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

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23 **Abstract**

24 Microbial methanogenesis in sediment plays a crucial role in CH_4 emission from
25 freshwater lake ecosystem. However, knowledge on the layer depth-related changes
26 of methanogen community structure and activities in freshwater lake sediment is still
27 limited. The present study was conducted to characterize the methanogenesis potential
28 in different sediment layer depths and the vertical distribution of microbial
29 communities in two freshwater lakes at different trophic status on the Yunnan Plateau
30 (China). Incubation experiments and inhibitor studies were carried out to determine
31 the methanogenesis potential and pathways. *McrA* and 16S rRNA genes were used to
32 investigate the abundance and structure of methanogen and archaeal communities,
33 respectively. Hydrogenotrophic methanogenesis was mainly responsible for methane
34 production in sediments of both freshwater lakes. The layer depth-related changes of
35 methanogenesis potential, and the abundance and community structure of
36 methanogens were observed in either Dianchi Lake or Erhai Lake. *mcrA* and archaeal
37 16S rRNA genes displayed the similar abundance change pattern in either lake, and
38 the relative abundance of methanogens decreased with increasing sediment layer
39 depth. Archaeal communities differed considerably in Dianchi Lake and Erhai Lake,
40 but methanogen communities showed a slight difference between in these two lakes.
41 However, methanogen communities illustrated a remarkable layer depth-related
42 change. Order *Methanomicrobiales* was the dominant methanogen group in all
43 sediments, while *Methanobacteriales* showed high proportion only in upper layer
44 sediments. The trophic status of lake might have a notable influence on the depth-

45 related change pattern of methangenesis activity, while the methanogen community
46 structure was mainly influenced by sediment depth.
47
48 **Keywords:** Methanogenesis pathways; Freshwater lake sediment; *Archaea*; *mcrA*;
49 Trophic status
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67 1. **Introduction**

68 Methane (CH_4) is an effective greenhouse gas in atmosphere, and lacustrine
69 ecosystems may be responsible for 6–16% of natural methane emission (Bastviken et
70 al., 2004). In anoxic sediment of freshwater lake, a large amount of methane can be
71 produced through microbial methanogenesis (Bastviken et al., 2008; Gruca-Rokosz
72 and Tomaszek, 2015). Biogenic methane is produced by the activity of methanogens,
73 a strictly anaerobic microbial group belonging to the archaeal phylum *Euryarchaeota*.
74 Methanogens from seven archaeal orders have been reported (Garcia, 1990; Paul et al.,
75 2012; Sakai et al., 2008), and their substrate is generally the end products of organic
76 matter degradation by fermentative bacteria and archaea (Borrel et al., 2011). In
77 freshwater lake, organic matter is fermented to acetate, CO_2 and H_2, which are further
78 converted to CH_4 by methanogens. There are two major methanogenic pathways,
79 namely hydrogenotrophic pathway (using H_2/CO_2) and acetoclastic pathway (using
80 acetate, i.e. the methyl group) (Conrad et al., 2010). The relative contribution of these
81 two pathways varies in different lakes (Conrad, 1999). To determine the contribution
82 of both methanogenic pathways, approaches including isotope labeling, $\delta^{13}C$ analysis
83 and inhibitor study have been applied (Conrad, 2005). Despite the theoretical ratio of
84 2:1 (acetoclastic pathway: hydrogenotrophic pathway) when carbohydrates or other
85 similar form of organic matter is degraded (Conrad, 1999), hydrogenotrophic pathway
86 can account for a considerable proportion of methane production in freshwater lake
87 sediment (Borrel et al., 2011). However, the relative significance of hydrogenotrophic
88 pathway remains unclear, because it can vary considerably with lake (Conrad, 1999).

89 To identify the methanogens involved in methanogenesis, both archaeal 16S rRNA
90 gene and functional *mcrA* gene have been used (Conrad et al., 2007; Luton et al.,
91 2002; Orphan et al., 2008). The dominance of *Methanomicrobiales* and
92 *Methanosarcinale* have been reported in a variety of freshwater lakes (Biderre-Petit et
93 al., 2011; Conrad et al., 2007; Youngblut et al., 2014). In freshwater lakes, both
94 methanogenesis pathway and methanogenic community structure can change with
95 sediment layer depth (Chan et al., 2005; Liu et al., 2016; Lofton et al., 2015).
96
97 Many previous studies have investigated methanogenesis in humic lakes (Youngblut
98 et al., 2014), oligotrophic lakes (Lofton et al., 2015), and meromictic lakes
99 (Biderre-Petit et al., 2011; Gies et al., 2014), while shallow meso- and eutrophic lakes
100 have attracted poor attention. Substrate plays an important role in methanogenesis,
101 and has a considerable effect on pathway preference (Liu et al., 2016; Nozhevnikova
102 et al., 2007). Methanogenesis in meso- and eutrophic lakes that have abundant
103 substrate might be different from that in other previously studied lakes. Therefore, for
104 sediments of mesotrophic and eutrophic lakes, the following questions have attracted
105 our attention, (1) How do the methanogenesis activity and contribution of different
106 pathways change with the increasing sediment layer depth? (2) How do methanogen
107 community structure and the dominant methanogens change along the sediment layer
108 depth? (3) Are these changing patterns similar in lakes at different trophic status?
109

110 2. **Materials and methods**

111 2.1. *Sampling sites and samples*

112 Dianchi Lake (with an area of 309 km^2 and an average water depth of 4.4 m) and
113 Erhai Lake (with an area of 250 km^2 and an average water depth of 10 m) are the first
114 and second largest freshwater lakes on Yunnan Plateau, China, and are of eutrophic
115 and mesotrophic status, respectively (Wang et al., 2015). The characteristics and
116 trophic status of these two lakes have been overviewed in detail by Wang (et al.,
117 2015), and some key features were listed in the Table 1.

118

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

126

127 The five replicate sediment cores were sliced into the layers (sample D1 or E1:0–5 cm,
128 sample D2 or E2:5–8 cm, sample D3 or E3:8–11 cm, sample D4 or E4:11–14 cm, and
129 sample D5 or E5:14–17 cm, sample D6 or E6:17–20 cm). Samples D1–D6 and E1–E6
130 were from Dianchi Lake and Erhai Lake, respectively. Replicate sediment samples
131 from the same layer depth in a given lake were mixed and then subsampled for
132 physicochemical and molecular analyses and incubation experiments. Sediment

133 samples for physicochemical and molecular analyses were stored at -20°C, while
134 those for incubation experiments were kept in gas-tight bottles under anoxic condition
135 at 4°C. The sediment samples were transported to laboratory within one week, which
136 would have no considerable effect on methanogenic activity (Nüsslein et al., 2001).
137
138 2.2. *Methane production potential measurement*
139 The incubation experiments for methane production measurement were performed
140 with reference to the standard procedure described in the literature (Conrad et al.,
141 2010). Uppermost sediment (0–5 cm) was centrifuged at 4000 rpm for 5 min to obtain
142 the similar water content with the samples from other layers. For each sediment layer,
143 a total of six sediment samples (1.5 g) and 8 mL bottom water were transferred into a
144 50-mL sterile serum bottle, flushed with N_2, and then closed with a butyl rubber
145 stopper. After incubation at 16°C overnight, the bottles were flushed with N_2 again,
146 and half of them were added with CH_3F (1 mL) to inhibit the aceticlastic
147 methanogenesis. Incubation was carried out at 16°C at 100 rpm for 28 days. At the
148 end of incubation, gas samples were taken from the headspace using a gas-tight
149 syringe, and then were analyzed using GC126 gas chromatography (INESA
150 instrument, Shanghai) with a flame ionization detector.
151
152 2.3 *Molecular analysis*
153 DNA was extracted using the Powersoil DNA extraction kit (Mobio Laboratories,
154 USA). The quality of DNA was checked using 1.0% agarose gel electrophoresis. For

155 quantitative PCR (qPCR), the primer sets mcrF

156 (5'-GGTGGTGTMGGATTCACACARTAYGCWACAGC-3') /mcrR

157 (5'-TCATTGCRTAGTTWGGRTAGTT-3') (Luton et al., 2002) and Arch344F

158 (5'-GYGCAGCAGGCGCGA-3') /Arch915R (5'-GTGCTCCCCCGCCAATTCCT-3')

159 (Casamayor et al., 2002; Conrad et al., 2014) were used for the quantification of *mcrA*

160 and archaeal 16S rRNA genes, respectively. The qPCR assay was carried out using an

161 ABI 7500 FAST (Applied Biosystems).The reaction mixture included 2×SYBR Green

162 PCR master mix (12.5 μL), 10 μM of each primer (1 μL), and template DNA (2 ng).

163 The PCR conditions were as described in the literatures (Casamayor et al., 2002;

164 Luton et al., 2002). Standard curves ranging from 10^3 to 10^7 gene copies/μL were

165 generated using serial dilutions of linearized plasmids (pGEM-T, Promega) containing

166 cloned target gene amplified from environmental DNA. The coefficient (r^2) for

167 archaeal 16S rRNA gene and *mcrA* gene were 0.9995 and 0.9998, respectively.

168 Significance was tested using one-way analysis of variance (ANOVA), followed by

169 S-N-K's post-hoc analysis (when the variances were homogenous) or Dunnett's

170 post-hoc analysis (when the variances were not homogenous).

171

172 For Illumina MiSeq sequencing, archaeal 16S rRNA gene was amplified using primer

173 set Arch519f (5'-CAGCCGCCGCGGTAA-3')/Arch915R

174 (5'-GTGCTCCCCCGCCAATTCCT-3') (He et al. 2016; Herfort et al., 2009; Long et

175 al. 2016). The PCR products from triplicate samples were mixed in equal amounts

176 and were subjected to Illumina MiSeq sequencing. Raw reads were merged and the

177 quality filtering was carried out using Trimmomatic (Bolger et al., 2014) and FLASH
178 (Magoc and Salzberg, 2011). The obtained raw reads were deposited in the NCBI
179 SRA (short-read archive) under accession SRP076837. After subsampling to the
180 lowest number of sequences, sequences from each sediment sample were checked for
181 chimeras and grouped into OTUs (operational taxonomic units) using Usearch
182 (version 7.1, http://drive5.com/uparse/) at 97% similarity. Diversity analysis was
183 carried out using the MOTHUR program (version v.1.30.1) (Schloss et al., 2011). The
184 taxonomic identities of representative sequences for each OTU were assigned
185 according to the Silva 16S rRNA database (Quast et al., 2013). For *mcrA* gene clone
186 library analysis, primer set mcrF/mcrR was used, and the PCR conditions were as
187 previously described (Luton et al., 2002). The obtained *mcrA* sequences were
188 deposited in the GenBank database under accession numbers KU997795-KU997842,
189 KX196972-KX197020 and KX093502-KX093920. The *mcrA* gene sequences were
190 grouped into OTUs at the similarity level of 89% (Webster et al., 2014) using the
191 MOTHUR program. OTU-based diversity was also calculated using the MOTHUR
192 program (Schloss et al., 2009). Phylogenetic analysis of the *mcrA* gene sequences was
193 carried out with the MEGA 6.0 software (Tamura et al., 2013). Weighted Unifrac
194 distance between samples was calculated using *R* library GUniFrac, and cluster
195 analysis were conducted based on Weighted Unifrac distance using R (version i386,
196 3.3.0).
197
198 ## 3. **Results**

199 3.1. *Total methane production*

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

208

209 The inhibited methane production (IMP) was measured under the condition of 2%
210 CH_3F. IMP showed a similar change trend with TMP in either of the studied two
211 freshwater lakes. With increasing depth, IMP generally increased in Dianchi Lake but
212 tended to decrease in Erhai Lake. Dianchi Lake had the IMP of 47.0–182.8
213 nmol/gDW, but Erhai Lake had a much lower IMP (9.5–38.1 nmol/gDW). Besides,
214 uninhibited methane production (mainly produced through aceticlastic pathway)
215 varied greatly in different layers (2.0-84.0 nmol/gDW in Dianchi Lake, 1.7–13.7
216 nmol/gDW in Erhai Lake), and showed a notable difference between these two
217 freshwater lakes.

218

219 3.2. *Community abundance of Archaea and methanogen*

220 The depth-related change pattern of either archaeal 16S rRNA or *mcrA* gene differed

221 in Dianchi Lake and Erhai Lake (Figure 2). The density of archaeal 16S rRNA gene

222 fluctuated between $2.9 \pm 0.1 \times 10^7$ and $8.4 \pm 0.8 \times 10^8$ copies/gDW in Dianchi Lake. In

223 Erhai Lake, the archaeal community abundance ranged from $2.7 \pm 0.1 \times 10^7$ to 3.4 ± 0.1

224 $\times 10^8$ copies/gDW, and showed an increase with depth followed by a significant

225 decrease ($P<0.05$), with the peak value in the 5–8-cm layer.

226

227 As for *mcrA* gene, its highest density was observed at the uppermost layer (0–5 cm) in

228 either Dianchi Lake ($1.06 \pm 0.06 \times 10^6$ copies/gDW) or Erhai Lake ($5.6 \pm 0.2 \times 10^5$

229 copies/gDW), while the lowest one occurred in Dianchi Lake sample D6 (17–20cm,

230 $2.5 \pm 0.3 \times 10^4$ copies/gDW) or Erhai Lake sample E5 (14–17 cm, $3.7 \pm 0.1 \times 10^4$

231 copies/gDW). The gene abundance generally decreased with increasing depth. In

232 addition, either *Archaea* or methanogens showed greater abundance in Dianchi Lake

233 than in Erhai Lake.

234

235 3.3. *Diversity of archaeal and methanogen communities*

236 In this study, after subsampling, a total of 16,028 archaeal sequences were retrieved

237 from each sediment sample. The number of OTUs in each library ranged between 547

238 and 1194, and library coverage was 98.07–99.64% (Table 2). Archaeal community

239 diversity of most sediment samples varied slightly (Shannon index=4.51–5.13),

240 whereas samples D2 and D3 had much lower Shannon diversity (3.98 or 3.88). The

241 samples from Erhai Lake showed higher archaeal diversity (Shannon index=4.81–

242 5.13) than those from Dianchi Lake (Shannon index=3.88–4.75).

243

244 A total of 516 *mcrA* gene sequences were retrieved from *mcrA* clone library, and

245 could be assigned into 30 OTUs (at 89% similarity). For each sample, the number of

246 sequences ranged from 35 to 49, while the number of OTUs varied between 6 and 11.

247 The coverage for each clone library was no less than 85.7%, indicating that the *mcrA*

248 OTUs of each sample had been well captured. The Shannon indices were 1.49–1.72

249 for Dianchi Lake and 1.25–1.84 for Erhai Lake, respectively. In Dianchi Lake, sample

250 D1 had the highest *mcrA* gene diversity, but sample D6 had the lowest diversity. In

251 Erhai Lake, sample E2 had the highest *mcrA* gene diversity followed by sample E1,

252 but E5 showed the lowest diversity.

253

254 3.4. *16S rRNA-based community composition*

255 *Bathyarchaota* (formally known as *Miscellaneous Crenarchaeotic_Group*, *MCG*) and

256 *Euryarchaeota* dominated the archaeal communities in both Dianchi Lake and Erhai

257 Lake (Figure 3). In the uppermost sediment layer in either lake, *Bathyarchaota*

258 accounted for nearly 20% of the total archaeal community. However, in Dianchi Lake,

259 with increasing layer depth, the proportion of *Bathyarchaota* showed a remarkable

260 decrease followed by a rise. *Bathyarchaota* organisms showed high proportion (44–

261 55%) in Dianchi Lake samples D4–D6 and Erhai Lake samples E2–E6. In contrast,

262 *Euryarchaeota* illustrated an opposite change trend. In Dianchi Lake, the proportion

263 of *Euryarchaeota* organisms was higher in samples D2 and D3 (63% or 67%) than in

264 sample D1 (47%), but displayed a notable decrease in deeper layers (26–27%). In

265 Erhai Lake, the *Euryarchaeota* proportion in sample E1 was 32.5% but became lower
266 in samples E2–E6 (9–23%). In addition, *Woesearchaeota* showed a greater abundance
267 in Dianchi Lake (8–13%) than in Erhai Lake (4–9%), while *Crenarchaeota* was
268 mainly distributed in Erhai Lake (3–29%).
269
270 Based on 16S rRNA gene analysis, methanogens comprised 3–17% of the total
271 archaeal community (Figure S1). The relative abundance of methanogens tended to
272 decrease with increasing sediment layer depth. Order *Methanobacteriales* dominated
273 the methanogen community in the uppermost layer samples (samples D1 or E1), and
274 also had a considerable proportion in samples D2 and D3. In contrast,
275 *Methanomicrobiales* was the dominant methanogen group in the lower layer
276 sediments (Dianchi Lake samples D2–D6 and Erhai Lake samples E2–E6).
277 *Methanocellales* and *Methanosarcinales* could also be detected in all sediment
278 samples, but their proportions were less than 2%. At genus level, *Methanobacterium*
279 (within *Methanobacteriales*) had the greatest proportion, followed by *Methanosaeta*
280 (within *Methanosarcinales*) and *Methanoregula* (within *Methanomicrobiales*), and the
281 proportion of each archaeal genus decreased with increasing layer depth in either lake
282 (data not shown).
283
284 3.5. *mcrA-based community composition*
285 In the present study, the 516 *mcrA* sequences from all sediment samples fell into 30
286 OTUs, 12 of which had only one sequence. The representative sequences from the

287 OTUs with at least two sequence members were further used to construct the
288 phylogenic tree with their close *mcrA* sequences reported in the NCBI database
289 (Figure 4). All of the sequences were grouped into five clusters (clusters 1–5). Cluster
290 1 contained most of the obtained *mcrA* sequences (440), and could be affiliated with
291 the *mcrA* sequences from *Methanomicrobiales*. Cluster 2 consisted of 29 *mcrA*
292 sequences related to those from *Thermoplasmatales* and *Methanoplasmatales*. Cluster
293 3 (with 13 sequence members) and cluster 5 (with 12 sequence members) were related
294 to *Methanobacteriales* and *Methanosarcinale*, respectively. Cluster 4 was the smallest
295 group and only contained 9 sequences. The sequences in this cluster were not related
296 to the *mcrA* sequences from known methanogens, but showed a close relation to an
297 uncultured sequence from paddy soil. In addition, the sequences affiliated to cluster 1
298 could be further divided into 3 clades. OTU7 was grouped together with the
299 sequences from two *Methanomicrobiaceae* strains. OTU8 showed a close relation to a
300 *Methanolinea*-like *mcrA* sequence, and OTU1, OTU2 and OTU6 were closely related
301 to *Methanoregula*-like *mcrA* sequence.
302

303 *Methanoregula*-like *mcrA* sequences (OTU1 and OTU2) were detected in all sediment
304 layer depths, but the proportion of OTU1 decreased with increasing layer depth
305 (Figure S2). *Methanomicrobiaceae*-like *mcrA* sequences (OTU7) were not detected in
306 Dianchi Lake samples D1, D2 and D3, but they dominated in the other samples and
307 their proportion increased with increasing sediment layer depth. *Methanoplasmatales*
308 -like *mcrA* sequences (OTU13) mainly existed in Dianchi Lake, but were also

309 detected in the uppermost sediment layer in Erhai Lake. *Methanolinea*-like *mcrA*
310 sequences (OTU8) were present in all sediment layers in both lakes, but their change
311 pattern was not evident. In addition, other genotypes of *mcrA* gene sequences
312 comprised less than 22% of the total sequences in each sample.
313
314 3.6. *Comparison of archaeal and methanogen communities*
315 The difference among archaeal assemblages was discriminated using
316 Weighted-Unifrac distance-based cluster analysis (Figure 5a). In either Dianchi Lake
317 or Erhai Lake, the sample from the uppermost layer (sample D1 or E1) was separated
318 from the samples in other sediment layers. In Dianchi Lake, samples D2 and D3 were
319 also grouped into one clade, and the other three samples fell into another one.
320 However, in Erhai Lake, samples E2, E3, E4, E5 and E6 were grouped together, and
321 the samples from neighboring layers tended to have a relatively similar archaeal
322 community structure.
323
324 For methanogen communities, the studied 12 sediment samples fell into two groups
325 (Figure 5b). Samples D1, D2, D3 and E1 were clustered together. For other samples,
326 samples at similar layer depth tended to have relatively similar methanogen
327 community structure. Moreover, the sediment samples from two lakes were not
328 clearly separated.
329
330 4. **Discussion**

331 4.1 *Methane production potential in freshwater lake sediment*

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

339

340 Several previous studies had investigated the inter-lake difference of methanogenesis.
341 Methane production rate could differ drastically between the two geomorphologically
342 similar oligotrophic lakes, and the quantity and quality of water dissolved organic
343 carbon (DOC) might be an influential factor (Lofton et al., 2015). Other
344 environmental factors, including geological constitute (e.g., calcareous or not),
345 geographical regions (Rinta et al., 2015) as well as water type (e.g., black water, clear
346 water or something else) (Conrad et al., 2014), were also found to have considerable
347 influences on methane production rate in freshwater lake sediment. Moreover, lake
348 characteristics could influence the methane production rate both directly and
349 indirectly (Borrel et al., 2011). In this study, the methanogenesis potential in Dianchi
350 Lake was found to be much higher than that in Erhai Lake. Dianchi Lake had a larger
351 amount of nutrient than Erhai Lake, and a high level of organic matter could exist in
352 lower sediment layers (Figure S3). TMP was found to be related to the availability of

353 organic matter (Liu et al., 2016; Lofton et al., 2015; Nozhevnikova et al., 2007), so
354 the abundant substrate in Dianchi Lake could favor higher TMP. In addition, although
355 methanogen communities in Dianchi Lake and Erhai Lake had similar structure, either
356 archaeal or bacterial community differed greatly in these two lakes (Dai et al., 2016;
357 Yang et al., 2016). Bacterial and non-methanogen archaeal community played
358 important roles in decomposing organic matter, and thus influenced the availability of
359 substrate.

360

361 In the current study, IMP accounted for only 24.2% of TMP, so it could be inferred
362 that hydrogenotrophic pathway might play a major role in Dianchi Lake. The result
363 was consistent with previous studies (Conrad et al., 2010; Liu et al., 2013; Liu et al.,
364 2016). However, in Erhai Lake, IMP was comparable to uninhibited methane
365 production. In this study, the layer depth-related difference in the relative contribution
366 of two methanogenic pathways was not clear, though acetoclastic methangenesis's
367 relative contribution was found to decrease with increasing lake sediment depth in
368 severtal studies (Chan et al., 2005; Liu et al., 2016). The depth-related change of
369 acetoclastic methangenesis's relative contribution might be attributed to the
370 decreasing availability of organic matter (Liu et al., 2016). However, in eutrophic
371 Dianchi Lake, organic matter could be at a high level even at the depth of 20 cm. As a
372 result, acetoclastic methangenesis might not decrease considerably with increasing
373 depth.

374

375 4.2. *Abundance of Archaea and methanogen in freshwater lake sediment*

376 In the current study, the density of archaeal 16S rRNA gene tended to decrease with
377 increasing depth in Erhai Lake but considerably fluctuated in Dianchi Lake.
378 According to the previous studies, the abundance of archaeal 16S rRNA gene
379 generally decreased with increasing depth in stratified lake sediments (Chan et al.,
380 2005; Zhu et al., 2012 (top 21 cm at 3 cm interval)). But in shallow and eutrophic
381 lakes, both archaeal 16S rRNA gene and *mcrA* gene abundance could fluctuate along
382 the sediment depth gradient (Ye et al., 2009 (top 30 cm at 5 cm interval); Zhu et al.,
383 2012). The water depth, as well as trophic status, might have an influence on the
384 depth-related change.

385

386 The abundance of methanogens could be assessed using either order-specific archaeal
387 16S rRNA gene primers or *mcrA* gene primers. Methanogen abundance was usually
388 found to rise at first followed by a decrease with the increasing depth in top 50 cm,
389 and its peak occurred at the surface layer or 5–10 cm beneath the sediment surface
390 (Borrel et al., 2012; Milferstedt et al., 2010; Zhu et al., 2012), which consisted with
391 the results found in this study.

392

393 In the current study, the abundance of archaeal 16S rRNA gene was comparable to
394 that reported in the literatures (about 1×10^7–2×10^9 copies/gDW in top 20 cm) (Borrel
395 et al., 2012; Zhu et al., 2012). However, for each sample, the *mcrA*/16S ratio was less
396 than 3% according to the results of qPCR, while the sequences affiliated with

397 methanogen-like organisms accounted for 3–17% of total 16S rRNA sequences. The
398 result suggested either the bias of amplification of *mcrA* gene or the difference
399 between the two archaeal 16S rRNA primer sets used in the current study.
400
401 4.3. *Diversity of archaeal and methanogen communities in freshwater lake sediment*
402 The diversity of archaeal community displayed a slight fluctuation along the sediment
403 layer depth gradient, which was in agreement with literatures considering different
404 sampling depth, e.g., top 14 cm sliced at 2–3 cm interval (Koizumi et al., 2004), top
405 180 cm sliced at 20 cm interval (Lim et al., 2011), and top 60 cm sliced at 3–9 cm
406 interval (Nam et al., 2008). To date, the diversity of *mcrA* gene was unclear, partly
407 due to the usage of order-specific primers of methanogens in the previous studies
408 (Borrel et al., 2012; Zhu et al., 2012). Moreover, information on the diversity of
409 methanogen 16S rRNA gene was lacking. In this study, relatively higher diversity of
410 *mcrA* gene was observed in the samples from upper layers (sample D1 in Dianchi
411 Lake and sample E2 in Erhai Lake), but *mcrA* gene diversity did not show a clear
412 change pattern. In addition, both community diversity and evenness of methanogens
413 could vary with lake (Milferstedt et al., 2010; Youngblut et al., 2014). In this current
414 study, the sediment samples from Erhai Lake had slightly lower *mcrA* gene diversity
415 than those from Dianchi Lake (Table 2).
416
417 4.4 *Composition of methanogen community in freshwater lake sediment based on 16S*
418 *rRNA gene*

419 In the present study, hydrogenotrophic *Methanomicrobiales* had high proportion in
420 sediment from each layer depth, which agreed with previous studies (Biderre-Petit et
421 al., 2011; Youngblut et al., 2014). It had been detected in a variety of environments,
422 and was the most frequently observed archaeal order in freshwater lake sediment.
423 *Methanobacteriales* was mainly distributed in Dianchi Lake samples D1, D2 and D3
424 and Erhai Lake sample E1. Abundance of *Methanobacteriales* has also been observed
425 in hypereutrophic Priest Pot (Earl et al., 2003) and eutrophic Taihu Lake (Ye et al.,
426 2009). The distribution of *Methanobacteriales* indicated that it might have a
427 preference to high levels of nutrients. *Methanosarcinales* showed lower proportion in
428 Dianchi Lake and Erhai Lake than in other previous reported freshwater lakes
429 (Biderre-Petit et al., 2011; Borrel et al., 2012; Zhu et al., 2012). Methanogens from
430 order *Methanosarcinales* mainly participated in reducing acetate and methyl
431 compounds (Borrel et al., 2011), so their low proportion might partly account for the
432 small contribution of hydrogenotrophic pathway.
433
434 In this study, the result of UniFrac-based cluster analysis (Figure 5a) indicated that
435 archaeal community structure differed evidently in Dianchi Lake and Erhai Lake. In
436 either Dianchi Lake or Erhai Lake, layer depth was found to be a key determinant to
437 archaeal community structure. However, the abrupt shift in archaeal community
438 structure occurred at different layer depths in these two lakes. In Dianchi Lake, the
439 structures of archaeal communities at layer depth 0–11 cm (including samples D1, D2
440 and D3) were much different from those at layer depth 11–20 cm (including samples

441 D4, D5 and D6). However, in Erhai Lake, a remarkable difference lay between the
442 sample from uppermost layer (D1) and those from other five layers. So far, only Ye et
443 al. (2009) documented the layer depth-related change of archaeal community structure
444 in Taihu Lake, while its change pattern was not clear.
445
446 4.5. *Composition of methanogen community in freshwater lake sediment based on*
447 *mcrA gene*
448 Based on *mcrA* gene clone library analysis, *Methanomicrobiales* was found to be the
449 dominant methanogen group in both lakes, which agreed with other studies
450 (Biderre-Petit et al., 2011; Youngblut et al., 2014). In the current study, the proportion
451 of *Methanoregula*-like *mcrA* sequences tended to decrease with increasing sediment
452 layer depth, while the proportion of *methanolinea*-like *mcrA* sequences did not show a
453 clear change pattern. *Methanoregula* and *Methanolinea*, affiliated within
454 *Methanomicrobiales*, were among the most frequently detected archaeal genera in
455 freshwater lake (Borrel et al., 2011). So far, the change pattern of methanogen
456 community at genus level along the layer depth gradient has not been addressed. In
457 this study, *Methanoplasmatales*-like methanogens were detected in both Dianchi Lake
458 and Erhai Lake, but the distribution pattern along sediment layer depth in these two
459 lakes was different. *Methanoplasmatales*-like methanogens were related to
460 *Thermoplasmatales* archaeons. It was usually present in termite guts and high-salinity
461 environments (e.g., marine sediment), and was the latest found order of methanogens
462 (Paul et al., 2012). Only several previous studies reported their existence in

463 freshewater lake sediment (Conrad et al., 2014; Liu et al., 2013; Webster et al., 2014).
464 *Methanosarcinales* and *Methanobacteriales* were also detected in different sediment
465 layers in both lakes. However, their change patterns were not clear because of their
466 low relative abundance. In addition, compared with other freshwater lake sediments
467 (Borrel et al., 2012; Zhu et al., 2012), sediments in Dianchi Lake and Erhai Lake
468 showed much higher *Methanomicrobiales* proportion but lower *Methanosarcinales*
469 proportion.
470
471 In this study, the results based on *mcrA* gene clone library were not always consistent
472 with that based on16S rRNA Illumina MiSeq sequencing. For an example,
473 *Methanosarcinales* organisms accounted for 7–20% of the total methanogens
474 according to 16S rRNA sequencing, but showed a very low proportion in *mcrA* clone
475 library (less than 3% in 11 out of 12 samples). *Methanobacteriales* was abundant in
476 uppermost layer sediments based on 16S rRNA sequencing, but it was a minor group
477 in *mcrA* clone library. Since the phylogeny of *mcrA* gene was congruent with that of
478 16S rRNA gene (Luton et al., 2002; Springer et al., 1995), the difference between the
479 results base on the two molecular approaches might be attributed to PCR bias.
480
481 UniFrac-based cluster analysis indicated that methanogen communities in sediments
482 of Dianchi Lake and Erhai Lake were not phylogenetically separated. Sediment depth
483 could shape methanogen community structure, although methanogen communities in
484 surface sediment were usually found to be lake-dependent (Milferstedt et al., 2010;

485 Youngblut et al., 2014).

486

487 ## 5. Conclusions

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

496

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502

503 ## Competing interests

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

505

506 ## References

507 Bastviken, D., Cole, J., Pace, M., and Tranvik, L.: Methane emissions from lakes:
508 Dependence of lake characteristics, two regional assessments, and a global
509 estimate, Global Biogeochem. Cy., 18, GB4009, 2004.
510 Bastviken, D., Cole, J. J., Pace, M. L., and Van de Bogert, M. C.: Fates of methane
511 from different lake habitats: Connecting whole-lake budgets and CH_4 emissions,
512 J. Geophys. Res., 113, G02024, 2008.
513 Biderre-Petit, C., Jézéquel, D., Dugat-Bony, E., Lopes, F., Kuever, J., Borrel, G.,
514 Viollier, E., Fonty, G., and Peyret, P.: Identification of microbial communities
515 involved in the methane cycle of a freshwater meromictic lake, FEMS Microbiol.
516 Ecol., 77, 533-545, 2011.
517 Bolger, A. M., Lohse, M., and Usadel, B.: Trimmomatic: a flexible trimmer for
518 Illumina sequence data, Bioinformatics, 30, 2114-2120, 2014.
519 Borrel, G., Jezequel, D., Biderre-Petit, C., Morel-Desrosiers, N., Morel, J. P., Peyret,
520 P., Fonty, G., and Lehours, A. C.: Production and consumption of methane in
521 freshwater lake ecosystems, Res. Microbiol., 162, 832-847, 2011.
522 Borrel, G., Lehours, A.-C., Crouzet, O., Je ́quel D, and Rockne K: Stratification of
523 Archaea in the Deep Sediments of a Freshwater Meromictic Lake: Vertical Shift
524 from Methanogenic to Uncultured Archaeal Lineages, PLoS ONE, 7, e3346,
525 2012.
526 Casamayor, E., Massana, R., Benlloch, S., Øvreås, L., Díez, B., Goddard, V., Gasol, J.,
527 Joint, I., Rodríguez-Valera, F., and Pedrós-Alió, C.: Changes in archaeal,
528 bacterial and eukaryal assemblages along a salinity gradient by comparison of

529 genetic fingerprinting methods in a multipond solar saltern, Environ. Microbiol., 4, 338-348, 2002.
530
531 Chan, O. C., Claus, P., Casper, P., Ulrich, A., Lueders, T., and Conrad, R.: Vertical
532 distribution of structure and function of the methanogenic archaeal community in
533 Lake Dagow sediment, Environ. Microbiol., 7, 1139-1149, 2005.
534 Conrad, R.: Contribution of hydrogen to methane production and control of hydrogen
535 concentrations in methanogenic soils and sediments, FEMS Microbiol. Ecol., 28,
536 193-202, 1999.
537 Conrad, R.: Quantification of methanogenic pathways using stable carbon isotopic
538 signatures: a review and a proposal, Org. Geochem., 36, 739-752, 2005.
539 Conrad, R., Chan, O.-C., Claus, P., and Casper, P.: Characterization of methanogenic
540 Archaea and stable isotope fractionation during methane production in the
541 profundal sediment of an oligotrophic lake (Lake Stechlin, Germany), Limnol.
542 Oceanogr., 53, 1393-1406, 2007.
543 Conrad, R., Klose, M., Claus, P., and Enrich-Prast, A.: Methanogenic pathway, C-13
544 isotope fractionation, and archaeal community composition in the sediment of
545 two clear-water lakes of Amazonia, Limnol. Oceanogr., 55, 689-702, 2010.
546 Conrad, R., Ji, Y., Noll, M., Klose, M., Claus, P., and Enrich-Prast, A.: Response of
547 the methanogenic microbial communities in Amazonian oxbow lake sediments to
548 desiccation stress, Environ. Microbiol., 16, 1682-1694, 2014.

549 Dai, Y., Yang, Y., Wu, Z., Feng, Q., Xie, S., and Liu, Y.: Spatiotemporal variation of
550 planktonic and sediment bacterial assemblages in two plateau freshwater lakes at
551 different trophic status, Appl. Microbiol. Biotechnol., 100, 4161-4175, 2016.
552 Dan, J., Kumai, T., Sugimoto, A., and Murase, J.: Biotic and abiotic methane releases
553 from Lake Biwa sediment slurry, Limnology, 5, 149-154, 2004.
554 Duc, N. T., Crill, P., and Bastviken, D.: Implications of temperature and sediment
555 characteristics on methane formation and oxidation in lake sediments,
556 Biogeochemistry, 100, 185-196, 2010.
557 Earl, J., Hall, G., Pickup, R., Ritchie, D., and Edwards, C.: Analysis of methanogen
558 diversity in a hypereutrophic lake using PCR-RFLP analysis of *mcr* sequences,
559 Microbiol. Ecol., 46, 270-278, 2003.
560 Garcia, J. L.: Taxonomy and ecology of methanogens, FEMS Microbiol. Rev., 87,
561 297-308, 1990.
562 Gies, E. A., Konwar, K. M., Beatty, J. T., and Hallam, S. J.: Illuminating Microbial
563 Dark Matter in Meromictic Sakinaw Lake, Appl. Environ. Microbiol., 80,
564 6807-6818, 2014.
565 Gruca-Rokosz, R., and Tomaszek, J. A.: Methane and Carbon Dioxide in the
566 Sediment of a Eutrophic Reservoir: Production Pathways and Diffusion Fluxes at
567 the Sediment–Water Interface, Water Air Soil Poll., 226, 16, 2015.
568 He, T., Guan, W., Luan, Z., Xie, S.: Spatiotemporal variation of bacterial and archaeal
569 communities in a pilot-scale constructed wetland for surface water treatment.
570 Appl. Microbiol. Biotechnol., 100, 1479-1488, 2016.

571 Herfort, L., Kim, J. H., Coolen, M. J. L., Abbas, B., Schouten, S., Herndl, G. J.,
572 Damste, J. S. S.: Diversity of *Archaea* and detection of crenarchaeotal *amoA*
573 genes in the rivers, Rhine and Tet, Aquat. Microb. Ecol., 55, 189-201, 2009.
574 Koizumi, Y., Takii, S., and Fukui, M.: Depth-related change in archaeal community
575 structure in a freshwater lake sediment as determined with denaturing gradient
576 gel electrophoresis of amplified 16S rRNA genes and reversely transcribed rRNA
577 fragments, FEMS Microbiol. Ecol., 48, 285-292, 2004.
578 Lim, J., Woodward, J., Tulaczyk, S., Christoffersen, P., and Cummings, S. P.: Analysis
579 of the microbial community and geochemistry of a sediment core from Great
580 Slave Lake, Canada, Anton. Leeuw., 99, 423-430, 2011.
581 Liu, Y., Yao, T., Gleixner, G., Claus, P., and Conrad, R.: Methanogenic pathways, ^{13}C
582 isotope fractionation, and archaeal community composition in lake sediments
583 and wetland soils on the Tibetan Plateau, J. Geophys. Res. Biogeo., 118, 650-664,
584 2013.
585 Liu, Y., Conrad, R., Yao, T., Gleixner, G., and Claus, P.: Change of methane
586 production pathway with sediment depth in a lake on the Tibetan plateau,
587 Paleogeogr. Paleoclimatol. Paleoecol., doi: 10.1016/j.palaeo.2016.06.021, 2016.
588 Lofton, D. D., Whalen, S. C., and Hershey, A. E.: Vertical sediment distribution of
589 methanogenic pathways in two shallow Arctic Alaskan lakes, Polar Biol., 38,
590 815-827, 2015.
591 Long, Y., Zhang, Z. , Pan, X., Li, B., Xie, S. , Guo, Q.: Substrate influence on
592 archaeal and bacterial assemblages in constructed wetland microcosm, Ecol.

593 Eng., 94, 437-442, 2016.

594 Luton, P. E., Wayne, J. M., Sharp, R. J., and Riley, P. W.: The *mcrA* gene as an
595 alternative to 16S rRNA in the phylogenetic analysis of methanogen populations
596 in landfill, Microbiology, 148, 3521-3530, 2002.

597 Magoc, T., and Salzberg, S.: FLASH Fast Length Adjustment of Short Reads to
598 Improve Genome, Bioinformatics, 27, 2957-2963, 2011.

599 Milferstedt, K., Youngblut, N. D., and Whitaker, R. J.: Spatial structure and
600 persistence of methanogen populations in humic bog lakes, ISME J., 4, 764-776,
601 2010.

602 Nüsslein, B., Chin, K.-J., Eckert, W., and Conrad, R.: Evidence for anaerobic
603 syntrophic acetate oxidation during methane production in the profundal
604 sediment of subtropical Lake Kinneret (Israel), Environ. Microbiol., 3, 460-470,
605 2001.

606 Nam, Y. D., Sung, Y., Chang, H. W., Roh, S. W., Kim, K. H., Rhee, S. K., Kim, J. C.,
607 Kim, J. Y., Yoon, J. H., and Bae, J. W.: Characterization of the depth-related
608 changes in the microbial communities in Lake Hovsgol sediment by 16S rRNA
609 gene-based approaches, J. Microbiol., 46, 125-136, 2008.

610 Nozhevnikova, A. N., Nekrasova, V., Ammann, A., Zehnder, A. J. B., Wehrli, B., and
611 Holliger, C.: Influence of temperature and high acetate concentrations on
612 methanogenensis in lake sediment slurries, FEMS Microbiol. Ecol., 62, 336-344,
613 2007.

614 Orphan, V. J., Jahnke, L. L., Embaye, T., Turk, K. A., Pernthaler, A., Summons, R. E.,
615 and DJ, D. E. S. M.: Characterization and spatial distribution of methanogens
616 and methanogenic biosignatures in hypersaline microbial mats of Baja California,
617 Geobiology, 6, 376-393, 2008.
618 Paul, K., Nonoh, J. O., Mikulski, L., and Brune, A.: "*Methanoplasmatales*,"
619 *Thermoplasmatales*-related archaea in termite guts and other environments, are
620 the seventh order of methanogens, Appl. Environ. Microbiol., 78, 8245-8253,
621 2012.
622 Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., and
623 Glockner, F. O.: The SILVA ribosomal RNA gene database project: improved
624 data processing and web-based tools, Nucleic Acids Res., 41, D590-596, 2013.
625 Rinta, P., Bastviken, D., van Hardenbroek, M., Kankaala, P., Leuenberger, M.,
626 Schilder, J., Stötter, T., and Heiri, O.: An inter-regional assessment of
627 concentrations and $\delta^{13}C$ values of methane and dissolved inorganic carbon in
628 small European lakes, Aquat. Sci., 77, 667-680, 2015.
629 Sakai, S., Imachi, H., Hanada, S., Ohashi, A., Harada, H., and Kamagata, Y.:
630 *Methanocella paludicola* gen. nov., sp. nov., a methane-producing archaeon, the
631 first isolate of the lineage 'Rice Cluster I', and proposal of the new archaeal order
632 *Methanocellales* ord. nov, Int. J. Evol. Syst. Micr., 58, 929-936, 2008.
633 Schloss, P. D., Gevers, D., and Westcott, S. L.: Reducing the Effects of PCR
634 Amplification and Sequencing Artifacts on 16S rRNA-Based Studies, PLoS
635 ONE, 6, e27310, 2011.

636 Schloss, P. D.,Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B.,
637 Lesniewski, R. A., Oakley, B. B., Parks, D. H., Robinson, C. J., Sahl, J. W.,
638 Stres, B., Thallinger, G. G., Van Horn, D. J., Weber, C. F.: Introducing mothur,
639 open-source, platform-independent, community-supported software for
640 describing and comparing microbial communities. Appl. Environ. Microbiol.,
641 75, 7537-7541, 2009.
642 Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular
643 evolutionary genetics analysis version 6.0. Mol. Biol. Evol., 30, 2725-2729,
644 2013.
645 Springer, E., Sacks, M., Woese, C., and Boone, D.: Partial gene sequences for the a
646 subunit of methyl-coenzyme M reductase (*mcrI*) as a phylogenetic tool for the
647 family *Methanosarcinaceae*, Int. J. Syst. Evol. Micr., 45, 554-559, 1995.
648 Tamura, K., Stecher, G., Peterson, D., Filipski, A., and Kumar, S.: MEGA6: molecular
649 evolutionary genetics analysis version 6.0, Mol. Biol. Evol., 30, 2725-2729,
650 2013.
651 Wang, S., Zheng, B., Chen, C., Dohmann, M., and Kolditz, O.: Thematic issue: water
652 of the Erhai and Dianchi Lakes, Environ. Earth. Sci., 74, 3685-3688, 2015.
653 Webster, G., O'Sullivan, L. A., Meng, Y., Williams, A. S., Sass, A. M., Watkins, A. J.,
654 Parkes, R. J., and Weightman, A. J.: Archaeal community diversity and
655 abundance changes along a natural salinity gradient in estuarine sediments,
656 FEMS Microbiol. Ecol., 91, 1-18, 2014.

657 Yang, Y., Dai, Y., Wu, Z., Xie, S., and Liu, Y.: Temporal and spatial dynamics of
658 archaeal communities in two freshwater lakes at different trophic status, Front.
659 Microbiol., 7, 451, 2016.
660 Ye, W., Liu, X., Lin, S., Tan, J., Pan, J., Li, D., and Yang, H.: The vertical distribution
661 of bacterial and archaeal communities in the water and sediment of Lake Taihu,
662 FEMS Microbiol. Ecol., 70, 107-120, 2009.
663 Youngblut, N. D., Dell'Aringa, M., and Whitaker, R. J.: Differentiation between
664 sediment and hypolimnion methanogen communities in humic lakes, Environ.
665 Microbiol., 16, 1411-1423, 2014.
666 Zhu, D.-L., Sun, C., and He, H.: Detection Methanogens in Newly Settled Sediments
667 from Xuanwu Lake in Nanjing, China, Curr. Microbiol., 64, 539-544, 2012.
668
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670
671
672 **Table 1** Characteristics and trophic stauts of Dianchi Lake and Erhai Lake. sTOC, sTN, sTP and wTOC, wTN, wTP were referred to average
673 levels of total organi carbon (TOC), total nitrogen (TN) and total phosphrous (TP) of sediment and water, respectively. The locations of
674 sampling sites were as illustrated in the literature (Yang et al, 2016).
675

Lake	Location	sTOC (g/kg)	sTN (mg/kg)	sTP (mg/kg)	wTOC (mg/L)	wTN (mg/L)	wTP (mg/L)	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	mesotrphic

676

677 **Table 2** diversity and library coverage of archaeal 16S rRNA and *mcrA* genes.
678 Samples D1–D6 and E1–E6 were retrieved from Dianchi Lake and Erhai Lake,
679 respectively. Digits “1” – “6” were referred to sediment depth 0–5, 5–8, 8–11, 11–14,
680 14–17 and 17–20 cm, respectively.
681

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

682

Figure captions

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

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

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" were referred to sediment depth 0–5, 5–8, 8–11, 11–14, 14–17 and 17–20 cm, respectively.

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 sample 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 1,000 resampled datasets. The values less than 0.5 are not listed. The bar represents 5% sequence divergence.

Figure 5 Environment clusters for archaeal 16S rRNA gene (a) and *mcrA* gene (b)

assemblages based on Unifrac distance. The scale represented Unifrac distance. Samples D1–D6 and E1–E6 were retrieved from Dianchi Lake and Erhai Lake, respectively. Digits “1” – “6” were referred to sediment depth 0–5, 5–8, 8–11, 11–14, 14–17 and 17–20 cm, respectively.

Figure 1

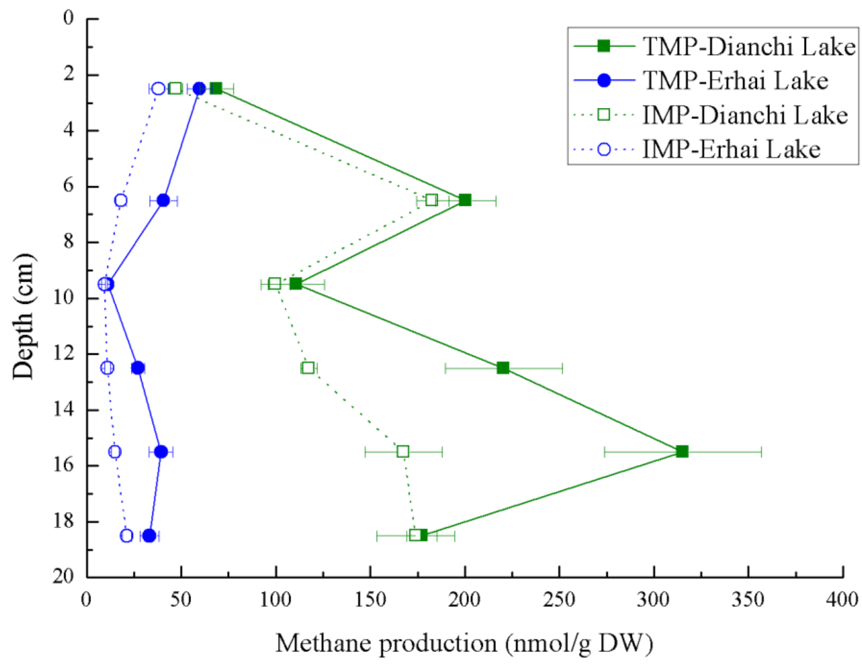


Figure 2

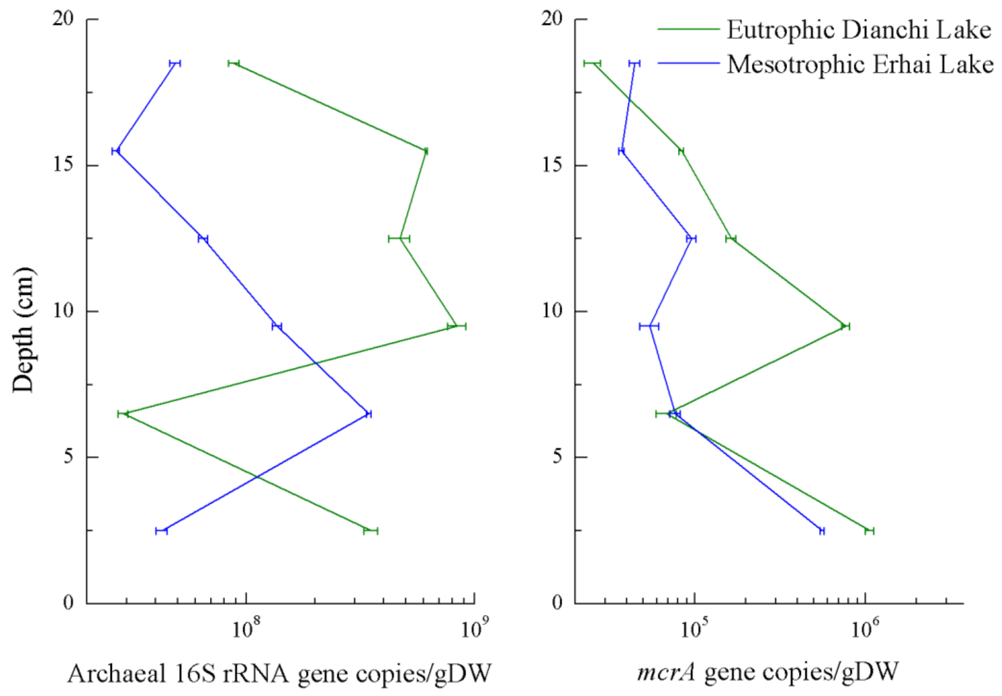


Figure 3

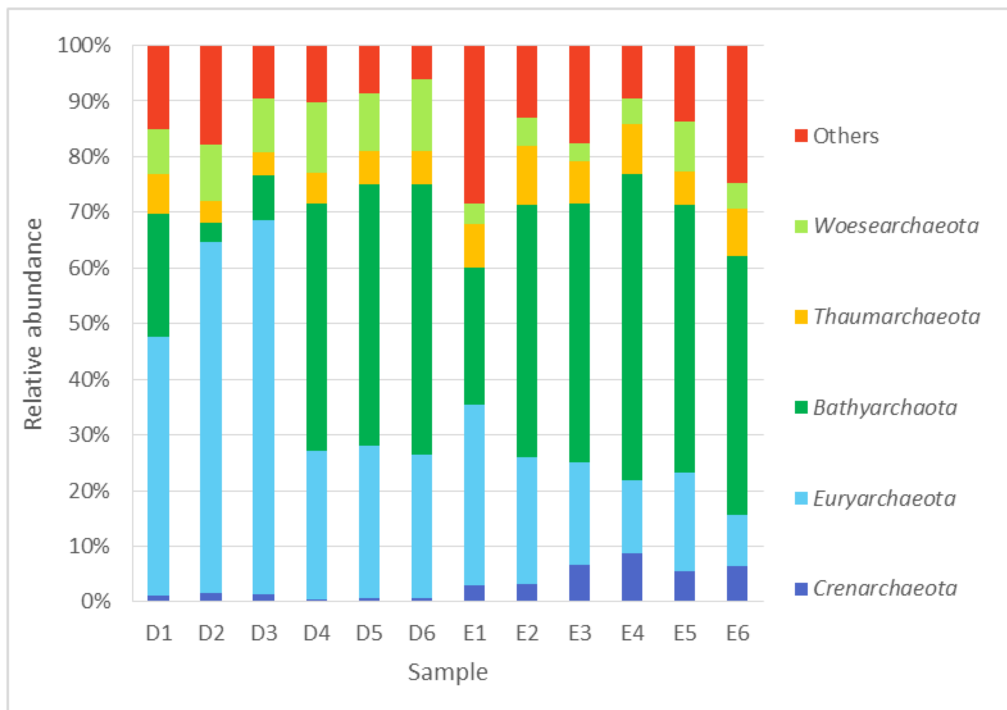


Figure 4

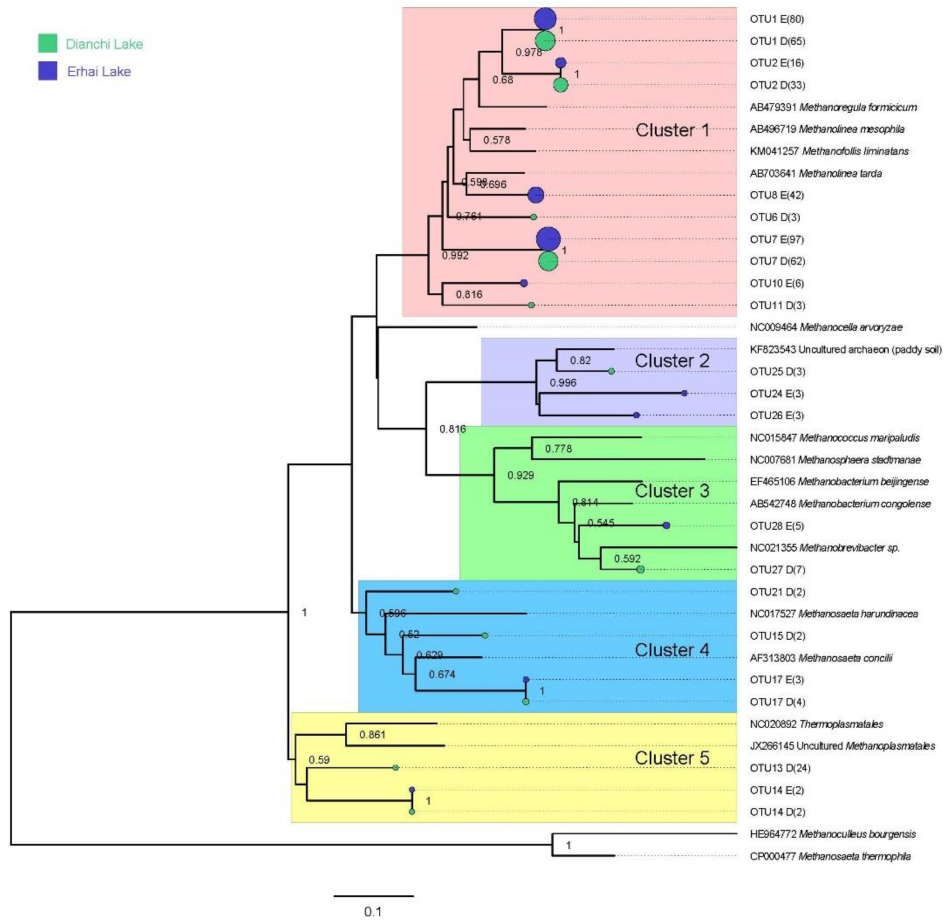
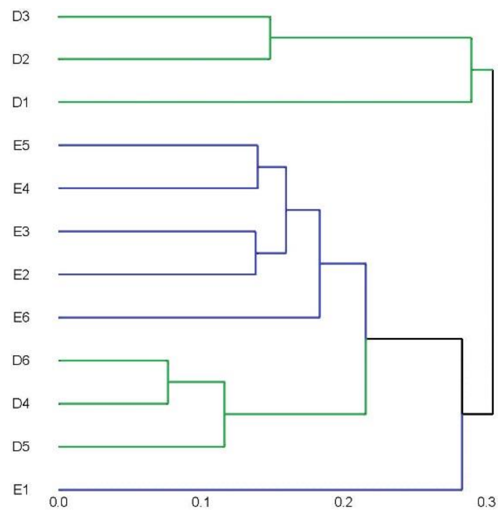


Figure 5

(a)



(b)