Anaerobic methane-oxidizing bacterial communities in sediments of a drinking reservoir, Beijing, China

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Abstract

Purpose: Nitrate/nitrite-dependent anaerobic methane oxidation (N-DAMO) connects the global cycle of carbon and nitrogen in aquatic ecosystems. The aims of this study were to investigate the spatiotemporal variation of N-DAMO bacteria and its driving factors in a drinking reservoir which are strongly affected by human consumption.

Methods: Cloning analyses were used to study the pmoA and 16S rRNA genes of N-DAMO bacteria. Sequencing and phylogenetic analyses were used to investigate the bacterial composition and structure. Redundancy analyses (RDA) and spearman’s correlations analyses were applied to analyze the relationships between bacterial distribution and environmental factors.

Result: There were remarkable spatial variations of N-DAMO bacteria in winter. Shannon biodiversity of 16S rRNA genes was higher in winter than that in summer. Compared with other freshwater ecosystems, there was higher diversity of N-DAMO bacteria in Miyun Reservoir. The N-DAMO bacterial communities (16S rRNA and pmoA genes) in sampling sites near to dam were different from the bacterial communities in other sampling sites. The N-DAMO bacterial community structure in dam areas may be associated with the water column depth in front of the dam. Moreover, Spearman’s correlations revealed that DO, NO$_3^-$-N, NO$_2^-$-N, and NH$_4^+$-N in interstitial water and sediment were potential determinant factors influencing the diversity of N-DAMO bacteria (16S rRNA and pmoA genes).

Conclusion: There were distinct seasonal variations in 16S rRNA and spatial variations in pmoA genes. N-DAMO bacterial communities contained novel Methylomirabilis oxyfera-like pmoA genes in Miyun Reservoir. Nitrogen (NO$_3^-$-N, NO$_2^-$-N, and NH$_4^+$-N) were the dominant factor influencing the N-DAMO bacterial community structure in the drinking reservoir. N-DAMO bacterial community structure in dam areas indicates that water depth and DO might be the dominant factor influencing the N-DAMO bacterial communities in the reservoir.

Keywords: N-DAMO bacteria, 16S rRNA, pmoA genes, Microbial community, Environmental factors, Miyun Reservoir
Introduction

Methane (CH$_4$) is the second most important greenhouse gas, which plays a key role in the carbon cycle (Shen et al. 2014a). Methane is estimated to contribute approximately 20% effect to the global warming, and the global warming potential caused by methane is about 25-fold higher than carbon dioxide (CO$_2$) on a per-molecule basis (Hu et al. 2014). Aquatic ecosystems in China have a much higher CH$_4$ emission flux than that in American and European, due to the higher nutrient enrichment and organic matter in the water (Yang et al. 2016). Previous studies revealed that approximately 76 ~ 90% of the CH$_4$ consumption relied on microbial oxidation by aerobic or anaerobic methanotrophic bacteria and archaea (Yan et al. 2015, Wang et al. 2016). CH$_4$ oxidation is the key process that can mitigate the methane emission production in anoxic sediment layers (Liu et al. 2015).

With the popular use of agricultural fertilizers, nitrate (NO$_3^{-}$) and nitrite (NO$_2^{-}$) became the major electron acceptors of nitrate/nitrite-dependent anaerobic methane oxidation (N-DAMO) bacteria in freshwater environments (Raghoebarsing et al. 2006, Ettwig et al. 2010, Yan et al. 2015). N-DAMO was catalyzed by "Candidatus Methylomirabilis oxyfera" (M. oxyfera) which is affiliated with the NC10 phylum (Ettwig et al. 2008, Hu et al. 2014). M. oxyfera bacteria is an intra-aerobic methanotroph that performs methanoxidizing through the “intra-aerobic” pathway which is a dismutation process with nitric oxide changes into dinitrogen gas and oxygen, and the oxygen can be used by the bacteria to oxidize methane with catalysis by the methane monooxygenase enzyme complex (Ettwig et al. 2010, Zhu et al. 2012, Ho et al. 2013, Liu et al. 2015). N-DAMO process constitutes a unique association between the two foremost global nutrient cycles including the carbon cycle and the nitrogen cycle (Steven et al. 2008, Shen et al. 2014a, b). Several studies revealed that N-DAMO could consume 4.1–6.1 Tg of CH$_4$ m$^{-2}$ per year in freshwater ecosystems under anaerobic conditions, which are roughly 2–6% of current worldwide CH$_4$ flux estimates for the freshwater ecosystem (Hu et al. 2014). Therefore, with the worldwide increasing in nitrogen pollution, N-DAMO has the potential to mitigate the release of methane in freshwater ecosystems.

With the development of specific PCR primer targeting, 16S rRNA (Ettwig et al. 2009) and pmoA genes (Luessen et al. 2011, Deutzmann et al. 2014) provided us new techniques to study the N-DAMO bacterial communities in freshwater ecosystems. Recently, the environmental N-DAMO bacteria was studied in many inland lake ecosystems, including Lake Constance in Germany (Deutzmann & Schink 2011), Lake Biwa in Japan (Kojima et al. 2012), and eutrophic lakes in Yunnan province, China (Liu et al. 2015, Yang et al. 2016). Moreover, many researchers recently investigated N-DAMO bacterial community distribution in many different ecosystems including paddy fields (Wang et al. 2012, Hu et al. 2014, Shen et al. 2014c), coastal mangrove wetlands (Chen et al. 2015), peatlands (Zhu et al. 2012), rivers (Shen et al. 2014a), estuaries (Shen et al. 2014b, Yan et al. 2015), and wastewater treatment sludge (Ho et al. 2013). The Ministry of Water Resources of the People’s Republic of China stated that there are over 80,000 reservoirs in China (Chen 2009, Yang et al. 2014), and the reservoir ecosystem was a hotspot for CH$_4$ emissions which contribute approximately 12% to the total natural methane emissions (Louis et al. 2000, Wang et al. 2016). In recent years, with the construction of dam, the reservoirs have become a potential clean energy source (Yang et al. 2014), while several studies also revealed that reservoirs are the hotspot areas of CH$_4$ emission (Yang et al. 2014). N-DAMO bacteria play an important role in regulating the impact of the reservoir on climate change because the bacteria can mediate the reaction of carbon and nitrogen. In recent years, researchers paid more attention on the study of reservoir methane oxidation bacteria and their ecosystem functions (Kinen et al. 2010, Li & Lu 2012, Bridgham et al. 2013). In order to develop strategies for controlling CH$_4$ emission, it is important to reveal the geographical distribution and spatial-temporal variation patterns of N-DAMO bacteria community in mesotrophic reservoirs.

Miyun Reservoir is an important available drinking water source in Beijing, and a series environmental protection measures were used to protect the water quality of the reservoir. Studies by Yang suggested that the construction of the Miyun Reservoir could have increased the regional CH$_4$ emission flux (Yang et al. 2014), while the study of N-DAMO bacteria in this reservoir was still limiting. The study has two objects: (1) investigate the spatial-temporal distribution for the N-DAMO bacteria in Miyun Reservoir and (2) determine major environmental determinants responsible for the structure of the bacterial community. The study of N-DAMO bacteria in Miyun Reservoir is essential to better understand the biogeochemical mechanism of methane in reservoirs.

Materials and methods

Study area

Miyun Reservoir (40° 31’ to 40° 45’ N, 115° 56’ to 117° 10’ E) was a drinking reservoir of Beijing located in the northern part of Beijing (Jiao et al. 2015) (Fig. 1). The total storage capacity of the reservoir is approximately 4375 billion m$^3$, and the total reservoir area is 188 km$^2$ (Li et al. 2016). Mean annual temperature in the watershed is 10.5 °C, average annual precipitation is 660 mm, and about 70–80% of the precipitation occurs in summer (from June to September) (Ou & Wang 2008, Jiao et al. 2015). With the rapid development of urbanization, Miyun Reservoir became an essential drinking source for more than two
million people in Beijing (Ding et al. 2016a), and nitrogen (NO$_3^-$-N) and phosphorus are the major nutrients to degrade the water quality of the reservoir (Wang et al. 2001, Li et al. 2016).

In Miyun Reservoir, sampling sites were grouped into two areas including western reservoir areas (1, 2, 3, and 4) and eastern reservoir area (5, 6, 7, and 8). Table S1 shows the characteristic of each sampling including depth of water column, characteristic of sediments, and the distance between the dams. The sediment sampling (0~5 cm) was collected using a columnar sediment sampler during summer (July in 2015) and winter (January in 2016) with the permission of Miyun Reservoir management office. Sediment of each sampling sites was sampled three times and mixed evenly, and then, the samples were stored in two sterile plastic bottles (1000 mL) and transported to the laboratory at 4 °C. We obtain the interstitial water using cryogenic high-speed centrifuge from one of the bottle samples following standard procedures: 5000 r min$^{-1}$ for 10 min. Sediment samples were divided into two subsamples: one subsample was homogenized and dried using a freeze dryer (Alpha 1-2 LD plus; Martin Christ, Germany) for physicochemical chemical analyses, and the other subsample was saved at − 80 °C used for DNA extraction.

At each sample site, water temperature (Temp), dissolved oxygen (DO), pH value, and conductivity (Cond) were measured in situ using a YSI Model 80 meter (Yellow Springs Instruments, Yellow Springs, Ohio). Altitude was measured using a GPS unit (Triton 500, Magellan, Santa Clara, CA). At each sample site, interstitial water samples were saved at 4 °C for chemical analyses. Total nitrogen (TN) was analyzed by ion chromatography after persulfate oxidation. Nitrate (NO$_3^-$-N) was determined by ion chromatography. Ammonium (NH$_4^+$-N) was analyzed using the indophenol colorimetric method. Total phosphorus (TP) was quantified using the ammonium molybdate method after oxidation. Total organic carbon (TOC) was analyzed using a

Table 1 The primers of 16S rRNA and pmoA genes, and thermal cycler procedures of polymerase chain reaction

| Primer         | Sequence (5’-3’)       | Specificity | Thermal profiles                                    | References                  |
|----------------|------------------------|-------------|----------------------------------------------------|-----------------------------|
| A189_b (PCR)   | GGNGACTGGGACTTYTGG     | N-DAMO pmoA | 4 min at 94 °C, followed by 35 cycles of 60 s at 94 °C, 60 s at 53 °C and 90 s at 72 °C, and finally 10 min at 72 °C | (Ettwig et al. 2009, Wang et al. 2012) |
| cmo682(PCR)    | AAAYCCGGACRAAGAACGA    | N-DAMO pmoA | 3 min at 95 °C, followed by 40 cycles of 60 s at 95 °C, 60 s at 63 °C and 90 s at 72 °C, and finally 5 min at 72 °C | (Luesken et al. 2011, Chen et al. 2015) |
| cmo182(PCR)    | TCACGTTGACGCCGATCC     | N-DAMO pmoA | 3 min at 95 °C, followed by 40 cycles of 60 s at 95 °C, 60 s at 63 °C and 90 s at 72 °C, and finally 5 min at 72 °C | (Luesken et al. 2011, Chen et al. 2015) |
| cmo568(PCR)    | GCACATACCTCCCCATC      | N-DAMO pmoA | 3 min at 95 °C, followed by 40 cycles of 60 s at 95 °C, 60 s at 63 °C and 90 s at 72 °C, and finally 5 min at 72 °C | (Luesken et al. 2011, Chen et al. 2015) |
| 202F(PCR)      | GACCAAAGGCGCGAGCG      | NC10 phylum 16S | 3 min at 95 °C, followed by 40 cycles of 60 s at 95 °C, 60 s at 63 °C and 90 s at 72 °C, and finally 5 min at 72 °C | (Luesken et al. 2011, Chen et al. 2015) |
| 1545R(PCR)     | CAKAAAGGAGTGTACCC      | Bacteria 16S | 3 min at 95 °C, followed by 40 cycles of 60 s at 95 °C, 60 s at 63 °C and 90 s at 72 °C, and finally 5 min at 72 °C | (Luesken et al. 2011, Chen et al. 2015) |
| qP1F(q)PCR     | GGGGTTGACATCCCGACAACCTG | N-DAMO 16S | 3 min at 95 °C, followed by 40 cycles of 60 s at 95 °C, 60 s at 63 °C and 90 s at 72 °C, and finally 5 min at 72 °C | (Luesken et al. 2011, Chen et al. 2015) |
| qP2R(q)PCR     | CTCAGCCGACTGAGTACAG    | N-DAMO 16S | 3 min at 95 °C, followed by 40 cycles of 60 s at 95 °C, 60 s at 63 °C and 90 s at 72 °C, and finally 5 min at 72 °C | (Luesken et al. 2011, Chen et al. 2015) |
Shimadzu TOC Analyzer (TOC-VCBH, Shimadzu Scientific Instruments, Columbia, Maryland).

DNA extraction and PCR amplification
Genomic DNA from sediment samples (5 g) were extracted using the Power Soil DNA Isolation Kit (Mo Bio Laboratories, Carlsbad, CA, USA) following the manufacturer’s protocols. The pm0A genes and 16S rRNA gene of N-DAMO bacteria were amplified using a nested approach: the pm0A genes first-step primer pair A189_b and cmo682, followed by primer pair cmo182 and cmo568, and the 16S rRNA gene first-step primer pair 202F and 1545R, followed by primer pair qP1F and qP2R (Ettwig et al. 2009, Luesken et al. 2011, Wang et al. 2012) (Table 1).

Cloning, sequencing, and phylogenetic analyses
PCR products were cloned using the pGEM-T Easy cloning kit (Promega) according to the manufacturer’s instructions. A maximum of about 50 positive clones were randomly selected for sequencing in The Sino Genome Research center Co., Ltd (Beijing, China). Sequences reported in this study are available from GenBank database under the accession numbers of KU199317-KU199676 (pm0A genes), KX082978-KX083338 (pm0A genes), KU213375-KU239099 (16S rRNA), and KX138657-KX138999 (16S rRNA). BLAST searches were performed against the sequences with the reference sequences databases in NCBI (National Center for Biotechnology Information) Genbank (http://www.ncbi.nlm.nih.gov/genbank/). Phylogenetic analyses of pm0A genes and 16S rRNA were performed using MEGA 5.1 software by neighbor-joining method based on the nucleotide sequences (Tamura et al. 2011), and bootstrap analyses with 1000 replicates were applied to examine the confidence level of the clustering of the trees.

Statistical analysis
Chimeric sequences were checked and filtered using the Mallard software (http://www.download32.com/mallard-software.html). Phylogeny-cluster analysis and principal coordinate analysis (PCoA) were conducted using R (version 3.3.2) (Daniel et al. 2014). Non-chimera sequences were clustered into operational taxonomic units (OTUs) with a complete linkage algorithm at 95% sequences identity level for pm0A genes and 98% sequences identity level for 16S rRNA. Shannon biodiversity, Chao1, and the coverage of the clone libraries were calculated using MOTHUR (Schloss et al. 2009). Redundancy analyses (RDA) was applied to analyze the distribution of the bacterial communities with respect to various environmental factors using R (version 3.3.2 and Vegan package 2.4) (Daniel et al. 2014). Spearman’s correlation analyses were employed to calculate the relationships between bacterial distribution and environmental factors using SPSS 20.0 software (SPSS, Chicago, IL, USA).

Results
N-DAMO bacterial community structure
A total of 697 16S rRNA sequences were obtained from Miyun Reservoir. The range of library coverage values from 0.96 to 1.00 suggesting the 16S rRNA gene sequences could represent in constructed clone libraries.

Table 2 Biodiversity of 16S rRNA and pm0A genes in Miyun Reservoir

| Samples | Sequence number | OTUs | Coverage | Shannon index | Simpson index | Chao1 |
|---------|----------------|------|----------|---------------|--------------|-------|
|         | pm0A | 16S  | pm0A | 16S | pm0A | 16S | pm0A | 16S | pm0A | 16S | pm0A | 16S |
| A1      | 47   | 42   | 4     | 4   | 0.96 | 1.00 | 0.83 | 0.99 | 0.49 | 0.49 | 5     | 4     |
| A2      | 45   | 49   | 1     | 2   | 1.00 | 1.00 | 0.00 | 0.23 | 0.57 | 0.88 | 1     | 2     |
| A3      | 45   | 49   | 3     | 3   | 1.00 | 0.96 | 1.01 | 0.20 | 0.38 | 0.92 | 3     | 4     |
| A4      | 45   | 40   | 4     | 4   | 0.96 | 1.00 | 0.64 | 0.56 | 0.66 | 0.62 | 5     | 2     |
| A5      | 50   | 46   | 2     | 3   | 1.00 | 1.00 | 0.29 | 0.86 | 0.84 | 0.46 | 2     | 3     |
| A6      | 48   | 49   | 2     | 3   | 0.98 | 1.00 | 0.10 | 0.59 | 0.96 | 0.68 | 2     | 3     |
| A7      | 41   | 51   | 2     | 2   | 1.00 | 1.00 | 0.37 | 0.58 | 0.78 | 0.60 | 2     | 2     |
| A8      | 37   | 47   | 3     | 3   | 1.00 | 0.98 | 0.86 | 0.60 | 0.44 | 0.64 | 3     | 3     |
| B1      | 42   | 37   | 1     | 3   | 1.00 | 1.00 | 0.00 | 0.85 | 1.00 | 0.45 | 1     | 3     |
| B2      | 40   | 38   | 2     | 2   | 1.00 | 1.00 | 0.18 | 0.55 | 0.92 | 0.63 | 2     | 2     |
| B3      | 32   | 44   | 3     | 2   | 0.97 | 1.00 | 0.72 | 0.54 | 0.54 | 0.64 | 3     | 2     |
| B4      | 53   | 50   | 3     | 3   | 0.98 | 1.00 | 0.25 | 0.81 | 0.89 | 0.52 | 3     | 3     |
| B5      | 46   | 47   | 4     | 4   | 1.00 | 0.96 | 1.17 | 0.86 | 0.33 | 0.45 | 4     | 5     |
| B6      | 42   | 37   | 8     | 2   | 0.98 | 1.00 | 1.75 | 0.58 | 0.70 | 0.59 | 8     | 2     |
| B7      | 46   | 35   | 5     | 4   | 0.98 | 1.00 | 1.15 | 0.97 | 0.38 | 0.46 | 5     | 4     |
| B8      | 55   | 36   | 4     | 4   | 0.98 | 0.98 | 1.09 | 0.96 | 0.34 | 0.44 | 4     | 4     |

A1–A8, sampling sites in summer; B1–B8, sampling sites in winter
Based on 2% genetic divergences, a total of 5 OTUs were detected in summer and 8 OTUs were observed in winter (Table 2). The Shannon biodiversity (0.54–0.97) presented stronger spatial variation in winter (Table 2). A total of 714 pmoA gene sequences were obtained in the reservoir. The range of library coverage value (0.96–1.00) indicated the availability of these sequences in our study (Table 2). Based on 5% genetic divergences, there were 5 OTUs obtained in summer and 16 OTUs obtained in winter. Shannon biodiversity of pmoA genes in winter (0.18–1.75) was higher than that in summer (0.10–1.01).

There were four clusters of 16S rRNA genes grouped indicating the seasonal variations (Fig. 2a). Sampling sites in summer (A6, A8, A2, and A3) and winter (B2, B5, and B8) were clustered separately. A1 was a dam sampling sites which were grouped as a cluster in the cluster analyses (Fig. 2a). In PCoA, sampling sites in summer clustered together and sampling sites in winter clustered together, and A1 was separated from other sampling sites (Fig. 2b). However, pmoA genes presented the spatial variation, A1 and B1, A3 and B3, A6 and B6, and A8 and B8 clustered together (Fig. 3a), and PCoA also demonstrated the spatial variation of the pmoA genes (Fig. 3b).

Phylogenetics of N-DAMO bacteria
In our study, 16S rRNA (Fig. 4) and pmoA (Fig. 5) gene sequences were used for phylogenetic analyses. The remarkable differences of 16S rRNA gene proportion between clusters indicated distinct spatial-temporal change in 16S rRNA genes of N-DAMO bacteria. Cluster I (A2, A3, A4, A5, A6, A8, B1, B3, B4, B5, B7, and B8) was the largest cluster and had a higher homology with 16S rRNA genes (100%), and the sequences in the cluster I
accounted for approximately 51% of 16S rRNA gene sequences. Cluster II was the second cluster which accounted for 41% of 16S rRNA gene sequences. Cluster III, cluster IV, cluster V, and cluster VI accounted for 11.6% of 16S rRNA gene sequences (Fig. 6a). Cluster V and cluster VI were obtained from sampling sites including A4, A5, A6, A8, B1, B3, B4, B5, B9, and B8.

Phylogenetic analyses showed that pmO genes were classified into five clusters (Fig. 5). Cluster I was the largest cluster which could be found in all sampling sites, and it covered 60.6% of the number of pmO genes sequences (Fig. 6b). Cluster II was mainly composed of pmO gene sequences from sampling sites including A2, A3, A6, A8, B2, B3, B4, B5, B6, B7, and B8, and this cluster accounted for 24.9% of the number of pmO gene sequences (Fig. 6b). Cluster III consisted of 22 pmO gene sequences from sampling sites of A3 and 6 gene sequences from B6. Cluster IV included pmO gene sequences from the sampling sites of A2, A7, B3, B5, and B7 (Fig. 5). Cluster V was the smallest group, which included pmO gene sequences from sampling sites of A5, A6, B4, and B7.

Environmental factors
As one of the main pollutants in Miyun Reservoir, TN (total nitrogen) concentration in water was two to five times higher than that indicated in the Chinese Environmental Quality Standards for Surface Water Grade V (≤ 2 mg-NL⁻¹). High TN content was also detected in winter, while high TP content was also detected in summer (Table S2). Spearman’s correlations and RDA were used to assess the potential effect of environmental variables on N-DAMO bacterial biodiversity (Table 3 and Fig. 7). NO₂⁻-N, NO₃⁻-N, and NH₄⁺-N in interstitial water and sediment were closely associated with Shannon biodiversity of pmO genes in summer and winter (Table 3, \( p < 0.05 \)). Water column depth of sampling sites was closely associated with Shannon biodiversity of pmO genes in summer (\( p < 0.05 \)), while there was no close relationship between water column depth and Shannon biodiversity of pmO genes in winter (Table 3). NO₃⁻-N in sediment was closely related to Shannon biodiversity of 16S rRNA genes (Table 3, \( p < 0.05 \)). RDA were used to evaluate the relationships of environmental variables and N-DAMO bacterial communities.
According to the Monte Carlo test, NO$_3^-$-N, TP, TOC, and C/N in sediment and NH$_4^+$-N, NO$_3^-$-N, and NO$_2^-$-N in interstitial water were significantly associated with 16S rRNA genes (two axes accounted for 89.67%, p < 0.05). The first axis was mainly defined by BS-TOC and BS-C/N in the positive direction, and the second axis was mainly defined by IW-NH$_4^+$-N and IW-NO$_3^-$-N in the positive direction and the BS-TP and IW-NO$_2^-$-N in the negative direction (Fig. 7a). NO$_2^-$-N, NH$_4^+$-N, and TP in interstitial water and NH$_4^+$-N, NO$_3^-$-N, NO$_2^-$-N, and TP in sediment were closely associated with pmoA genes (two axes accounted for 82.60%, p < 0.05). BS-NH$_4^+$-N, BS-NO$_3^-$-N, BS-TP, and IW-NO$_2^-$-N defined the positive direction of the first axis, and IW-TP, BS-NO$_2^-$-N, BS-TOC, and IW-NH$_4^+$-N defined the negative direction of the first axis (Fig. 7b).

Discussion

N-DAMO bacterial communities

N-DAMO bacteria were widespread in many natural ecosystems (Shen et al. 2014a, b, c, Yan et al. 2015, Ding et al. 2016b, Wang et al. 2016). In our study, 16S rRNA genes obtained from the Miyun reservoir sediment were closely associated with the N-DAMO bacteria in other ecosystems. The pmoA genes in cluster I were closely associated with the gene sampling from reservoirs, estuaries, rivers, and lakes (Shen et al. 2014a, Yan et al. 2015, Wang et al. 2016). The pmoA genes in cluster II were closely associated with the gene sampling from dam areas in reservoir and lake (Deutzmann & Schink 2011, Kojima et al. 2012), peatland (Zhu et al. 2012), and sewage treatment plant sludge (Luesken et al. 2011). Moreover, pmoA genes in cluster III were affiliated with the genes from Jiaojiang Estuary, Shahe River, and Shangqiu.
The pmoA genes in cluster IV were mainly obtained from paddy and water level fluctuation zone soil (Zhou et al. 2014, Ding et al. 2016b), rivers, and lakes (Liu et al. 2015). In addition, the pmoA genes in cluster V were affiliated with uncultured ones from Tarim River and Ditch (Shen et al. 2014c). Moreover, researchers found that the pmoA genes from Lake Constance and Biwa were closely similar to Candidatus M. oxyfera affiliating to the NC10 bacteria (Deutzmann & Schink 2011, Sakai et al. 2013), and the pmoA genes obtained in Qiantang River and Yellow River were also closely similar to the genes of Candidatus M. oxyfera (Shen et al. 2014a, Yan et al. 2015). However, pmoA genes in Miyun Reservoir were not similar to the genes of Candidatus M. oxyfera, which illustrated that there are novel pmoA genes in the sediment of the reservoir. Moreover, the close relationship between pmoA genes in Miyun Reservoir and the genes in other ecosystems could suggest that the novel pmoA genes might be widespread in many ecosystems.

There were seasonal variations in N-DAMO bacterial communities in Miyun Reservoir. N-DAMO bacteria community structures in Yellow River Estuary and Yunnan Plateau lake also showed seasonal variation (Chen et al. 2015, Yang et al. 2016). Moreover, the Shannon biodiversity...
of N-DAMO bacteria also showed seasonal change in wetland and paddy soil (Chen et al. 2015, Wang et al. 2015, Zhou et al. 2015). Temperature is the dominant factor for bacterial seasonal changes because N-DAMO bacteria belong to intermediate temperature micro-organisms, the suitable temperature of these bacteria is from 30 to 35 °C, and the activity of bacteria was higher at 30~35 °C than that at the temperature below 20 °C (Yan et al. 2015). In addition, DO is an important factor influencing N-DAMO bacteria because the lower concentration of DO at the 35 °C could restrain the activity of the bacteria (Luesken et al. 2012). Many studies indicated that ammonia-oxidizing bacteria also showed seasonal variation (Li et al. 2011, Sher et al. 2013, Lu et al. 2015). N-DAMO bacteria and anammox bacteria could influence methane emission because these two bacteria could co-exist and remove jointly the nitrate and methane, with ammonium and methane as the electron donor (Haroon et al. 2013, Shi et al. 2013, Xia et al. 2015). In Miyun reservoir, methane emission flux showed seasonal variation and the emission flux in summer was higher than in other seasons (Yang et al. 2014), and the high methane emission in summer might result from the high N-DAMO bacterial richness and diversity. However, the effect mechanism of aerobic methane-oxidizing bacteria and anammox bacterial on the methane emission was still unclear. The direct measurement of N-DAMO and anammox bacterial activity is necessary to enable sound conclusions on the importance of this process in the Miyun Reservoir in the further study.

Fig. 6 Relative abundance of 16S rRNA gene (a) and the pmoA genes (b) grouped into cluster I, cluster II, cluster III, cluster IV, cluster V, and cluster VI, based on phylogenetic tree
Environmental factors associated with the N-DAMO bacteria

The environmental characteristics of water and sediment play a major role in affecting N-DAMO bacterial distribution and biodiversity. Previous studies mainly focused on the physicochemical characteristic of sediment (Ding et al. 2006b, He et al. 2016), while a few studies have extended to study the influence of interstitial water on the bacteria distribution. RDA result showed that nitrogen (NO$_2^-$-N, NO$_3^-$-N and NH$_4^+$-N) in interstitial water and sediment were closely associated with N-DAMO bacterial distribution, and this result was similar to the relationships between N-DAMO bacteria and environmental factors in Qiantang River (Shen et al. 2014a), Mai Po sediments (Chen et al. 2015), and Yellow River Estuary (Yan et al. 2015). The effect of NO$_2^-$-N and NO$_3^-$-N on N-DAMO bacteria is closely related to the methane-oxidizing process which based on an “intra-aerobic” pathway (Ettwig et al. 2009). This pathway is an intra-aerobic methanotroph pathway which could generate oxygen and dinitrogen gas from the dismutation of NO$_3^-$-N and NO$_2^-$-N, and this oxygen could be used to oxidize methane with methane monooxygenase enzyme complex (Ho et al. 2013, Liu et al. 2015). In addition, NH$_4^+$-N and the molar ratio of NH$_4^+$-N to NO$_3^-$-N and NO$_2^-$-N were closely related to N-DAMO bacterial community structure (Shen et al. 2014a, b, Chen et al. 2015, Liu et al. 2018). The effect of NH$_4^+$-N to N-DAMO bacteria was driven by the methane monooxygenase, because the similar structure of NH$_4^+$ and CH$_4$ could cause NH$_4^+$ substitute CH$_4$ completely in methane oxidation (Yang et al. 2015). Moreover, many studies suggested that NH$_4^+$-N could drive the N-DAMO bacteria cooperate or compete with other bacteria, which could drive the reduction of nitrate to nitrite (Hu et al. 2011, Haroon et al. 2013). It is speculated that the effect of environmental factors on N-DAMO bacteria community structure is multiple and complex, and nutritional status of the M i y u n Reservoir was the crucial factor influencing the methane oxidation.

Many studies suggested that bacterial communities in freshwater ecosystems were influenced by dam setting (Liu et al. 2016, Chen et al. 2017). In our study, there was the sampling site (A1 and B1) near the dam (Baihe Dam), and N-DAMO bacteria (16 s rRNA and pmoA genes) at this site were distinctively different from other sample sites. The dam-specific effects of the bacterial composition may be due to the higher water column depth in front of the dam position (Chen et al. 2017). In our study, the water column depth of the dam samples was greater than 30 m with lower oxygen content, and the dam-specific characteristics of N-DAMO bacteria were mainly driven by the low concentration of DO at dam sampling site. In addition, Liu et al. (2016) examined the effect of dam setting on ammonia-oxidizing

| Table 3 | Spearman’s correlation between abiotic environment factors and biodiversity of N-DAMO bacteria (16s rRNA and pmoA genes) in Miyun Reservoir |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Environment factors | Number of OTUs | Shannon | Number of OTUs | Shannon |
| | pmoA-A | 16s-A | pmoA-B | 16s-B | pmoA-A | 16s-A | pmoA-B | 16s-B |
| Temperature | −0.72$^b$ | −0.56 | −0.44 | 0.44 | − | − | − | − |
| WD | 0.61$^a$ | 0.32 | −0.73$^a$ | 0.23 | 0.13 | 0.13 | 0.04 | 0.04 |
| pH | 0.42 | −0.14 | 0.26 | −0.33 | −0.47 | −0.86$^b$ | −0.31 | −0.90$^b$ |
| DO | −0.15 | 0.29 | 0.64$^a$ | −0.25 | −0.29 | −0.50 | −0.69$^a$ | −0.63$^a$ |
| Salinity | 0.40 | −0.06 | 0.46 | −0.44 | −0.88$^b$ | −0.58 | −0.87$^b$ | −0.55 |
| IW-NO$_2^-$-N | −0.96$^b$ | −0.15 | −0.92$^b$ | 0.16 | 0.85$^a$ | 0.04 | 0.66$^a$ | 0.51 |
| IW-NO$_3^-$-N | 0.94$^b$ | −0.14 | 0.96$^b$ | −0.31 | −0.83$^a$ | 0.22 | −0.67$^a$ | −0.49 |
| IW-NO$_4^+$-N | −0.32 | −0.10 | −0.58 | 0.26 | 0.44 | −0.32 | 0.80$^a$ | −0.52 |
| IW-TP | −0.11 | 0.08 | 0.23 | −0.30 | −0.21 | −0.05 | −0.19 | 0.33 |
| BS-NO$_2^-$-N | −0.61 | −0.14 | 0.62$^a$ | −0.25 | 0.84$^b$ | 0.63$^a$ | 0.83$^b$ | 0.62$^a$ |
| BS-NO$_3^-$-N | −0.52 | −0.91$^b$ | 0.55 | −0.91$^b$ | −0.67$^a$ | −0.44 | −0.76$^a$ | −0.43 |
| BS-NO$_4^+$-N | 0.272 | 0.34 | −0.82$^a$ | 0.14 | 0.66$^b$ | 0.70$^a$ | 0.50 | 0.68$^a$ |
| BS-TP | 0.09 | −0.44 | 0.26 | −0.44 | −0.156 | 0.06 | −0.19 | 0.02 |
| BS-TN | 0.321 | −0.14 | 0.24 | −0.19 | 0.48 | 0.01 | 0.31 | 0.19 |
| BS-TOC | −0.05 | 0.21 | 0.74$^a$ | 0.25 | 0.86$^b$ | 0.38 | 0.74$^a$ | 0.45 |
| BS-C/N | 0.22 | −0.69 | 0.28 | −0.31 | −0.37 | −0.51 | −0.29 | −0.47 |

WD water depth, DO dissolved oxygen, NO$_2^-$-N nitrate, NO$_3^-$-N nitrate, NH$_4^+$-N ammonium, TP total phosphorus, TN total nitrogen, TOC total organic carbon, C/N total organic carbon/total nitrogen, IW interstitial water, BS bottom sediment

$^a$Correlation is significant at the 0.05 level

$^b$Correlation is significant at the 0.01 level
bacterial communities in North Canal (Beijing, China); the result suggested that the operation of the dam influenced the activity of ammonia-oxidizing bacteria. Moreover, other studies also suggested that the operation of the dam could affect the release of methane gas, and the opening of the dam promoted the methane release from the water (Kinen et al. 2010). We could deduce that N-DAMO bacterial activity would decrease when dams were opened, because the increase of DO with the dam open might destroy the anaerobic environment that favors N-DAMO growth, which results in increasing the quantity of methane emission. The 16S rRNA and pmoA genes of N-DAMO bacteria were dam-specific, which may be associated with the higher water column depth in front of the dam. The effect of the dam on N-DAMO bacteria was complex, and dam samples were collected during the closing of the dam, while the effect of the operation of a dam on N-DAMO bacteria was still unclear. Thus, further study could focus on links between the operation of dam and N-DAMO bacterial activity and methane emission.

**Conclusions**

This study represented an attempt to investigate the spatial and temporal patterns of N-DAMO bacteria in reservoir sediment in Beijing. There was obvious temporal variation in 16S rRNA genes and spatial variation in pmoA genes. The pmoA genes in Miyun Reservoir were different from *Candidatus M. oxyfera* bacteria which are observed in other ecosystems. The diversity of
N-DAMO bacteria communities in Miyun Reservoir was relatively higher than in other ecosystems, and the biodiversity was much higher in winter than in summer. Nitrogen (NO$_2^-$-N, NO$_3^-$-N, and NH$_4^+$-N) in interstitial water and sediment was the determined factors influencing N-DAMO bacterial biodiversity. Moreover, the composition of N-DAMO bacteria was dam-specific, which may be related to water column depth in front of the dam, while the relationship of the water depth and environmental properties of interstitial water and sediment was unclear. Further study should be focused on the effect of water depth on N-DAMO bacteria. This study improved our knowledge of N-DAMO bacterial communities in Miyun reservoir, which helped to better understand the biogeochemical mechanism of methane oxidation in drinking reservoir.

Supplementary information

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Authors’ contributions

YL and XYW did the analyses and prepared the manuscript; XYW reviewed and revised the manuscript. YL, XYW, and YJC designed the study. YL, LRZ, KLX, and YD performed the field work and laboratory analyses; XYW and YJC gave suggestions during the whole work. The authors read and approved the final manuscript.

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Availability of data and materials

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Ethics approval and consent to participate

This article does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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