

Abundance and community structure of ammonia-oxidizing bacteria in activated sludge from different geographic regions in China

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ABSTRACT

Detailed ecological information on ammonia-oxidizing bacteria (AOB) in activated sludge of wastewater treatment plants (WWTPs) is very important to improve the efficiency of wastewater treatment. In this study, activated sludge samples were collected from seven municipal WWTPs located in seven cities in China, and real-time quantitative polymerase chain reaction (qPCR), as well as construction of clone libraries combined with correlation-based data analysis was performed. Further, the effect of geographic distribution and some water quality parameters on the ecological distribution of AOB in activated sludge from WWTPs were investigated. The geographic distribution, the influent concentration of total nitrogen (TN) and ammonia nitrogen ($\text{NH}_4^+\text{-N}$) had significant effects on the abundance of AOB ($P < 0.05$). However, the community structure of AOB were not significantly affected by geographic distribution, but by water quality parameters including the concentrations of TN and $\text{NH}_4^+\text{-N}$. *N. oligotropha* lineage was the dominant AOB group in the wastewater treatment systems. The results obtained in this study provide useful information to understand some aspects of the ecological information and influencing factors of AOB in geographically distributed WWTPs.

Key words | abundance, activated sludge, ammonia-oxidizing bacteria (AOB), community structure, wastewater treatment plant

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INTRODUCTION

As the first and rate-limiting step of the nitrification process, ammonia oxidation plays an important role in the treatment of activated sludge containing organic nitrogen wastewater process (Zhao *et al.* 2014a). This process is mainly completed by ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) (Beman & Francis 2006). But many previous studies have indicated that the abundance of AOA is much lower than those of AOB in activated sludge samples of wastewater treatment plants (WWTPs) (Siripong & Rittmann 2007; Li *et al.* 2013). Li *et al.* (2013) suggested that AOA may be a major contributor to ammonia oxidation in natural habitats but play minor roles in highly aerated activated sludge. Limpiyakorn *et al.* (2011) also indicated that the archaeal *amoA* gene is below the limit of detection in WWTPs with higher influent ammonium levels. Therefore, AOB received more attention in studies of nitrification in activated sludge samples. The abundance and community structure of AOB in WWTPs have been previously studied

(Ottawa *et al.* 2006; Limpiyakorn *et al.* 2011; Lu *et al.* 2014; Fitzgerald *et al.* 2015). Nevertheless, little information on the relationships between AOB communities and water quality parameters have been reported, and the characterization of AOB communities in WWTPs from different geographic locations has never been considered.

Unlike other environmental microorganisms, the abundance and structure of AOB communities in WWTPs are widely considered to be associated with various plant-specific parameters. For instance, Wells *et al.* (2011) found that the operation and wastewater conditions were related to the abundance of AOB. Therefore, most previous studies paid attention to the distribution of AOB in plant-specific wastewater conditions or water quality parameters. Limpiyakorn *et al.* (2011) investigated the abundance of archaeal and bacterial *amoA* genes in activated sludge of full-scale WWTPs, and found a fourfold difference among the two groups in favor of AOB. Fitzgerald *et al.* (2015)

investigated the microbial communities in reactors with efficient nitrification at low concentrations of dissolved oxygen (DO) and concluded that *Pseudomonas*, *Xanthomonadaceae*, *Rhodococcus*, and *Sphingomonas* are involved in nitrification under low DO conditions. However, these studies did not consider the effects of other water quality parameters, especially geographical localization of WWTPs. Sloan *et al.* (2006) and Lozupone (2007) indicated that the local microbial communities in influent wastewater might also affect microbial communities in WWTPs, because they were selected by local environmental conditions according to neutral processes. In addition, different operational parts in a similar WWTP may also affect microbial communities, where the activated sludge post-processing and mechanical purifying sections had the highest amount of bacteria (Kowalski *et al.* 2017). In recent years, few studies have investigated the difference of bacterial communities in geographic distribution WWTPs (Zhang *et al.* 2012; Zhao *et al.* 2014a; Niu *et al.* 2016). We hypothesized that the geographic distribution has relevant effects on the abundance and community structure of AOB in activated sludge of distinct WWTPs. To confirm this hypothesis, activated sludge samples were collected from different regions to reveal the relationships between geographic distribution and other water quality parameters with the abundance and community structure of AOB in activated sludge of WWTPs.

In this study, activated sludge samples were collected from seven WWTPs in seven cities of China. These sampling sites are located in different regions of eastern, central and western China. Real-time quantitative polymerase chain reaction (qPCR), as well as construction of clone libraries combined with correlation-based data analysis, were employed. We are trying to investigate: (1) if the abundance and community structure of AOB in activated sludge samples differ in geographically distinct WWTPs in China; (2) the relationships between water quality parameters and ecological distribution of AOB.

MATERIALS AND METHODS

WWTPs and activated sludge sampling

Samples of activated sludge were collected from the aeration tanks of seven municipal WWTPs in seven cities in China, including Wuxi (WX), Ma'anshan (MAS), Hefei (HF), Nanjing (NJ), Taiyuan (TY), Xi'an (XA) and Urumqi (ULMQ). These sampling sites are located in different parts of China, of which WX, MAS, HF, NJ are located in the eastern part; XA,

TY are located in the central part; and ULMQ is located in the western part. All the samples were collected in July and August of 2012. Most WWTPs of this study mainly treat domestic wastewater, but HF and XA also process of industrial wastewater (ca. 30–40% of total). Details of the seven WWTPs, such as wastewater treatment processes, wastewater influent parameters and effluent parameters, are shown in Table S1 (available with the online version of this paper).

Each activated sludge sample (about 1 L) was collected in sterile plastic bottles, and transported on dry ice to the laboratory within 24 h. The samples were dried using a freeze dryer (ALPHA1-2, CHRIST, Germany) and stored at -80 °C for further analysis.

Preparation of the samples and DNA extraction

For each sample, about 0.3 g dry activated sludge was collected in triplicate according to a method previously described (Zhou *et al.* 1996) for DNA extraction. DNA was extracted from each sample (triplicate) using the Power Soil DNA Isolation kit (MoBio Laboratories, Solana Beach, CA, USA) according to the manufacturer's instructions. The extracted DNA from the triplicate samples was combined. Then, they were verified by electrophoresis in 0.8% (w/v) agarose gel. Finally, the quality of the extracted DNA was determined using a Bio Photometer (Eppendorf, Hamburg, Germany).

Real-time quantitative PCR

In order to investigate the abundance of AOB in activated sludge samples, real-time qPCR was employed to quantify the bacterial *amoA* gene copies in triplicate in different activated samples.

The fluorescent dyes of SYBR Green I were used to react on the IQ5 Thermocycler (RG65HD, Corbett, Australia). The primers *amoA*-1F/*amoA*-2R were used for the bacterial *amoA* PCR amplification (Rotthauwe *et al.* 1997). A series of 10 times dilutions of plasmid DNA were used to construct the standard curves, covering a known concentration range (1.59×10^2 – 1.59×10^8 of bacterial *amoA* gene copies per microliter).

The 20 μ L reaction mixtures for bacterial *amoA* PCR amplification, included 5 ng DNA template, 1 \times SYBR Premix Ex TaqTM buffer (Takara, Japan), 0.2 μ M forward and reverse primers. Thermalcycler protocol was set at: 95 °C for 3 min; 45 cycles of 95 °C for 30 s, 50 °C for 1 min, 72 °C for 20 s, and a final extension at 72 °C for 7 min.

The specificity of the PCR products was checked with melting curve analysis and 2% (w/v) agarose gel electrophoresis. Data analysis was carried out using the RotorGene 6,000 software package. The amplification efficiencies of bacterial *amoA* gene were 0.95–1.04 ($R^2 = 0.994$ – 0.996). The obtained data were normalized with the mass of the samples to determine the number of bacterial *amoA* gene copies/g dry activated sludge.

Clone library construction and phylogenetic analysis

DNA extracted from activated sludge samples of each WWTP were used to construct clone libraries. The primers described above were used to perform the PCR amplification reaction of the bacterial *amoA* gene. The 25- μ L PCR products from triplicate reactions were pooled, the gel bands were purified using the Axygen PCR cleanup purification kit, and cloned using the pGEM-T vector (Promega, Madison, WI, USA). The ligation products were transformed into competent *Escherichia coli* cells (DH5a, Takara, Japan) and grown overnight on LB agar plates containing 100 μ g/mL ampicillin, 40 μ g/mL X-Gal and 24 μ g/mL IPTG. Positive clones were selected and detected by PCR amplification using vector primers (T7 and SP6). Then, they were sequenced at the Shanghai Majorbio Bio-technology Co., Ltd.

The DNASTar software package was used to remove the vector sequences by bioinformatics analysis. Then we blasted all the obtained sequences with the online GenBank BLAST program according to Altschul *et al.* (1997), and the highest match sequences were retrieved. Operational taxonomic units (OTUs) were defined as sequences with <3% nucleotide differences. Multiple sequence alignments of the most related environmental sequences and clone sequences were carried out by ClustalX (Thompson *et al.* 1997). The diversity indices (Shannon-Weiner, *H*) and non-parametric richness estimator (bias-corrected Chao1 (S_{Chao1})) were calculated for each library by the software program DOTUR (Schloss & Handelsman 2005). The library coverage was calculated according to Mullins *et al.* (1995). A representative sequence with highest quality for each OTU was exported for phylogenetic analysis. Neighbor-joining phylogenetic trees based on Jukes–Cantor distances were built by MEGA 4.0 (Tamura *et al.* 2007).

Nucleotide sequence accession numbers

All the bacterial *amoA* gene sequences obtained in this study have been deposited in the GenBank database under the accession numbers JX999156–JX999365.

Data analysis

Correlation analysis (two-tailed Pearson correlation coefficients) between the bacterial *amoA* gene copies and water quality parameters was performed with the SPSS 13.0 software (SPSS, Chicago, IL). One-way analysis of variance (ANOVA) and post-hoc comparisons were used to test statistical differences of the wastewater parameters and bacterial *amoA* gene abundance among samples with software SPSS 13.0. Statistical significance was defined at $P < 0.05$. In order to investigate the relationships between the differences in AOB community structures and the geographic distribution among sampling WWTPs, Mantel test was carried out with the *vegan* package in R 2.15.0 environment with permutation number of 999. Canonical correspondence analysis (CCA) was performed using the *vegan* package in R (version: 2.15.0) to investigate the effects of wastewater parameters on AOB community structure in activated sludge samples of WWTPs (Oksanen *et al.* 2010).

RESULTS

Abundance of bacterial *amoA* genes

Duncan's multiple range test method was used to analyze the results of qPCR. As the results show in Table 1, the highest bacterial *amoA* gene abundance (1.85×10^7 copies/g of dry activated sludge) was detected in the HF sample, whereas sample collected from TY showed the lowest abundance of bacterial *amoA* gene (6.58×10^5 copies/g of dry activated sludge). Among the different samples, we observed significant differences in the bacterial *amoA* gene abundance ($P < 0.05$). To be exact, the abundance of bacterial

Table 1 | Copy numbers of the bacterial *amoA* gene in activated sludge samples collected from the seven municipal wastewater treatment plants

Samples	Bacterial <i>amoA</i> gene copies/g dry activated sludge
WX	$6.06 \times 10^6 \pm 1.55 \times 10^5$ c
MAS	$9.38 \times 10^6 \pm 9.00 \times 10^4$ b
HF	$1.85 \times 10^7 \pm 1.80 \times 10^6$ a
NJ	$8.22 \times 10^6 \pm 7.90 \times 10^5$ b/c
TY	$6.58 \times 10^5 \pm 4.45 \times 10^4$ d
XA	$2.73 \times 10^6 \pm 1.30 \times 10^5$ d
ULMQ	$2.16 \times 10^6 \pm 2.20 \times 10^5$ d

WX: Wuxi; MAS: Ma'anshan; HF: Hefei; NJ: Nanjing; TY: Taiyuan; XA: Xi'an; ULMQ: Urumchi.

amoA gene in the HF sample was significantly higher than other samples and the samples collected from TY, XA and ULMQ were significantly lower than the other four samples ($P < 0.05$).

Diversity analysis of AOB

A total of 210 bacterial *amoA* sequences of seven clone libraries were identified in the present study, which could be divided into 36 OTUs. The library of each sample included 26–32 sequences (Table 2). OTUs were identified based on <3% nucleotide differences by using the DOTUR software package (Beman & Francis 2006). The number of OTUs in each clone library varied from four to 15. Shannon–Weiner (H) and S_{Chao1} richness estimators were calculated with the OTU data using DOTUR to characterize the diversity of AOB and shown in Table 2. The coverage of the seven clone libraries ranged from 61.5% to 93.8%, demonstrating adequate sequencing depth. According to the number of OTUs and Shannon–Weiner (H) index, the TY sample had the highest bacterial *amoA* gene diversity, followed by NJ sample, whereas the MAS sample had the lowest bacterial *amoA* gene diversity.

Relationships between water quality parameters and the bacterial *amoA* gene abundance

In Table 3, Pearson's correlation coefficients were employed to estimate that how the abundance of bacterial *amoA* gene related to water quality parameters. Significant negative correlations were detected between the bacterial *amoA* gene abundance and the influent

Table 2 | Richness and diversity indices of bacterial *amoA* gene in the seven activated sludge samples

Samples	Number of clones	Number of OTUs	C (%)	H	S_{Chao1}
WX	30	6	86.7	1.08	12
MAS	32	4	93.8	0.65	5
HF	32	10	78.1	1.64	31
NJ	30	12	73.3	1.95	26
TY	26	15	61.5	2.51	30
XA	29	9	82.8	1.50	12.3
ULMQ	31	10	80.6	1.67	17.5
Total	210	36	90.5	2.67	67.7

WX: Wuxi; MAS: Ma'anshan; HF: Hefei; NJ: Nanjing; TY: Taiyuan; XA: Xi'an; ULMQ: Urumchi.
H: Shannon–Weiner, S_{Chao1} : richness estimator.

Table 3 | Correlations between environmental factors and the abundance and diversity of the bacterial *amoA* gene

Environmental variables	Pearson correlation coefficients	
	Bacterial <i>amoA</i> gene copies/g dry activated sludge	Number of OTUs of AOB
Influent $\text{NH}_4^+\text{-N}$	−0.801*	0.519
Effluent $\text{NH}_4^+\text{-N}$	−0.605	0.340
$\text{NH}_4^+\text{-N}$ removal rate	0.245	−0.153
Influent TN	−0.773*	0.393
Influent TP	−0.553	0.411
Influent COD_{Cr}	−0.661	0.349

$\text{NH}_4^+\text{-N}$: ammonia nitrogen; TN: total nitrogen; TP: total phosphorus; COD_{Cr} : chemical oxygen demand.

* $P < 0.05$.

Duncan's multiple range test method was used to analyze the results and different letters indicate significant differences between samples ($P < 0.05$, $n = 4$).

concentrations of total nitrogen (TN) and ammonia nitrogen ($\text{NH}_4^+\text{-N}$) in the activated sludge samples ($P < 0.05$). However, the number of OTUs was not significantly correlated to any water quality parameters. Furthermore, the $\text{NH}_4^+\text{-N}$ removal rate also showed insignificant correlations with the abundance and diversity of bacterial *amoA* gene.

Phylogenetic analysis of AOB

The phylogenetic tree of bacterial *amoA* gene sequences is shown in Figure S1 (available with the online version of this paper). All the sequences collected from the seven samples could be assigned into three lineages: *Nitrosospira*, *N. oligotropha* and *N. europaeal/Nc. mobilis* lineages. The relative abundance of different bacterial *amoA* gene groups in the seven activated sludge samples are shown in Figure 1. In all the samples, *N. oligotropha* lineage was the superior group, which covered 43.33–96.88% of bacterial *amoA* gene sequences in each clone library. All sequences in WX and HF samples were assigned to the *N. oligotropha* lineage, except for one sequence affiliated with *N. europaea* and *Nc. mobilis* lineages. In the MAS sample and the XA sample, only a few sequences belonging to *Nitrosospira* lineage were detected except for the *N. oligotropha* lineage, with the relative percentages of 3.12% and 10.34%, respectively. The sequences recovered from NJ, TY and ULMQ sample fell into all three lineages, and the *N. oligotropha* lineage had the same relative percentages (43.33%) as the *Nitrosospira* lineage in the sequences isolated from NJ.

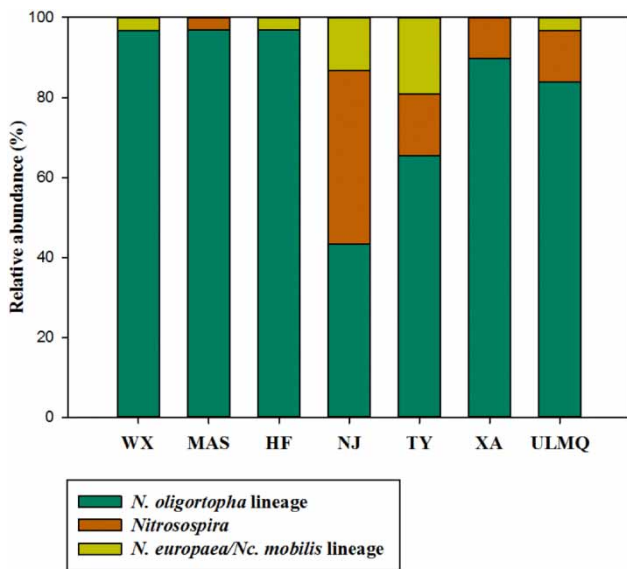


Figure 1 | Relative abundance of the different bacterial *amoA* gene groups in the seven activated sludge samples. WX: Wuxi; MAS: Ma'anshan; HF: Hefei; NJ: Nanjing; TY: Taiyuan; XA: Xi'an; ULMQ: Urumchi.

Effects of water quality parameters on the community structure of AOB

A Mantel test was employed to investigate the relationships between AOB community structure and geographic distribution of the WWTPs by Euclidean distance. According to the results, geographic distribution showed no significantly correlation with the variations of bacterial community structure (Spearman's rank correlation coefficients, $R = 0.2137$, $P = 0.108$). Thus, we speculated that the community structure of AOB in activated sludge of WWTPs might be affected by other water quality parameters.

In order to investigate which water quality indicators had the greatest impact on community structure, CCA was carried out (Figure 2). The results indicate that the influent concentration of TN and $\text{NH}_4^+\text{-N}$ were the dominated effects of factors on the AOB communities in the activated sludge samples of this study, followed by the influent concentration of total phosphorus (TP) and the removal rate of $\text{NH}_4^+\text{-N}$. The influent concentration of chemical oxygen demand (COD_{Cr}) had the least effect on the AOB community structure.

DISCUSSION

In the present study, the abundance of bacterial *amoA* gene showed significant differences in distinct geographical areas,

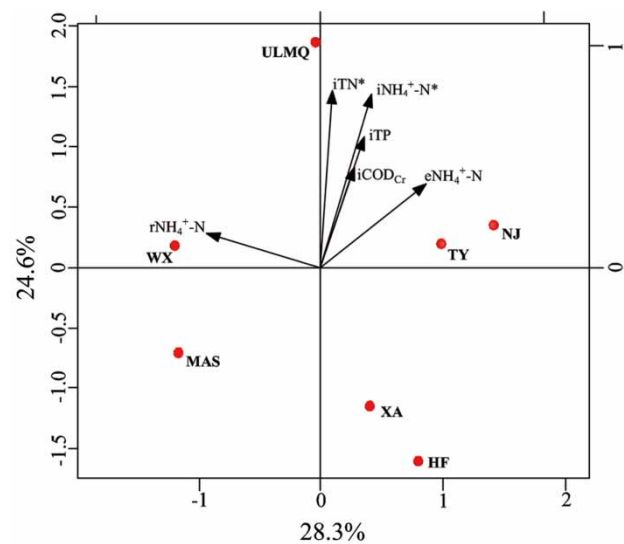


Figure 2 | CCA of the relationships between the community structure of AOB and water quality indicators in the seven activated sludge samples. WX: Wuxi; MAS: Ma'anshan; HF: Hefei; NJ: Nanjing; TY: Taiyuan; XA: Xi'an; ULMQ: Urumchi. $\text{iNH}_4^+\text{-N}$: influent concentration of ammonia nitrogen; iTN^* : influent concentration of total nitrogen; iCOD_{Cr} : influent concentration of COD_{Cr} ; iTP : influent concentration of total phosphorus; $\text{eNH}_4^+\text{-N}$: effluent concentration of ammonia nitrogen; $\text{rNH}_4^+\text{-N}$: removal rate of ammonia nitrogen. * Environmental factors that have the significant impact on AOB community structure.

and the differences mainly exhibited that the copy numbers of bacterial *amoA* gene of activated sludge samples in the eastern of China (HF, MAS, WX, NJ) were significantly higher than those in the central and western regions (TY, XA, ULMQ) ($P < 0.05$). Although there were no direct conclusions in previous studies that could be compared to the present study, Zhao et al. (2014a) indicated that both the source microorganisms and the resident microorganisms in the system would be affected by the local environment and climate, which explains why the geographical distance had an impact on the abundance of AOB. Niu et al. (2016) also found that nitrogen removal associated bacteria in high altitude WWTPs showed a great sensitivity to water quality parameters. In this study, the bacterial *amoA* gene abundance in the samples collected from high-altitude plants (TY, XA, ULMQ) was significantly lower than those collected from low-altitude plants (HF, MAS, WX, NJ) ($P < 0.05$), which is consistent with the result of Niu et al. (2016).

A significantly negative correlation was found between the influent concentrations of TN and $\text{NH}_4^+\text{-N}$ and the bacterial *amoA* gene abundance ($P < 0.05$). Although little information on the relationship between water quality parameters and the abundance of AOB has been previously reported in activated sludge, the significant correlation of AOB abundance with environmental variables, as dissolved

oxygen (DO) (Fitzgerald *et al.* 2015), temperature (T) (Zeng *et al.* 2014) and nitrate nitrogen (NO_3^- -N) (Zhao *et al.* 2014a), have been discussed for other habitats. For instance, previous studies showed that ammonia concentrations positively influence the bacterial *amoA* gene abundance in soil ecosystem (Zhao *et al.* 2015; Wang *et al.* 2009). However, a negative correlation was found between the concentrations of TN and NH_4^+ -N and the bacterial *amoA* gene abundance in a eutrophic lake (Zhao *et al.* 2014b) and the sediments of Taihu (Zeng *et al.* 2012). Ammonia maintain probably positive effects on the abundance and diversity of AOB as for the substrate for ammonia oxidizers (Adair & Schwartz 2008), but further water quality parameters not considered in this study could also play different roles, such as temperature (T), which could cause both inhibitory and stimulative effects on the abundance of bacterial *amoA* gene (Zeng *et al.* 2014).

According to the results of Mantel test, geographic distribution had no significant effects on AOB communities. However, in this study, we observed that the samples collected from different WWTPs had different community structure of AOB (Figure 1). There might be a number of factors that can cause this result, such as treatment process and treatment scale. Dytczak *et al.* (2008) found that the different reactors are dominated by different AOB communities. In this study, MAS, HF and XA were using oxidation ditch (OD) process, and they had similar AOB community structure, which supported the results mentioned. Rowan *et al.* (2003) analyzed the AOB community structure in different scale WWTPs and found that there were similarities in AOB structure in the two wastewater treatment systems, but the AOB community structure of the large-scale WWTP was more stable and more diverse. In the present study, TY had the largest scale and the highest diversity of AOB community, indicating that the scale of the wastewater treatment plant might have a certain impact on AOB community structure.

Except for the treatment process and treatment scale, the influent water quality of WWTPs could also affect the AOB community structure. Sangchul *et al.* (2005) showed that the community structure of AOB in municipal WWTPs were more diverse than that in industrial WWTPs. Among all WWTPs investigated in this study, only HF and XA involved industrial wastewater (ca. 30–40% of total). And the samples of HF and XA did show lower diversity than others (except for WX and MAS), which is consistent with Sangchul *et al.* (2005). Otawa *et al.* (2006) and Limpiyakorn *et al.* (2011) found that the concentration of ammonia nitrogen (NH_4^+ -N) in the original wastewater had a certain effect on AOB

communities in the activated sludge, in which the *Nitrosomonas europaea-eutropha* cluster was the main cluster at high NH_4^+ -N concentrations, whereas the *Nitrosomonas ureae oligotropha-marina* cluster was the main cluster at low NH_4^+ -N concentrations. In this study, there was no significant difference in the concentration of ammonia nitrogen in the considered WWTPs, but a significant difference of the community structure of AOB still existed among WWTPs as determined by Mantel test ($P < 0.05$). We hypothesized that the water quality parameters influencing the community structure of the AOB in the WWTPs were multifaceted rather than singular, as AOB communities may have different responses to different water quality parameters and the superposition of these water quality parameters may result in the final community structure of the AOB. The results of the CCA suggested that this was the case, and the influent concentrations of TN and NH_4^+ -N were the most significant influencing factors, followed by TP and NH_4^+ -N removal rate.

The results shown in Figure 1 revealed that *N. oligotropha* lineage dominated the AOB communities of each library. The lineage had previously been reported from many ecosystems such as WWTPs, fresh water, sediments, soil and others (Regan *et al.* 2002; Hallin *et al.* 2005; Limpiyakorn *et al.* 2005; LaPara & Ghosh 2006; Qin *et al.* 2008; Zhao *et al.* 2013). Zhao *et al.* (2013) also found that the three lineages existed in the sediments of a eutrophic lake, and *N. oligotropha* lineage was the dominated lineage. A previous study showed that *N. oligotropha* lineage has the advantage of fast growth, and a low saturation constant (K_s) for free ammonia, indicating that it is suitable for survival in the environment with low ammonia nitrogen (Lydmark *et al.* 2007). In this study, the influent NH_4^+ -N of the seven WWTPs were low, except for TY, which explain why *N. oligotropha* lineage was the dominant population of AOB. However, in the industrial WWTPs that have a high concentration of NH_4^+ -N, *N. europaeal* and *Nc. mobilis* lineages may be the dominant populations (Koops & Andreas 2001).

The data obtained in this study demonstrated that geographic distribution did have an effect on the distribution of AOB in WWTPs. However, this was rarely discussed in previous studies. Although geographic distribution only affects the abundance of AOB, the effect can-not be ignored. At the same time, the results further clarified which water quality parameters of the WWTP have the most significant effects on the abundance and community structure of AOB.

The information obtained in this study would be useful to elucidate the abundance and community structure of AOB in activated sludge. Further investigations are needed

to explore the factors affecting the ecological distribution of AOB in activated sludge samples. This will need to include more WWTPs in further areas of China, as well as further water quality parameters such as dissolved oxygen (DO) or nitrate nitrogen (NO_3^- -N).

CONCLUSIONS

In this study, we investigated the abundance and community structure of AOB in the activated sludge of seven WWTPs in seven cities throughout China, and the influence of various water quality parameters on the abundance and community structure of AOB were examined. It was observed that the geographic distribution of WWTPs had a significant effect on the abundance of the bacterial *amoA* gene. The community structure of AOB was not significantly affected by geographic distribution, but most probably by the treatment process, treatment scale and influent wastewater quality, especially by the concentrations of TN and NH_4^+ -N. *N. oligotropha* lineage was the superior AOB population in the considered wastewater treatment system in this study.

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