Diversity and abundance of comammox bacteria in the sediments of an urban lake

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Abstract:

Aims: Although comammox have been discovered in a variety of ecosystems, there are few studies in urban lakes. This paper attempted to confirm whether this microbe exists in urban lakes and to

determine the factors influencing its existence.

Methods and Results: This study investigated the diversity and abundance of comammox bacteria in sediments of a typical urban lake in China, and the ecological relationship with other ammonia-oxidizing microorganisms. The phylogenetic analysis indicated that there was only clade A in the comammox strain in the sediment of Lake Donghu. The comammox co-existed with ammonia-oxidizing archaea (AOA), ammonia-oxidizing bacteria (AOB) and anaerobic ammonia-oxidizing (anammox) bacteria in the sediment of this lake. The abundances of the ammonia monooxygenase genes for comammox, AOA, AOB, and anammox 16S rRNA were 2.43×10^8 , 1.07×10^8 , 3.24×10^7 , and 3.21×10^{11} copies g⁻¹ dry sediment, respectively. Moreover, the *amoA* gene abundance of comammox was positively correlated with that of AOA and AOB. The redundancy analysis (RDA) showed that the abundance of the comammox *amoA* gene was negatively correlated with various nitrogen related indicators in both the sediment and the water column, indicating that eutrophication may inhibit the growth of comammox.

Conclusions: Comammox bacteria play an important ecological role in the nitrogen cycle of urban lake sediments.

Significance and Impact of the Study: Our results indicated comammox bacteria were widespread in urban lakes and eutrophication may inhibit their growth.

Keywords

Comammox; *Nitrospira*; Diversity; Abundance; Lake Sediment

Introduction

Nitrification is a key component of the nitrogen cycle in lakes. Traditional nitrification processes had been generally considered involving two steps (Shiozaki *et al.* 2016), ammonia-oxidizing archaea

(AOA) and ammonia-oxidizing bacteria (AOB) first oxidize ammonia to nitrite, then nitrite-oxidizing bacteria (NOB) oxidize nitrite to nitrate (Konneke *et al.* 2005). However, Costa *et al.* (2006) speculated from the perspective of the kinetic theory that there was probably a nitrifying microorganism that can directly oxidize ammonia to nitrate and grow in a low ammonium environment. The discovery of complete ammonia oxidizers (comammox) confirmed the presence of this microbe (Daims *et al.* 2015; van Kessel *et al.* 2015). The genome of this microbe contains both gene encoding ammonia monooxygenase, hydroxylamine dehydrogenase and nitrite oxidoreductase, indicating that they have the potential for the full-scale ammoxidation (Daims *et al.* 2015; van Kessel *et al.* 2015). This discovery has profoundly expanded our understanding of the ammonia oxidation process. Therefore, the existence and ecological effects of this oxidation process in natural ecosystems need to be further evaluated.

Although comammox was discovered just a few years ago, it has been found to co-exist with AOA, and AOB, and widely distributed in different ecosystems using molecular biology techniques, such as in drinking water treatment plants (DWTPs), atmospheric particulate matter, soil, estuarine sediments, and wastewater treatment plants (WWTPs) (Gao *et al.* 2016; Wang *et al.* 2017; Fowler *et al.* 2018; Wang *et al.* 2018; Yu *et al.* 2018). AOA were previously thought to dominate in environments with low ammonium concentrations, such as oceans or oligotrophic lakes (Herrmann *et al.* 2011; Hou *et al.* 2013), and AOB have a higher abundance in nutrient-rich waters and soils (Dang *et al.* 2010; Prosser and Nicol 2012). However, with further research on comammox community and its environments, it showed that comammox may have advantages over AOA and AOB in low ammonium environments (Kits *et al.* 2017), and ammonium concentrations could also affect the diversity of commomox community (Yu *et al.* 2018). In some drinking water influent, river, groundwater and urban waterway

sediment samples, the proportion of comammox in the total number of ammonia-oxidizing microorganisms was higher than 70 % (Xia *et al.* 2018). And it may be an important functional community in the nitrification process in low dissolved oxygen (DO) environments (Roots *et al.* 2019). The similar results were also found in the study of tidal sediments in which middle and lower sediments with a sharp drop in dissolved oxygen concentration provided better growth conditions for comammox bacteria than surface sediments (Jiang *et al.* 2019).

Based on the similarity of the *amoA* gene, comammox can be divided into two major branches, clade A and clade B (Daims *et al.* 2015; van Kessel *et al.* 2015). Many studies have shown that these two branches may have ecological differentiation in nature. Pjevac *et al.* (2017) designed a PCR primer to amplify the comammox *amoA* gene to verify the distribution of comammox in various environments (Freshwater biofilm; Forest soil; Rice paddy soil; WWTPs; DWTPs *et al.*), and the results showed that the abundance of the two branches in varying samples were significantly different. High-throughput sequencing of river sediments revealed that the concentration of comammox clade A was higher than that of clade B (Xia *et al.* 2018), while comammox in the groundwater-fed rapid sand filter samples was dominated by clade B (Fowler *et al.* 2018). Studies in forest soils have found that the abundances of comammox clade A and AOA were comparable when the soil is alkaline, but in the acidic soil, comammox clade A is more abundant than AOA (Hu and He 2017). The rate of nitrogen deposition also has an effect on ammonia-oxidizing microorganisms. With the increased of nitrogen deposition rate, the *amoA* gene abundance of comammox *Nitrospira* clade B was significantly increased (Shi *et al.* 2018). When applying nutrients to the soil, the relative abundance of comammox *Nitrospira* clade A increases with the increasing nutrient input gradient, while clade B was the opposite. This may be due to the different physiological characteristics between the comammox *Nitrospira* clade (Wang *et al.*

2019a).

Anaerobic ammonia-oxidizing (anammox) bacteria can also oxidize ammonia using nitrite as an electron acceptor under anaerobic or anoxic conditions (Mulder *et al.* 1995). Therefore, AOA and AOB can provide the growth substrate of nitrite for anammox bacteria (Zhao *et al.* 2019a). It has been found that AOA, AOB, and anammox bacteria can co-exist in habitats, such as soils, rivers, lake sediments, and marine sediments (Kuypers *et al.* 2003; Chen and Gu 2017; Wang *et al.* 2019b). Some studies indicated that anammox bacteria can also co-exist with comammox bacteria (Annavajhala *et al.* 2018), and these four types of microorganisms were both detected in the sequencing batch reactor (SBR) (Pamela *et al.* 2017). However, these microorganisms all require ammonium as a substrate for their reaction, and thus there may be a complex competition-collaboration relationship between them. The study using SBR to treat sludge digester liquor found that AOB, NOB, comammox bacteria and anammox bacteria could coexist, and the removal efficiency of $\text{NH}_4^+\text{-N}$ was as high as 98% (Wu *et al.* 2019). The proportion of partial-nitrification, comammox, and anammox removal of $\text{NH}_4^+\text{-N}$ in this study was 55.11%, 25.43%, and 18.28%, respectively. And the one-step nitrification process of comammox bacteria avoids the production of nitrous oxide which was often comes from the AOA and AOB-dominated ammoxidation processes (Daims *et al.* 2015; van Kessel *et al.* 2015). Therefore, comammox may play a very important role in the ecosystem nitrogen cycle.

The nitrogen content of many urban lakes in the middle and lower reaches of the Yangtze River in China is usually high (Chen *et al.* 2009; Wu *et al.* 2010; Hou *et al.* 2013). This high nitrogen concentrations could changed the community structure of the AOA and AOB (Yang *et al.* 2016a). However, the existence and diversity of comammox bacteria in these urban lakes, and its relationships with other ammonia-oxidizing microorganisms are still unclear. We hypothesized that the nutritional

status of the water would have an effect on the growth of comammox bacteria. Therefore, we selected a typical urban lake, Lake Donghu, and investigated the comammox bacteria diversity in sediments, the correlation of comammox bacteria abundance with environmental factors, and its ecological relationships with AOA, AOB, and anammox bacteria.

Materials and methods

Sample Collection

Lake Donghu (30°33'N, 114°23'E) is a shallow lake (average depth: 2.5 m; surface area 32 km²) in the middle reaches of the Yangtze River, located in the urban area of Wuhan city, China. In this study, samples were collected from five sub-lakes in Lake Donghu, namely, Lake Guozheng (LGZ), Lake Tuan (LT), Lake Tangling (LTL), Lake Hou (LH), and Lake Miao (LM) (Fig. 1). The water quality of the first three sub-lakes was better than that of the last two sub-lakes (Ji *et al.* 2019). Sediments around the lake have been polluted by domestic sewage and non-point sources, while sediments in the center of the lake were less disturbed by humans. Thus, we collected the lake sediments of each sub-lake. At each sampling sites, the pH, DO, and water temperature (T) of the overlying water were recorded using the YSI EXO2 Multi-Parameter Water Quality Analyzer (YSI Inc., Yellow Springs, Ohio, USA) in July 2018. At the same time, both overlying water and the surface sediment samples were collected. The surface 10 cm sediment samples were collected using a cylindrical sampler and stored in sterile ziplock bags. Then, these sediments were taken back to the laboratory on ice. A portion of the sediment sample was stored at 4 °C for the determination of basic physical and chemical parameters, and the rest was stored in a -80 °C ultra-low temperature freezer for DNA extraction.

Environmental Physicochemical Analyses

The water column total phosphorus (TP), total nitrogen (TN), and chemical oxygen demand (COD)

were measured according to the Chinese national standard methods (GB/T 11893-1989, GB/T 11894-1989, China) and national environmental protection standard method (HJ/T 399-2007, China), respectively. The concentrations of ammonia nitrogen ($\text{NH}_4^+\text{-N}$), nitrite nitrogen ($\text{NO}_2^-\text{-N}$), and nitrate nitrogen ($\text{NO}_3^-\text{-N}$) in the sediment after KCl extraction was measured determined according to Bao (1981). The pH of the sediment after KCl extraction was measured using a pH meter (METTLER TOLEDO, Switzerland). The sediment organic content was measured by the Walkley-Black chromic acid wet oxidation method (Bao 1981). The sediment TP, and TN were determined by the perchloric acid-sulfuric acid method and the Kjeldahl method (Bao 1981), respectively.

DNA extraction and PCR amplification

Approximately 0.4 g of the sediment sample was placed in a Lysing Matrix E tube to extract the total genomic DNA in the sediment using the Fast DNA[®] Spin for Soil kit (MPBIO, USA) according to the manufacturer's protocols. The purity and concentration of the extracted DNA samples were examined using a super differential spectrophotometer (NanoPhotometer-N60, IMPLEN, Germany). The extracted DNA samples were stored at -20 °C for subsequent analysis. PCR amplification was performed in a total volume of 30 μL containing: 3 μL of 10 $\times$ TransTaq[®]-T Buffer (Trans Gen, China), 2 μL of dNTPs (2.5 mM, Trans Gen, China), 1 μL of each primer (10 μM), 0.2 μL of Trans Taq[®]-T DNA Polymerase (Trans Gen, China), and 2 μL of template DNA. Comammox gene in sediments was amplified using the specific primers comamoA AF (AGGNGAYTGGGAYTTCTGG) and comamoA SR (CCGVACATACATRAAGCCCAT) (Wang *et al.* 2018). PCR amplification procedures consisted of initial denaturation at 96 °C for 5 min, followed by 12 cycles of 96 °C for 30 s, 62 °C for 30 s, 72 °C for 30 s, and then 37 cycles of 96 °C for 30 s, 50 °C for 30 s, 72 °C for 30 s, followed by a final extension at 72 °C for 10 min. The expected length of the PCR product was 436 bp.

Clone Library Construction and Phylogenetic Analysis

The purified DNA was ligated with PMD18-T (TAKARA, Dalian, China), and the ligated product was transformed into *Escherichia coli* competent cells, then coated with X-gal, IPTG, and ampicillin-resistant LB plates. These coated plates were incubated at 37 °C in the incubator for 16 h. After incubation, 69 positive clones were picked out, inoculation cultured and extracted for the plasmid. Plasmids with the desired fragments were sequenced using an ABI 3730x1 DNA Sequencer (Applied Biosystems, USA). The obtained sequences were assigned to the same operational taxonomic unit (OTU) using the MOTHUR (version 1.34.1, Schloss *et al.* 2009) program with a maximum distance of 3 %. The adjacency method using MEGA 6.0 software (Tamura *et al.* 2013) was used for the phylogenetic tree construction, and 1000 guided analyses was repeated to estimate the confidence values of the tree nodes.

Quantitative PCR

Quantitative PCR (q-PCR) was performed on QuantStudio™ 6 Flex Real Time PCR System (Thermo Fisher Scientific, Singapore) to estimate the abundance of comammox *amoA*, AOA *amoA*, AOB *amoA*, and anammox 16S rRNA genes, with three replicates each. Primers used for comammox *amoA*, AOA *amoA*, AOB *amoA*, and anammox 16S rRNA genes were Ntsp-*amoA* 359R/Ntsp-*amoA* 162F (Fowler *et al.* 2018), GenAOAF/GenAOAR (Meinhardt *et al.* 2015), *amoA*-1Fmod/GenAOBR (Meinhardt *et al.* 2015), and AMX809F/AMX1066R (Ji *et al.* 2012), respectively (Table S1). The quantification of each sample was based on the fluorescent dye SYBR Green I, which bound to the double-stranded DNA during PCR amplification. The quantitative PCR reactions for comammox *amoA*, AOA *amoA*, AOB *amoA*, and anammox 16S rRNA genes were performed in a 10 µL reactions mixture which included 5 µL of T5 Fast qPCR Mix (2 ×), 0.4 µL of each primer (10 µM), 0.2 µL of ROX

Reference Dye II (50 ×), 3 µL of distilled deionized water (ddH₂O), and 1 µL of template DNA. The quantitative PCR amplification conditions for comammox *amoA* gene was: 95 °C for 1 min; followed by 40 cycles of 95 °C for 10 s, 52 °C for 20 s, 72 °C for 30 s; AOA *amoA* gene was: 95 °C for 1 min; followed by 40 cycles of 95 °C for 10 s, 53 °C for 15 s, 72 °C for 30 s; AOB *amoA* gene was: 95 °C for 1 min; followed by 40 cycles of 95 °C for 10 s, 52 °C for 15 s, 72 °C for 30 s; and anammox 16S rRNA gene was: 95 °C for 1 min; followed by 40 cycles of 95 °C for 10 s, 53 °C for 20 s, 72 °C for 30 s. The genes to be amplified were separately cloned into the pGEM[®]-T vector (Promega, Madison, WI, USA) to generate a standard plasmid, and the obtained plasmid DNA was serially diluted by 6 - 8 gradients to prepare a standard curve. Then, the qPCR standard curve was repeated three times, and the amplification efficiencies of the four genes were 83.30 %, 91.33 %, 91.22 %, and 92.59 %, respectively, and the correlation coefficients (r^2) were all greater than 0.99. A negative control containing no template DNA was performed after the same qPCR method.

Statistical analyses

One-way ANOVA and Tukey test ($P < 0.05$) were performed using SPSS 20.0 software (Hulsen *et al.* 2008) to determine the difference of physicochemical parameters amongst sampling locations both in sediments and the overlying water, and significant differences of microbial abundances amongst sampling locations. In the detrended correspondence analysis (DCA), the lengths of gradient in AOA, AOB, comammox bacteria, and anammox bacteria were shorter than 3.0. So, redundancy analysis (RDA) were used to analyze the relationship between the community structure of the ammoxidation microbes and environmental factors by Canoco 4.5 software (Lepš and Šmilauer 2003).

Nucleotide sequence accession numbers

The nucleotide sequences of comammox *amoA* obtained in this study were deposited in the

GenBank database under accession MK948223-MK948291.

Results

Physicochemical Characteristics of Samples

The concentration of DO, TP, TN and COD in the overlying water of Lake Miao were higher than those of other four sub-lakes ($P < 0.05$; Table 1.). The concentration of TP and COD in the overlying water of Lake Hou were also higher than Lake Guozheng, Lake Tuan, and Lake Tangling, and the value of pH and NO_2^- -N content in the sediments of this lake were the highest ($P < 0.05$) in the five sub-lakes. Whereas, the highest content of TP and organic matter (OM) in sediments was found in Lake Guozheng ($P < 0.05$).

Diversity of the Comammox *amoA* gene

A total of 69 comammox *amoA* gene sequences were obtained from the constructed cloned library. Using Mothur software, the sequences with similarity greater than 97 % were classified into one OTU, and a total of six OTUs and three clusters were obtained, namely *Nitrosa Cluster*, *Nitrificans Cluster*, and *Inopinata Cluster* (Fig. 2). Although the phylogenetic tree divided *Nitrospira nitrosa* and *Nitrospira sp* into two branches, they belonged to the *Nitrosa Cluster*. And *Candidatus Nitrospira nitrosa* has low homology with *Nitrospira sp* (< 50 %, 1000 replicates).

All three Clusters belong to comammox clade A. Among them, OTU 1 and OTU 5 belonged to *Candidatus Nitrospira nitrosa*, and OTU 2 was located on the *Nitrospira sp* branch. A total of 48 sequences of these three OTUs accounted for 69.6 % of all comammox *amoA* gene sequences. And these sequences were highly homologous to the drinking water system, paddy soil, and tidal sediments in the database (> 95 %, 1000 replicates). OTU 3 was assigned to the *Nitrificans Cluster*, and it shared a high degree of homology (> 95 %, 1000 replicates) with *Candidatus Nitrospira nitrificans* and river

sediments. *Nitrificans Cluster* and *Inopinata Cluster* contained approximately the same number of sequences, accounting for 15.9 % and 14.5 % of all comammox *amoA* gene sequences, respectively. The OTU 4 and OTU 6 in the *Inopinata Cluster* have higher homology to samples from drinking water plants and ocean sediments.

Gene abundance of Comammox *amoA*, AOA *amoA*, AOB *amoA* and Anammox 16S rRNA

The abundance of the comammox *amoA* gene was not significantly different amongst LGZ, LT, LTL, and LH sites (approx. 10^8 copies g⁻¹ dry sediment), but they were all significantly higher than that of the LM site (10^7 copies g⁻¹ dry sediment; Fig. 3). Comammox *amoA* gene abundance in the five sub-lakes was higher than that of AOA *amoA* gene and AOB *amoA* gene.

The average of AOA *amoA* gene abundance was $(1.07 \pm 1.1) \times 10^8$ copies g⁻¹ dry sediment, with the highest detected at the LTL site (2.79×10^8 copies g⁻¹ dry sediment), while the lowest at the LM site (2.73×10^5 copies g⁻¹ dry sediment). And the average of AOB *amoA* gene copy number was $(3.24 \pm 1.8) \times 10^7$ copies g⁻¹ dry sediment. There had significant difference in the AOB *amoA* gene abundance at these five sub-lakes. The maximum value of AOB *amoA* gene abundance observed at LGZ was 5.20×10^7 copies g⁻¹ dry sediment, the lowest at the LM (2.42×10^6 copies g⁻¹ dry sediment).

The average of abundance of anammox 16S rRNA gene was $(3.24 \pm 0.45) \times 10^{11}$ copies g⁻¹ dry sediment, which was much higher than that of the other three aerobic strains *amoA*. The gene abundance of anammox 16S rRNA at LTL was up to 3.85×10^{11} copies g⁻¹ dry sediment, and the lowest was found at LH.

Effect of environmental variables on abundance of Comammox, AOA, AOB and Anammox

The gene abundance of these four bacteria and environmental factors were analyzed using RDA. The first two RDA axes together explained 99.4 % of the total variance for the gene abundance (Fig. 4).

Among them, the distance between LGZ, LT and LTL sites were relatively close, indicating that there was a small difference between them and the water quality was relatively close. The abundance of comammox *amoA* gene was positively correlated with that of the AOA *amoA* and AOB *amoA* gene. The abundance of AOA *amoA* gene was positively correlated with OM and TN in sediments, while AOB *amoA* gene was negatively correlated with NO₃⁻-N, TP of the sediments. Anammox 16S rRNA was positively correlated with TP, TN, and OM. Comammox *amoA* gene was positively correlated with sediment OM and TN, but negatively correlated with NO₃⁻-N, TP, and NH₄⁺-N in sediments and overlying water indicators.

Discussion

This study investigated the diversity and abundance of the comammox in the sediments of an urban lake and effects of the water quality and sediment properties on comammox distribution. *Candidatus Nitrospira nitrosa* was the dominant comammox in the sediment of Lake Donghu, followed by *Candidatus Nitrospira nitrificans*, and then *Candidatus Nitrospira inopinata*. These genus of comammox were also widely found in other ecosystems, such as wastewater treatment systems (Wang *et al.* 2018; Zheng *et al.* 2019), drinking water treatment systems (Pinto *et al.* 2016; Wang *et al.* 2017), and tidal wetlands (Yu *et al.* 2018). In the study of the sewage treatment plant, most of the samples were mainly composed of *Candidatus Nitrospira nitrosa* (Keene-Beach and Noguera 2018). *N. nitrosa* cluster was also found to be dominant in heavily polluted river sediments (Zhao *et al.* 2019b), which was similar to our study. These results indicate that *N. nitrosa* can adapt to higher substrate concentrations and may have an important role in eutrophic waters.

The comammox genus found in this study all belong to clade A, indicating comammox clade A may play an important ecological role in the nitrogen cycle of lake sediments in the middle reaches of

the Yangtze River. Most of the current research found comammox clade A and clade B co-existed in rapid gravity sand filter, WWTPs, Vercelli rice rhizosphere, SBR, and soil (Palomo *et al.* 2016; Pjevac *et al.* 2017; Roots *et al.* 2019; Wang *et al.* 2019a). However, only comammox clade A was found in our study and in lake sediment, freshwater biofilms and some artificial ecosystems, and only comammox clade B was found in the forest soil (Pjevac *et al.* 2017). Comammox clade A and clade B were detected in agricultural soils samples (Zhao *et al.* 2019b). The presence of clade A and clade B in different habitats suggests that niche differentiation have occurred between clade A and B on horizontal habitat dimensions. Some studies found that clade B lived in an oligotrophic environment mainly because it has Amt-type transporters with higher affinity for ammonia (Palomo *et al.* 2018; Zhao *et al.* 2019b). But Clade A was more inclined to eutrophic environment because clade A has low ammonia affinity and high absorptive Rh-type transporters (Palomo *et al.* 2018; Zhao *et al.* 2019b). Transporters differences may be an important reason for their niche differentiation, and it may also be the reason that only clade A existed in the eutrophic Lake Donghu.

Many studies have shown that the abundance of AOA and AOB may be affected by the nutritional status of lakes (Yang *et al.* 2016b; Pollet *et al.* 2018). However, whether the nutritional status of the lake can affect the abundance of comammox is still unclear. In this study, the three sub-lakes of LGZ, LT and LTL were clustered into one group in the RDA analysis, which all had better water quality conditions and higher abundance of comammox (averaged at 3.11×10^8 copies g⁻¹ dry sediment), compared to the sub-lakes of LM and LH. Additionally, the water quality of Lake LM was the worst, and its comammox *amoA* gene abundance was also the lowest (1.40×10^7 copies g⁻¹ dry sediment). This indicated that the abundance of comammox *amoA* gene seemed to be affected by the water quality of Lake Donghu, with the higher level of eutrophication reducing the abundance of comammox *amoA*

gene. It has been demonstrated that comammox has a strong affinity for ammonia and may be more competitive at low ammonium concentrations (Daims *et al.* 2015; van Kessel *et al.* 2015). Therefore, the lower ammonium environment in the three sub-lakes of LGZ, LT and LTL in this study might have favored the growth of comammox, compared to the other two sub-lakes, which might be the reason for their higher abundance.

The comammox *amoA* gene abundance of the sediment in this study was $1.40 \times 10^7 \sim 3.38 \times 10^8$ copies g⁻¹ dry sediment, which were close to those filtered drinking water in the four drinking water systems (Wang *et al.* 2017) and biofilter in the freshwater recirculating aquaculture systems (Bartelme *et al.* 2017). However, it was lower than the rice paddy soil and WWTPs (10^{11} copies g⁻¹ dry sediment) (Pjevac *et al.* 2017). The abundance of the comammox *amoA* gene in fertilized soils (Wang *et al.* 2019a) was close to that in the river and lake sediments (Yu *et al.* 2018). However, it was lower than that in the acidic forest soils (Hu and He 2017), and the highest abundance value was found in groundwater wells (Pjevac *et al.* 2017). These findings suggest that the proportion of comammox in N bacteria community might differ in different ecosystems, thus its contribution to the nitrogen cycle may also differ in various environments. Therefore, comammox bacteria may have some selectivity to habitats in the process of ammonia conversion in ecosystems.

Comammox bacteria has been found to co-exist with AOA and AOB in various habitats such as soil, tidal flat sediments, and WWTPs (Shi *et al.* 2018; Jiang *et al.* 2019; Roots *et al.* 2019), and it can also co-exist with anammox bacteria (Annavaiahala *et al.* 2018). The present study confirmed that comammox bacteria can co-exist with AOA, AOB, and anammox bacteria in lake sediments. Moreover, there was a significant positive correlation between the abundance of comammox bacteria and AOA/AOB, indicating that there might be a synergistic relationship between these bacteria. This

synergistic relationship is likely due to the complex interaction between these bacteria from utilizing ammonia, nitrite and organic matter in the N cycling (Daims *et al.* 2015; van Kessel *et al.* 2015). We hypothesized that the presence of comammox bacteria might be negatively correlated with anammox bacteria, as comammox directly oxidizes ammonia to nitrate, which reduces the source of nitrite supply to anammox bacteria and may adversely affect the growth of anammox bacteria (Daims *et al.* 2015; van Kessel *et al.* 2015; Annavajhala *et al.* 2018). However, the correlation between comammox and anammox bacteria were not significant in the present study. This might be due to the fact that comammox bacteria use of organic matter and total nitrogen may affect the deamination of anammox bacteria. The competition-collaboration mechanism and driving factors among the four bacteria still need further research.

RDA analysis in the present study showed that the abundance of the comammox *amoA* gene was positively correlated with OM and TN in sediments, which was similar to the results in tidal sediments (Yu *et al.* 2018). Studies have shown that comammox has a high affinity for $\text{NH}_4^+\text{-N}$ and was suitable for growth at low $\text{NH}_4^+\text{-N}$ concentrations (Kits *et al.* 2017). In this study, the $\text{NH}_4^+\text{-N}$ concentration of the sediment has a negative correlation with comammox *amoA* abundance, which was similar to the findings of a study of tidal flat sediments in the Yangtze River estuary (Jiang *et al.* 2019). Moreover, $\text{NH}_4^+\text{-N}$ has no significant correlation with AOA *amoA* or AOB *amoA* gene, which was similar to the study of rhizosphere sediments of riparian zone (Wang *et al.* 2019c). This was likely due to the relatively high $\text{NH}_4^+\text{-N}$ concentration in lake sediments ($19.12 \sim 44.13 \text{ mg kg}^{-1}$) in the present study, compared to that in soils ($7 \sim 20 \text{ mg kg}^{-1}$) (Shi *et al.* 2018). Such high $\text{NH}_4^+\text{-N}$ concentrations can supply sufficient reaction substrates, rather than being a limiting factor, for aerobic ammonia oxidizing bacteria.

The half-saturation constants (K_m) of *Ca. N. inopinata* was determined to be 49 nM by pure culture method (Daims *et al.* 2017; Kits *et al.* 2017). And the AOA K_m was 3.6 nM ~ 4 μ M (Kits *et al.* 2017). Compared with comammox *Nitrospira* and AOA, AOB has higher half-saturation constants and lower substrate affinity. Its K_m (NH_3) ranged from 6 to 11 μ M (Lehtovirta-Morley 2019; Prosser and Nicol 2012). At present, there is no clear conclusion on the rate of ammonia oxidation of comammox bacteria. However, when the total ammonia nitrogen concentration of *Ca. N. inopinata* was as low as 5 $\mu\text{mol L}^{-1}$, the maximum oxidation rate of total ammonium (V_{max}) can reach 14.8 $\mu\text{mol N (mg}\cdot\text{protein}\cdot\text{h)}^{-1}$ (Daims *et al.* 2017; Kits *et al.* 2017). In future studies, more attention should be paid to the ecological role of comammox in different habitats, especially in low ammonia nitrogen environment, and its contribution to nitrification rate in the nitrogen cycle.

In this study, three genus of comammox bacteria were found in an urban eutrophic lake sediments, but they were all classified as comammox clade A. The average abundance of the comammox bacteria was 2.43×10^8 copies g^{-1} dry sediment in the urban Lake Donghu. However, the comammox bacteria abundance in the five sub-lakes showed differences due to various water quality conditions. Interestingly, better water quality favored the growth of comammox bacteria. In addition, the abundance of the comammox *amoA* gene in each sub-lake was higher than that of the AOA *amoA*, and AOB *amoA* gene. The abundance of comammox *amoA* gene was positively correlated with AOA *amoA*, and AOB *amoA* gene abundance, but weakly correlated with anammox 16S rRNA gene. Comammox also had a positive correlation with sediment OM and TN, but negatively correlated with water column NO_3^- -N, TP and NH_4^+ -N. These findings suggested that comammox should play an important ecological role in the nitrogen cycle of urban lake sediments.

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Conflict of Interest

The authors state that they have no conflict of interest to declare.

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Table Legends

Table 1. Physicochemical characteristics (mean $\pm$ SD.) of the overlying water and sediments in the five sampling sub-lakes.

Table S1. Quantitative PCR primers used in this study.

Figure Legends

Fig. 1 Sampling sites in Lake Donghu.

Fig. 2 Phylogenetic tree of representative comammox *amoA* sequences in the lake sediment and reference sequences from Genbank. The software MEGA 6.0 was applied to construct the phylogenetic tree using the neighbor-joining method. In the 1000 re-samplings, the bootstrap value appear on the branch nodes of the evolution distance tree in different color.

Fig. 3 Abundances of four functional genes in the five sub-lakes of Lake Donghu. Error bars represented the standard deviations of the independent triplicate qPCR reactions. Different letters for the same gene indicate the significant difference amongst five sub-lakes at $P < 0.05$ (Tukey's test).

Fig. 4 RDA diagram of the relationship between four functional genes with environmental factors in all samples. LGZ, Lake Guozheng; LT, Lake Tuan; LTL, Lake Tangling; LH, Lake Hou; LM, Lake Miao; S, Sedimentary environmental factor; W, Overlying water environment factor.

Table 1. Physicochemical characteristics (mean $\pm$ SD) of the overlying water and sediments in the five sampling sub-lakes.

Sub-lake ID	Overlying water				Sediments						
	DO (mg L ⁻¹)	TP (mg L ⁻¹)	TN (mg L ⁻¹)	COD (mg L ⁻¹)	pH	NO ₂ ⁻ -N (mg kg ⁻¹ dw)	NO ₃ ⁻ -N (mg kg ⁻¹ dw)	NH ₄ ⁺ -N (mg kg ⁻¹ dw)	OM (g kg ⁻¹ dw)	TN (g kg ⁻¹ dw)	TP (g kg ⁻¹ dw)
LGZ	9.4 $\pm$ 0.1b	0.094 $\pm$ 0.01c	0.65 $\pm$ 0.06b	18.04 $\pm$ 0.39d	7.53 $\pm$ 0.01b	0.15 $\pm$ 0.01ab	13.11 $\pm$ 0.94b	27.89 $\pm$ 1.61bc	89.71 $\pm$ 0.57a	4.18 $\pm$ 0.03a	1.25 $\pm$ 0.01b
LT	8.2 $\pm$ 0.01e	0.049 $\pm$ 0.01d	0.39 $\pm$ 0.05b	16.27 $\pm$ 1.15d	7.40 $\pm$ 0.10b	0.13 $\pm$ 0.02b	12.84 $\pm$ 1.84b	24.89 $\pm$ 0.42bc	78.59 $\pm$ 0.92ab	3.47 $\pm$ 0.13ab	0.61 $\pm$ 0.01d
LTL	8.5 $\pm$ 0.01d	0.12 $\pm$ 0.01bc	1.15 $\pm$ 0.04b	24.71 $\pm$ 1.45c	8.01 $\pm$ 0.05a	0.15 $\pm$ 0.02ab	13.10 $\pm$ 0.48b	19.12 $\pm$ 1.07c	70.10 $\pm$ 2.71b	2.89 $\pm$ 0.05b	0.77 $\pm$ 0.01c
LH	8.7 $\pm$ 0.03c	0.13 $\pm$ 0.01b	0.91 $\pm$ 0.03b	28.98 $\pm$ 0.78b	8.03 $\pm$ 0.03a	0.21 $\pm$ 0.01a	16.73 $\pm$ 1.95b	35.63 $\pm$ 5.13b	56.26 $\pm$ 0.28c	2.81 $\pm$ 0.04b	0.82 $\pm$ 0.02a
LM	10.9 $\pm$ 0.05a	0.23 $\pm$ 0.02a	2.36 $\pm$ 0.09a	36.48 $\pm$ 1.64a	7.88 $\pm$ 0.01a	0.16 $\pm$ 0.01ab	28.10 $\pm$ 4.90a	44.13 $\pm$ 6.20a	72.82 $\pm$ 8.90b	3.52 $\pm$ 0.57ab	2.82 $\pm$ 0.01c

LGZ: Lake Guozheng. LT: Lake Tuan. LTL: Lake Tangling. LH: Lake Hou. LM: Lake Miao.

SD: standard deviation, dw: dry weight.

Different letters indicate significant differences amongst sub-lakes ($P < 0.05$).

Table S1. Quantitative PCR primers used in this study

Target genes	Primers	Primer sequences (5'-3')	Annealing Temperature (°C)	Reference
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Comammox <i>amoA</i>	Ntsp- <i>amoA</i> 162F	GGATTTCTGGNTSGATTGGA	52	(Fowler <i>et al.</i> 2018)
	Ntsp- <i>amoA</i> 359R	WAGTTNGACCACCASTACCA		
AOA <i>amoA</i>	GenAOAF	ATAGAGCCTCAAGTAGGAAAGTTCTA	53	(Meinhardt <i>et al.</i> 2015)
	GenAOAR	CCAAGCGGCCATCCAGCTGTATGTCC		
AOB <i>amoA</i>	<i>amoA</i> -1Fmod	CTGGGGTTTCTACTGGTGGTC	52	(Meinhardt <i>et al.</i> 2015)
	GenAOBR	GCAGTGATCATCCAGTTGCG		
Anammox 16S rRNA	AMX809F	GCCGTA AACGATGGGCACT	53	(Ji <i>et al.</i> 2012)
	AMX1066R	AACGTCTCACGACACGAGCTG

Nitrosa Cluster

Nitrificans Cluster

Inopinata Cluster