone libraries were constructed and eight OTUs were obtained from the AOA *amoA* gene sequences at the 3% nucleotide cut-off. The relative abundance of OTUs in the samples collected from the heights of 2.0 and 3.5 m was over 60%, and OTUb and OTUc were the dominant types in samples collected between the heights of 4.0 and 5.0 m. The relative abundance of both kinds of OTU types was around 40%. OTUb was the most prevalent OTU type among the eight OTUs in the BAF, being present in all samples (Fig. 7). The value of *C* ranged between 78.6 and 100%,

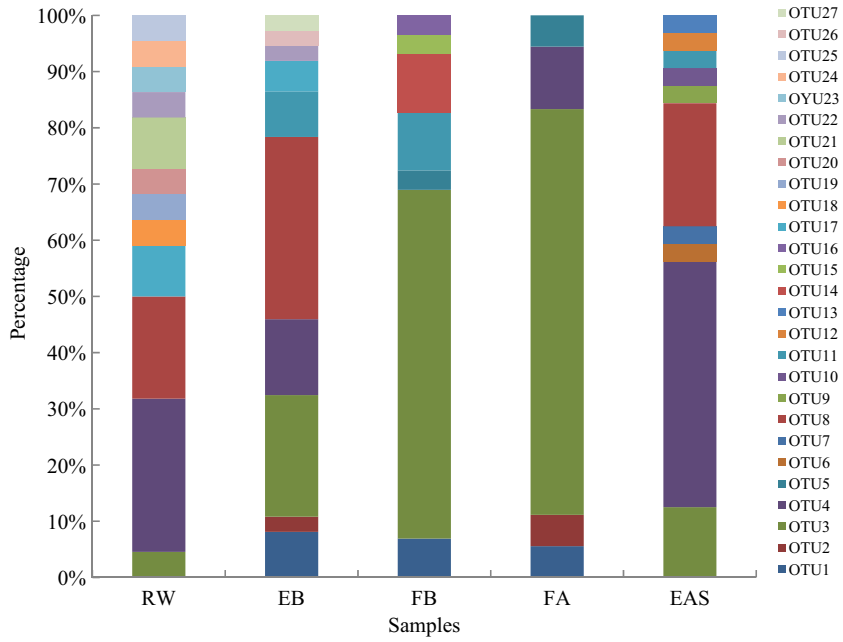

Fig. 6 Distribution and relative abundance of the AOA *amoA* gene in the collected samples. *OTU* Operational taxonomic unit

Table 2 Diversity of each clone library of ammonia oxidizing archaea *amoA* genes in the five water samples

Samples	No. of clones	OTUs	*C*	*J*	*H*	*S*
RW	37	12	78.4%	0.65	1.63	21.78
EB	18	8	68.2%	0.85	1.95	11.50
FB	29	7	90.0%	0.67	1.30	8.88
FA	32	5	90.6%	0.34	0.54	6.88
EAS	22	10	77.8%	1.19	2.47	34.5

OTU, Operational taxonomic unit; *C*, library coverage; *H,* Shannon–Weiner diversity index; *J*, Pielou's evenness index; *S* Chao 1 richness estimate

Table 3 Diversity of each clone library of ammonia oxidizing archaea *amoA* genes at different heights of the biological aerated filter

Height (m)	No. of clones	OTUs	*C*	*J*	*H*	*S*
2	9	2	100%	6.77	0.64	2.00
2.5	23	3	100%	0.69	0.75	3.00
3	14	4	78.6%	0.54	0.75	8.5
3.5	24	4	95.8%	0.69	0.96	4.50
4	7	3	85.7%	0.91	1.00	3.50
5	28	6	92.8%	0.75	1.35	6.11

suggesting that AOA communities covered the whole clone libraries of these samples (Table 3). However, the *H* value increased gradually from 0.64 to 1.35 with increasing of height from 2.0 to 5.0 m, indicating that the increase of AOA diversity was related to the height of filter layer in BAF.

Phylogenetic analysis of AOA

The phylogenetic tree of AOA *amoA* genes in the municipal WWTPs is shown in Fig. 8. Based on the results, the obtained AOA *amoA* sequences could be grouped into two major clusters. Most of the OTUs in one of the major clusters were strongly correlated with sequences from the natural environment, such as rivers, lakes, estuary sediments and soils. The OTUs in the second major cluster had many similarities to the sequences from WWTPs. The most abundant OTU type in the RW and EAS samples, OTU4, showed high similarities to the sequence HZNAOA7 from Dongjiang River sediment, which belongs to *Nitrosopumilus* cluster (Sun et al. 2013). OTU27 from the EB sample was related to the sequences of *Nitrosopumilaceae archaeon* MY1 which fall phylogenetically within the *Nitrosopumilus* cluster (Jung et al. 2011). OTU8, another dominant OTU type in the RW and EAS samples, correlated with sequences collected from the Mississippi River. OTU10 in the EAS sample was seen to be closely related to the sequences from WWTPs, which also belong to the *Nitrosopumilus* cluster (Bai et al. 2012; Gao et al. 2014). OTU3, which was dominant in the filter layer of BAF, showed a similarity to the AOA species detected in bench-scale airlift reactor for wastewater treatment. OTU26 was strongly correlated with AOA sequences collected from rice soil. OTU14 from the FB sample showed a high similarity to sequence WG6612 from Yellowstone hot spring (Zhang et al. 2008). OTU1 was present in the EB, FB and FA samples and was correlated to SL-37 from the Stanley WWTP that falls into the *Nitrososphaera* sister subcluster (Zhang et al. 2009).

Eight OTUs also fell into two major clusters, as shown in the AOA phylogenetic tree of the BAF (Fig. 9). OTUd, which was the dominant OTU type in the BAF filter between the height at 2.0 and 3.5 m, was strongly correlated with the sequences belonging to sediments of Dongjiang River. OTUb and OTUc, the dominant types in the BAF between 4.0 and 5.0 m, were similar to the AOA clones detected in a

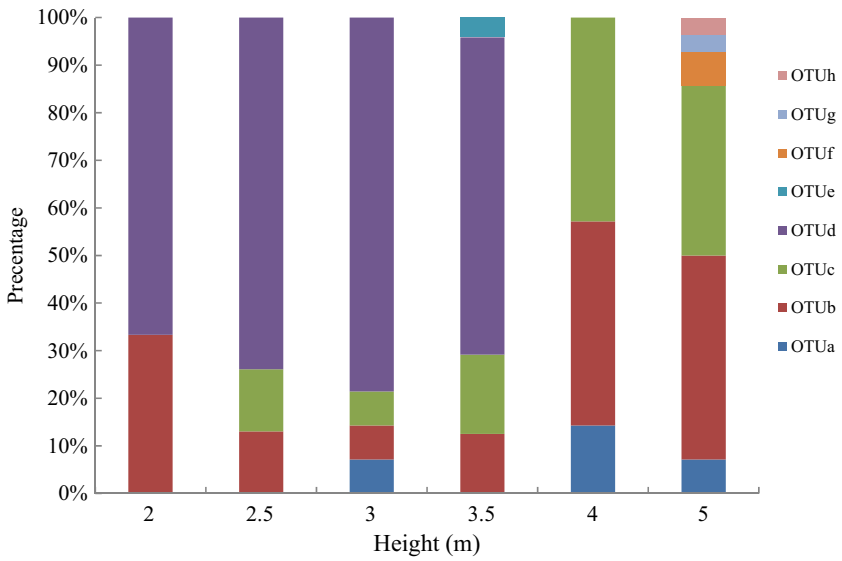

Fig. 7 Distribution and relative abundance of AOA *amoA* gene of the filter sample at different heights of the BAF

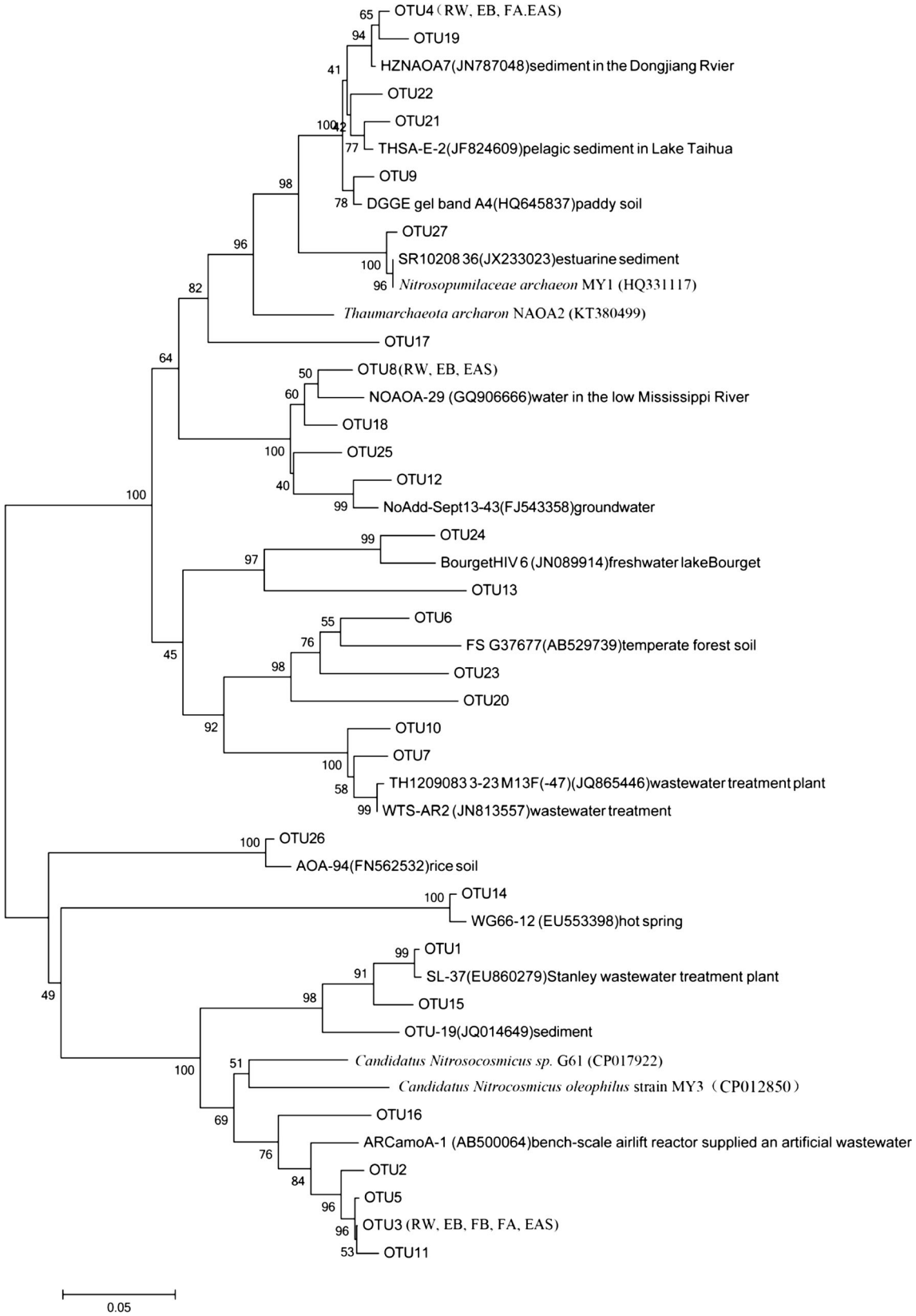

Fig. 8 Phylogenetic tree of AOA *amoA* gene sequence from different samples in the municipal wastewater treatment plants

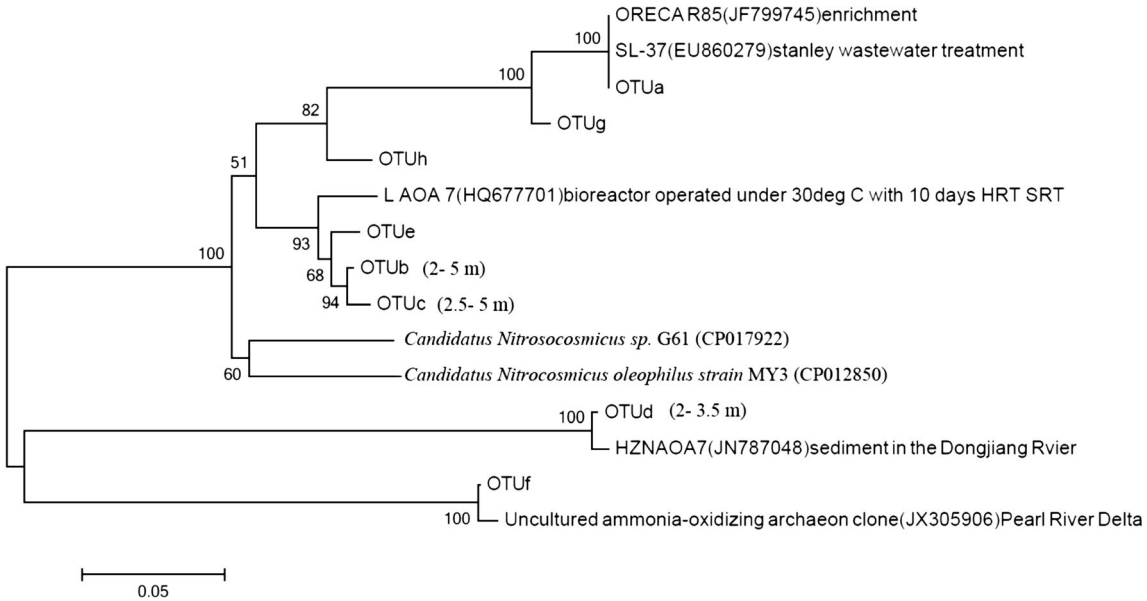

Fig. 9 Phylogenetic tree of AOA *amoA* gene sequences from different samples in the filter layer of the BAF

wastewater treatment bioreactor operating at 30 °C for 10 days. Most OTUs obtained from the filter layer of BAF fell into the *Nitrosocomicus* cluster. These results led us to conclude that the AOA near the inlet of the BAF were similar to the ones from natural environments, while the AOA near the outlet of BAF were similar to the ones from wastewater treatment systems.

Correlations between AOA community structure and environmental factors

We used RDA to evaluate the correlations between the AOA community structures in the BAF and environmental factors. The results are shown in Fig. 10, where Axis1 and Axis2 correspond to 73.0 and 10.9% of the cumulative variance of the AOA community–environment relationship, respectively. The location of the red arrows of COD, pH and DO near Axis1 suggest that these factors had significant effects on the community structures of AOA from the filter layer of the BAF. The AOA near the bottom of filter layer (between the height of 2 and 2.5 m) in the BAF were mainly distributed along pH and DO gradients, indicating that the AOA from the bottom of the filter layer were strongly correlated with pH and DO concentrations. The communities of AOA at the top of the filter layer (at the height of 5 m) in the BAF were significantly affected by COD, NH_4^+-N and NO_2^--N. In addition, the DO concentration showed a strong negative correlation with the concentrations of COD, NH_4^+-N and NO_2^--N. NH_4^+-N concentration also showed a strong negative correlation with NO_3^--N concentration.

Discussion

A number of studies have demonstrated the presence of AOA *amoA* genes in WWTPs, but the abundances of AOA and AOB *amoA* genes have remained a focus of controversy. AOB *amoA* genes have been reported to be the dominant type in WWTPs (Wells et al. 2009; Jin et al. 2010; Tong et al. 2011; Bai et al. 2012; Gao et al. 2013). Yapsakli et al. (2011) investigated AOA and AOB abundance in a full-scale leachate

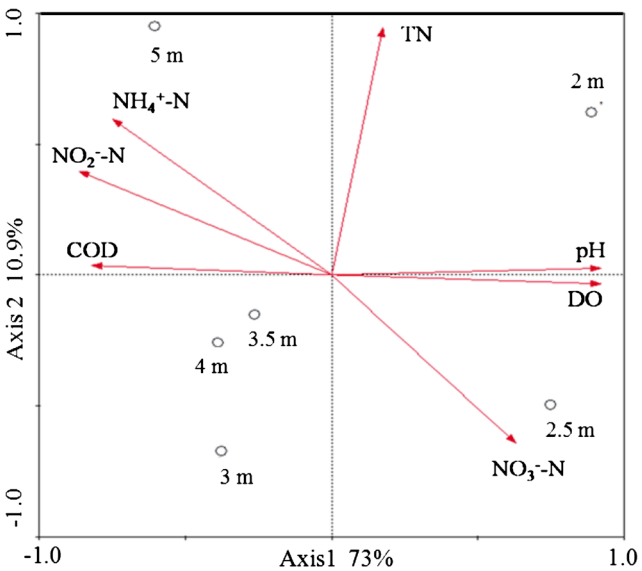

Fig. 10 Ordination plots generated by redundancy analysis. *Red arrows* Environmental variables (pH, DO, COD, TN, NO_2^--N, NO_3^--N, and NH_4^+-N), *circles and numbers* filter samples collected at different heights from the BAF

treatment plant with high concentration of COD (5000 mg O_2/L) and TN (2000 mg N/L) and found that AOB were dominant in the treatment plant, with an AOB/AOA ratio range of one to three orders of magnitude. These results were confirmed by Gao et al. (2014) who reported that AOB *amoA* genes outnumbered AOA in ten WWTPs. In contrast, Kayee et al. (2011)found that AOA *amoA* genes outnumbered AOB *amoA* genes in eight municipal WWTPs, and Bai et al. (2012) demonstrated that AOA were dominant in three municipal WWTPs, while AOB were dominant in three industrial WWTPs. A relatively higher amount of AOA *amoA* genes was also found by Muβmann et al. (2011) as well as Sonthiphand and Limpiyakorn (2011). In the present study, we investigated a full-scale municipal WWTP with a BAF as the main processing unit. The WWTP had a high capacity for treating pollutants, with the concentrations of COD and SS in wastewater decreasing from 171.25 mg O_2/L and 90 mg/L to 18.90 mg O_2/L and 4 mg/L, respectively (Table 1). AOA and AOB *amoA* genes were detected in the collected samples from different sections of the WWTP, and our results showed that AOA were dominant in the BAF processing unit. Across the flow path of the treatment system, the abundance of AOA *amoA* genes in wastewater increased from 26.00 to 217.27 copies/ng DNA, and the NH_4^+-N concentration decreased from 16.21 to 3.13 mg N/L (Table 1). The abundance of AOB *amoA* genes in the wastewater also increased from 4.18 to 133.97 copies/ng DNA during the process.

AOA abundance showed a strong, positive association with temperature and NO_3^--N concentration and a significant, negative correlation with NH_4^+-N and NO_2^--N concentrations, respectively, and pH value (Table 4). Since NO_3^--N was mainly converted from NH_4^+-N oxidation in the BAF, NO_3^--N concentrations increased concomitantly with the decrease in NH_4^+-N concentrations. In addition, NO_2^--N concentrations were always very low, at <0.4 mg N/L. The pH value and NH_4^+-N concentration decreased with increasing height of the filter, with a simultaneous increase in AOA and AOB *amoA* genes. We therefore surmised that NH_4^+-N concentration and pH value were the main critical influences on the abundance of AOA. In terms of AOB abundance, the pH value and NH_4^+-N concentration had a significant negative influence. As such, AOA and AOB *amoA* genes shared common influencing factors. As an electron acceptor, DO is also considered to be an important factor in the processing of wastewater. It has been suggested that some types of AOA may only be able to survive under low DO concentrations; thus low DO concentrations may be a crucial factor for the presence of AOA (Erguder et al. 2009). However, the results obtained in our study are no in agreement with this speculation. In our study, the amount of AOA *amoA* genes increased with increasing DO concentration in the filter layer. One possible explanation for this phenomenon is the possibility that the AOA types in the BAF were able to adapt to the environment of a relatively high DO level. It is also possible that the oxygen was consumed quickly by heterotrophic microorganisms on the surface of biofilm and that AOA may be present in the depths of biofilm at low DO levels. In addition, with the increase in the height of the BAF from 2.0 to 5.0 m, COD and NH_4^+-N concentrations decreased while at the same time there was a significant increase of AOA *amoA* genes. This phenomenon suggests that low COD and NH_4^+-N levels may be favorable for the growth of AOA in BAF. DO concentrations and pH values, which changed regularly along the direction of filtration, may also be important influencing factors.

AOA have been divided into five major clusters based on the published AOA *amoA* gene sequences (Pester et al. 2012): *Nitosopumilis* cluster, *Nitrososphaera* cluster, *Nitrosocaldus* cluster, *Nitrosotalea* cluster and *Nitrosophaera* sister cluster. In the present study, five clone libraries of AOA were constructed with samples from the WWTP. The obtained AOA *amoA* gene sequences fell into two major clusters. OTU4 and OTU8, which were dominant in the wastewater samples, grouped into Cluster A, showing a highly similarity with sequences from natural environments, which belonged to the *Nitrosopumilus* cluster. OTU3 from FB fell into Cluster B, with most sequences obtained from WWTPs. Many studies have found that most AOA obtained from WWTPs belong to the *Nitrososphaera* cluster (Muβmann et al. 2011; Tong et al. 2011; Yapsakli et al. 2011; Sauder et al. 2012; Gao et al. 2013), but other studies have reported that *Nitrosopumilus* cluster is dominant in WWTPs. A novel AOA strain SAT1 enriched from activated sludge has been affiliated with the *Nitrosopumilus* cluster (Li et al. 2016). Similar results were also reported by Wu et al. (2013).

The abundance and diversity of AOA *amoA* genes can be significantly influenced by environmental factors. The increase in DO concentrations could promote the degradation of organic pollutants, and the nitrification process in which

Table 4 Statistical analysis of physicochemical parameters and the abundance of *amoA* genes of AOA and AOB

Parameters	NH_4^+-N	NO_2^--N	NO_3^--N	TN	COD	DO	pH	Temperature
AOA *amoA* genes	−0.685**	−0.623**	0.536**	−0.116	−0.252	0.355	−0.555**	0.596**
AOB *amoA* genes	−0.450*	−0.316	0.166	−0.387	−0.078	0.380*	−0.572**	0.226

**Highly significant at $P \leq 0.01$; *significant at $P \leq 0.05$

NH_4^+-N and NO_2^--N is converted to NO_3^--N correlated negatively to the concentrations of COD, NH_4^+-N and NO_2^--N. The negative correlation between NH_4^+-N and NO_3^--N may be attributed to the conversion of NH_4^+-N into NO_3^--N in the BAF. The minimal angles between DO, pH, COD and Axis 1 also confirmed that the AOA community structure in BAF was significantly influenced by DO concentrations, pH value and COD concentrations (Fig. 10).

In summary, in this study, we investigated the abundance and diversity of AOA in a full-scale WWTP with BAF as the main processing unit. We found that AOA *amoA* genes outnumbered AOB *amoA* genes in the WWTP, especially in the filter layer of the BAF. The abundance of AOA *amoA* genes in the BAF varied from 6.32×10^3 to 3.8×10^4 copies/ng DNA, while the highest abundance of AOB *amoA* genes was 1.05×10^2 copies/ng DNA. AOA were dominant in the BAF, suggesting the important role of this type of microorganisms on nitrogen removal. The AOA sequences obtained from the WWTP were grouped into two major clusters, with one cluster strongly correlating with AOA from natural environments and the other cluster showing a high similarity to the AOA from WWTPs. The AOA in the WWTP were mostly related to the *Nitrosopumilus* cluster. pH and NH_4^+-N were critical environmental factors with a major effect on the abundance of AOA and AOB *amoA* genes. AOA community structure was significantly influenced by DO concentrations, pH value and COD concentration.

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Compliance with ethical standards

Disclosure No conflict of interest was reported by the authors.

References

Bai Y, Sun Q, Wen D, Tang X (2012) Abundance of ammonia-oxidizing bacteria and archaea in industrial and domestic wastewater treatment systems. FEMS Microbiol Ecol 80:323–330

Bao T, Chen T, Tan J, Wille M, Zhu D, Chen D, Xi Y (2016) Synthesis and performance of iron oxide-based porous ceramsite in a biological aerated filter for the simultaneous removal of nitrogen and phosphorus from domestic wastewater. Sep Purif Technol 167:154–162

Caffrey JM, Bano N, Kalanetra K, Hollibaugh JT (2007) Ammonia oxidation and ammonia-oxidizing bacteria and archaea from estuaries with differing histories of hypoxia. ISME J 1:660–662

Chao A (1987) Estimating the population size for capture–recapture data with unequal catchability. Biometrics 43:783–791

Chen Y, Xu Z, Hu H, Hu Y, Hao Z, Jiang Y, Chen B (2013) Responses of ammonia-oxidizing bacteria and archaea to nitrogen fertilization and precipitation increment in a typical temperate steppe in Inner Mongolia. Appl Soil Ecol 68:36–45

Chen C, Zhang RC, Xu XJ, Fang N, Wang AJ, Ren NQ, Lee DJ (2017) Enhanced performance of denitrifying sulfide removal process at high carbon to nitrogen ratios under micro-aerobic condition. Biores Technol 232:417–422

Erguder TH, Boon N, Wittebolle L, Marzorati M, Verstraete W (2009) Environmental factors shaping the ecological niches of ammonia-oxidizing archaea. FEMS Microbiol Rev 33(5):855–869

Ferrera I, Sánchez O (2016) Insights into microbial diversity in wastewater treatment systems: how far have we come? Biotechnol Adv 34: 790–802

Gao J, Luo X, Wu G, Li T, Peng Y (2014) Abundance and diversity based on *amoA* genes of ammonia-oxidizing archaea and bacteria in ten wastewater treatment systems. Appl Microbiol Biotechnol 98:3339–3354

Gao JF, Luo X, Wu GX, Li T, Peng YZ (2013) Quantitative analyses of the composition and abundance of ammonia-oxidizing archaea and ammonia-oxidizing bacteria in eight full-scale biological wastewater treatment plants. Bioresour Technol 138C:285–296

Good IJ (1953) The population frequencies of species and the estimation of population parameters. Biometrika 40:237–264

Hill TC, Walsh KA, Harris JA, Moffett BF (2003) Using ecological diversity measures with bacterial communities. FEMS Microbiol Ecol 43:1–11

Hou J, Song C, Cao X, Zhou Y (2013) Shifts between ammonia-oxidizing bacteria and archaea in relation to nitrification potential across trophic gradients in two large Chinese lakes (Lake Taihu and Lake Chaohu). Water Res 47:2285–2296

Jin T, Zhang T, Yan Q (2010) Characterization and quantification of ammonia-oxidizing archaea (AOA) and bacteria (AOB) in a nitrogen-removing reactor using T-RFLP and qPCR. Appl Microbiol Biotechnol 87:1167–1176

Jung MY, Park SJ, Min D, Kim JS, Rijpstra WIC, Damsté JSS, Kim GJ, Madsen EL, Rhee SK (2011) Enrichment and characterization of an autotrophic ammonia-oxidizing archaeon of mesophilic Crenarchaeal group I.1a from an agricultural soil. Appl Environ Microbiol 77:8635–8647

Kayee P, Sonthiphand P, Rongsayamanont C, Limpiyakorn T (2011) Archaeal amoA genes outnumber bacterial *amoA* genes in municipal wastewater treatment plants in Bangkok. Microb Ecol 62:776–788 (retracted article)

Kowalchuk GA, Stephen JR (2001) Ammonia-oxidizing bacteria: a model for molecular microbial ecology. Annu Rev Microbiol 55:485–529

Li Y, Ding K, Wen X, Zhang B, Shen B, Yang Y (2016) A novel ammonia-oxidizing archaeon from wastewater treatment plant: its enrichment, physiological and genomic characteristics. Sci Rep Uk 6:23747

Limpiyakorn T, Fürhacker M, Haberl R, Chodanon T, Srithep P, Sonthiphand P (2013) *amoA*-encoding archaea in wastewater treatment plants: a review. Appl Microbiol Biotechnol 97:1425–1439

Lv ZLWJ (2013) Abundance and diversity of ammonia-oxidizing archaea in response to various habitats in Pearl River Delta of China, a subtropical maritime zone. J Environ Sci (China) 25:1195–1205

Mußmann M, Brito I, Pitcher A, Damsté JSS, Hatzenpichler R, Richter A, Nielsen JL, Nielsen PHR, Müller A, Daims H (2011) Thaumarchaeotes abundant in refinery nitrifying sludges express *amoA* but are not obligate autotrophic ammonia oxidizers. Proc Natl Acad Sci USA 108:16771

NEPA Chinese (2002) Water and wastewater monitoring methods. Chinese Environmental Science Publishing House, Beijing

Park HD, Wells GF, Bae H, Criddle CS, Francis CA (2006) Occurrence of ammonia-oxidizing archaea in wastewater treatment plant bioreactors. Appl Environ Microbiol 72:5643–5647

Park SJ, Park BJ, Rhee SK (2008) Comparative analysis of archaeal 16S rRNA and *amoA* genes to estimate the abundance and diversity of ammonia-oxidizing archaea in marine sediments. Extremophiles 12: 605–615

Pester M, Rattei T, Flechl S, Gröngröft A, Richter A, Overmann J, Reinhold-Hurek B, Loy A, Wagner M (2012) *amoA*-based consensus phylogeny of ammonia-oxidizing archaea and deep sequencing of amoA genes from soils of four different geographic regions. Environ Microbiol 14:525–539

Rotthauwe JH, Witzel KP, Liesack W (1997) The ammonia monooxygenase structural gene *amoA* as a functional marker: molecular fine-scale analysis of natural ammonia-oxidizing populations. Appl Environ Microbiol 63:4704

Sakami T (2012) Distribution of ammonia-oxidizing archaea and bacteria in the surface sediments of Matsushima Bay in relation to environmental variables. Microbes Environ 27:61–66

Santoro AE, Casciotti KL (2011) Enrichment and characterization of ammonia-oxidizing archaea from the open ocean: phylogeny, physiology and stable isotope fractionation. ISME J 5:1796–1808

Sauder LA, Peterse F, Schouten S, Neufeld JD (2012) Low-ammonia niche of ammonia-oxidizing archaea in rotating biological contactors of a municipal wastewater treatment plant. Environ Microbiol 14:2589

Sonthiphand P, Limpiyakorn T (2011) Change in ammonia-oxidizing microorganisms in enriched nitrifying activated sludge. Appl Microbiol Biotechnol 89:843–853

Sun W, Xia C, Xu M, Guo J, Wang A, Sun G (2013) Distribution and abundance of archaeal and bacterial ammonia oxidizers in the sediments of the Dongjiang River, a drinking water supply for Hong Kong. Microbes Environ 28:457

Tong Z, Lin Y, Yan TAH, Shao MF, Si L (2011) Ammonia-oxidizing archaea and ammonia-oxidizing bacteria in six full-scale wastewater treatment bioreactors. Appl Microbiol Biotechnol 91:1215–1225

Wang Q, Wei W, Gong Y, Yu Q, Li Q, Sun J, Yuan Z (2017) Technologies for reducing sludge production in wastewater treatment plants: State of the art. Sci Total Environ

Wells GF, Park HD, Yeung CH, Eggleston B, Francis CA, Criddle CS (2009) Ammonia-oxidizing communities in a highly aerated full-scale activated sludge bioreactor: betaproteobacterial dynamics and low relative abundance of Crenarchaea. Environ Microbiol 11:2310

Wu Y, Whang L, Fukushima T, Chang S (2013) Responses of ammonia-oxidizing archaeal and betaproteobacterial populations to wastewater salinity in a full-scale municipal wastewater treatment plant. J Biosci Bioeng 115:424–432

Yan L, Li Z, Wang G, Gao Y, Wang Y, Gu J, Wang W (2016) Diversity of ammonia-oxidizing bacteria and archaea in response to different aeration rates during cattle manure composting. Ecol Eng 93:46–54

Yapsakli K, Aliyazicioglu C, Mertoglu B (2011) Identification and quantitative evaluation of nitrogen-converting organisms in a full-scale leachate treatment plant. J Environ Manag 92:714–723

Ye L, Zhang T (2011) Ammonia-oxidizing bacteria dominates over ammonia-oxidizing archaea in a saline nitrification reactor under low DO and high nitrogen loading. Biotechnol Bioeng 108:2544–2552

Yu Q, Huang H, Ren H, Ding L, Geng J (2016) In situ activity recovery of aging biofilm in biological aerated filter: surfactants treatment and mechanisms study. Bioresour Technol 219:403

Zhang CL, Ye Q, Huang Z, Li W, Chen J, Song Z, Zhao W, Bagwell C, Inskeep WP, Ross C (2008) Global occurrence of archaeal *amoA* genes in terrestrial hot springs. Appl Environ Microbiol 74:6417

Zhang T, Jin T, Yan Q, Shao M, Wells G, Criddle C, Hh PF (2009) Occurrence of ammonia-oxidizing archaea in activated sludges of a laboratory scale reactor and two wastewater treatment plants. J Appl Microbiol 107:970–977