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ENVIRONMENTAL BIOTECHNOLOGY

Sediment nitrite-dependent methane-oxidizing microorganisms temporally and spatially shift in the Dongjiang River

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Abstract Nitrite-dependent anaerobic methane oxidation (n-damo) process can play an important role in the methane mitigation in the environment. However, the distribution of n-damo organisms in freshwater sediment ecosystem and the associated environmental factors remain essentially unclear. The present study investigated the temporal and spatial dynamics of sediment n-damo community in the freshwater Dongjiang River using quantitative PCR assay and clone library analysis targeting n-damo *pmoA* gene. Sediment samples were collected at nine locations along the Dongjiang River in May and August in 2015. The remarkable temporal and spatial changes of sediment n-damo community abundance, richness, diversity, and structure occurred in the Dongjiang River and its tributaries. The n-damo *pmoA* gene in sediments of the Dongjiang River and its tributaries varied from 9.07×10^4 to 3.02×10^6 copies per gram dry sediment. Compared to the stem of the Dongjiang River, tributaries had relatively higher sediment n-damo community size. Sediment n-damo community abundance was higher in August than in May, while an opposite trend was observed for n-damo community richness and diversity. Sediment n-damo community structure showed a great difference between in May and August. Sediment nitrite nitrogen content was positively correlated to n-damo community abundance, but negatively to richness and diversity. Ammonia nitrogen content showed a positive correlation to n-damo community abundance, while n-damo community diversity was negatively correlated to the ratio of total organic carbon to total nitrogen (C/N). In addition, nitrite nitrogen as well as C/N might influence n-damo community structure.

Keywords Freshwater · Nitrite-dependent anaerobic methane oxidation · Nitrogen · Sediment · River

Introduction

As an important greenhouse gas, methane is of critical significance to global climate change (IPCC 2007). Methane emission from anoxic environment in freshwater ecosystems contributes to a considerable part of total natural methane emission (Bastviken et al. 2011; Chowdhury and Dick 2013; Rahalkar et al. 2009). However, most of the methane produced in anoxic environment can be mitigated before it reaches the atmosphere (Deutzmann et al. 2014). The mitigation of methane in freshwater aquatic ecosystems can be performed by both aerobic and anaerobic methane-oxidizing microorganisms (Liu et al. 2015; Yang et al. 2016). Nitrite-dependent anaerobic methane oxidation (n-damo) process was firstly discovered in an enrichment culture (Raghoebarsing et al. 2006). The n-damo process is known to be performed by NC10 bacterium *Candidatus Methylomirabilis oxyfera* (*M. oxyfera*) through an "intraaerobic" pathway, where oxygen is produced from the reduction of nitrite to nitric oxide and then used to oxidize

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methane using the methane monooxygenase enzyme complex (Ettwig et al. 2010, 2012; Liu et al. 2015). The n-damo process has received increasing attention, because it can be an important methane sink in natural ecosystems and constitutes a relationship between carbon and nitrogen cycles (Shen et al. 2015a). So far, a number of previous studies have reported the presence (or abundance) and diversity of n-damo microorganisms in freshwater ecosystems, such as lake (Deutzmann and Schink 2011; Kojima et al. 2012; Liu et al. 2015), reservoir (Han and Gu 2013), river (Shen et al. 2014a), wetland (Shen et al. 2015b; Wang et al. 2016), and paddy field (Han and Gu 2013; Shen et al. 2015c; Wang et al. 2012). These previous studies illustrated that n-damo community could vary with sampling site and soil/sediment layer depth, yet the environmental factors driving the spatial change of n-damo community remain poorly understood. For examples, Liu et al. (2015) revealed that freshwater sediment n-damo community might be influenced by the ratio of organic matter to total nitrogen, while Shen et al. (2014a) suggested that freshwater sediment n-damo community might be influenced by a variety of environmental factors, such as total inorganic nitrogen, ammonium nitrogen, and organic matter. Moreover, information on the temporal variation of n-damo community in natural environment is still very limited. Only few previous studies have documented the temporal changes of n-damo community abundance and diversity in soil ecosystem (Wang et al. 2016; Zhou et al. 2014).

The freshwater Dongjiang River originates from the Jiangxi Province, but it is mainly located in the subtropical Guangdong Province (southern China). This main stem of the Dongjiang River extends 562 km long from northeast to southwest (Ding et al. 2016). The mean annual precipitation and air temperature in its watershed area are about 1800 mm and 21 °C, respectively (Ding et al. 2016). The Dongjiang River is the drinking water source not only for several local cities in the Guangdong Province but also for Hong Kong. To date, several previous studies have investigated sediment ammonia-oxidizing microorganisms in the Dongjiang River (Sun et al. 2013, 2014), yet information on the n-damo organisms in the Dongjiang River is still lacking. Therefore, the main aim of the present study was to investigate the temporal and spatial changes of sediment n-damo community in the Dongjiang River. The links between n-damo community and environmental factors were also explored.

Materials and methods

Study locations and sampling

In May (early rainy season with small precipitation) and August (rainy season with large precipitation) in 2015, sediment cores in triplicate were collected from nine locations along the Dongjiang River (Fig. S1) using self-made gravity stainless steel columnar sediment sampler (patent number ZL201420490790.1). To avoid contamination, each sediment core was sampled using a new sampler. The sampling sites were accessed in a small boat. Sampling sites D1 (23° 31′ 14″ N, 114° 41′ 22″ E), D2 (23° 23′ 18″ N, 114° 35′ 51″ E), D3 (23° 6′ 20″ N, 114° 23′ 56″ E), D4 (23° 4′ 33″ N, 114° 5′ 10″ E), and D5 (23° 6′ 40″ N, 113° 52′ 59″ E) were located in the stem of the Dongjiang River. Sites XF (23° 44′ 44″ N, 114° 41′ 4″ E), QX (23° 24′ 40″ N, 114° 38′ 9″ E), XZ (23° 4′ 59″ N, 114° 25′ 11″ E), and SM (23° 1′ 20″ N, 114° 6′ 17″ E) were located in the tributaries Xingfeng River, Qiuxiang River, Xizhijiang River, and Shima River, respectively. These sediment cores were immediately transported back to the laboratory in iceboxes after collection and were then sliced into layers. In this study, the upper layer (0–10 cm) sediments were used for further chemical and molecular analyses. In this study, samples MD1 and AD1, MD3 and AD3, MD4 and AD4, and MD5 and AD5 represent the May and August sediment samples from sites D1, D3, D4, and D5 in the stem of the Dongjiang River, respectively. Sample MD2 represent the May sediment samples from sites D2 in the stem of the Dongjiang River. Samples MXF and AXF, MQX and AQX, MXZ and AXZ, and MSM and ASM represent the May and August sediment samples from the tributaries Xingfeng River, Qiuxiang River, Xizhijiang River and Shima River, respectively.

Sediment pH was determined using an IQ150 pH meter. Sediment total organic carbon (TOC), total phosphorus (TP), ammonium nitrogen (NH_4^+-N), nitrite nitrogen (NO_2^--N), nitrate nitrogen (NO_3^--N), and total nitrogen (TN) were determined using potassium dichromate titration method, molybdenum blue colorimetry method, Nessler's reagent method, naphthalene ethylenediamine spectrophotometry method, phenol disulphonic acid colorimetric method, and Kjeldahl method, respectively (Wang 2012). The detailed physicochemical parameters of the river sediment samples are listed in Table 1.

Quantitative PCR assay

Sediment total genomic DNA was extracted using Powersoil DNA extraction kit (Mobio Laboratories, USA) according to the manufacturer's instructions. The DNA quality was checked using 1.0 % agarose gel electrophoresis. The DNA concentration was determined using a biophotometer (Eppendorf, Hamburg, Germany). The number of river sediment n-damo *pmoA* gene was quantified using the primer pair cmo182/cmo568 using the same amplification reactions, as previously described (Yan et al. 2015). All sediment samples were analyzed in triplicate. Standard curves ranging from 10^2

Table 1 Physicochemical features of sediment samples from the Dongjiang River and its tributaries

Sample	NH_4^+-N (mg/kg)	NO_3^--N (mg/kg)	NO_2^--N (mg/kg)	TN (mg/kg)	TP (mg/kg)	TOC (g/kg)	pH	C/N[a]
MD1	8.59	0.29	0.22	424.4	362.85	6.26	7.71	14.8
MD2	24.07	1.99	0.11	297.13	721.95	10.94	5.8	36.8
MD3	27.86	10.91	0.17	1036.16	698.25	14.37	5.98	13.9
MD4	24.08	1.03	0.04	1459	908	6.47	6.57	4.4
MD5	18.37	0.62	0.07	876.85	579.59	14.75	6.76	16.8
MQX	13.72	1.61	0.17	375.74	695.52	13.57	5.75	36.1
MSM	24.44	17.44	0.16	446.64	281.61	6.9	7.77	15.4
MXF	23.26	0.73	0.1	389.19	166.91	6.91	6.98	17.8
MXZ	58.61	0.91	0.16	260.86	218.91	8.36	5.2	32
AD1	91.11	0.73	1.08	756.35	482.43	8.22	6.28	10.9
AD3	144.40	2.33	0.36	918.95	997.36	28.83	6.15	31.4
AD4	159.1	2.62	0.82	1114.24	281.41	48.29	5.84	43.3
AD5	40.95	0.11	0.17	397.18	624.25	29.33	5.76	73.8
AQX	93.35	1.33	0.77	532	336.34	10.68	5.11	20.1
ASM	230.6	1.53	2.65	555.83	355.18	10.46	7.95	18.8
AXF	18.75	0.79	0.98	45.22	612.92	2.11	7.26	46.7
AXZ	72.62	1.77	0.98	159.81	120.66	9.34	5.94	58.4

[a] The ratio of TOC to TN

to 10^7 gene copies/mL were generated with serial dilutions of a known number of plasmid DNA containing the target gene. The amplification efficiency and coefficient (r^2) for n-damo *pmoA* gene were 95 % and 0.993, respectively.

Clone library analysis

For the construction of clone library, the n-damo *pmoA* gene was amplified using a nested approach (first-step primer pair A189_b/cmo682 and second-step primer pair cmo182/cmo568) according to the literatures (Luesken et al. 2011; Wang et al. 2016). The PCR reactions were performed with the initial melting step at 94 °C for 4 min, followed by 40 cycles of denaturation at 94 °C for 1 min, annealing at 56 °C for 1 min, elongation at 72 °C for 1.5 min, and a final elongation step at 72 °C for 10 min. PCR products were purified using QIAquick PCR Purification Kit (Qiagen Inc.) following the manufacturer's instructions. The purified amplicons from triplicate river sediment samples were pooled in equal amounts and cloned into pMD19-T vector (Takara Corp, Japan). The obtained chimera-free *pmoA* gene sequences in the current study were deposited in the GenBank database under accession numbers KX000960–KX001302 and KX001578–KX001760. Sequences from each sediment sample were assigned into operational taxonomic units (OTUs) with 97 % similarity and then α-diversity (Chao1 richness estimator and Shannon index) was generated using the MOTHUR program (Schloss et al. 2009). Phylogenetic analysis of river sediment *pmoA* gene sequences was conducted using the software MEGA 6.0 (Tamura et al. 2013) using the neighbor-joining method. Moreover, the microbial similarity between sediment samples was assessed using the OTU-based Bray–Curtis similarity matrices. Relative abundance of each *pmoA* OTU equaled the ratio of the sequence number of each OTU to the total sequences in a given sediment sample. Sample clustering was performed with unweighted pair group method with arithmetic mean (UPGMA) using the software PRIMER 5.0 (Clarke and Warwick 2001).

Statistical analysis

In the present study, the links between n-damo *pmoA* gene and sediment physicochemical properties were determined with Pearson's correlation analysis using the software SPSS 20.0. To identify the correlations between the overall n-damo *pmoA* gene composition and the environmental factors, detrended correspondence analysis (DCA) was carried out to choose the suitable ordination analysis method. The longest DCA axis had a gradient less than 3 standard deviation units, so redundancy analysis (RDA) was performed (Lepš and Šmilauer 2003) using the software CANOCO 4.5. Relative abundance of *pmoA* gene sequence in each OTU was assigned as species input, while sediment physicochemical parameters were used as input for environmental variables. The significance test of Monte Carlo permutations was conducted to select a suitable model of the microbe–environment relationship.

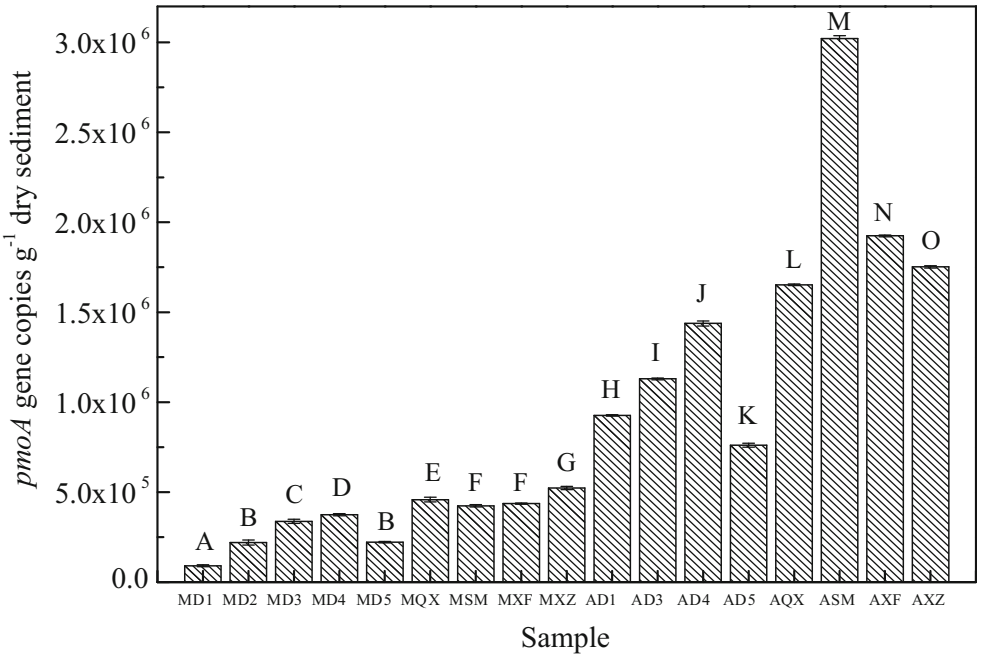


Fig. 1 Abundance of n-damo *pmoA* gene in the different river sediment samples. *Different letters* above the *columns* indicate the significant differences ($P < 0.05$)

Results

Abundance of river sediment n-damo community

The August sediment at sampling site D2 in the Dongjiang River was below quantitative PCR detection limit. For the other studied river sediments, n-damo *pmoA* gene density was 9.07×10^4–5.23×10^5 and 7.61×10^5–3.02×10^6 copies per gram dry sediment in May and August, respectively (Fig. 1). In either May or August, sediment n-damo *pmoA* gene abundance in tributaries was significantly greater than that in the stem of the Dongjiang River ($p < 0.05$). Significant difference in n-damo *pmoA* gene abundance was also found among the sediments either from the stem of the Dongjiang River or from its four tributaries ($p < 0.05$). Moreover, the August sediments had much greater n-damo *pmoA* gene abundance than the May ones ($p < 0.05$).

River sediment n-damo community richness and diversity

In this study, the August sediment sample from sampling site D2 in the Dongjiang River was not successfully amplified. A total of 526 chimera-free n-damo *pmoA* gene sequences was retrieved from sediments in the Dongjiang River and its four tributaries (Table 2). Each n-damo *pmoA* gene clone library included 20–43 chimera-free sequences. The May sediment samples were composed of 9–18 OTUs, while the August sediment samples comprised 1–13 OTUs. The Good's coverage estimator revealed that usually less than 70 % of the n-damo *pmoA* OTUs were obtained for the May river sediment samples, which indicated further sequencing would have resulted in more OTUs. In contrast, the August sediment samples usually had a relatively higher Good's coverage estimator (70.8–95.2 %), suggesting that the indicated OTUs of most of *pmoA* gene clone libraries had been well captured. The Chao1 richness estimators and Shannon diversity indices of river sediment n-damo *pmoA* gene clone libraries were 1–27 and 0–2.58, respectively. In either May or August, the evident spatial changes of sediment n-damo *pmoA* OTU

Table 2 Diversity indices of each river sediment n-damo *pmoA* gene clone library

Sample	Sequences	OTUs	Chao1 estimator	Shannon index	Good's coverage (%)
MD1	35	13	16.8	2.19	62.9
MD2	38	16	19.5	2.49	59.0
MD3	43	12	27	1.95	72.1
MD4	39	15	20.3	2.39	61.5
MD5	29	11	13	2.22	62.1
MQX	43	18	33	2.58	58.1
MSM	43	12	13	2.16	72.1
MXF	31	10	11	2.07	67.7
MXZ	42	9	9	1.97	78.6
AD1	23	13	20	2.36	43.5
AD3	24	9	10.5	1.85	62.5
AD4	23	6	6	1.48	73.9
AD5	24	5	5	1.49	79.2
AQX	24	7	13	1.56	70.8
ASM	20	2	2	0.42	90.0
AXF	24	2	2	0.42	91.7
AXZ	21	1	1	0	95.2

number, Chao1 richness, and Shannon diversity occurred in the stem and tributaries of the Dongjiang River. In addition, at each sampling site, the May river sediment generally had more OTUs and higher Chao1 richness and Shannon diversity than the corresponding August one.

Phylogeny of n-damo pmoA gene sequences

Figure 2 displays the phylogenetic relationship of the representative n-damo *pmoA* gene sequences of the major OTUs (including at least three sequences in all n-damo *pmoA* clone libraries) with their close relatives reported in GenBank database. The n-damo *pmoA* gene sequences from the major OTUs were assigned into seven clusters (clusters I, II, III, IV, V, VI, and VII). The proportion of each n-damo *pmoA* cluster varied with both sampling site and time (Fig. 3). Cluster I was the largest n-damo *pmoA* group, and it included a total of 165 sequences that could be related to several uncultured *pmoA* sequences retrieved from river and reservoir sediments. Cluster I-like n-damo *pmoA* gene sequences showed high proportion in a number of sediment samples, including samples MD3, MD4, MD5, MSN, AD3, AD4, AD5, AQX, AXF, and AXZ (accounting for 38–100 %). Cluster II was the second largest n-damo group that contained 79 sequences. These *pmoA* sequences could be related to those from river and estuary sediments. Cluster II-like n-damo *pmoA* gene sequences were mainly distributed in samples MD1, MD2, MD3, MD4, MQX, MXF, MXZ, AD1, AD3, AD4, and AXF (15–44 %). Cluster III was a 63-member n-damo *pmoA* group. The sequences in this cluster were related to the n-damo *pmoA* gene sequences from reservoir sediment and rice soil. Cluster III-like gene sequences were mainly distributed in several May sediments (MD1, MD2, MD5, MQX, and MXF) (17–36 %) and one August sediment (AD1) (38 %). Cluster VI also included 63 n-damo *pmoA* gene sequences that could be grouped together with those from reservoir sediment. Cluster VI-like gene sequences predominated in samples ASM (85 %) and MXZ (62 %) but became much less abundant in other sediments (0–29 %). Moreover, cluster IV was composed of 57 n-damo *pmoA* sequences that could be grouped with those from river and reservoir sediments. Cluster IV-like sequences were mainly detected in sediment samples MD4, MQX, AD1, AD5, AQX, and ASM (15–33 %). In addition, clusters V and VII were the smallest n-damo *pmoA* groups. Both of them comprised 22 n-damo *pmoA* gene sequences. The members in cluster V were related to several reservoir sediment *pmoA* gene sequences. Cluster VII-like sequences were mainly distributed in samples MD2 (17 %) and AD5 (26 %). The members in cluster VII could be grouped with the n-damo *pmoA* gene sequences, retrieved from peatland soil and lake and reservoir sediments.

UPGMA clustering analysis of n-damo community composition

Figure 4 illustrates the dendrogram constructed for the composition of sediment n-damo *pmoA* gene sequences in the Dongjiang River and its four tributaries. The May river samples were distantly separated from the August ones, suggesting the remarkable temporal shift in sediment n-damo *pmoA* gene composition. Moreover, the May river samples could be further clustered into four groups. Sample MSM alone formed a cluster, and sample MXF alone formed another cluster. These two samples were clearly separated from other May river sediments. Samples MD1, MD2, and MQX were clustered together. In addition, the August river samples could be further clustered into five clades. Sample ASM was distantly separated from other river samples. Sample AD1 alone formed a clade, while sample AXZ alone formed another clade. Samples AD5 and AQX were grouped together, but they were separated from samples AD3, AD4, and AXF. These results suggested the evident spatial change of n-damo *pmoA* gene composition in both May and August sediments.

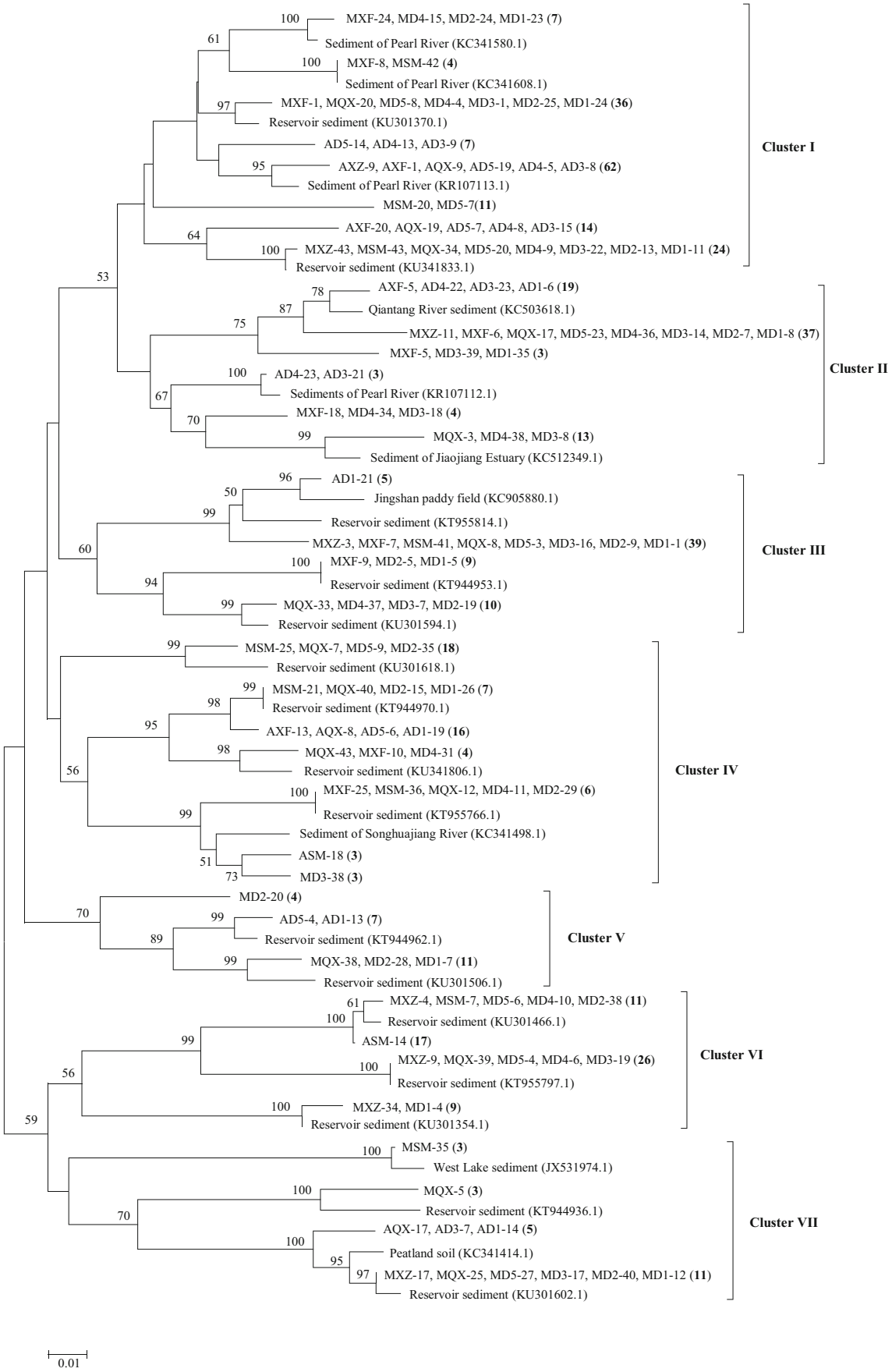
Influential factors regulating n-damo organisms

The result of Pearson's correlation analysis indicated that n-damo *pmoA* gene abundance was positively correlated to the levels of sediment NH_4^+-N and NO_2^--N ($p < 0.01$), while n-damo *pmoA* Chao1 richness showed a negative correlation with sediment NO_2^--N ($p < 0.05$) (Table 3). In addition, n-damo *pmoA* Shannon diversity was found to be negatively correlated to sediment NO_2^--N ($p < 0.01$) and C/N (the ratio of TOC to TN) ($p < 0.05$). The environmental factors in the first two RDA axes, respectively, accounted for 25.2 and 15.2 % of the total variance in sediment n-damo *pmoA* OTU composition (Fig. 5). NO_2^--N ($F = 3.504$, $P = 0.002$, 999 Monte Carlo permutations) and C/N($F = 3.263$, $P = 0.006$, 999 Monte Carlo permutations) were found to significantly contribute to the n-damo organism–environment relationship (Table S1).

Discussion

Change of n-damo community abundance in natural ecosystem

Several previous studies displayed the change of sediment or soil n-damo community abundance with sampling site in both saline ecosystems (Chen et al. 2015b; Shen et al. 2014b) and


MXF-24, MD4-15, MD2-24, MD1-23 (7)
Sediment of Pearl River (KC341580.1)
MXF-8, MSM-42 (4)
Sediment of Pearl River (KC341608.1)
MXF-1, MQX-20, MD5-8, MD4-4, MD3-1, MD2-25, MD1-24 (36)
Reservoir sediment (KU301370.1)
AD5-14, AD4-13, AD3-9 (7)
AXZ-9, AXF-1, AQX-9, AD5-19, AD4-5, AD3-8 (62)
Sediment of Pearl River (KR107113.1)
MSM-20, MD5-7(11)
AXF-20, AQX-19, AD5-7, AD4-8, AD3-15 (14)
MXZ-43, MSM-43, MQX-34, MD5-20, MD4-9, MD3-22, MD2-13, MD1-11 (24)
Reservoir sediment (KU341833.1)
Cluster I
AXF-5, AD4-22, AD3-23, AD1-6 (19)
Qiantang River sediment (KC503618.1)
MXZ-11, MXF-6, MQX-17, MD5-23, MD4-36, MD3-14, MD2-7, MD1-8 (37)
MXF-5, MD3-39, MD1-35 (3)
AD4-23, AD3-21 (3)
Sediments of Pearl River (KR107112.1)
MXF-18, MD4-34, MD3-18 (4)
MQX-3, MD4-38, MD3-8 (13)
Sediment of Jiaojiang Estuary (KC512349.1)
Cluster II
AD1-21 (5)
Jingshan paddy field (KC905880.1)
Reservoir sediment (KT955814.1)
MXZ-3, MXF-7, MSM-41, MQX-8, MD5-3, MD3-16, MD2-9, MD1-1 (39)
MXF-9, MD2-5, MD1-5 (9)
Reservoir sediment (KT944953.1)
MQX-33, MD4-37, MD3-7, MD2-19 (10)
Reservoir sediment (KU301594.1)
Cluster III
MSM-25, MQX-7, MD5-9, MD2-35 (18)
Reservoir sediment (KU301618.1)
MSM-21, MQX-40, MD2-15, MD1-26 (7)
Reservoir sediment (KT944970.1)
AXF-13, AQX-8, AD5-6, AD1-19 (16)
MQX-43, MXF-10, MD4-31 (4)
Reservoir sediment (KU341806.1)
MXF-25, MSM-36, MQX-12, MD4-11, MD2-29 (6)
Reservoir sediment (KT955766.1)
Sediment of Songhuajiang River (KC341498.1)
ASM-18 (3)
MD3-38 (3)
Cluster IV
MD2-20 (4)
AD5-4, AD1-13 (7)
Reservoir sediment (KT944962.1)
MQX-38, MD2-28, MD1-7 (11)
Reservoir sediment (KU301506.1)
Cluster V
MXZ-4, MSM-7, MD5-6, MD4-10, MD2-38 (11)
Reservoir sediment (KU301466.1)
ASM-14 (17)
MXZ-9, MQX-39, MD5-4, MD4-6, MD3-19 (26)
Reservoir sediment (KT955797.1)
MXZ-34, MD1-4 (9)
Reservoir sediment (KU301354.1)
Cluster VI
MSM-35 (3)
West Lake sediment (JX531974.1)
MQX-5 (3)
Reservoir sediment (KT944936.1)
AQX-17, AD3-7, AD1-14 (5)
Peatland soil (KC341414.1)
MXZ-17, MQX-25, MD5-27, MD3-17, MD2-40, MD1-12 (11)
Reservoir sediment (KU301602.1)
Cluster VII
0.01

◀ **Fig. 2** Phylogenetic tree of representative n-damo *pmoA* sequences and reference sequences from GenBank. The obtained *pmoA* sequences beginning with "MD1" and "AD1," "MD3" and "AD3," "MD4" and "AD4," and "MD5" and "AD5" were referred to those retrieved from the May and August sediment samples from sites D1, D3, D4, and D5 in the stem of the Dongjiang River, respectively. The obtained *pmoA* sequences beginning with "MD2" were referred to those retrieved from the May sediment samples from sites D2 in the stem of the Dongjiang River. The obtained *pmoA* sequences beginning with "MXF" and "AXF," "MQX" and "AQX," "MXZ" and "AXZ," and "MSM" and "ASM" were referred to those retrieved from the May and August sediment samples from the tributaries Xingfeng River, Qiuxiang River, Xizhijiang River, and Shima River, respectively. The *bold numbers* in *parentheses* represent the total sequences from all sediment samples in the same OTU. *Numbers* at the nodes indicate the levels of bootstrap support based on neighbor-joining analysis of 1000 resampled datasets. The values less than 50 are not listed. The *bar* represents 1 % sequence divergence

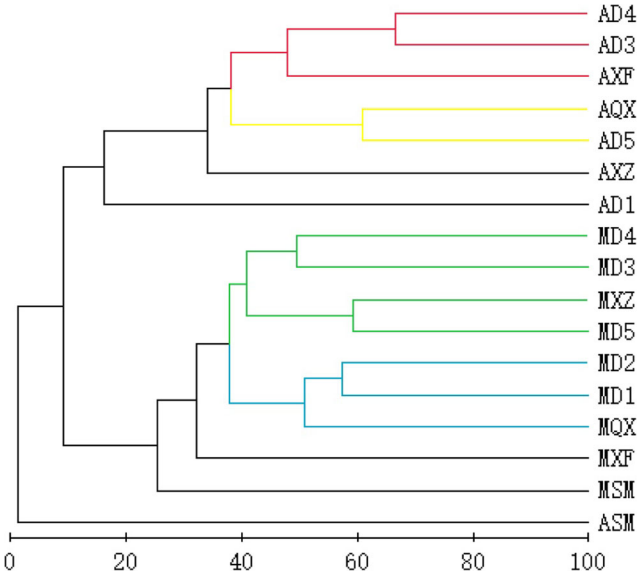


Fig. 4 UPGMA cluster diagram of n-damo *pmoA* gene composition similarity values for river sediment samples. Similarity levels are indicated *below* the diagram

freshwater ecosystems (Kojima et al. 2012; Shen et al. 2014a; Wang et al. 2016). The n-damo community abundance also differed considerably in profundal sediments of various small freshwater lakes on the Yunnan Plateau (Liu et al. 2015). These previous studies determined n-damo community abundance based on 16S ribosomal RNA (rRNA) gene. Information on n-damo community abundance based on *pmoA* gene is still very limited. Yan et al. (2015) illustrated the horizontal change of n-damo community abundance in saline Yellow River estuary sediment based on both *pmoA* gene and 16S rRNA gene. Moreover, limited information existed on the temporal change of n-damo community abundance in the environment. Two previous studies reported the temporal variation of n-damo community abundance in freshwater wetland soil (Wang et al. 2016) and paddy soil (Zhou et al. 2014). In this study, quantitative PCR targeting n-damo *pmoA* gene was applied to determine the abundance of sediment n-damo community in the Dongjiang River and its tributaries. The density of n-damo *pmoA* gene in river sediments ranged between 9.07×10^4 and 3.02×10^6 copies per gram dry sediment, generally much greater than that reported in Yellow River estuary sediments (Yan et al. 2015). In both May and August, sediment n-damo community in tributaries was more abundant than that in the stem of the Dongjiang River. The spatial heterogeneity of sediment n-damo community abundance also occurred in either the stem of the Dongjiang River or among its tributaries. These results displayed a remarkable spatial change of sediment n-damo community abundance in river ecosystems. This was in agreement with the result of the previous study on freshwater Qiantang River (Shen et al. 2014a). In addition, river sediment n-damo community abundance was found to be much greater in August than in May,

Fig. 3 Compositions of n-damo *pmoA* clusters in river sediments

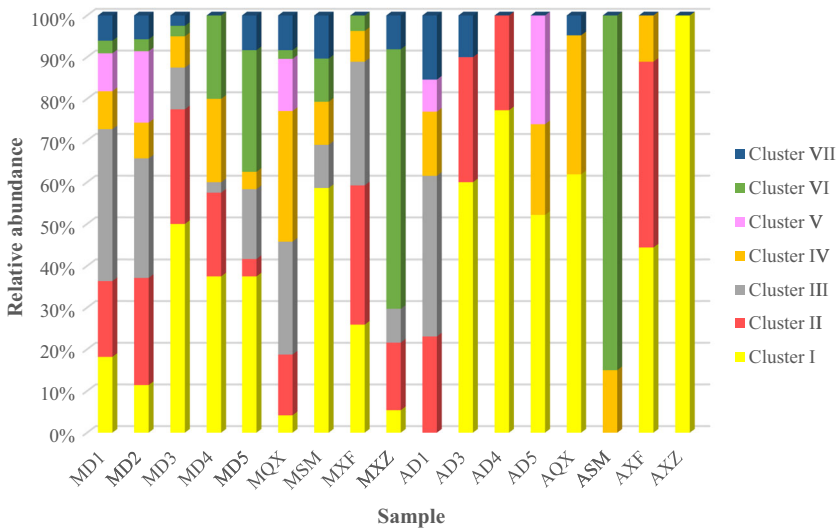


Table 3 Pearson's correlation analysis of n-damo community with the determined sediment environmental factors

	NH_4^+-N	NO_3^--N	NO_2^--N	TN	TP	TOC	pH	C/N[a]
Abundance	0.772**	−0.191	0.916**	−0.174	−0.243	0.096	0.143	0.252
Chao1	−0.456	0.204	−0.489*	0.317	0.444	−0.173	−0.201	−0.477
Shannon	−0.460	0.137	−0.691**	0.362	0.358	−0.020	−0.152	−0.494*

[a] The ratio of TOC to TN

***Correlation is significant at the 0.05 level; correlation is significant at the 0.01 level

illustrating a remarkable temporal shift. Hence, the present study provided the first evidence for the temporal variation of freshwater sediment n-damo community abundance.

So far, little is known about the environmental factors influencing n-damo community abundance in natural environment. Several previous studies indicated that sediment or soil n-damo community abundance might be influenced by organic carbon (Shen et al. 2014a, b), TP/TN (Yan et al. 2015), and C/N (Wang et al. 2016). However, in this study, the result of Pearson's correlation analysis suggested that river sediment n-damo community abundance was positively influenced by the increase of NH_4^+-N and NO_2^--N levels. NO_2^--N is one of the substrates of *M. oxyfera*-like bacteria. High levels of NO_2^--N can support a larger number of *M. oxyfera*-like organisms. Moreover, archaeal and bacterial ammonia oxidizers were found to be abundant in the sediments of the Dongjiang River (Sun et al. 2013). The abundance of these ammonia oxidizers might effectively convert sediment NH_4^+-N into NO_2^--N, which further increased the river sediment n-damo community abundance.

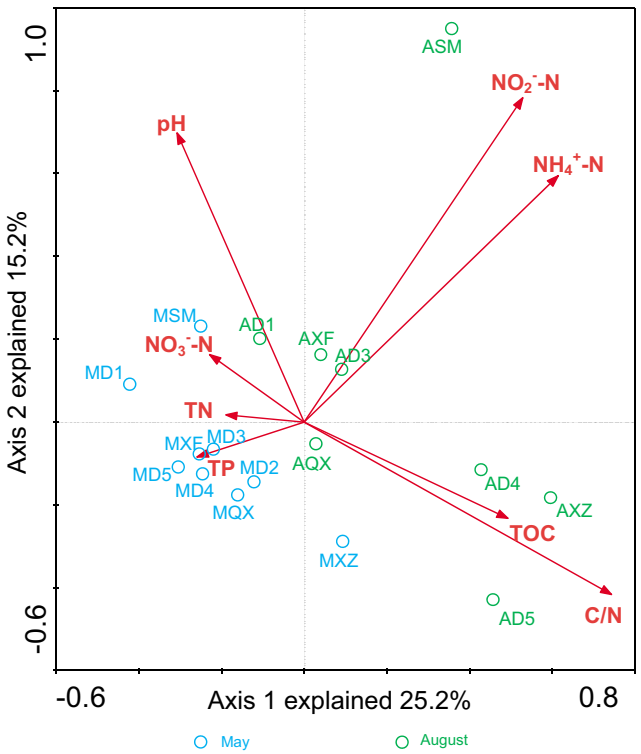


Fig. 5 RDA ordination plot for the first two principal dimensions of the relationship between n-damo *pmoA* OTU composition and the determined environmental factors

Change of n-damo community richness and diversity in natural ecosystem

The remarkable horizontal changes of the richness and diversity of n-damo community based on *pmoA* gene or/and 16S rRNA gene have been found in a variety of natural ecosystems, such as marine sediment (Chen et al. 2014, 2015a), coastal intertidal wetland sediment (Chen et al. 2015b), marine estuary sediment (Shen et al. 2014b), river estuary sediment (Yan et al. 2015), and freshwater wetland soil (Wang et al. 2016). Moreover, n-damo community richness and diversity illustrated the considerable difference in profundal sediments of freshwater lakes on the Yunnan Plateau (Liu et al. 2015). However, to date, only Shen et al. (2014a) reported the changes of n-damo community richness and diversity with sampling location in freshwater river sediment ecosystem. In this study, in either May or August 2015, the evident changes of sediment n-damo *pmoA* gene richness and diversity with sampling site were also found in the Dongjiang River and its tributaries. To date, the temporal shifts in the richness and diversity of n-damo community have received poor attention. Only two previous studies revealed the variations of n-damo community richness and diversity with sampling time in freshwater wetland soil (Wang et al. 2016) and paddy soil (Zhou et al. 2014). In this study, river sediment n-damo *pmoA* richness and diversity tended to be higher in May than in August, in contrary to the trend for n-damo community abundance. Therefore, the present study provided the evidence for the first time that n-damo community richness and diversity could vary with sampling time in freshwater sediment ecosystem.

The environmental factors influencing n-damo community richness and diversity remain essentially unclear. However, some previous studies suggested the potential roles of nitrogen compounds on n-damo community richness and diversity. Chen et al. (2014) suggested that sediment n-damo community richness and diversity in South China Sea might be regulated by the levels of NO_2^--N and NO_3^--N. In contrast, Shen et al. (2014a) suggested that sediment n-damo community richness and diversity in freshwater Qiantang River might be positively influenced by the increase of NH_4^+-N and total inorganic nitrogen (TIN). In this study, the result of

Pearson's correlation analysis suggested that sediment NO_2^--N might be play an important role in determining sediment n-damo *pmoA* gene richness and diversity in freshwater water river system. High levels of NO_2^--N could reduce river sediment n-damo community richness and diversity. Moreover, high levels of organic carbon content favored n-damo community richness and diversity in Jiaojiang estuarine sediment (Shen et al. 2014b). The increase of sediment C/N might promote n-damo community diversity in freshwater lakes on the Yunnan Plateau (Liu et al. 2015). However, in this study, the result of Pearson's correlation analysis suggested that n-damo community diversity was negatively influenced by the increase of C/N.

Change of n-damo community structure in natural ecosystem

The horizontal shift in n-damo community structure has been reported in various saline natural environments (Chen et al. 2014, 2015a, b; Shen et al. 2014b; Yan et al. 2015), while information on the change of n-damo community structure with sampling site in freshwater ecosystem is still scant. Only Shen et al. (2014a) and Wang et al. (2016) reported the horizontal variation of n-damo community structure in freshwater river sediment and wetland soil, respectively. Moreover, little is known about the temporal change of n-damo community structure in natural ecosystem. Only two previous studies revealed the temporal variation of soil n-damo community structure (Wang et al. 2016; Zhou et al. 2014). In this study, the results of both UPGMA clustering and phylogenetic analyses illustrated that sediment n-damo community structure in freshwater river changed considerably with both sampling site and time. So far, the links between n-damo community structure and environmental factors remain elusive. Several previous studies suggested that sediment n-damo community structure in saline environment might be influenced by NH_4^+-N (Chen et al. 2014, 2015b; Yan et al. 2015), the sum of NO_2^--N and NO_3^--N (Chen et al. 2014), organic content (Shen et al. 2014b; Yan et al. 2015), pH (Yan et al. 2015), and salinity (Yan et al. 2015), while Shen et al. (2014a) suggested that sediment n-damo community structure in freshwater Qiantang River might be influenced by NH_4^+-N and TIN. In this study, the result of RDA suggested that NO_2^--N and C/N might shape sediment n-damo community structure in freshwater river. Therefore, the present study expanded the knowledge on the links between environmental factors and freshwater sediment n-damo community structure.

In conclusion, in the Dongjiang River and its tributaries, sediment n-damo community abundance, richness, diversity, and structure varied considerably with both sampling site and time. The temporal change pattern of sediment n-damo community abundance was different from those of richness and diversity. High levels of sediment nitrite nitrogen favored n-damo community abundance but had a negative impact on richness and diversity. Moreover, nitrite nitrogen played an important role in shaping sediment n-damo community and influencing its distribution in river ecosystem.

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Compliance with ethical standards

Ethical statement I would like to declare on behalf of my co-authors that the work described was an original research that has not been published previously, and not under consideration for publication elsewhere, in whole or in part.

Conflict of interest The authors declare that they have no conflict of interest.

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