UNIVERSITÀ
DEGLI STUDI
DI MILANO

ORIGINAL ARTICLE   **Open Access**

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

Yang Liu[1,2,3,4], Xiaoyan Wang[1*], Yongjuan Chen[1], Lingrong Zhang[1], Kangli Xu[1] and Yi Du[1]

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_2^--N, NO_3^--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

* Correspondence: wangxy@cnu.edu.cn
[1]College of Resource Environment and Tourism, Capital Normal University, Beijing 100048, China
Full list of author information is available at the end of the article

BMC

Liu *et al. Annals of Microbiology*   (2020) 70:42

Page 2 of 13

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 methane oxidizing 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 (Luesken et al. 2011, Deutzmann et al. 2014) provided us many 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
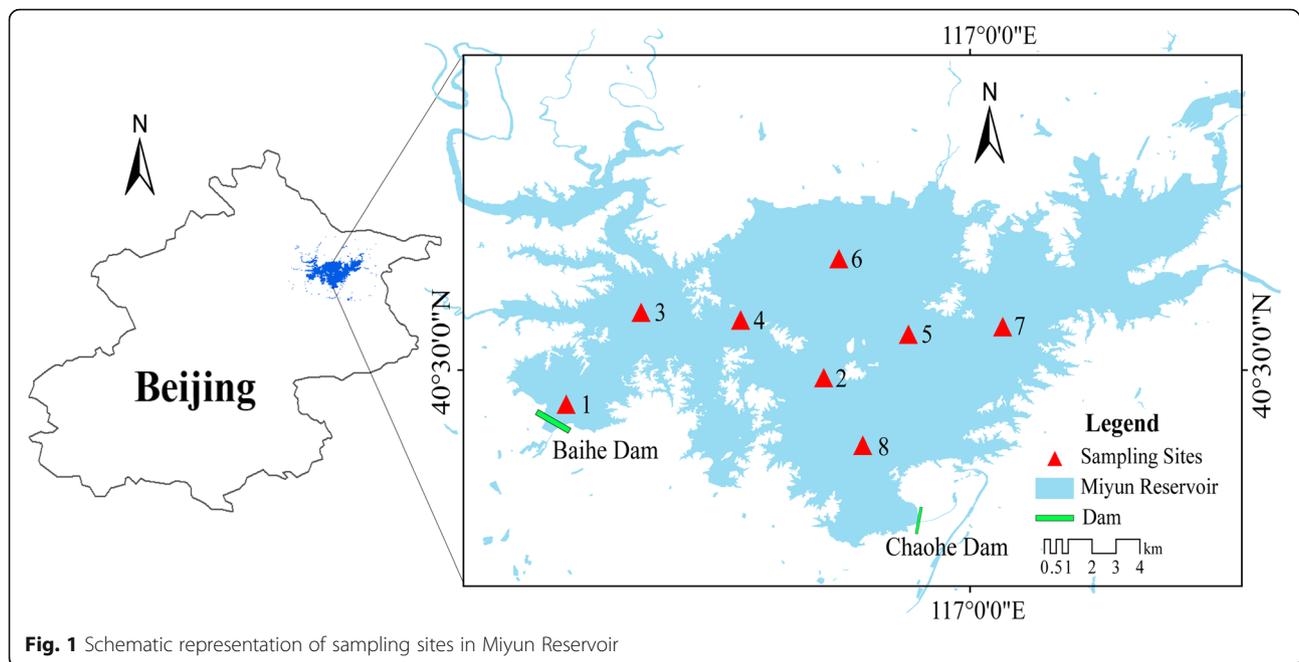
Fig. 1 Schematic representation of sampling sites in Miyun Reservoir

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)	AAAYCCGGCRAAGAACGA	N-DAMO *pmoA*		
cmo182(PCR)	TCACGTTGACGCCGATCC	N-DAMO *pmoA*		
cmo568(PCR)	GCACATACCCATCCCCATC	N-DAMO *pmoA*		
202F(PCR)	GACCAAAGGGGGCGAGCG	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)	CAKAAAGGAGGTGATCC	Bacteria 16S		
qP1F((*q*)PCR)	GGGCTTGACATCCCACGAACCTG	N-DAMO 16S		
qP2R((*q*)PCR)	CTCAGCGACTTCGAGTACAG	N-DAMO 16S		

Shimadzu TOC Analyzer (TOC-VCPH, 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 *pmoA* genes and 16S rRNA gene of N-DAMO bacteria were amplified using a nested approach: the *pmoA* 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 (*pmoA* genes), KX082978-KX083338 (*pmoA* 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 *pmoA* 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 *pmoA* 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 *pmoA* genes in Miyun Reservoir

Samples	Sequence number		OTUs		Coverage		Shannon index		Simpson index		Chao1	
	pmoA	16S	*pmoA*	16S	*pmoA*	16S	*pmoA*	16S	*pmoA*	16S	*pmoA*	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	2	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	0.20	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

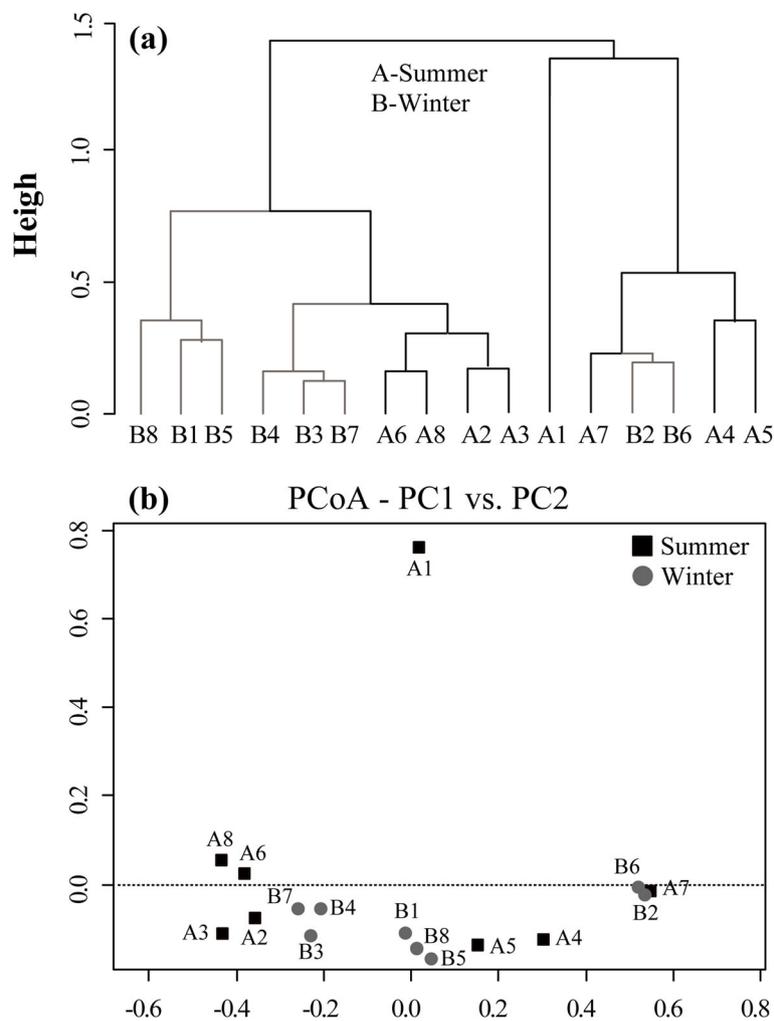A1–A8, sampling sites in summer; B1–B8, sampling sites in winter

Fig. 2 a Complete cluster analysis showing the differences in the 16S rRNA genes between summer and winter based on Bray Curtis distance. **b** Principal coordinate analysis (PCoA) for 16S rRNA gene assemblages using Bray Curtis distance. Bray Curtis distances were calculated using the relative abundance of the genes. The letter *A* represents 16S rRNA genes in summer, and the letter *B* represents the genes 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
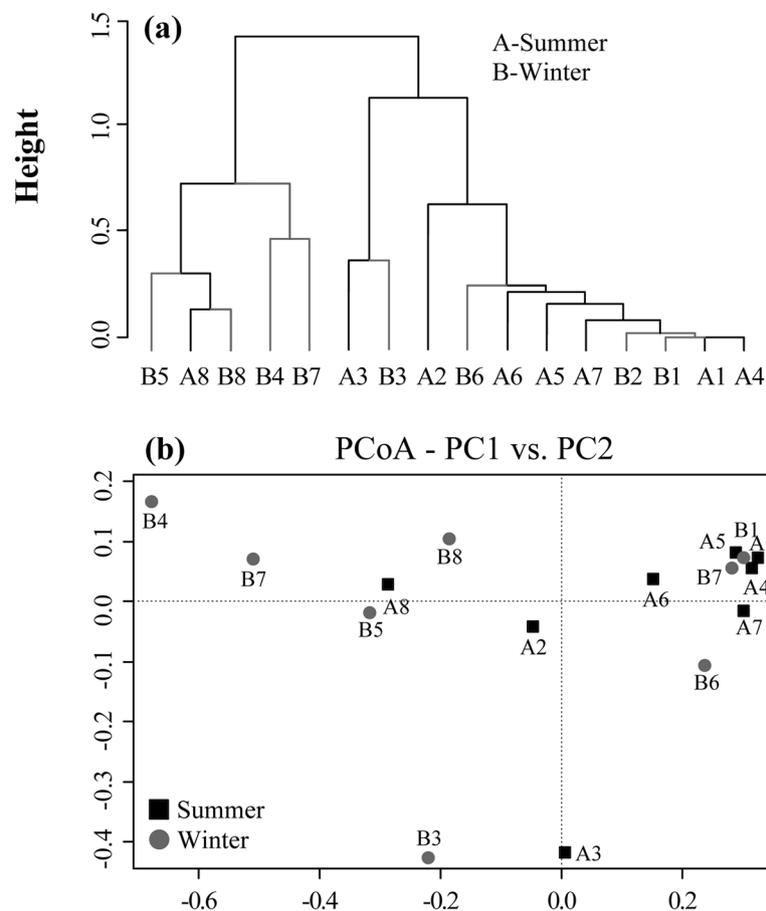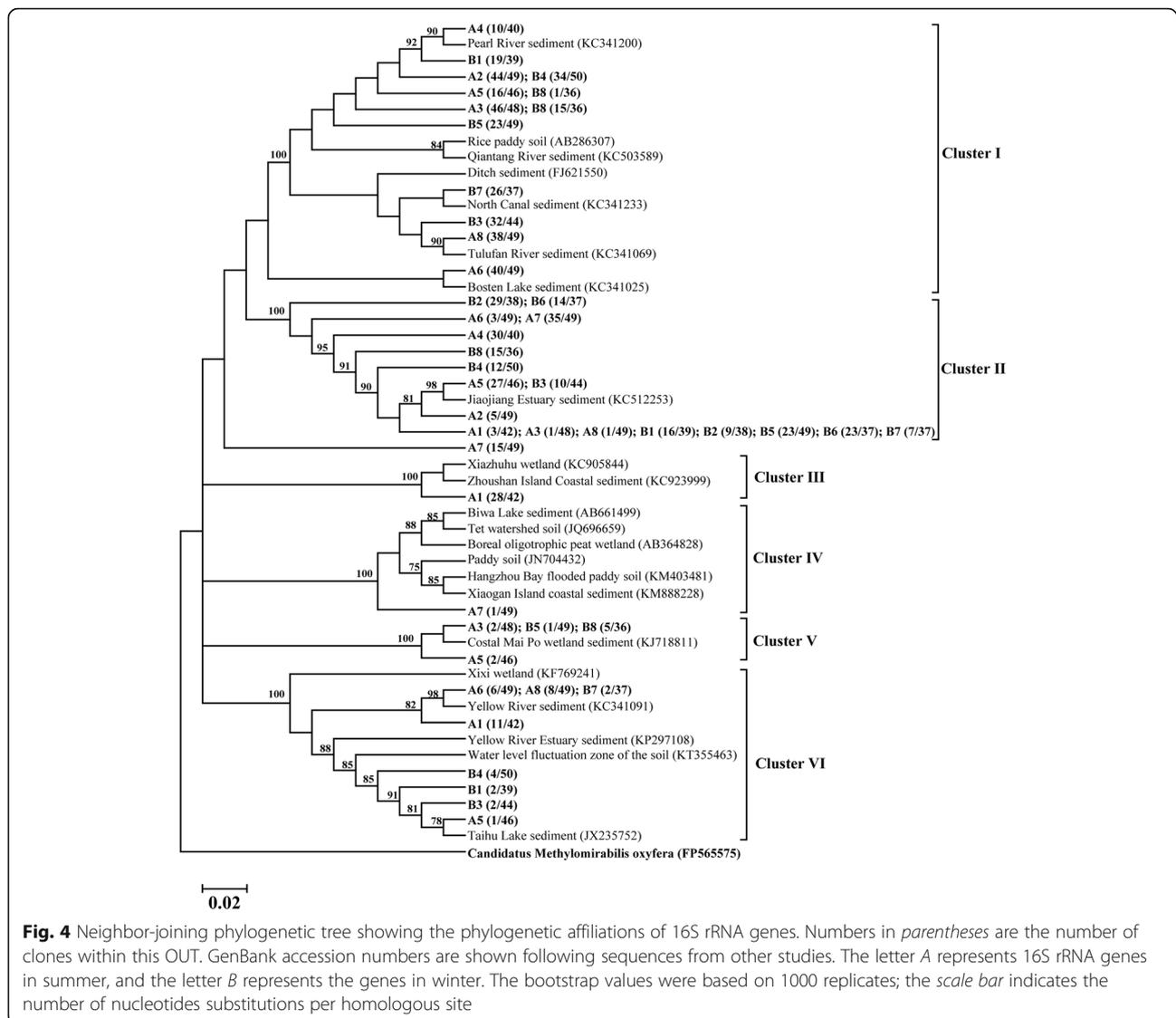
Fig. 3 a Complete cluster analysis showing the differences in the *pmoA* genes between summer and winter based on Bray Curtis distance. **b** Principal coordinate analysis (PCoA) for *pmoA* genes using Bray Curtis distance. Bray Curtis distances were calculated using the relative abundance of the genes. The letter *A* represents *pmoA* genes in summer, and the letter *B* represents the genes winter

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, and B8.

Phylogenetic analyses showed that *pmoA* 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 *pmoA* gene sequences (Fig. 6b). Cluster II was mainly composed of *pmoA* 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 *pmoA* gene sequences (Fig. 6b). Cluster III consisted of 22 *pmoA* gene sequences from sampling sites of A3 and 6 gene sequences from B6. Cluster IV included *pmoA* gene sequences from the sampling sites of A2, A7, B3, B5, and B7 (Fig. 5). Cluster V was the smallest group, which included *pmoA* 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 ($\leq$ 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). NO_2^--N, NO_3^--N, and NH_4^+-N in interstitial water and sediment were closely associated with Shannon biodiversity of *pmoA* genes in summer and winter (Table 3, $p < 0.05$). Water column depth of sampling sites was closely associated with Shannon biodiversity of *pmoA* genes in summer ($p < 0.05$), while there was no close relationship between water column depth and Shannon biodiversity of *pmoA* genes in winter (Table 3). NO_3^--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

Fig. 4 Neighbor-joining phylogenetic tree showing the phylogenetic affiliations of 16S rRNA genes. Numbers in *parentheses* are the number of clones within this OUT. GenBank accession numbers are shown following sequences from other studies. The letter *A* represents 16S rRNA genes in summer, and the letter *B* represents the genes in winter. The bootstrap values were based on 1000 replicates; the *scale bar* indicates the number of nucleotides substitutions per homologous site

(Fig. 7a). 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

Fig. 5 Neighbor-joining phylogenetic tree showing the phylogenetic affiliations of the *pmoA* genes. Numbers in *parentheses* are the number of clones within this OUT. GenBank accession numbers are shown following sequences from other studies. The letter *A* represents 16S rRNA genes in summer, and the letter *B* represents the genes in winter. The letter *A* represents *pmoA* genes in summer, and the letter *B* represents the genes in winter. The bootstrap values were based on 1000 replicates; the *scale bar* indicates the number of nucleotides substitutions per homologous site

Reservoir (Shen et al. 2014b). 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
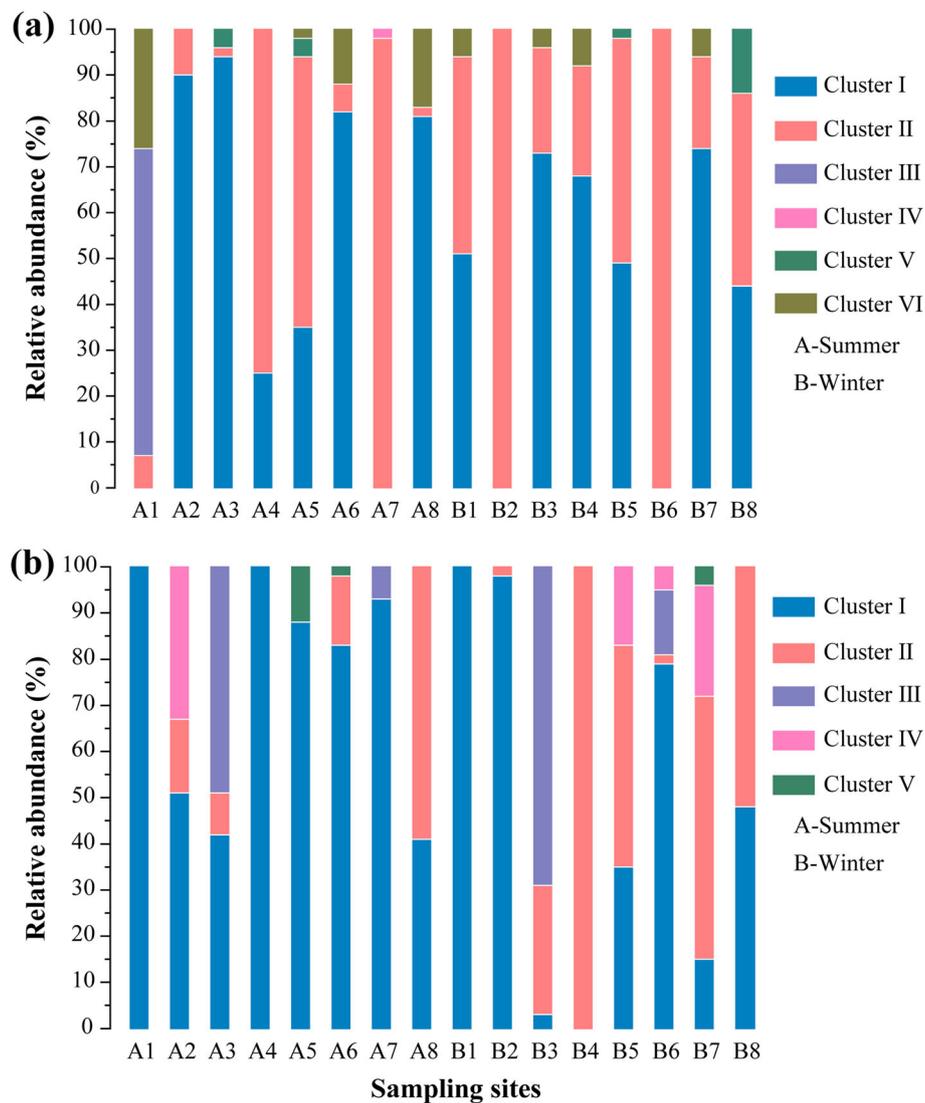
Fig. 6 Relative abundance of 16S rRNA gene (**a**) and the *pmoA* genes (**b**) grouped into cluster I, cluster II, cluster III, cluster IV, cluster V, and cluster VI, based on phylogenetic tree

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 ℃, and the activity of bacteria was higher at 30~ 35 ℃ than that at the temperature below 20 ℃ (Yan et al. 2015). In addition, DO is an important factor influencing N-DAMO bacteria because the lower concentration of DO at the 35 ℃ 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.

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*-A	16s-A	*pmoA*-B	16s-B	*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

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_3^--N and NO_2^--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 Miyun 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
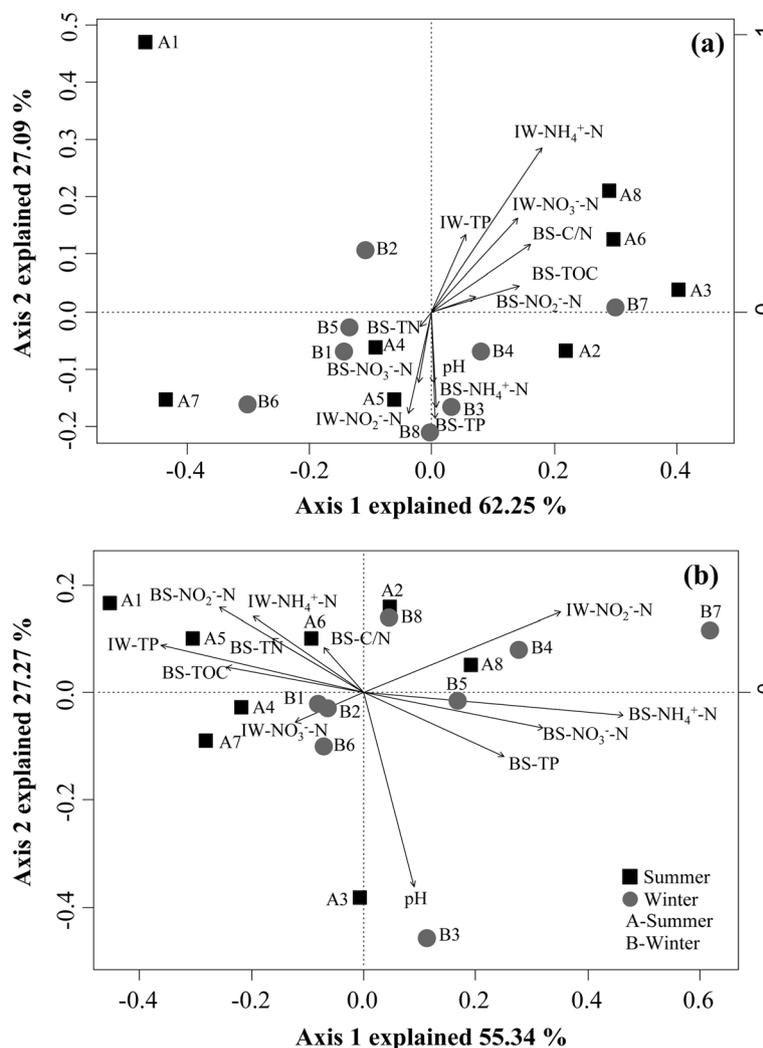
Fig. 7 Redundancy analyses (RDA) of 16S rRNA (**a**) and nutrient variables in summer and winter. Redundancy analyses (RDA) of *pmoA* genes (**b**) and nutrient variables in summer and winter. Correlations between environmental factors and RDA axes are represented by the length and angle of *arrows*. T, temperature; NO_2^--N, nitrite; 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, summer; B, winter

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 favor for 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

Liu *et al. Annals of Microbiology* (2020) 70:42

Page 12 of 13

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

Supplementary information accompanies this paper at https://doi.org/10.1186/s13213-020-01578-5.

Additional file 1. Supplementary materials.

Author details
[1]College of Resource Environment and Tourism, Capital Normal University, Beijing 100048, China. [2]State Key Laboratory of Simulation and Regulation of Water Cycle in River Basin, China Institute of Water Resources and Hydropower Research, Beijing 100038, China. [3]Department of Water Environment, China Institute of Water Resources and Hydropower Research, Beijing 100038, China. [4]College of Hydrology and Water Resources, Hohai University, Nanjing 210098, China.

References
Bridgham SD, Cadillo-Quiroz H, Keller JK, Zhuang Q (2013) Methane emissions from wetlands: biogeochemical, microbial, and modeling perspectives from local to global scales. Global Change Biol 19:1325–1346

Chen J, Zhou Z, Gu J (2015) Complex community of nitrite-dependent anaerobic methane oxidation bacteria in coastal sediments of the Mai Po wetland by PCR amplification of both 16S rRNA and pmoA genes. Appl Microbiol Biot 99: 1463-1473. 290

Chen X (2009) Gazette of the ministry of water resources of the People's Republic of China. Ministry of Water Resources of the People's 3: 91, Beijing.

Chen Y, Liu Y, Wang X (2017) Spatiotemporal variation of bacteria and archaeal communities in sediments of a drinking resercoir, Beijing, China. Appl Microbiol Biot 101:3379–3391

Daniel B, Francois G, Pierre L, Lan J (2014) Numerical Ecology with R. Higher Education Press, Beijing

Deutzmann JS, Schink B (2011) Anaerobic oxidation of methane in sediments of Lake Constance, an oligotrophic freshwater lake. Appl Environ Microb 77: 4429–4436

Deutzmann JS, Stief P, Brandes J, Schink B (2014) Anaerobic methane oxidation coupled to denitrification is the dominant methane sink in a deep lake. P Natl Acad Sci USA 111:18273–18278

Ding H, Ji H, Tang L, Zhang A, Guo X, Li C, Gao Y, Briki M (2016a) Heavy metals in the gold mine soil of the upstream area of a metropolitan drinking water source. Environ Sci Pollut R 23:2831–2847

Ding J, Fu L, Ding Z, Lu Y, Cheng SH, Zeng RJ (2016b) Environmental evaluation of coexistence of denitrifying anaerobic methane-oxidizing archaea and bacteria in a paddy field. Appl Microbiol Biot 100:439–446

Ettwig KF, Butler MK, Le Paslier D, Pelletier E, Mangenot S, Kupers MMM, Schreiber F, Dutilh BE, Zedelius J, De Beer D, Gloerich J, Wessels HJCT, van Alen T, Luesken F, Wu ML, van de Pas-Schoonen KT, Op den Camp HJM, Janssen-Megens EM, Francoijs KJ, Stunnenberg H, Weissenbach J, Jetten MSM, Strous M (2010) Nitrite-driven anaerobic methane oxidation by oxygenic bacteria. Nature 464:543–548

Ettwig KF, Shima S, Van de Pas-Schoonen KT, Kahnt J, Mederma MH, HJM ODC, MSM J, Strous M (2008) Denitrifying bacteria anaerobically oxidize methane in the absence of Archaea. Environ Microbiol 10:3164–3173. 308

Ettwig KF, van Alen T, van de Pas-Schoonen KT, Jetten MSM, Strous M (2009) Enrichment and molecular detection of denitrifying methanotrophic bacteria of the NC10 phylum. Appl Environ Microb 75:3656–3662

Haroon MF, Hu S, Shi Y, Imelfort M, Keller J, Hugenholtz P, Yuan Z, Tyson GW (2013) Anaerobic oxidation of methane coupled to nitrate reduction in a novel archaeal lineage. Nature 500:567–570

He Z, Geng S, Wang L, Cai C, Wang J, Liu J, Zheng P, Xu X, Hu B (2016) Improvement of mineral nutrient concentrations and pH control for the nitrite-dependent anaerobic methane oxidation process. Sep Purif Technol 162:148–153

Ho A, Vlaeminck SE, Ettwig KF, Schneider B, Frenzel P, Boon N (2013) Revisiting methanotrophic communities in sewage treatment plants. Appl Environ Microb 79:2841–2846

Hu BL, Shen LD, Lian X, Zhu Q, Liu S, Huang Q, He Z, Geng S, Cheng D, Lou L, Xu X, Zheng P, He Y (2014) Evidence for nitrite-dependent anaerobic methane oxidation as a previously overlooked microbial methane sink in wetlands. P Natl Acad Sci USA 111:4495–4500

Hu S, Zeng RJ, Keller J, Lant PA, Yuan Z (2011) Effect of nitrate and nitrite on the selection of microorganisms in the denitrifying anaerobic methane oxidation process. Env Microbiol Rep 3:315–319

Jiao J, Du P, Lang C (2015) Nutrient concentrations and fluxes in the upper catchment of the Miyun Reservoir, China, and potential nutrient reduction strategies. Environ Monit Assess 187:1–15

Kinen KM, Khan S, Moore D, Dore J, Gyawali D (2010) Policy considerations for greenhouse gas emissions from freshwater reservoirs. Water Altern 3:91–105

Kojima H, Tsutsumi M, Ishikawa K, Iwata T, Mußmann M, Fukui M (2012) Distribution of putative denitrifying methane oxidizing bacteria in sediment of a freshwater lake, Lake Biwa. Syst Appl Microbiol 35:233–238

Li D, Liang J, Di Y, Gong H, Guo X (2016) The spatial-temporal variations of water quality in controlling points of the main rivers flowing into the Miyun Reservoir from 1991 to 2011. Environ Monit Assess 188:1–12

Li M, Cao H, Hong Y, Gu J (2011) Seasonal dynamics of anammox bacteria in estuarial sediment of the Mai Po Nature Reserve revealed by analyzing the 16S rRNA and hydrazine oxidoreductase (hzo) genes. Microbes Environ 26: 15-22. 338

Li S, Lu XX (2012) Uncertainties of carbon emission from hydroelectric reservoirs. Natural Hazards 62:1343–1345

Liu Y, Chen YJ, Wang XY, Xu KL (2018) Influences of anthropogenic activities on the community structure of N-DAMO bacteria in the North Canal. Environ Sci 39:3677–3688

Liu Y, Yu Y, Wang XY, Bao LL (2016) Nitrification activity and Ammonia oxidizing bacteria in water around the Gate Dams in Beiyun River. Acta Scientiae Circumstantiae 36:4044–4052

Liu Y, Zhang J, Zhao L, Li Y, Yang Y, Xie SG (2015) Aerobic and nitrite-dependent methane-oxidizing microorganisms in sediments of freshwater lakes on the Yunnan Plateau. Appl Microbiol Biot 99:2371–2381

Louis VLS, Kelly CA, Duchemin E (2000) Reservoir surfaces as sources of greenhouse gases to the atmosphere: a global estimate. Bioscience 50: 766–775

Lu S, Liao M, Xie C, He X, Li D, He L, Chen J (2015) Seasonal dynamics of ammonia-oxidizing microorganisms in freshwater aquaculture ponds. Ann Microbiol 5:651–657

Luesken F, Wu M, Op DCH (2012) Effect of oxygen on the anaerobic methanotroph'Candidatus Methylomirabilis oxyfera: Kinetic and transcriptional analysis. Environ Microb 14:1024–1034

Luesken FA, Zhu B, van Alen TA, Butler MK, Diaz MR, Song B, Op den Camp HJM, Jetten MSM, Ettwing KF (2011) *pmoA* Primers for detection of anaerobic methanotrophs. Appl Environ Microb 77:3877–3880

Ou Y, Wang X (2008) Identification of critical source areas for non-point source pollution in Miyun reservoir watershed near Beijing, China. Water Sci Technol 58:2235–2241

Raghoebarsing A, Pol A, van de Pas-Schoonen KT, Smolders AJP, Ettwig KF, Rijpstra WIC, Schouten S, Damste JSS, Op den Camp HJM, Jetten MS, Strous M (2006) A microbial consortium couples anaerobic methane oxidation to denitrification. Nature 440:918–921

Sakai Y, Karube Z, Takeyama T, Kohzu A, Yoshimizu C, Nagata T, Tayasu I, Okuda N (2013) Seasonal and site-specific variability in terrigenous particulate organic carbon 3 concentration in near-shore waters of Lake Biwa, Japan. Limnology 2:167–177

Schloss PD, Westcott SL, Ryabin T, Hall JR, Hartmann M, Hollister EB, Lesniewski RA, Oakley BB, Parks DH, Robinson CJ, Sahl JW, Stres B, Thallinger GG, Hom DJV, Weber CF (2009) Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. Appl Environ Microb 75:7537-7541. 368

Shen LD, Liu S, Zhu Q, Li X, Cai C, Cheng D, Lou L, Xu X, Zheng P, Hu B (2014a) Distribution and diversity of nitrite-dependent anaerobic methane-oxidising bacteria in the sediments of the Qiantang River. Environ Microbiol 67:341–349

Shen LD, Zhu Q, Liu S, Du P, Zeng J, Cheng D, Xu X, Zheng P, Hu B (2014b) Molecular evidence for nitrite-dependent anaerobic methane-oxidising bacteria in the Jiaojiang Estuary of the East Sea (China). Appl Microbiol Biot 98:5029–5038

Shen LD, Liu S, Huang Q, Lian X, He Z, Geng S, Jin R, He Y, Lou L, Xu X, Zheng P, Hu B (2014c) Evidence for the cooccurrence of nitrite-dependent anaerobic ammonium and methane oxidation processes in a flooded paddy field. Appl Environ Microb 80:7611–7619

Sher Y, Zaady E, Nejidat A (2013) Spatial and temporal diversity and abundance of ammonia oxidizers in semi-arid and arid soils: indications for a differential seasonal effect on archaeal and bacterial ammonia oxidizers. FEMS Microbiol Ecol 86:544–556

Shi Y, Hu S, Lou J, Lu P, Keller J, Yuan Z (2013) Nitrogen removal from wastewater by coupling anammox and methane-dependent denitrification in a membrane biofilm reactor. Environ Sci Technol 47:11577–11583

Steven B, Pollard WH, Greer CW, Whyte LG (2008) Microbial diversity and activity through a permafrost/ground ice core profile from the Canadian high Arctic. Environ Microbiol 10:3388–3403

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood,
evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28: 2731–2739

Wang J, Song Y, Peng J, Yuan L (2015) Spatial-temporal distribution and community structure analysis of nitrite-dependent anaerobic methane-oxidizing bacteria in the sediments of the Hun River. Research of Environmental Sciences 28:1670–1676

Wang X, Li T, Xu Q, He W (2001) Study of the distribution of non-point source pollution in the watershed of the Miyun Reservoir, Beijing, China. Water Sci Technol 44:35–40

Wang Y, Huang P, Ye F, Jiang Y, Song L, Op den Camp HJM, Zhu G, Wu S (2016) Nitrite-dependent anaerobic methane oxidizing bacteria along the water level fluctuation zone of the Three Gorges Reservoir. Appl Microbiol Biot 100: 1977–1986

Wang Y, Zhu G, Harhangi HR, Zhu B, Jetten MS, Yin C, Op DCH (2012) Co-occurrence and distribution of nitrite-dependent anaerobic ammonium and methane-oxidizing bacteria in a paddy soil. FEMS Microbiol Lett 336:79–88

Xia C, Zhu G, Zou Y, Zhou R, Zhao S (2015) Distribution of anaerobic ammonia oxidizing bacteria and nitrite dependent anaerobic methane oxidizing bacteria in soil profile in the riparian zone. Acta Scientiae Circumstantiae 35: 3965–3975

Yan P, Li M, Wei G, Li H, Gao Z (2015) Molecular fingerprint and dominant environmental factors of nitrite-dependent anaerobic methane-oxidizing bacteria in sediments from the Yellow River Estuary, China. Plos One 10: e137996

Yang M, Geng X, Grace J, Lu C, Zhu Y, Zhou Y, Lei G (2014) Spatial and seasonal CH4 flux in the littoral zone of Miyun Reservoir near Beijing: the effects of water level and its fluctuation. Plos One 9:e94275

Yang SS, Chen IC, Ching-Pao L (2015) Carbon dioxide and methane emissions from Tanswei River in Northern Taiwan. Atmos Pollut Res 6:52–61

Yang Y, Zhao Q, Cui Y, Wang Y, Xie S, Liu Y (2016) Spatio-temporal variation of sediment methanotrophic microorganisms in a large eutrophic lake. Microb Ecol 71:9–17

Zhou L, Wang Y, Long XE, Guo J, Zhu G (2014) High abundance and diversity of nitrite-dependent anaerobic methane-oxidizing bacteria in a paddy field profile. FEMS Microbiol Lett 360:33–41

Zhou R, Zhu G, Zhou L, Zhou Y, Wang C (2015) Spatial-temporal distribution and community structure analysis of nitrite- dependent anaerobic methane oxidizing bacteria in paddy soil. Acta Scientiae Circumstantiae 35:729–737

Zhu B, van Dijk G, Fritz C, Smolders AJP, Pol A, Jetten MSM, Ettwig KF (2012) Anaerobic oxidization of methane in a minerotrophic peatland: enrichment of nitrite-dependent methane-oxidizing bacteria. Appl Environ Microbiol 78: 8657–8665

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.