Effects of dam building on Niche differentiation of Comammox Nitrospira in the main stream of the Three Gorges Reservoir area

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Article

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Abstract

Comammox *Nitrospira* can completely oxidize ammonia nitrogen to nitrate, and the various habitats of comammox *Nitrospira* are an important guarantee for its survival. This study investigated comammox in river sediments in the Three Gorges Reservoir area. Comammox clade A and comammox clade B were detected in all samples, in which comammox clade A was dominant, and the OUTs detected from comammox clade B (14 OTUs) were more than those detected from comammox clade A. 1 (11 OTUs) and comammox clade A.2 (4 OTUs). The abundance of comammox *Nitrospira* clade A and clade B amoA genes in the main stream of the Three Gorges Reservoir indicated an increasing trend along the river stream, and reached the maximum in the of the reservoir area. The highest abundance of AOA amoA genes appeared in the upper stream section of the reservoir area. Comammox *Nitrospira* clade A exhibited the highest abundance \((3.00 \times 10^4 \pm 8782.37 \text{ copies/g})\), followed by comammox *Nitrospira* clade B \((1.83 \times 10^3 \pm 1019.82 \text{ copies/g})\), AOB \((1.28 \times 10^3 \pm 574.69 \text{ copies/g})\), AOA \((1.73 \times 10^2 \pm 48.05 \text{ copies/g})\). Both the abundances of comammox clade A and B were positively correlated with sediment pH, indicating that pH was an important environmental factor affecting the growth of comammox bacteria. The Mantel test chart indicated that overlying water dissolved oxygen (DO) in the reservoir area had a significant effect on the differentiation of comammox clade A into comammox clade A.1 and comammox clade A.2. This study confirms that the construction of the Three Gorges Dam has an impact on the niche differentiation of comammox *Nitrospira* in the main stream of the Three Gorges Reservoir area.

1 Introduction

Nitrification is a key link in the transformation of nitrogen in water\(^1\). Nitrification generally includes two steps, namely, ammoxidation and nitrite oxidation. As the rate-limiting step, ammoxidation is completed by ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA), and the other step nitrite oxidation is completed by nitrite-oxidizing bacteria (NOB)\(^2,3\). Before 2015, it was generally agreed that the traditional nitrification was completed in two steps. Daims and van Kessel in this year found that there was a microorganism that could directly oxidize ammonia nitrogen \((\text{NH}_4^+\)) to nitrate nitrogen \((\text{NO}_3^-)\), which was called complete ammonia oxidizer (comammox) microorganism\(^4,5\). These discoveries changed the traditional two-step nitrification theory.

There is competition for ammonia between traditional ammonia-oxidizing microorganisms and comammox *Nitrospira*, which may lead to their niche differentiation. Farmland soil studies have shown that there are niche differences between AOA and AOB\(^6\). The study of soil aggregates has revealed that comammox *Nitrospira* can coexist with both AOA and AOB, and it is more similar in the ecological differentiation to AOA\(^7\). Another study found that branch A and branch B of the comammox *Nitrospira* exhibit the differences in nitrogen affinity and ecological conditions\(^8\), and thus branch A and B may also be differentiated. Recent study has shown that not only branch A and branch B are differentiated in farmland soil and tidal flats in the Yangtze River estuary, but also branch A is differentiated into three
sub-branches A.1, A.2 and A.3. When the water environment changes spatially, the various branches of comammox bacteria may produce different adaptabilities, resulting in the phenomenon of differentiation. It remains unclear how the comammox \textit{Nitrospira} clades compete with each other and coexist.

Cascade dam construction alters the transport of nutrients in the river, making it easier for sediment to settle down in the reservoir area, resulting in the accumulation of nitrogen and phosphorus in front of the reservoir\textsuperscript{13,14}. Previous study has shown that dam construction and impoundment will lead to the accumulation of nitrogen and phosphorus in the river and the enrichment of nutrients in the river, thus changing the living environment of microorganisms\textsuperscript{15}. The Three Gorges Reservoir (TGR) is the largest reservoir in the world, and it is located in the upper reaches of the Yangtze River in China with a total water storage capacity of 39.3 billion m\textsuperscript{3}. The total length of the Three Gorges Reservoir is 663 km and the water area reaches 1084 km\textsuperscript{2}\textsuperscript{16}. Every year, the Three Gorges Reservoir undergoes a periodic storage and discharge process\textsuperscript{17}. Water is stored in the dry season and drained in the rainy season. The upstream sediments are easily deposited and bring a large amount of nutrients\textsuperscript{18}, thus making the main stream in the reservoir area change spatially along the river stream.

The construction of the Three Gorges Dam has resulted in spatial changes in the mainstream sediments, which may have further led to the differentiation of comammox bacteria. However, there is still a lack of understanding whether comammox bacteria have differentiation in the mainstream of the Three Gorges Reservoir area and what the main influencing factors are. This study proposed a hypothesis that the construction of Three Gorges Dam might have caused niche differentiation of the comammox \textit{Nitrospira} in the reservoir area. To test this hypothesis, we investigated the abundance, diversity, and distribution of comammox \textit{Nitrospira} in the mainstream sediments of the Three Gorges Reservoir area of the Yangtze River, as well as their competition relationship with traditional ammonia-oxidizing microorganisms.

2 Materials And Methods

2.1 Sample collection

The study area is the mainstream of the Three Gorges Reservoir area (108°39'27.34"~108°42'52.91"E, 30°56'57.24"~31°5'47.89"N). It has a typical subtropical monsoon climate with warm less-rainy winters and warm rainy summers. In this study, 9 sampling points (M1-9) were selected from the mainstream of the Three Gorges Reservoir region covering the area from the beginning to the end of the reservoir (Fig. 1). In December 2019, temperature, pH, and DO of the overlying water were measured with a YSI water quality meter (EXO2, America). Three samples of 10 cm surface sediments were collected from each sampling point, packed in sterile ziplock bags, stored on ice, and transported to the laboratory. Each sediment sample was divided into two parts with one part used for the determination of physicochemical properties, and the other part stored at -80°C for the determination of molecular biology.

2.2 Determination of soil physiochemical properties
The fresh sediment was dried at 105°C to a constant temperature to measure the moisture content. The sediment pH was determined with a pH meter (METTLER TOLEDO, Switzerland). The air-dried ground sediment was leached with 2 mol/L KCl, and then ammonia nitrogen (NH$_4^+$), nitrate nitrogen (NO$_3^-$), and nitrite nitrogen (NO$_2^-$) were determined. Total carbon (TC) and total nitrogen (TN) in the sediments were determined with an elemental analyzer (Elementar Vario PYRO cube, Germany).

### 2.3 DNA extraction and PCR amplification

Microbial DNA was extracted from 0.4 g sediment sample using the Fast DNA Spin kit for soil (MPBIO, USA) according to kit manufacturers’ instruction. The quality of the DNA extract was inspected with a super differential spectrophotometer (NanoPhotometer-N60, IMPLEN, Germany).

The specific primers Com-amoA_1_R (CGAGATCATGGTGCTGTGAC) and pmoA-189b F (GNGACTGGGACTTTYTGG) were used to amplify the sediment comammox amoA gene$^{19}$. The 25 µL of the total PCR amplification system contained 2µL of template DNA, 5 µL of 5×reaction buffer, 2 µL of dNTP (2.5 mM, TransGen, China), 1µL of each primer (10 µM), 5 µL of 5×GC buffer, 0.25 µL of Q5® High-Fidelity DNA Polymerase (New England Biolabs, USA), and 8.75 µL of ddH$_2$O. PCR amplification program was as follows: at 98 °C for 2 min, followed by 30 cycles at 98 °C for 15 s, 55 °C for 30 s, and 72 °C for 30 s, and a final extension at 72 °C for 5 min. The specificity of the amplified final product was detected by 1.2% agarose gel electrophoresis.

### 2.4 Amplification sequencing and phylogenetic tree construction

The PCR-amplified products were sequenced on the Illumina NovaSeq PE250 platform (Shanghai Personal Biotechnology Co., Ltd). The raw data were processed with Vsearch (v2.13.4_linux_x86_64). The reference sequences with the highest similarity to the representative sequences of the main OTUs were retrieved from Genbank. Phylogenetic tree was constructed by neighbor-joining method using MEGA 5 with the bootstrap set as 1000 times. The reliability of phylogenetic tree topology was evaluated$^{20,21}$.

### 2.5 Real-time fluorescence quantitative PCR

The QuantStudio™ 6 Flex (Thermo Fisher Scientific, Singapore) was used to determine the bacterial copy number of comammox clade A, comammox clade B, ammonia oxidizing bacteria (AOB), and ammonia oxidizing archaea (AOA). The primers used for the amplification of the amoA genes of comammox clade A, comammox clade B, AOB, and AOA were presented in Table 1.
Table 1
Primer information table in this study

<table>
<thead>
<tr>
<th>Primer</th>
<th>Primer sequences(5’-3’)</th>
<th>Target genes</th>
<th>Fragment length</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arch-amoA F</td>
<td>STAATGGTCTGGCTTAGACG</td>
<td>AOA</td>
<td>635 bp</td>
<td>22</td>
</tr>
<tr>
<td>Arch-amoA R</td>
<td>GCGGCCATCCATCTGTATGT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>amoA-1F9</td>
<td>GGGTTTCTACTGGTGTTG</td>
<td>AOB</td>
<td>491 bp</td>
<td>23</td>
</tr>
<tr>
<td>amoA-2R5</td>
<td>CCCCTCKGSAAAGCCTTCTTC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CA3771f</td>
<td>GTGGTGCTGGTGCBAAYTA</td>
<td>comammox clade A</td>
<td>514 bp</td>
<td>24</td>
</tr>
<tr>
<td>C576r</td>
<td>GAAGCCCATRTARTCNGCC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CB377f</td>
<td>GTACTGGTGGGCBAAYTT</td>
<td>comammox clade B</td>
<td>514bp</td>
<td>24</td>
</tr>
<tr>
<td>C567r</td>
<td>GAAGCCCATRTARTCNGCC</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The amplification system contained 0.2 µl of forward primers, 0.2 ul reverse primers, 0.4 µl of ROX, 5 µl of T5 Fast qPCR Mix, and 1µl of 10-fold serially diluted DNA template, and amplification system volume was supplemented to 10µl with 3.2 µl ddH₂O.

AOA and AOB quantitative PCR amplification procedures were as follows: pre-denaturation at 95°C for 30 s, 35 cycles of denaturation at 95°C for 10 s, annealing at 53°C (AOA) or 55°C (AOB) for 30 s, and extension at 72°C for 35 s, and the final extension at 72°C for 1 min.\(^4\)\(^5\). Comammox A quantitative PCR amplification program was as follows: pre-denaturation at 95°C for 5 min, 35 cycles of annealing at 94°C for 45 s and extension at 53°C for 60 s, and final extension at 72°C for 56 s. Comammox B quantitative PCR amplification program was as follows: pre-amplification denaturation at 95°C for 5 min, followed by 45 cycles of annealing at 94°C for 10 s and extension at 58°C for 10 s, ending up with a final extension at 72°C for 13 s.\(^2\)\(^4\) The copy number of plasmids was counted after 10-fold serial dilution, and 8 standard samples and DNA samples were amplified. The experiment was conducted in triplicates for each sample. The amplification rate of the standard samples was between 90% and 110%, and the correlation of the standard curve was greater than 0.99.

2.6 Data statistical analysis

R (version 4.02) was used to process the data, and Duncan's and ANOVA tests (P < 0.05) were used to evaluate the significant differences in environmental physicochemical indicators and microbial abundance. Microbial abundance was calculated using QuantStudioTM Real-Time PCR Software (version 1.2). Diversity indices were calculated using QIIME2.\(^2\)\(^5\) RDA analysis of environmental parameters and abundance data was performed using Canoco 5. "Hmisc" and "corrplot" packages in R (version 4.02) were used to perform Spearman correlation analysis of abundance, diversity and environmental parameters and to plot (P < 0.05). The "circlize" package was employed to visualize the
dominant OUTs to plot chordal graph, and the "ggcor" package was employed to plot significant correlation between the comammox branch and each physicochemical index (P < 0.05). The "SpiecEasi" package was used to plot the contribution rate of dominant OTUs in the comammox branch and the significant correlation between various dominant OUTs (p < 0.05).

2.7 Serial number upload

The nucleotide sequence of Comamox *Nitrospira* amoA obtained in this study was submitted to the GenBank database with accession numbers of ON130361-ON130389.

3. Results

3.1 Geochemical characteristics of overlying water and sediments

The average pH of the overlying water in the mainstream of the Three Gorges Reservoir area was 8.33 ± 0.24; the bottom water temperature was 15.76 ± 1.41°C; the dissolved oxygen in the water was 7.57 ± 0.33 mg/L, and the water depth of the sampling point ranged from 22.78 m to 75.047 m (Supplementary Table S1).

The sediment physicochemical parameters were shown in Table 2. The sediment pH was 7.41–7.8. The NO$_2^-$ content ranged from 0.001 to 0.014 mg/kg with the highest NO$_2^-$ content at sampling site M8 and the lowest NO$_2^-$ content at M1. NO$_3^-$ and NH$_4^+$ content exhibited little change. The highest NO$_3^-$ content was observed at M3 (8.14 ± 2.10 mg/kg), which was 1.64 times that at M4 with the lowest NO$_3^-$ content (4.97 ± 0.63 mg/kg). The lowest content of NH$_4^+$ at M4 (0.46 ± 0.06 mg/kg) was one third of the highest content at M1 (1.38 ± 0.64 mg/kg). Total carbon (TC) varied from 1.08 ± 0.03 g/kg (M2) to 1.54 ± 0.07 g/kg at M9. The difference in TC content was small (20.32 ± 0.26 g/kg ~ 25.79 ± 1.46 g/kg). The difference in sediment moisture content was significant, and the minimum (33.77%) at M2 was about half of the maximum (58.27%) at M9 (Table.2).
Table 2

<table>
<thead>
<tr>
<th>Sampling sites</th>
<th>MC</th>
<th>pH</th>
<th>NO$_2^-$ (mg/kg)</th>
<th>NO$_3^-$ (mg/kg)</th>
<th>NH$_4^+$ (mg/kg)</th>
<th>TN (g/kg)</th>
<th>TC (g/kg)</th>
<th>C:N</th>
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</thead>
<tbody>
<tr>
<td>M1</td>
<td>48.75± 2.64b</td>
<td>7.6±0.04abc</td>
<td>0.001±0.000b</td>
<td>7.14±3.23bc</td>
<td>1.38±0.64a</td>
<td>1.46±0.06ab</td>
<td>25.76±0.76a</td>
<td>17.66±0.81c</td>
</tr>
<tr>
<td>M2</td>
<td>33.77±1.37d</td>
<td>7.80±0.03a</td>
<td>0.002±0.001b</td>
<td>5.62±0.38bc</td>
<td>0.49±0.13ab</td>
<td>1.08±0.03d</td>
<td>21.36±0.32c</td>
<td>19.86±0.88a</td>
</tr>
<tr>
<td>M3</td>
<td>39.76±4.76c</td>
<td>7.48±0.15bc</td>
<td>0.007±0.006ab</td>
<td>8.14±2.10a</td>
<td>0.66±0.42b</td>
<td>1.27±0.03c</td>
<td>21.08±0.18c</td>
<td>16.56±0.53bc</td>
</tr>
<tr>
<td>M4</td>
<td>48.29±8.79b</td>
<td>7.62±0.03abc</td>
<td>0.004±0.000b</td>
<td>4.97±0.63c</td>
<td>0.46±0.06b</td>
<td>1.42±0.03b</td>
<td>20.32±0.26c</td>
<td>14.28±0.44d</td>
</tr>
<tr>
<td>M5</td>
<td>50.38±0.68b</td>
<td>7.52±0.25bc</td>
<td>0.009±0.004ab</td>
<td>6.13±0.57bc</td>
<td>0.51±0.18b</td>
<td>1.51±0.03ab</td>
<td>20.92±1.45c</td>
<td>13.87±1.11d</td>
</tr>
<tr>
<td>M6</td>
<td>50.27±3.14b</td>
<td>7.65±0.03ab</td>
<td>0.006±0.001ab</td>
<td>6.23±1.01bc</td>
<td>0.41±0.01b</td>
<td>1.53±0.03a</td>
<td>21.64±0.06c</td>
<td>14.11±0.22d</td>
</tr>
<tr>
<td>M7</td>
<td>48.77±9.53b</td>
<td>7.56±0.13bc</td>
<td>0.018±0.021b</td>
<td>5.49±2.13b</td>
<td>0.42±0.06b</td>
<td>1.47±0.07ab</td>
<td>20.49±1.26c</td>
<td>13.99±1.02d</td>
</tr>
<tr>
<td>M8</td>
<td>52.07±1.13b</td>
<td>7.41±0.06c</td>
<td>0.014±0.005a</td>
<td>5.65±1.36c</td>
<td>0.57±0.16b</td>
<td>1.46±0.07ab</td>
<td>23.39±1.56b</td>
<td>16.02±0.71c</td>
</tr>
<tr>
<td>M9</td>
<td>58.27±1.07a</td>
<td>7.45±0.08bc</td>
<td>0.007±0.004b</td>
<td>5.93±0.85bc</td>
<td>0.94±0.62b</td>
<td>1.54±0.07a</td>
<td>21.22±1.26c</td>
<td>13.82±1.48d</td>
</tr>
</tbody>
</table>

Using analysis of one-way ANOVA (P < 0.05, Duncan test), Different lowercase letters in the same column indicate significant differences.

3.2 Abundance of comammox bacteria and traditional ammonia oxidizing bacteria

The abundances of comammox *Nitrospira* clade A and clade B amoA genes in the main stream of the Three Gorges Reservoir area showed an upward trend along the river stream, reaching the maximum in the middle part of the reservoir area and decreasing in the tail part of the reservoir area. The highest abundance of clade A and clade B amoA genes was 4.50×10$^4$±9734.79 copies/g (M7) and 4.25×10$^3$±1050.50 copies/g (M4), respectively. The lowest abundance of clade A and clade B amoA genes was 1.86×10$^4$±4582.10 copies/g (M5) and 4.96×10$^2$±350.39 copies/g (M1), respectively. The
abundance of amoA genes in AOA was higher in the upper section of the reservoir area (Fig. 2a), and exhibited a gradual downward trend along the river stream. No significant spatial difference in the abundance of AOB was observed.

The abundance of comammox *Nitrospira* Clade A amoA gene was $3.00 \times 10^4 \pm 8782.37$ copies/g, which was significantly higher than that of the other three functional genes. The amoA gene abundance of AOA was $1.73 \times 10^2 \pm 48.05$ copies/g, and the amoA gene abundance of AOB was $1.28 \times 10^3 \pm 574.69$ copies/g. The amoA gene abundance of comammox *Nitrospira* clade B was $1.83 \times 10^3 \pm 1019.82$ copies/g (Fig. 2b), and that of AOA from each sampling point was the lowest. There was no significant difference in abundance between AOB and comammox *Nitrospira* clade B amoA (Fig. 2b).

Species richness and Shannon index exhibited similar spatial variation trend, and both indices were increased rapidly from M1, and then showed a gradual downward trend. 1/Simpson index was the highest at M1, and then showed a downward trend, but it exhibited a high value at M7.

### 3.3 Correlation between Comammox abundance and soil physiochemical properties

The abundance of comammox Clade A genes was positively correlated with pH ($r = 0.47$, $n = 9$), which was extremely significantly correlated with NH$^+$ ($r = 0.75$, $p < 0.05$, $n = 9$) (Fig. 3). The abundance of comammox clade B genes was positively correlated with pH ($r = 0.4$, $n = 9$). AOA amoA gene abundance was negatively correlated with NO$_2^-$ ($r = -0.24$, $n = 9$). AOB amoA gene abundance was negatively correlated with pH ($r = -0.32$, $n = 9$) and TN ($r = -0.33$, $n = 9$). Both AOA and AOB amoA gene abundances were positively correlated with TC ($r = 0.22$, $n = 9$) ($P < 0.05$).

### 3.4 Phylogenetic tree of Comammox bacteria

A total of 727,323 comammox gene sequences were obtained by high-throughput sequencing with sequences per sample ranging from 56,789 to 101,426 for community analysis. In total, 29 OTUs with relatively high abundance were screened (accounting for 65.34% of the comammox *Nitrospira* sequence) for phylogenetic analysis. These 29 OTUs fell into three clades comammox clade A.1 (11 OTUs), comammox clade A.2 (4 OTUs), and comammox clade B (14 OTUs). The OTUs of comammox clade A.1 accounted for 18.69%, followed by comammox clade A.2 (18.58%) and comammox clade B (14.3%) (Fig. 4a). The dominant OUT of comammox clade A.1 was OTU24 (3.57%); that of comammox clade A.2 was OTU1 (11.18%); and that of comammox clade B was OTU7 (8.38%) (Fig. 4a).

At sampling point M8, the number of OUT was the largest (70762 OTUs). At sampling point M3, the number of OUTs was the smallest (36179 OTUs). Branches A.1, A.2 and B were most abundant at sampling points M2, M9, and M2, respectively (Fig. 4b).
3.5 Interaction between environment and Comammox bacteria

Dissolved oxygen (DO) had a significant effect on comammox clade A.2 and comammox clade B communities (Fig. 5a), but had no effect on comammox clade A.1. The community of comammox clade A.2 was affected by environmental factor NO$_3^-$, but the concentration of NO$_3^-$ had little effect on comammox clade B and comammox clade A.1.

The two axes of RDA jointly explained 99.60% of the species information change and species-environment relationship (Fig. 5b). The abundance of amoA genes in the two clades of comammox *Nitrospira* showed a significant positive correlation ($r = 0.26, p < 0.05, n = 9$). The abundance of comammox *Nitrospira* clade A amoA gene was positively correlated with NO$_3^-$ ($r = 0.23, p < 0.05, n = 9$) and DO ($r = 0.17, p < 0.05, n = 9$). The abundances of comammox *Nitrospira* clade B amoA and AOB amoA genes were significantly positively correlated with TN ($r = 0.01, p < 0.05, n = 9$), TC ($r = 0.15, p < 0.05, n = 9$), and pH ($r = 0.15, p < 0.05, n = 9$). AOA amoA was positively correlated with NH$_4^+$ ($r = 0.14, p < 0.05, n = 9$).

As shown in the network plot, positive correlations was more than negative correlations in the network ($p < 0.05$) (Fig. 5c) comammox clade A.1 accounted for 24.88% in all samples, and comammox clade A.2 accounted for 33.15%. Comammox clade B exhibited the highest percentage in all the samples, accounting for 45.98%. In the environment, comammox clade B displayed the highest abundance, whereas comammox Clade A.1 exhibited the lowest abundance.

4 Discussion

4.1 Comammox *Nitrospira* clade differentiation

In different environments, the proportion of each branch of comammox *Nitrospira* differs$^{10}$. In this study, our phylogenetic tree showed that the comammox bacteria in the sample fell into three branches, namely, comammox clade A.1, comammox clade A.2, and comammox clade B. Comammox clade A was more widely distributed than comammox clade B, but the distribution difference was not significant, which might be due to the scouring of the middle and lower reaches of the riverbed and the coarsening of particles, which affects the microbial niche and diversity$^{13,26}$, so that comammox clade A and comammox clade B could coexist in large quantities. Consistently, many studies have also found that comammox clade A is more widely distributed than comammox clade B, and that comammox clade A.1 clades are more widespread than comammox Clade $^{10,27}$.

Comammox clade A.1 was widely distributed at sampling site M2, mainly from estuarine wetlands, river reservoir, and tidal flat sediments, freshwater river sediments, sewage treatment plants$^{10,28,29}$. Comammox clade A.2 was most abundant at sampling site M9, and it was mainly distributed in estuarine
tidal flat wetlands. Comammox Clade B accounted for the highest proportion (28.07%) at M2, mainly from paddy soil, forest soil, wetland soil, lake bottom sediments, and wastewater plants.

This study found that the abundance of comammox clade A and comammox clade B was higher in the middle section of the reservoir area, indicating that comammox had adaptability to the water environment of the reservoir area. Some studies have found that after damming, physical changes in rivers will cause sediment deposition. Along the direction of water flow, there is a high deposition rate at the tail of the reservoir, and deposition decreases rapidly with the decreasing distance from the dam. Large particles settle down first, and small particles settle down later or have difficulty in settling. Dam building also leads to the changes in the chemical and biological composition of the reservoir. Previous studies have found that in the sediments of the main stream of the Yangtze River, comammox Nitrospira have large-scale niche differentiation along the altitude, and climate, topography, and landform are the main influencing factors. Our results showed that the sediment subsidence in the Three Gorges Reservoir area caused by the dam construction and the resultant water environment changes may be mainly responsible for the differentiation of comammox bacteria in the entire reservoir area.

4.2 Coexistence and differentiation of Comammox and traditional ammonia oxidizing bacteria

Comammox, AOA, and AOB have been reported to exist in various environments such as soil and tidal flats. Consistently, comammox, AOA, and AOB were detected in this study, indicating that comammox bacteria were widely present in the main stream of Three Gorges reservoir area. The abundance of comammox clade A genes was higher than that of AOA and AOB genes in this study area. Our result is similar to the previous study report that in coastal waters and river sediments, the abundance of comammox amoA gene is higher than that of AOA amoA and AOB amoA genes. The existing studies have shown that comammox bacteria have a lower nitrous oxide emission rate than AOB, and its emission rate is relatively close to that of AOA. Our data indicated that the abundance of clade A gene in the mainstream of the Three Gorges Reservoir area was much higher than that of AOA and AOB, which might be beneficial to the reduction in the nitrous oxide production in the reservoir area.

Most of the existing studies have shown that comammox has a higher transcription rate than AOA and AOB, and it has a higher affinity to ammonia, and thus it has a competitive advantage in a low-ammonia environment. Several studies have found that comammox clade A can adapt to eutrophic conditions. Our data showed no significant positive correlation between the abundance of comammox clade A or clade B genes and NH$_4^+$, indicating that in this study area, the ammonia content in the sediment was not low enough to endow comammox bacteria with the competitive advantage.

Our results showed that species richness and Shannon indices were similar in the space variation, and that 1/Simpson representing species diversity showed a change trend opposite to species richness and Shannon indices. The highest species richness and diversity of comammox were observed in the middle part of the reservoir area, which might be due to the fact that the relatively stable water environment in
the middle of the reservoir area was more conducive to the species reproduction of comammox bacteria. In the upper part of the reservoir area, the large particles of sediment in the river continued to settle down at the bottom of the reservoir. The continuous discharge of water from the Three Gorges Dam resulted in the rapid flow of the river water in the lower part of the reservoir area.

4.3 Influence of geographical environment on Comammox and traditional ammonia oxidizing microorganisms

Ammonia is the common substrate of AOA\AOB\comammox bacteria, and these three ammonia-oxidizing bacteria generally compete for ammonia. This study found that NH$_4^+$ concentration was negatively correlated with comammox cladeA amoA gene abundance, but positively correlated with AOA amoA gene abundance. There was also a negative correlation between NH$_4^+$ concentration and comammox cladeA amoA gene abundance in tidal flat sediments of the Yangtze River$^{24}$. This study also found no significant correlation between NH$_4^+$ concentration and AOB amoA gene abundance. Xu et al. (2020) have found high ammonia concentrations might provide sufficient reaction substrates for aerobic AOB$^{44}$. These findings suggest that the effects of ammonia on these ammonia-oxidizing microorganisms are complex, and that there is not necessarily a competition for ammonia among these microorganisms.

pH has long been recognized as a major factor affecting the activity and distribution of ammonia-oxidizing microorganisms in ecosystems$^{45}$. Some sediment and soil studies have found that comammox clade A prefers high pH environments$^{9,44}$. In soil studies, pH has been reported to be an important factor affecting the niche differentiation of AOA and AOB$^{46}$. In this study, pH was positively correlated with comammox clade B amoA and AOB, but pH exhibited no significant correlation with the abundance of AOA amoA gene and comammox clade A amoA gene. The pH, T, and NO$_2^-$ had more significant effects on the niche differentiation of comammox clade A and comammox clade B than on that of AOA and AOB.

5 Conclusion

In this study, we studied comammox in river sediments in the Three Gorges Reservoir area, and comammox clade A, comammox clade B, AOA and AOB were detected in all the samples. The abundance of comammox clade A amoA gene was higher than that of AOA amoA gene and AOB amoA gene, and comammox *Nitrospira* was the dominant species among ammonia oxidizing microorganisms. The abundance of comammox *Nitrospira* clade A and clade B amoA genes in the main stream of the Three Gorges Reservoir showed an upward trend along the river stream, and reached the maximum in the middle part of the reservoir area, whereas the highest AOA amoA gene abundance value appeared in the upper part of the reservoir area. The abundance of comammox *Nitrospira* clade A was the highest (3.00×10$^4±32.72$copies/g), followed by comammox *Nitrospira* clade B, AOB, and AOA. Comammox clade B had the highest percentage, followed by comammox clade A.1 and comammox clade A.2. The Mantel
test chart showed that DO overlying water in the reservoir area had a significant effect on the
differentiation of comammox clade A into comammox clade A.1 and comammox clade A.2. Redundancy
analysis (RDA) analysis showed that DO, NO$_3^-$, TC had significant effects on camammox clade A, while
NO$_2^-$ and pH had significant effects on comammox clade B. This study confirms that the construction of
the Three Gorges Dam had an impact on the niche differentiation of comammox bacteria in the main
stream of reservoir area.

**Declarations**

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Conceptualization, Mingming Hu and Jianwei Zhao; Data curation, Shuang Liu; Funding acquisition,
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**Availability of data and materials**

The nucleotide sequence of comammox *Nitrospira* amoA gene obtained in this study are available in the
GenBank, Accession Number(s): ON130361-ON130389.

**Ethics approval and consent to participate**

Not applicable

**Consent for publication**

Not applicable.

**Conflicts of interest**
The authors declare no conflict of interest.

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Figures
Figure 1

Locations of the sampling sites
Figure 2

(a) Abundance of AOA, AOB, comammox clade A and comammox clade B amoA at M1, M2, M3, M4, M5, M6, M7, M8, and M9. (b) Total abundance of AOA, AOB, comammox clade A and comammox clade B amoA in samples.
Figure 3

Heat map of correlation between AOA, AOB, comammox clade A, comammox clade B amoA, and environmental physiochemical indexes. Blue represents positive correlation; red indicates negative correlation. Only the significant correlations (P < 0.05) are labeled in circles. Scale bars indicate correlation coefficients.
Figure 4

(a) Phylogenetic analysis of Comammox amoA gene. The percentage in parentheses (behind OTU) indicates the proportion of each OTU to the total number of Comammox amoA genes. Only the obtained OTU with more than 0.52% Comammox amoA gene sequence was displayed in the phylogenetic tree. The reference sequence comes from GenBank. The letters and numbers behind the sequence are the submission serial number. The circle, gray dot, black dot at the branch nodes represent 50%~70%, 70%~90%, and 90%~100% sequence similarity, respectively. The percentage in parentheses behind OTU indicates the proportion of each OTU in the total sequence of comammox amoA genes. The calculation method is the maximum composite likelihood method. The scale bar represents 20% of the sequence difference. (b) The distribution of 29 dominant OTUs at different sampling sites. The 9 sampling points and 29 OTUs are represented by different colors.
Figure 5

(a) Correlation between comammox A.1, comammox A.2 or comammox B branches and environmental physicochemical indexes. (b) Redundancy analysis of comammox A, comammox B, AOA, and AOB amoA in sampling sites. (c) Contribution analysis of Comammox A.1, comammox A.2, and comammox B branch networks. The blue line represents positive correlation, whereas the red line represents negative correlation (P < 0.05.)

Supplementary Files

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- Schedule1.xlsx