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Vertical profiles of sediment methanogenic potential and communities in two plateau freshwater lakes

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Abstract. Microbial methanogenesis in sediment plays a crucial role in CH_4 emission from freshwater lake ecosystems. However, knowledge of the layer-depth-related changes of methanogen community structure and activities in freshwater lake sediment is still limited. The present study was conducted to characterize the methanogenesis potential in different sediment-layer depths and the vertical distribution of microbial communities in two freshwater lakes of different trophic status on the Yunnan Plateau (China). Incubation experiments and inhibitor studies were carried out to determine the methanogenesis potential and pathways. 16S rRNA and *mcrA* genes were used to investigate the abundance and structure of methanogen and archaeal communities, respectively. Hydrogenotrophic methanogenesis was mainly responsible for methane production in sediments of both freshwater lakes. The layer-depth-related changes of methanogenesis potential and the abundance and community structure of methanogens were observed in both Dianchi Lake and Erhai Lake. Archaeal 16S rRNA and *mcrA* genes displayed a similar abundance change pattern in both lakes, and the relative abundance of methanogens decreased with increasing sediment-layer depth. Archaeal communities differed considerably in Dianchi Lake and Erhai Lake, but methanogen communities showed a slight difference between these two lakes. However, methanogen communities illustrated a remarkable layer-depth-related change. Order *Methanomicrobiales* was the dominant methanogen group in all sediments, while *Methanobacteriales* showed a high proportion only in upper layer sediments. The trophic status of the lake might have a notable influence on the depth-related change pattern of methanogenesis activity, while the methanogen community structure was mainly influenced by sediment depth.

1 Introduction

Methane (CH_4) is an effective greenhouse gas in atmosphere, and lacustrine ecosystems may be responsible for 6–16 % of natural methane emission (Bastviken et al., 2004). In anoxic sediment of a freshwater lake, a large amount of methane can be produced through microbial methanogenesis (Bastviken et al., 2008; Gruca-Rokosz and Tomaszek, 2015). Biogenic methane is produced by the activity of methanogens, a strictly anaerobic microbial group belonging to the archaeal phylum *Euryarchaeota*. Methanogens from seven archaeal orders have been reported (Garcia, 1990; Paul et al., 2012; Sakai et al., 2008), and their substrate is generally the end product of organic matter degradation by fermentative bacteria and Archaea (Borrel et al., 2011). In freshwater lakes, organic matter is fermented to acetate, CO_2, and H_2, which are further converted to CH_4 by methanogens. There are two major methanogenic pathways, namely hydrogenotrophic pathways (using H_2/CO_2) and acetoclastic pathways (using acetate, i.e., the methyl group) (Conrad et al., 2010). The relative contribution of these two pathways varies in different lakes (Conrad, 1999). To determine the contribution of both methanogenic pathways, approaches including isotope labeling, $\delta^{13}C$ analysis, and inhibitor study have been applied (Conrad, 2005). Despite the theoretical ratio of 2 : 1 (ace-

toclastic pathways : hydrogenotrophic pathways) when carbohydrates or other similar forms of organic matter are degraded (Conrad, 1999), hydrogenotrophic pathways can account for a considerable proportion of methane production in freshwater lake sediment (Borrel et al., 2011). However, the relative significance of hydrogenotrophic pathways remains unclear, because it can vary considerably by lake (Conrad, 1999). To identify the methanogens involved in methanogenesis, both the archaeal 16S rRNA gene and functional *mcrA* gene have been used (Conrad et al., 2007; Luton et al., 2002; Orphan et al., 2008). The dominance of *Methanomicrobiales* and *Methanosarcinales* has been reported in a variety of freshwater lakes (Biderre-Petit et al., 2011; Conrad et al., 2007; Youngblut et al., 2014). In freshwater lakes, both methanogenesis pathways and methanogenic community structure can change with sediment-layer depth (Chan et al., 2005; Liu et al., 2016; Lofton et al., 2015).

Many previous studies have investigated methanogenesis in humic lakes (Youngblut et al., 2014), oligotrophic lakes (Lofton et al., 2015), and meromictic lakes (Biderre-Petit et al., 2011; Gies et al., 2014), while shallow mesotrophic and eutrophic lakes have attracted poor attention. Substrate plays an important role in methanogenesis, and has a considerable effect on pathway preference (Liu et al., 2016; Nozhevnikova et al., 2007). Methanogenesis in mesotrophic and eutrophic lakes that have abundant substrate might be different from that in other previously studied lakes. Therefore, for sediments of mesotrophic and eutrophic lakes, the following questions have attracted our attention: (1) how do the methanogenesis activity and contribution of different pathways change with the increasing sediment-layer depth? (2) How do methanogen community structure and the dominant methanogens change along the sediment-layer depth? (3) Are these changing patterns similar in lakes at different trophic status?

2 Materials and methods

2.1 Sampling sites and samples

Dianchi Lake (with an area of 309 km^2 and an average water depth of 4.4 m) and Erhai Lake (with an area of 250 km^2 and an average water depth of 10 m) are the first- and second-largest freshwater lakes on Yunnan Plateau, China, and are of eutrophic and mesotrophic status, respectively (Wang et al., 2015). The characteristics and trophic status of these two lakes have been overviewed in detail by Wang et al. (2015), and some key features are listed in the Table 1.

In this study, five replicate sediment cores (0–20 cm) were collected at the profundal areas of both Dianchi Lake and Erhai Lake with a columnar sediment sampler in December 2015. The water depths at the sampling sites were 6.4 and 11 m in Dianchi Lake and Erhai Lake, respectively. The in situ sediment temperature were 16.4 °C in Dianchi Lake and 14.8 °C in Erhai Lake. Meanwhile, a total of 500 mL bottom water at each sampling site was collected for the subsequent incubation experiments.

The five replicate sediment cores were sliced into layers (sample D1 or E1: 0–5 cm, sample D2 or E2: 5–8 cm, sample D3 or E3: 8–11 cm, sample D4 or E4: 11–14 cm, and sample D5 or E5: 14–17 cm, sample D6 or E6: 17–20 cm). Samples D1–D6 and E1–E6 were from Dianchi Lake and Erhai Lake, respectively. Replicate sediment samples from the same layer depth in a given lake were mixed and then subsampled for physicochemical and molecular analyses and incubation experiments. Sediment samples for physicochemical and molecular analyses were stored at −20 °C, while those for incubation experiments were kept in gas-tight bottles under anoxic condition at 4 °C. The sediment samples were transported to a laboratory within 1 week, which would have no considerable effect on methanogenic activity (Nüsslein et al., 2001).

2.2 Methane production potential measurement

The incubation experiments for methane production measurement were performed with reference to the standard procedure described in the literature (Conrad et al., 2010). Uppermost sediment (0–5 cm) was centrifuged at 4000 rpm for 5 min to obtain similar water content to the samples from other layers. For each sediment layer, a total of six sediment samples (1.5 g) and 8 mL bottom water were transferred into a 50 mL sterile serum bottle, flushed with N_2, and then closed with a butyl rubber stopper. After incubation at 16 °C overnight, the bottles were flushed with N_2 again, and half of them were added with CH_3F (1 mL) to inhibit the acetoclastic methanogenesis. Incubation was carried out at 16 °C at 100 rpm for 28 days. At the end of incubation, gas samples were taken from the headspace using a gas-tight syringe, and were then analyzed using GC126 gas chromatography (INESA instrument, Shanghai) with a flame ionization detector.

2.3 Molecular analysis

DNA was extracted using the PowerSoil DNA extraction kit (Mobio Laboratories, USA). The quality of DNA was checked using 1.0 % agarose gel electrophoresis. For quantitative PCR (qPCR), the primer sets mcrF (5′-GGTGGTGTMGGATTCACACARTAYGCWACAGC-3′)/mcrR (5′-TCATTGCRTAGTTWGGRTAGTT-3′) (Luton et al., 2002) and Arch344F (5′-GYGCAGCAGGCGCGA-3′)/Arch915R (5′-GTGCTCCCCCGCCAATTCCT-3′) (Casamayor et al., 2002; Conrad et al., 2014) were used for the quantification of *mcrA* and archaeal 16S rRNA genes, respectively. The qPCR assay was carried out using an ABI 7500 Fast system (Applied Biosystems). The reaction mixture included 2 × SYBR Green PCR master mix (12.5 µL), 10 µM of each primer (1 µL), and template DNA (2 ng). The PCR conditions were as described in the literature

Table 1. Characteristics and trophic status of Dianchi Lake and Erhai Lake. sTOC, sTN, sTP and wTOC, wTN, wTP refer to average levels of total organic carbon (TOC), total nitrogen (TN), and total phosphorus (TP) of sediment and water, respectively. The locations of sampling sites were as illustrated in the literature (Yang et al., 2016).

Lake	Location	sTOC ($g\,kg^{-1}$)	sTN ($mg\,kg^{-1}$)	sTP ($mg\,kg^{-1}$)	wTOC ($mg\,L^{-1}$)	wTN ($mg\,L^{-1}$)	wTP ($mg\,L^{-1}$)	Trophic status
Dianchi	102°42′ E, 24°51′ N	0.13	1027.44	320.44	4.4	1.65	0.114	eutrophic
Erhai	100°11′ E, 25°45′ N	0.10	818.15	183.79	6.4	0.56	0.018	mesotrophic

(Casamayor et al., 2002; Luton et al., 2002). Standard curves ranging from 10^3 to 10^7 gene copies μL^{-1} were generated using serial dilutions of linearized plasmids (pGEM-T, Promega) containing cloned target gene amplified from environmental DNA. The coefficients (r^2) for the archaeal 16S rRNA gene and *mcrA* gene were 0.9995 and 0.9998, respectively. Significance was tested using one-way analysis of variance (ANOVA), followed by Student–Newman–Keul's post hoc analysis (when the variances were homogenous) or Dunnett's post hoc analysis (when the variances were not homogenous).

For Illumina MiSeq sequencing, the archaeal 16S rRNA gene was amplified using primer set Arch519f (5′-CAGCCGCCGCGGTAA-3′)/Arch915R (5′-GTGCTCCCCCGCCAATTCCT-3′) (He et al., 2016; Herfort et al., 2009; Long et al., 2016). The PCR products from triplicate samples were mixed in equal amounts and were subjected to Illumina MiSeq sequencing. Raw reads were merged and the quality filtering was carried out using Trimmomatic (Bolger et al., 2014) and FLASH (Magoc and Salzberg, 2011). The obtained raw reads were deposited in the NCBI SRA (short-read archive) under accession SRP076837. After subsampling to the lowest number of sequences, sequences from each sediment sample were checked for chimeras and grouped into OTUs (operational taxonomic units) using USEARCH (version 7.1, http://drive5.com/uparse/) at 97 % similarity. Diversity analysis was carried out using the MOTHUR program (version v.1.30.1) (Schloss et al., 2011). The taxonomic identities of representative sequences for each OTU were assigned according to the SILVA 16S rRNA database (Quast et al., 2013). For *mcrA* gene clone library analysis, the primer set mcrF/mcrR was used, and the PCR conditions were as previously described (Luton et al., 2002). The obtained *mcrA* sequences were deposited in the GenBank database under accession numbers KU997795-KU997842, KX196972-KX197020, and KX093502-KX093920. The *mcrA* gene sequences were grouped into OTUs at the similarity level of 89 % (Webster et al., 2014) using the MOTHUR program. OTU-based diversity was also calculated using the MOTHUR program (Schloss et al., 2009). Phylogenetic analysis of the *mcrA* gene sequences was carried out with the MEGA 6.0 software (Tamura et al., 2013). Weighted UniFrac distance between samples was calculated using R library GUniFrac, and cluster analyses were conducted based on Weighted UniFrac distance using R (version i386, 3.3.0).

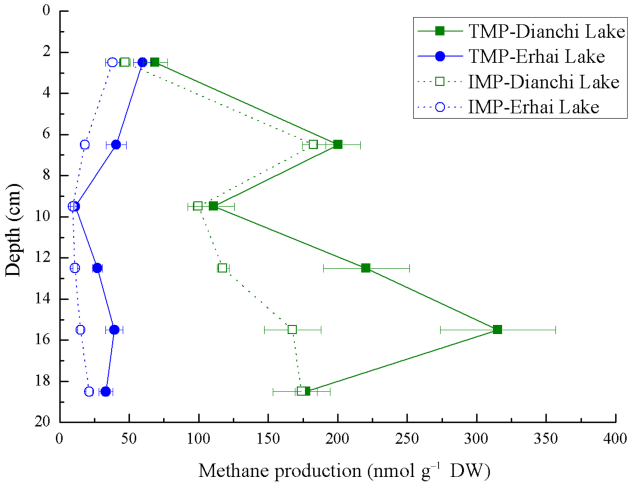

Figure 1. Uninhibited (TMP) and 2 % CH_3F inhibited (IMP) methane production potential at different depths. Error bars represent standard deviation of mean ($n = 3$).

3 Results

3.1 Total methane production

In this study, total methane production (TMP) varied remarkably with both lake and sediment depth except for the uppermost sediments layers, which were remarkably similar between Dianchi Lake and Erhai Lake (Fig. 1). The change pattern of TMP differed in the two lakes. In Dianchi Lake, the TMP generally increased with increasing depth, varying from 68.9 to 315.3 $nmol\,gDW^{-1}$ in a 5–20 cm layer (DW stands for dry weight, which was determined gravimetrically), while in Erhai Lake, the MPP decreased from 59.6 to 11.2 $nmol\,gDW^{-1}$. The sediment samples from Dianchi Lake showed a significantly higher TMP than those from Erhai Lake ($P < 0.05$).

The inhibited methane production (IMP) was measured under the condition of 2 % CH_3F. IMP showed a similar change trend with TMP in either of the two freshwater lakes

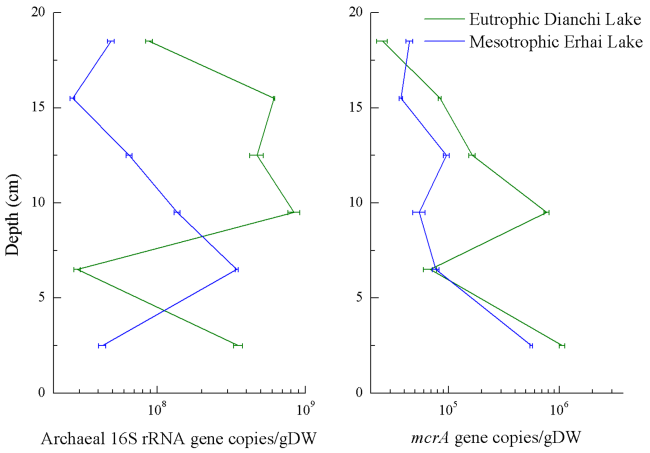

Figure 2. Abundance of archaeal 16S rRNA and *mcrA* genes at different depths. Error bars represent standard deviation of mean ($n = 3$).

studied. With increasing depth, IMP generally increased in Dianchi Lake but tended to decrease in Erhai Lake. Dianchi Lake had the IMP of 47.0–182.8 nmol gDW^{-1}, but Erhai Lake had a much lower IMP (9.5–38.1 nmol gDW^{-1}). Besides, uninhibited methane production (mainly produced through acetoclastic pathways) varied greatly in different layers (2.0–84.0 nmol gDW^{-1} in Dianchi Lake, 1.7–13.7 nmol gDW^{-1} in Erhai Lake), and showed a notable difference between these two freshwater lakes.

3.2 Community abundance of Archaea and methanogen

The depth-related change pattern of either archaeal 16S rRNA or *mcrA* gene differed in Dianchi Lake and Erhai Lake (Fig. 2). The density of the archaeal 16S rRNA gene fluctuated between $2.9 \pm 0.1 \times 10^7$ and $8.4 \pm 0.8 \times 10^8$ copies gDW^{-1} in Dianchi Lake. In Erhai Lake, the archaeal community abundance ranged from $2.7 \pm 0.1 \times 10^7$ to $3.4 \pm 0.1 \times 10^8$ copies gDW^{-1}, and showed an increase with depth followed by a significant decrease ($P < 0.05$), with the peak value in the 5–8 cm layer.

As for the *mcrA* gene, its highest density was observed at the uppermost layer (0–5 cm) in both Dianchi Lake ($1.06 \pm 0.06 \times 10^6$ copies gDW^{-1}) and Erhai Lake ($5.6 \pm 0.2 \times 10^5$ copies gDW^{-1}), while the lowest one occurred in Dianchi Lake sample D6 (17–20 cm, $2.5 \pm 0.3 \times 10^4$ copies gDW^{-1}) or Erhai Lake sample E5 (14–17 cm, $3.7 \pm 0.1 \times 10^4$ copies gDW^{-1}). The gene abundance generally decreased with increasing depth. In addition, both *Archaea* or methanogens showed greater abundance in Dianchi Lake than in Erhai Lake.

3.3 Diversity of archaeal and methanogen communities

In this study, after subsampling, a total of 16 028 archaeal sequences were retrieved from each sediment sample. The number of OTUs in each library ranged between 547 and 1194, and library coverage was 98.07–99.64 % (Table 2). Archaeal community diversity of most sediment samples varied slightly (Shannon index = 4.51–5.13), whereas samples D2 and D3 had much lower Shannon diversity (3.98 or 3.88). The samples from Erhai Lake showed higher archaeal diversity (Shannon index = 4.81–5.13) than those from Dianchi Lake (Shannon index = 3.88–4.75).

A total of 516 *mcrA* gene sequences were retrieved from the *mcrA* clone library, and could be assigned into 30 OTUs (at 89 % similarity). For each sample, the number of sequences ranged from 35 to 49, while the number of OTUs varied between 6 and 11. The coverage for each clone library was no less than 85.7 %, indicating that the *mcrA* OTUs of each sample had been well captured. The Shannon indices were 1.49–1.72 for Dianchi Lake and 1.25–1.84 for Erhai Lake, respectively. In Dianchi Lake, sample D1 had the highest *mcrA* gene diversity, but sample D6 had the lowest diversity. In Erhai Lake, sample E2 had the highest *mcrA* gene diversity followed by sample E1, but E5 showed the lowest diversity.

3.4 The 16S rRNA-based community composition

Bathyarchaeota (formally known as *Miscellaneous Crenarchaeotic_Group*, *MCG*) and *Euryarchaeota* dominated the archaeal communities in both Dianchi Lake and Erhai Lake (Fig. 3). In the uppermost sediment layer in both lakes, *Bathyarchaeota* accounted for nearly 20 % of the total archaeal community. However, in Dianchi Lake, with increasing layer depth, the proportion of *Bathyarchaeota* showed a remarkable decrease followed by a rise. *Bathyarchaeota* organisms showed a high proportion (44–55 %) in Dianchi Lake samples D4–D6 and Erhai Lake samples E2–E6. In contrast, *Euryarchaeota* illustrated an opposite change trend. In Dianchi Lake, the proportion of *Euryarchaeota* organisms was higher in samples D2 and D3 (63 or 67 %) than in sample D1 (47 %), but displayed a notable decrease in deeper layers (26–27 %). In Erhai Lake, the *Euryarchaeota* proportion in sample E1 was 32.5 % but became lower in samples E2–E6 (9–23 %). In addition, *Woesearchaeota* showed a greater abundance in Dianchi Lake (8–13 %) than in Erhai Lake (4–9 %), while *Crenarchaeota* was mainly distributed in Erhai Lake (3–29 %).

Based on 16S rRNA gene analysis, methanogens comprised 3–17 % of the total archaeal community (Fig. S1 in the Supplement). The relative abundance of methanogens tended to decrease with increasing sediment-layer depth. The order *Methanobacteriales* dominated the methanogen community in the uppermost layer samples (samples D1 or E1), and also had a considerable proportion in samples D2 and D3. In contrast, *Methanomicrobiales* was the dominant methanogen

Table 2. Diversity and library coverage of archaeal 16S rRNA and *mcrA* genes. Samples D1–D6 and E1–E6 were retrieved from Dianchi Lake and Erhai Lake, respectively. Digits "1"–"6" refer to sediment depth 0–5, 5–8, 8–11, 11–14, 14–17, and 17–20 cm, respectively.

Sample	Coverage		Number of OTUs		Shannon index	
	Arch 16S	*mcrA*	Arch 16S	*mcrA*	Arch 16S	*mcrA*
D1	98.35 %	97.92 %	1120	8	4.51	1.72
D2	98.70 %	91.30 %	897	8	3.98	1.50
D3	98.45 %	94.87 %	995	6	3.88	1.54
D4	98.38 %	91.43 %	1060	8	4.73	1.59
D5	98.14 %	85.71 %	1194	11	4.75	1.65
D6	98.53 %	90.00 %	959	9	4.53	1.49
E1	98.12 %	93.88 %	1261	8	5.00	1.51
E2	98.17 %	90.00 %	1256	10	5.13	1.84
E3	98.07 %	95.65 %	1234	6	4.88	1.32
E4	98.39 %	93.02 %	1104	7	4.81	1.31
E5	98.33 %	93.18 %	1162	6	4.99	1.25
E6	99.64 %	95.45 %	547	8	5.03	1.60

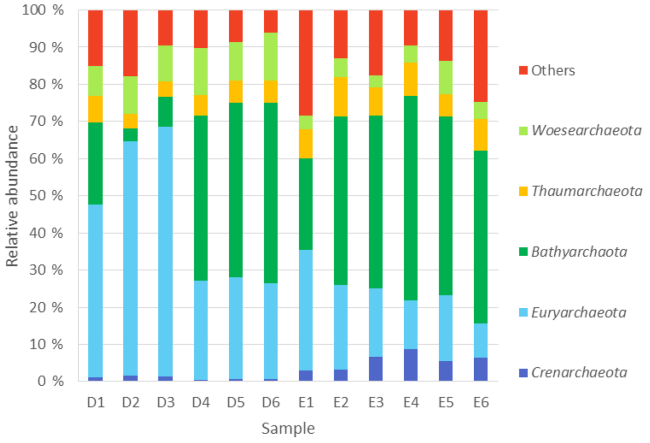

Figure 3. Compositions of archaeal communities at phylum level based on 16S rRNA gene. Samples D1–D6 and E1–E6 were retrieved from Dianchi Lake and Erhai Lake, respectively. Digits "1"–"6" refer to sediment depth 0–5, 5–8, 8–11, 11–14, 14–17, and 17–20 cm, respectively.

group in the lower-layer sediments (Dianchi Lake samples D2–D6 and Erhai Lake samples E2–E6). *Methanocellales* and *Methanosarcinales* could also be detected in all sediment samples, but their proportions were less than 2 %. At genus level, *Methanobacterium* (within *Methanobacteriales*) had the greatest proportion, followed by *Methanosaeta* (within *Methanosarcinales*) and *Methanoregula* (within *Methanomicrobiales*), and the proportion of each archaeal genus decreased with increasing layer depth in either lake (data not shown).

3.5 The *mcrA*-based community composition

In the present study, the 516 *mcrA* sequences from all sediment samples fell into 30 OTUs, 12 of which had only 1 sequence. The representative sequences from the OTUs with at least two sequence members were further used to construct the phylogenic tree with their close *mcrA* sequences reported in the NCBI database (Fig. 4). All of the sequences were grouped into five clusters (clusters 1–5). Cluster 1 contained most of the obtained *mcrA* sequences (440), and could be affiliated with the *mcrA* sequences from *Methanomicrobiales*. Cluster 2 consisted of 29 *mcrA* sequences related to those from *Thermoplasmatales* and *Methanoplasmatales*. Cluster 3 (with 13 sequence members) and cluster 5 (with 12 sequence members) were related to *Methanobacteriales* and *Methanosarcinales*, respectively. Cluster 4 was the smallest group and only contained 9 sequences. The sequences in this cluster were not related to the *mcrA* sequences from known methanogens, but showed a close relation to an uncultured sequence from paddy soil. In addition, the sequences affiliated to cluster 1 could be further divided into 3 clades. OTU7 was grouped together with the sequences from two *Methanomicrobiaceae* strains. OTU8 showed a close relation to a *Methanolinea*-like *mcrA* sequence, and OTU1, OTU2, and OTU6 were closely related to *Methanoregula*-like *mcrA* sequence.

Methanoregula-like *mcrA* sequences (OTU1 and OTU2) were detected in all sediment-layer depths, but the proportion of OTU1 decreased with increasing layer depth (Fig. S2). *Methanomicrobiaceae*-like *mcrA* sequences (OTU7) were not detected in Dianchi Lake samples D1, D2, and D3, but they dominated in the other samples and their proportion increased with increasing sediment-layer depth. *Methanoplasmatales*-like *mcrA* sequences (OTU13) mainly existed in Dianchi Lake, but were also detected in the uppermost sediment layer in Erhai Lake. *Methanolinea*-like *mcrA* sequences (OTU8) were present in all sediment layers in both lakes, but their change pattern was not evident. In addition,

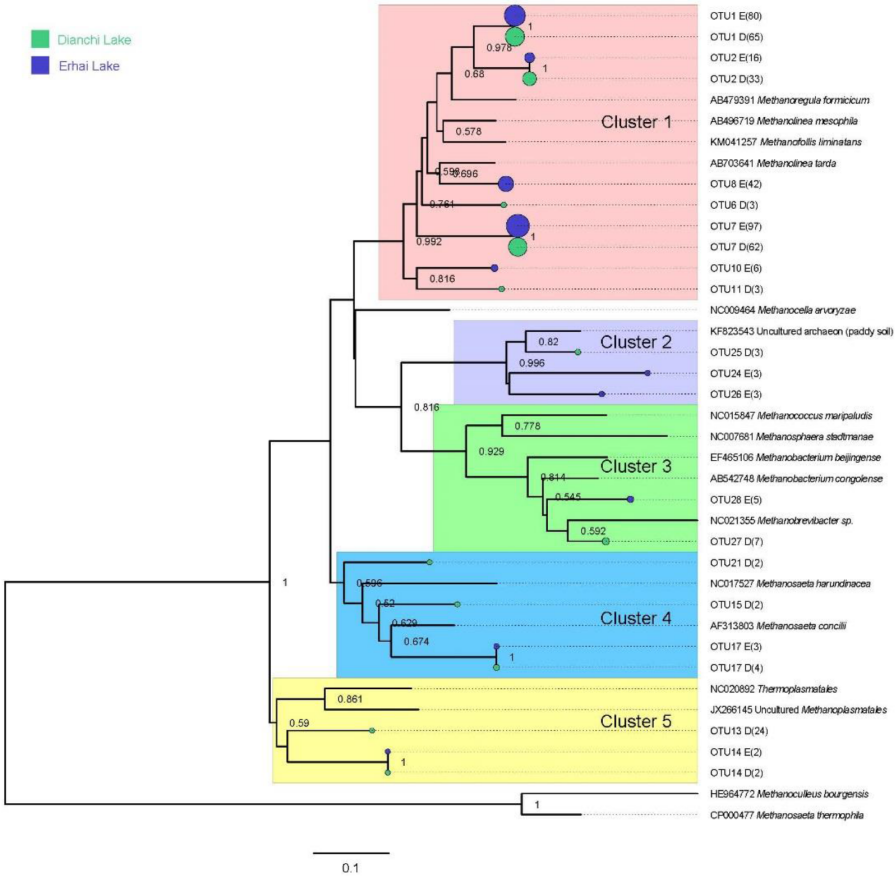

Figure 4. Phylogenetic tree of representative archaeal *mcrA* sequences and reference sequences from GenBank. The sequences beginning with "D1"–"D6" and "E1"–"E6" were referred to the sequences retrieved from samples D1–D6 and E1–E6, respectively. The number in parentheses, as well as the size of circle, represents the numbers of the total sequences in the libraries of a certain lake. Numbers at the nodes indicate the levels of bootstrap support based on neighbor-joining analysis of 1000 resampled datasets. The values less than 0.5 are not listed. The bar represents 5 % sequence divergence.

other genotypes of *mcrA* gene sequences comprised less than 22 % of the total sequences in each sample.

3.6 Comparison of archaeal and methanogen communities

The difference among archaeal assemblages was discriminated using weighted-UniFrac distance-based cluster analysis (Fig. 5a). In either Dianchi Lake or Erhai Lake, the sample from the uppermost layer (sample D1 or E1) was separated from the samples in other sediment layers. In Dianchi Lake, samples D2 and D3 were also grouped into one clade, and the other three samples fell into another one. However, in Erhai Lake, samples E2, E3, E4, E5, and E6 were grouped together, and the samples from neighboring layers tended to have a relatively similar archaeal community structure.

For methanogen communities, the studied 12 sediment samples fell into two groups (Fig. 5b). Samples D1, D2, D3, and E1 were clustered together. For other samples, samples at similar layer depth tended to have relatively similar methanogen community structure. Moreover, the sediment samples from two lakes were not clearly separated.

4 Discussion

4.1 Methane production potential in freshwater lake sediment

The TMP in Erhai Lake decreased with increasing sediment depth, which was in accordance with the literature (Chan et al., 2005 (top 20 cm sliced at 3–5 cm intervals); Liu et al., 2016 (top 50 cm depth sliced at 5 cm intervals); Lofton et al., 2015 (top 5 cm sliced at 1 cm intervals)). Lofton et al. (2015) also documented a slight TMP shift followed by a sharp decrease in vertical sediment layers. However, to the authors' knowledge, Dianchi Lake was the first lake that illustrated the increase of TMP with increasing sediment-layer depth.

Several previous studies have investigated the inter-lake difference of methanogenesis. The methane production rate

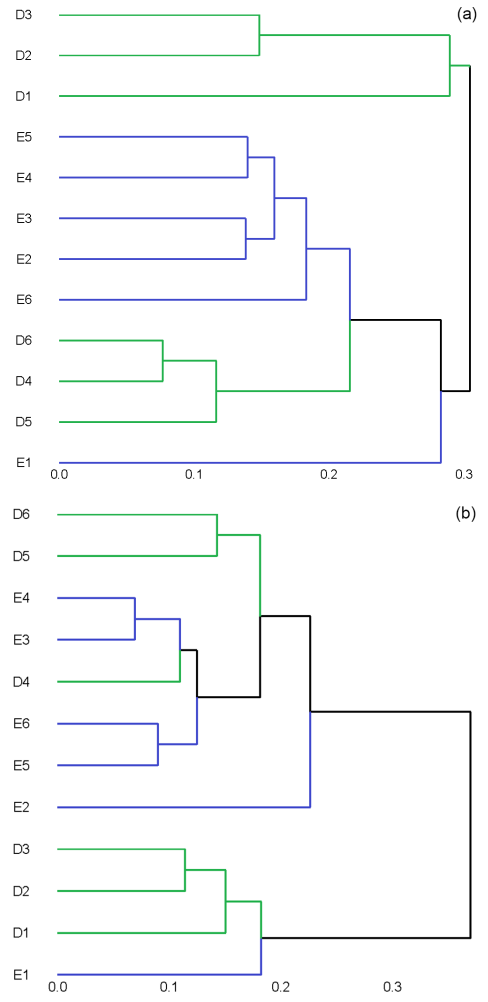

Figure 5. Environment clusters for archaeal 16S rRNA gene **(a)** and *mcrA* gene **(b)** assemblages based on UniFrac distance. The scale represents UniFrac distance. Samples D1–D6 and E1–E6 were retrieved from Dianchi Lake and Erhai Lake, respectively. Digits "1"–"6" refer to sediment depth 0–5, 5–8, 8–11, 11–14, 14–17, and 17–20 cm, respectively.

could differ drastically between the two geomorphologically similar oligotrophic lakes, and the quantity and quality of water-dissolved organic carbon (DOC) might be an influential factor (Lofton et al., 2015). Other environmental factors, including geological constitution (e.g., calcareous or not), geographical regions (Rinta et al., 2015), as well as water type (e.g., black water, clear water, or something else) (Conrad et al., 2014) were also found to have considerable influence on the methane production rate in freshwater lake sediment. Moreover, lake characteristics could influence the methane production rate both directly and indirectly (Borrel et al., 2011). In this study, the methanogenesis potential in Dianchi Lake was found to be much higher than that in Erhai Lake. Dianchi Lake had a larger amount of nutrients than Erhai Lake, and a high level of organic matter could exist in lower sediment layers (Fig. S3). TMP was found to be related to the availability of organic matter (Liu et al., 2016; Lofton et al., 2015; Nozhevnikova et al., 2007), so the abundant substrate in Dianchi Lake could favor higher TMP. In addition, although methanogen communities in Dianchi Lake and Erhai Lake had similar structure, both archaeal or bacterial communities differed greatly in these two lakes (Dai et al., 2016; Yang et al., 2016). Bacterial and non-methanogen archaeal communities played important roles in decomposing organic matter, and thus influenced the availability of substrate.

In the current study, IMP accounted for only 24.2 % of TMP, so it could be inferred that hydrogenotrophic pathways might play a major role in Dianchi Lake. The result was consistent with previous studies (Conrad et al., 2010; Liu et al., 2013, 2016). However, in Erhai Lake, IMP was comparable to uninhibited methane production. In this study, the layer-depth-related difference in the relative contribution of two methanogenic pathways was not clear, though acetoclastic methanogenesis's relative contribution was found to decrease with increasing lake sediment depth in several studies (Chan et al., 2005; Liu et al., 2016). The depth-related change of acetoclastic methanogenesis's relative contribution might be attributed to the decreasing availability of organic matter (Liu et al., 2016). However, in eutrophic Dianchi Lake, organic matter could be at a high level even at the depth of 20 cm. As a result, acetoclastic methanogenesis might not decrease considerably with increasing depth.

4.2 Abundance of Archaea and methanogen in freshwater lake sediment

In the current study, the density of the archaeal 16S rRNA gene tended to decrease with increasing depth in Erhai Lake but fluctuated considerably in Dianchi Lake. According to the previous studies, the abundance of the archaeal 16S rRNA gene generally decreased with increasing depth in stratified lake sediments (Chan et al., 2005; Zhu et al., 2012 (top 21 cm at 3 cm intervals)). But in shallow and eutrophic lakes, both archaeal 16S rRNA gene and *mcrA* gene abundance could fluctuate along the sediment-depth gradient (Ye et al., 2009 (top 30 cm at 5 cm intervals); Zhu et al., 2012). The water depth, as well as trophic status, might have an influence on the depth-related change.

The abundance of methanogens could be assessed using either order-specific archaeal 16S rRNA gene primers or *mcrA* gene primers. Methanogen abundance was usually found to rise at first, followed by a decrease with the increasing depth in top 50 cm, and its peak occurred at the surface layer or 5–10 cm beneath the sediment surface (Borrel et al., 2012; Milferstedt et al., 2010; Zhu et al., 2012), which are consistent with the results found in this study.

In the current study, the abundance of the archaeal 16S rRNA gene was comparable to that reported in the literature (about 1×10^7–2×10^9 copies gDW^{-1} in top 20 cm) (Borrel

et al., 2012; Zhu et al., 2012). However, for each sample, the *mcrA* / 16S ratio was less than 3 % according to the results of qPCR, while the sequences affiliated with methanogen-like organisms accounted for 3–17 % of total 16S rRNA sequences. The result suggested either the bias of amplification of the *mcrA* gene or the difference between the two archaeal 16S rRNA primer sets used in the current study.

4.3 Diversity of archaeal and methanogen communities in freshwater lake sediment

The diversity of archaeal communities displayed a slight fluctuation along the sediment-layer depth gradient, which was in agreement with literature considering different sampling depth, e.g., top 14 cm sliced at 2–3 cm intervals (Koizumi et al., 2004), top 180 cm sliced at 20 cm intervals (Lim et al., 2011), and top 60 cm sliced at 3–9 cm intervals (Nam et al., 2008). To date, the diversity of the *mcrA* gene was unclear, partly due to the usage of order-specific primers of methanogens in the previous studies (Borrel et al., 2012; Zhu et al., 2012). Moreover, information on the diversity of the methanogen 16S rRNA gene was lacking. In this study, relatively higher diversity of the *mcrA* gene was observed in the samples from upper layers (sample D1 in Dianchi Lake and sample E2 in Erhai Lake), but *mcrA* gene diversity did not show a clear change pattern. In addition, both community diversity and evenness of methanogens could vary with lake (Milferstedt et al., 2010; Youngblut et al., 2014). In this current study, the sediment samples from Erhai Lake had slightly lower *mcrA* gene diversity than those from Dianchi Lake (Table 2).

4.4 Composition of methanogen community in freshwater lake sediment based on 16S rRNA gene

In the present study, hydrogenotrophic *Methanomicrobiales* had a high proportion in sediment from each layer depth, which agreed with previous studies (Biderre-Petit et al., 2011; Youngblut et al., 2014). It had been detected in a variety of environments, and was the most frequently observed archaeal order in freshwater lake sediment. *Methanobacteriales* was mainly distributed in Dianchi Lake samples D1, D2, and D3 and Erhai Lake sample E1. Abundance of *Methanobacteriales* has also been observed in hypereutrophic Priest Pot (Earl et al., 2003) and eutrophic Taihu Lake (Ye et al., 2009). The distribution of *Methanobacteriales* indicated that it might have a preference to high levels of nutrients. *Methanosarcinales* showed a lower proportion in Dianchi Lake and Erhai Lake than in other previous reported freshwater lakes (Biderre-Petit et al., 2011; Borrel et al., 2012; Zhu et al., 2012). Methanogens from the order *Methanosarcinales* mainly participated in reducing acetate and methyl compounds (Borrel et al., 2011), so their low proportion might partly account for the small contribution of hydrogenotrophic pathways.

In this study, the result of a UniFrac-based cluster analysis (Fig. 5a) indicated that archaeal community structure differed evidently in Dianchi Lake and Erhai Lake. In either Dianchi Lake or Erhai Lake, layer depth was found to be a key determinant in archaeal community structure. However, the abrupt shift in archaeal community structure occurred at different layer depths in these two lakes. In Dianchi Lake, the structures of archaeal communities at layer depth 0–11 cm (including samples D1, D2, and D3) were much different from those at layer depth 11–20 cm (including samples D4, D5, and D6). However, in Erhai Lake, a remarkable difference lay between the sample from uppermost layer (D1) and those from other five layers. So far, only Ye et al. (2009) documented the layer-depth-related change of archaeal community structure in Taihu Lake, while its change pattern was not clear.

4.5 Composition of methanogen community in freshwater lake sediment based on the *mcrA* gene

Based on *mcrA* gene clone library analysis, *Methanomicrobiales* was found to be the dominant methanogen group in both lakes, which agreed with other studies (Biderre-Petit et al., 2011; Youngblut et al., 2014). In the current study, the proportion of *Methanoregula*-like *mcrA* sequences tended to decrease with increasing sediment-layer depth, while the proportion of *Methanolinea*-like *mcrA* sequences did not show a clear change pattern. *Methanoregula* and *Methanolinea*, affiliated with *Methanomicrobiales*, were among the most frequently detected archaeal genera in freshwater lakes (Borrel et al., 2011). So far, the change pattern of methanogen community at genus level along the layer-depth gradient has not been addressed. In this study, *Methanoplasmatales*-like methanogens were detected in both Dianchi Lake and Erhai Lake, but the distribution pattern along sediment-layer depth in these two lakes was different. *Methanoplasmatales*-like methanogens were related to *Thermoplasmatales* Archaea. It was usually present in termite guts and high-salinity environments (e.g., marine sediment), and was the latest found order of methanogens (Paul et al., 2012). Only a few previous studies reported their existence in freshwater lake sediment (Conrad et al., 2014; Liu et al., 2013; Webster et al., 2014). *Methanosarcinales* and *Methanobacteriales* were also detected in different sediment layers in both lakes. However, their change patterns were not clear because of their low relative abundance. In addition, compared with other freshwater lake sediments (Borrel et al., 2012; Zhu et al., 2012), sediments in Dianchi Lake and Erhai Lake showed a much higher *Methanomicrobiales* proportion but lower *Methanosarcinales* proportion.

In this study, the results based on the *mcrA* gene clone library analysis were not always consistent with those based on 16S rRNA Illumina MiSeq sequencing. For an example, *Methanosarcinales* organisms accounted for 7–20 % of the total methanogens according to 16S rRNA sequencing, but

showed a very low proportion in the *mcrA* clone library (less than 3 % in 11 out of 12 samples). *Methanobacteriales* was abundant in uppermost layer sediments based on 16S rRNA sequencing, but it was a minor group in the *mcrA* clone library. Since the phylogeny of the *mcrA* gene was congruent with that of the 16S rRNA gene (Luton et al., 2002; Springer et al., 1995), the difference between the results based on the two molecular approaches might be attributed to PCR bias.

UniFrac-based cluster analysis indicated that methanogen communities in sediments of Dianchi Lake and Erhai Lake were not phylogenetically separated. Sediment depth could shape methanogen community structure, although methanogen communities in surface sediment were usually found to be lake-dependent (Milferstedt et al., 2010; Youngblut et al., 2014).

5 Conclusions

The MPP and abundance of sediment methanogens differed greatly in Dianchi Lake and Erhai Lake, while these two lakes had a similar methanogen community structure, with a dominance of *Methanomicrobiales* and *Methanobacteriales*. Hydrogenotrophic methanogenesis was the major methane production pathway in sediments of both lakes. The layer depth-related changes of methanogenesis potential and the abundance and community structure of methanogens were observed in both Dianchi Lake and Erhai Lake. Sediment methanogen community and activity might be influenced by lake trophic status.

6 Data availability

The *mcrA* sequences were deposited in the GenBank database under accession numbers KU997795-KU997842,KX196972-KX197020, and KX093502-KX093920, while the raw archaeal 16S rRNA gene reads were deposited in the NCBI SRA (short-read archive) under accession SRP076837.

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Competing interests. The authors declare that they have no conflict of interest.

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