Biogeosciences Discuss., doi:10.5194/bg-2016-321, 2016
Manuscript under review for journal Biogeosciences
Published: 9 August 2016
© Author(s) 2016. CC-BY 3.0 License.

Biogeosciences Discussions
Open Access
EGU

1 Vertical profiles of sediment methanogenic potential and communities in two plateau
2 freshwater lakes
3
4
5 Yuyin Yang[1], Bingxin Li[1], Shuguang Xie[1,*], Yong Liu[2,*]
6
7 [1]State Key Joint Laboratory of Environmental Simulation and Pollution Control,
8 College of Environmental Sciences and Engineering, Peking University, Beijing
9 100871, China
10
11 [2]Key Laboratory of Water and Sediment Sciences (Ministry of Education), College of
12 Environmental Sciences and Engineering, Peking University, Beijing 100871, China
13
14
15 * Corresponding author. Tel: 86-10-62751923. Fax: 86-10-62751923.
16 Email: xiesg@pku.edu.cn (S Xie); yongliu@pku.edu.cn (Y Liu)
17
18
19
20
21
22

Biogeosciences Discuss., doi:10.5194/bg-2016-321, 2016
Manuscript under review for journal Biogeosciences
Published: 9 August 2016
© Author(s) 2016. CC-BY 3.0 License.

Biogeosciences Discussions Open Access EGU

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

44

© Author(s) 2016. CC-BY 3.0 License.

45 **Keywords:** Methanogenesis pathways; Freshwater lake sediment; Vertical profile;

46 *Archaea*; *mcrA*

47

48

49

50

51

52

53

54

55

56

57

58

59

60

61

62

63

64

65

66

Biogeosciences Discuss., doi:10.5194/bg-2016-321, 2016
Manuscript under review for journal Biogeosciences
Published: 9 August 2016
© Author(s) 2016. CC-BY 3.0 License.

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 archaeal phylum *Euryarchaeota*.
74 Methanogens from various archaeal orders have been reported (Garcia, 1990; Paul et
75 al., 2012; Sakai et al., 2008), and their substrate is generally the end products of
76 organic matter degradation by fermentative bacteria and archaea (Borrel et al., 2011).
77 Methanogens produce methane through either hydrogenotrophic (using H_2/CO_2) or
78 acetoclastic pathway (using acetate, i.e. the methyl group) (Conrad et al., 2010). To
79 determine the contribution of both methanogenic pathways, approaches including
80 isotope labeling, $\delta^{13}C$ analysis and inhibitor study have been applied (Conrad, 2005).
81 Despite the theoretical ratio of 2:1 (acetoclastic pathway: hydrogenotrophic pathway)
82 (Conrad, 1999), most methane in freshwater lake is produced through
83 hydrogenotrophic pathway (Borrel et al., 2011). However, the relative significance of
84 hydrogenotrophic pathway remains unclear, because it can vary considerably with
85 lake (Conrad, 1999). To identify the methanogens involved in methanogenesis, both
86 archaeal 16S rRNA gene and functional *mcrA* gene have been used (Conrad et al.,
87 2007; Luton et al., 2002; Orphan et al., 2008). The dominance of *Methanomicrobiales*
88 and *Methanosarcinale* have been reported in a variety of freshwater lakes (Biderre-

Biogeosciences Discuss., doi:10.5194/bg-2016-321, 2016
Manuscript under review for journal Biogeosciences
Published: 9 August 2016
© Author(s) 2016. CC-BY 3.0 License.

89 Petit et al., 2011; Conrad et al., 2007; Youngblut et al., 2014). In freshwater lakes,
90 both methanogenesis pathway and methanogenic community structure can change
91 with sediment layer depth (Chan et al., 2005; Liu et al., 2016; Lofton et al., 2015).
92
93 Many previous studies have investigated methanogenesis in humic lakes (Youngblut
94 et al., 2014), oligotrophic lakes (Lofton et al., 2015), and meromictic lakes (Biderre-
95 Petit et al., 2011; Gies et al., 2014), while shallow meso- and eutrophic lakes have
96 attracted poor attention. Substrate plays an important role in methanogenesis, and has
97 a considerable effect on pathway preference (Liu et al., 2016; Nozhevnikova et al.,
98 2007). Methanogenesis in meso- and eutrophic lakes that have abundant substrate
99 might be different from that in other previously studied lakes. Therefore, the
100 following questions attracted our attention: in sediments of mesotrophic and eutrophic
101 lakes, (1) How do the methanogenesis activity and contribution of different pathways
102 change with the increasing sediment layer depth? (2) How do methanogen community
103 structure and the dominant methanogens change along the sediment layer depth? (3)
104 Are these changing patterns similar in lakes at different trophic status?
105
106 2. **Materials and methods**
107 2.1. *Sampling sites and samples*
108 Dianchi Lake is a highly eutrophic lake with an area of 309 km^2 and the average water
109 depth of 4.4 m, while mesotrophic Erhai Lake has an area of 250 km^2 and the average
110 water depth of 10 m (Wang et al., 2015). In this study, five replicate sediment cores

© Author(s) 2016. CC-BY 3.0 License.

111 (0–20 cm) were collected at the profundal area of both Dianchi Lake and Erhai Lake
112 with a columnar sediment sampler in December 2015. The water depths at the
113 sampling sites were 6.4 and 11 m in Dianchi Lake and Erhai Lake, respectively. The
114 in-situ sediment temperature were 16.4°C in Dianchi Lake and 14.8 °C in Erhai Lake,
115 respectively. Meanwhile, a total of 500 mL bottom water at each sampling site was
116 collected for the subsequent incubation experiments.
117
118 The five replicate sediment cores were sliced into the layers: 0–5, 5–8, 8–11, 11–14,
119 14–17, and 17–20 cm. Replicate sediment samples from the same layer depth in a
120 given lake were mixed and then subsampled for physicochemical and molecular
121 analyses and incubation experiments. Sediment samples for physicochemical and
122 molecular analyses were stored at -20°C, while those for incubation experiments were
123 kept in gas-tight bottles under anoxic condition at 4°C. The sediment samples were
124 transported to laboratory within one week, which would have no considerable effect
125 on methanogenic activity (Nüsslein et al., 2001). The levels of sediment total organic
126 carbon (TOC), total nitrogen (TN), nitrate nitrogen (NO_3^--N), ammonium nitrogen
127 (NH_4^+-N), and total phosphorus (TP) were shown in Figure S1.
128
129 2.2. *Methane production potential measurement*
130 The incubation experiments for methane production potential (MPP) measurement
131 were performed with reference to the standard procedure described in the literature
132 (Conrad et al., 2010). Uppermost sediment (0–5 cm) was centrifuged at 4000 rpm for

Biogeosciences Discuss., doi:10.5194/bg-2016-321, 2016
Manuscript under review for journal Biogeosciences
Published: 9 August 2016
© Author(s) 2016. CC-BY 3.0 License.

133 5 min to obtain the similar water content with the samples from other layers. For each
134 sediment layer, a total six sediment samples (1.5 g) and 8 mL bottom water were
135 transferred into a 50-mL sterile serum bottle, flushed with N_2, and then closed with a
136 butyl rubber stopper. After incubation at 16°C overnight, the bottles were flushed with
137 N_2 again, and half of them were added with CH_3F (1 mL) to inhibit the aceticlastic
138 methanogenesis. Incubation was carried out at 16°C at 100 rpm for 28 days. At the
139 end of incubation, gas samples were taken from the headspace using a gas-tight
140 syringe, and then were analyzed using GC126 gas chromatography (INESA
141 instrument, Shanghai) with a flame ionization detector.
142
143 2.3 *Molecular analysis*
144 DNA was extracted using the Powersoil DNA extraction kit (Mobio Laboratories,
145 USA). The quality of DNA were checked using 1.0% agarose gel electrophoresis. For
146 quantitative PCR (qPCR), the primer sets mcrF (5'-
147 GGTGGTGTMGGATTCACACARTAYGCWACAGC-3') /mcrR (5'-
148 TCATTGCRTAGTTWGGRTAGTT-3') (Luton et al., 2002) and Arch344F (5'-
149 GYGCAGCAGGCGCGA-3') /Arch915R (5'-GTGCTCCCCCGCCAATTCCT-3')
150 (Casamayor et al., 2002; Conrad et al., 2014) were used for the quantification of
151 archaeal 16S rRNA and *mcrA* genes, respectively. The qPCR assay was carried out
152 using an ABI 7500 FAST (Applied Biosystems).The reaction mixture included
153 2×SYBR Green PCR master mix (12.5 μL), 10 μM of each primer (1 μL), and
154 template DNA (2 ng). The PCR conditions were as described in the literatures

Biogeosciences Discuss., doi:10.5194/bg-2016-321, 2016
Manuscript under review for journal Biogeosciences
Published: 9 August 2016
© Author(s) 2016. CC-BY 3.0 License.

155 (Casamayor et al., 2002; Luton et al., 2002). Standard curves ranging from 10^3 to 10^7
156 gene copies/mL were generated using serial dilutions of linearized plasmids (pGEM-
157 T, Promega) containing cloned target gene amplified from environmental DNA. The
158 coefficient (r^2) for archaeal 16S rRNA gene and *mcrA* gene were 0.9995 and 0.9998,
159 respectively. Significance was tested using one-way analysis of variance (ANOVA),
160 followed by S-N-K's post-hoc analysis (when the variances were homogenous) or
161 Dunnett's post-hoc analysis (when the variances were not homogenous).
162
163 For Illumina MiSeq sequencing, archaeal 16S rRNA gene was amplified using primer
164 set Arch519f (5'-CAGCCGCCGCGGTAA-3')/Arch915R (5'-
165 GTGCTCCCCCGCCAATTCCT-3') (He et al. 2016; Herfort et al., 2009; Long et al.
166 2016). The PCR products from triplicate samples were mixed in equal amounts and
167 were subjected to Illumina MiSeq sequencing. Raw reads were merged and the
168 quality filtering was carried out using Trimmomatic (Bolger et al., 2014) and FLASH
169 (Magoc and Salzberg, 2011). The obtained raw reads were deposited in the NCBI
170 SRA (short-read archive) under accession SRP076837. After subsampling to the
171 lowest number of sequences, sequences from each sediment sample were grouped into
172 OTUs (operational taxonomic units) using Usearch (version 7.1,
173 http://drive5.com/uparse/) at 97% similarity. Diversity analysis was carried out using
174 the MOTHUR program (version v.1.30.1) (Schloss et al., 2011). The taxonomic
175 identities of representative sequences for each OTU were assigned using the Silva 16S
176 rRNA database (Quast et al., 2013). For *mcrA* gene clone library analysis, primer set

Biogeosciences Discuss., doi:10.5194/bg-2016-321, 2016
Manuscript under review for journal Biogeosciences
Published: 9 August 2016
© Author(s) 2016. CC-BY 3.0 License.

177 mcrF/mcrR was used, and the PCR conditions were as previously described (Luton et
178 al., 2002). The obtained *mcrA* sequences were deposited in the GenBank database
179 under accession numbers KU997795-KU997842, KX196972-KX197020 and
180 KX093502-KX093920. The chimera-free *mcrA* gene sequences were grouped into
181 OTUs at the similarity level of 89% (Webster et al., 2014) using the MOTHUR
182 program. OTU-based diversity was also calculated using the MOTHUR program
183 (Schloss et al., 2009). Phylogenetic analysis of the *mcrA* gene sequences was carried
184 out with the MEGA 6.0 software (Tamura et al., 2013). Weighted Unifrac distance
185 between samples was calculated using *R* library GUniFrac, and PCoA (Principal
186 Coordinate Analysis) and environment clusters analysis were conducted based on
187 Weighted Unifrac distance using R (version i386, 3.3.0).
188
189 3. **Results**
190 3.1. *Methane production potential*
191 In this study, MPP varied remarkably with both lake and sediment layer depth (Figure
192 1). In contrast, MPP differed slightly in the uppermost layer sediments (0–5 cm) of the
193 studied two lakes. The change pattern of MPP differed in the two lakes. In Dianchi
194 Lake, the MPP generally increased with increasing layer depth (varying from 2.46 to
195 11.26 nmol/gDW/day in 5–20 cm layer), while in Erhai Lake, the MPP decreased
196 from 2.13 to 0.40 nmol/gDW/day. The sediment samples from Dianchi Lake showed a
197 significantly higher MPP than those from Erhai Lake (P<0.05).
198

Biogeosciences Discuss., doi:10.5194/bg-2016-321, 2016
Manuscript under review for journal Biogeosciences
Published: 9 August 2016
© Author(s) 2016. CC-BY 3.0 License.

199 The hydrogenotrophic methanogenesis potential (HMP) was measured under the
200 condition of 2% CH_3F. HMP showed a similar change trend with MPP in either of the
201 studied two freshwater lakes. With increasing layer depth, MPP generally increased in
202 Dianchi Lake but tended to decrease in Erhai Lake. Dianchi Lake had the HMP of
203 1.68–6.53 nmol/gDW/day, but Erhai Lake had a much lower HMP (0.34–1.36
204 nmol/gDW/day). Besides, methane production through aceticlastic pathway varied
205 greatly in different layers (0.07–3.00 nmol/gDW/day in Dianchi Lake, 0.04–0.49
206 nmol/gDW/day in Erhai Lake), and showed a notable difference between in these two
207 freshwater lakes.
208
209 3.2. *Community abundance of Archaea and methanogen*
210 The depth-related change pattern of either archaeal 16S rRNA or *mcrA* gene differed
211 in Dianchi Lake and Erhai Lake (Figures 2a and 2b). The density of archaeal 16S
212 rRNA gene fluctuated between $2.9 \pm 0.1 \times 10^7$ and $8.4 \pm 0.8 \times 10^8$ copies/g dry weight in
213 Dianchi Lake. However, in Erhai Lake, the archaeal community abundance ranged
214 from $2.7 \pm 0.1 \times 10^7$ to $3.4 \pm 0.1 \times 10^8$ copies/g dry weight, and showed an increase
215 followed by a significant decrease ($P<0.05$), with the peak value in the 5–8-cm layer.
216
217 As for *mcrA* gene, its highest density was observed at the uppermost layer (0–5 cm) in
218 either Dianchi Lake ($1.06 \pm 0.06 \times 10^6$ copies/g dry weight) or Erhai Lake ($5.6 \pm$
219 0.2×10^5 copies/g dry weight), while the lowest one occurred in Dianchi Lake sample
220 D6 ($2.5 \pm 0.3 \times 10^4$ copies/g dry weight) or Erhai Lake sample E5 ($3.7 \pm 0.1 \times 10^4$ copies/g

Biogeosciences Discuss., doi:10.5194/bg-2016-321, 2016
Manuscript under review for journal Biogeosciences
Published: 9 August 2016
© Author(s) 2016. CC-BY 3.0 License.

221 dry weight). The gene abundance generally decreased with increasing layer depth. In
222 addition, either *Archaea* or methanogens showed greater abundance in Dianchi Lake
223 than in Erhai Lake.
224
225 3.3. *Diversity of archaeal and methanogen communities*
226 In this study, after normalization, a total of 16,028 archaeal sequences were retrieved
227 from each sediment sample. The number of OTUs in each library ranged between 547
228 and 1194, and library coverage was 98.07–99.64% (Table 1). Archaeal community
229 diversity of most sediment samples varied slightly (Shannon index=4.51–5.13),
230 whereas samples D2 and D3 had much lower Shannon diversity (3.98 or 3.88). The
231 samples from Erhai Lake showed higher archaeal diversity (Shannon index=4.81–
232 5.13) than those from Dianchi Lake (Shannon index=3.88–4.75).
233
234 A total of 516 *mcrA* gene sequences were retrieved from *mcrA* clone library, and
235 could be assigned into 30 OTUs (at 89% similarity). For each sample, the number of
236 sequences ranged from 35 to 49, while the number of OTUs varied between 6 and 11.
237 The coverage for each clone library was no less than 85.7%, indicating that the *mcrA*
238 OTUs of each sample had been well captured. The Shannon indices were 1.49–1.72
239 for Dianchi Lake and 1.25–1.84 for Erhai Lake, respectively. In Dianchi Lake, sample
240 D1 had the highest *mcrA* gene diversity, but sample D6 had the lowest diversity. In
241 Erhai Lake, sample E2 had the highest *mcrA* gene diversity followed by sample E1,
242 but E5 showed the lowest diversity.

Biogeosciences Discuss., doi:10.5194/bg-2016-321, 2016
Manuscript under review for journal Biogeosciences
Published: 9 August 2016
© Author(s) 2016. CC-BY 3.0 License.

Biogeosciences Discussions Open Access EGU

243

244 3.4. *16S rRNA-based community composition*

245 *Bathyarchaota* (formally known as *Miscellaneous Crenarchaeotic_Group*, *MCG*) and
246 *Euryarchaeota* dominated the archaeal communities in both Dianchi Lake and Erhai
247 Lake (Figure 3). In the uppermost sediment layer in either lake, *Bathyarchaota*
248 accounted for nearly 20% of the total archaeal community. However, in Dianchi Lake,
249 with increasing layer depth, the proportion of *Bathyarchaota* showed a remarkable
250 decrease followed by a rise. *Bathyarchaota* organisms showed high proportion (44–
251 55%) in Dianchi Lake samples D4–D6 and Erhai Lake samples E2–E6. In contrast,
252 *Euryarchaeota* illustrated an opposite change trend. In Dianchi Lake, the proportion
253 of *Euryarchaeota* organisms was higher in samples D2 and D3 (63% or 67%) than in
254 sample D1 (47%), but displayed a notable decrease in deeper layers (26–27%). In
255 Erhai Lake, the *Euryarchaeota* proportion in sample E1 was 32.5% but became lower
256 in samples E2–E6 (9–23%). In addition, *Woesearchaeota* showed a greater abundance
257 in Dianchi Lake (8–13%) than in Erhai Lake (4–9%), while *Crenarchaeota* was
258 mainly distributed in Erhai Lake (3–29%).

259

260 Based on 16S rRNA gene analysis, methanogens comprised 3–17% of the total
261 archaeal community (Figure S2). The relative abundance of methanogens tended to
262 decrease with increasing sediment layer depth. Order *Methanobacteriales* dominated
263 the methanogen community in the uppermost layer samples (samples D1 or E1), and
264 also had a considerable proportion in samples D2 and D3. In contrast,

© Author(s) 2016. CC-BY 3.0 License.

265 *Methanomicrobiales* was the dominant methanogen group in the lower layer
266 sediments (Dianchi Lake samples D2–D6 and Erhai Lake samples E2–E6).
267 *Methanocellales* and *Methanosarcinales* could also be detected in all sediment
268 samples, but their proportions were less than 2%. At genus level, *Methanobacterium*
269 had the greatest proportion, followed by *Methanosaeta* and *Methanoregula*, and the
270 proportion of each archaeal genus decreased with increasing layer depth in either lake
271 (data not shown).
272
273 3.5. *mcrA-based community composition*
274 In the present study, the 516 *mcrA* sequences from all sediment samples fell into 30
275 OTUs, 12 of which had only one sequence. The representative sequences from the
276 OTUs with at least two sequence members were further used to construct the
277 phylogenic tree with their close *mcrA* sequences reported in the NCBI database
278 (Figure 4). All of the sequences were grouped into five clusters (clusters 1–5). Cluster
279 1 contained most of the obtained *mcrA* sequences (440), and could be affiliated with
280 the *mcrA* sequences from *Methanomicrobiales*. Cluster 2 consisted of 29 *mcrA*
281 sequences related to those from *Thermoplasmatales* and *Methanoplasmatales*. Cluster
282 3 (with 13 sequence members) and cluster 5 (with 12 sequence members) were related
283 to *Methanosarcinale* and *Methanobacteriales*, respectively. Cluster 4 was the smallest
284 group and only contained 10 sequences. The sequences in this cluster were not related
285 to the *mcrA* sequences from known methanogens. In addition, the sequences affiliated
286 to cluster 1 could be further divided into 3 clades. OTU7 was grouped together with

Biogeosciences Discuss., doi:10.5194/bg-2016-321, 2016
Manuscript under review for journal Biogeosciences
Published: 9 August 2016
© Author(s) 2016. CC-BY 3.0 License.

287 the sequences from two *Methanomicrobiaceae* strains. OTU8 showed a close relation
288 to a *Methanolinea*-like *mcrA* sequence, and OTU1, OTU2 and OTU6 were closely
289 related to *Methanoregula*-like *mcrA* sequence.
290
291 *Methanoregula*-like *mcrA* sequences (OTU1 and OTU2) were detected in all sediment
292 layer depths, but the proportion of OTU1 decreased with increasing layer depth
293 (Figure S3). *Methanomicrobiaceae*-like *mcrA* sequences (OTU7) were not detected in
294 Dianchi Lake samples D1, D2 and D3, but they dominated in the other samples and
295 their proportion increased with increasing sediment layer depth. *Methanoplasmatales*
296 -like *mcrA* sequences (OTU13) mainly existed in Dianchi Lake, but were also
297 detected in the uppermost sediment layer in Erhai Lake. *Methanolinea*-like *mcrA*
298 sequences (OTU8) were present in all sediment layers in both lakes, but their change
299 pattern was not evident. In addition, other genotypes of *mcrA* gene sequences
300 comprised less than 22% of the total sequences in each sample.
301
302 3.6. *Comparison of archaeal and methanogen communities*
303 The difference among archaeal assemblages was discriminated using Weighted-
304 Unifrac distance-based cluster analysis (Figure 5a). In either Dianchi Lake or Erhai
305 Lake, the sample from the uppermost layer (sample D1 or E1) was separated from the
306 samples in other sediment layers. In Dianchi Lake, samples D2 and D3 were also
307 grouped into one clade, and the other three samples fell into another one. However, in
308 Erhai Lake, samples E2, E3, E4, E5 and E6 were grouped together, and the samples

© Author(s) 2016. CC-BY 3.0 License.

309 from neighboring layers tended to have a relatively similar archaeal community
310 structure.
311
312 For methanogen communities, the studied 12 sediment samples fell into two groups
313 (Figure 5b). Samples D1, D2, D3 and E1 were clustered together. For other samples,
314 samples at similar layer depth tended to have relatively similar methanogen
315 community structure. Moreover, the sediment samples from two lakes were not
316 clearly separated.
317
318 4. **Discussion**
319 4.1 *Methane production potential in freshwater lake sediment*
320 The methanogenesis potential varied in a wide range (Dan et al., 2004; Duc et al.,
321 2010; Lofton et al., 2015), from less than 1 nmol CH_4/gDW day to more than one
322 thousand nmol CH_4/gDW day, and the methanogenesis potential obtained in the
323 current study fell in this range. Numerous previous studies showed that MPP
324 decreased with increasing sediment layer depth (Chan et al., 2005; Liu et al., 2016;
325 Lofton et al., 2015), or displayed a slight shift followed by a sharp decrease (Lofton et
326 al., 2015). The change pattern of MPP in Erhai Lake was similar to that reported in
327 these literatures. However, to the authors' knowledge, Dianchi Lake was the first lake
328 that illustrated the increase of MPP with increasing sediment layer depth.
329
330 Several previous studies had investigated the inter-lake difference of methanogenesis.

© Author(s) 2016. CC-BY 3.0 License.

331 Methane production rate could differed drastically between in two
332 geomorphologically similar oligotrophic lakes, and the quantity and quality of water
333 dissolved organic carbon (DOC) might be an influential factor (Lofton et al., 2015).
334 Other environmental factors, including geological constitute, geographical regions
335 (Rinta et al., 2015) as well as water type (Conrad et al., 2014), were also found to
336 have considerable influences on methane production rate in freshwater lake sediment.
337 Moreover, lake characteristics could influence the methane production rate both
338 directly and indirectly (Borrel et al., 2011). In this study, the methanogenesis potential
339 in Dianchi Lake was found to be much higher than that in Erhai Lake. Dianchi Lake
340 had a larger amount of nutrient than Erhai Lake, and a high level of organic matter
341 could exist in lower sediment layers (Figure S1). MPP was found to be related to the
342 availability of organic matter (Liu et al., 2016; Lofton et al., 2015; Nozhevnikova et
343 al., 2007), so the abundant substrate in Dianchi Lake could favor higher MPP. In
344 addition, although methanogen communities in Dianchi Lake and Erhai Lake had
345 similar structure, either archaeal or bacterial community differed greatly in these two
346 lakes (Dai et al., 2016; Yang et al., 2016). Bacterial and non-methanogen archaeal
347 community played important roles in decomposing organic matter, and thus
348 influenced the availability of substrate.
349
350 By comparing the uninhibited and the inhibited methanogenesis potential, it could be
351 inferred that hydrogenotrophic pathway played a major role in Dianchi Lake (75.8 %
352 of total methane production potential). The result was consistent with the previous

Biogeosciences Discuss., doi:10.5194/bg-2016-321, 2016
Manuscript under review for journal Biogeosciences
Published: 9 August 2016
© Author(s) 2016. CC-BY 3.0 License.

353 studies (Conrad et al., 2010; Liu et al., 2013; Liu et al., 2016). However, in Erhai
354 Lake, the methanogenesis potential through aceticlastic pathway was comparable to
355 that through hydrogenotrophic pathway. Moreover, acetoclastic methangenesis was
356 found to decrease with increasing lake sediment layer depth (Chan et al., 2005; Liu et
357 al., 2016), which could be attributed to the decreasing availability of organic matter
358 (Liu et al., 2016). In this study, the layer depth-related difference in the contribution
359 of two methanogenic pathways was not clear. This might be partly due to the
360 fluctuation of acetoclastic methangenesis with sediment layer depth. This also might
361 suggest the excess substrate for methanogenesis in sediments of eutrophic lake.
362
363 4.2. *Abundance of Archaea and methanogen in freshwater lake sediment*
364 According to the previous studies, the abundance of archaeal 16S rRNA gene
365 generally decreased with increasing layer depth in stratified lake sediments (Chan et
366 al., 2005; Zhu et al., 2012). However, in shallow and eutrophic lakes, both archaeal
367 16S rRNA gene and *mcrA* gene abundance could fluctuate along the sediment depth
368 gradient (Ye et al., 2009; Zhu et al., 2012). In the current study, the density of archaeal
369 16S rRNA gene tended to decrease with increasing layer depth in Erhai Lake but
370 considerably fluctuated in Dianchi Lake, which might be attributed to the difference
371 of substrate supply and lake water depth.
372
373 The abundance of methanogens could be assessed using either archaeal 16S rRNA or
374 *mcrA* gene. Mthanogen abundance was usually found to rise at first followed by a

Biogeosciences Discuss., doi:10.5194/bg-2016-321, 2016
Manuscript under review for journal Biogeosciences
Published: 9 August 2016
© Author(s) 2016. CC-BY 3.0 License.

375 decrease with increasing layer depth, and its peak occurred at the surface layer or 5–
376 10 cm beneath the sediment surface (Borrel et al., 2012; Milferstedt et al., 2010; Zhu
377 et al., 2012), which consisted with the results found in this study.
378
379 In the current study, the abundance of archaeal community was comparable to that
380 reported in the literatures (Borrel et al., 2012; Zhu et al., 2012). However, for each
381 sample, the *mcrA*/16S ratio was less than 3%, while the sequences affiliated with
382 methanogen-like organisms accounted for 3–17% of total 16S rRNA sequences. The
383 result might suggest either the bias of amplification of *mcrA* gene or the numerical
384 difference between organisms and functional gene.
385
386 4.3. *Diversity of archaeal and methanogen communities in freshwater lake sediment*
387 The diversity of archaeal community usually displayed a slight fluctuation along the
388 sediment layer depth gradient (Koizumi et al., 2004; Lim et al., 2011; Nam et al.,
389 2008). This was in agreement with the result found in this study. To date, the diversity
390 of *mcrA* gene was unclear, partly due to the usage of order-specific primers of
391 methanogens in the previous studies (Borrel et al., 2012; Zhu et al., 2012). Moreover,
392 information on the diversity of methanogen 16S rRNA gene was lacking. In this
393 study, relatively higher diversity of *mcrA* gene was observed in the samples from
394 upper layers (sample D1 in Dianchi Lake and sample E2 in Erhai Lake), but the
395 change pattern of *mcrA* gene diversity was not clear. In addition, both community
396 diversity and evenness of methanogens could vary with lake (Milferstedt et al., 2010;

Biogeosciences Discuss., doi:10.5194/bg-2016-321, 2016
Manuscript under review for journal Biogeosciences
Published: 9 August 2016
© Author(s) 2016. CC-BY 3.0 License.

397 Youngblut et al., 2014). In this current study, the sediment samples from Erhai Lake
398 had slightly lower *mcrA* gene diversity than those from Dianchi Lake.
399
400 4.4 *Composition of methanogen community in freshwater lake sediment based on 16S*
401 *rRNA gene*
402 Hydrogenotrophic *Methanomicrobiales* was detected in a variety of environments,
403 and was the most frequently observed archaeal order in freshwater lake sediment
404 (Biderre-Petit et al., 2011; Youngblut et al., 2014). In the present study,
405 *Methanomicrobiales* had high proportion in sediment from each layer depth, which
406 agreed with these two previous studies. Moreover, *Methanobacteriales* was usually
407 found in the ecosystems with high levels of nutrition and substrate, such as
408 hypereutrophic Priest Pot (Earl et al., 2003) and eutrophic Taihu Lake (Ye et al.,
409 2009). In this study, *Methanobacteriales* was mainly distributed in Dianchi Lake
410 samples D1, D2 and D3 and Erhai Lake sample E1. In addition, methanogens from
411 order *Methanosarcinales* mainly participated in reducing acetate and methyl
412 compounds (Borrel et al., 2011) that were relatively abundant and labile in lake
413 ecosystems. However, *Methanosarcinales* showed lower proportion in Dianchi Lake
414 and Erhai Lake than in other previous reported freshwater lakes (Biderre-Petit et al.,
415 2011; Borrel et al., 2012; Zhu et al., 2012).
416
417 Ye et al. (2009) documented the layer depth-related change of archaeal community
418 structure in Taihu Lake, while its change pattern was not clear. In this study, the result

Biogeosciences Discuss., doi:10.5194/bg-2016-321, 2016
Manuscript under review for journal Biogeosciences
Published: 9 August 2016
© Author(s) 2016. CC-BY 3.0 License.

419 of UniFrac-based cluster analysis indicated that archaeal community structure differed
420 remarkably in Dianchi Lake and Erhai Lake. In either Dianchi Lake or Erhai Lake,
421 layer depth was found to be a key determinant to archaeal community structure.
422 However, the abrupt shift in archaeal community structure occurred at different layer
423 depths in these two lakes. In Dianchi Lake, the structures of archaeal communities at
424 layer depth 0–11 cm (including samples D1, D2 and D3) were much different from
425 those at layer depth 11–20 cm (including samples D4, D5 and D6). However, in Erhai
426 Lake, a remarkable difference lay between the sample from uppermost layer (D1) and
427 those from other five layers.
428
429 4.5. *Composition of methanogen community in freshwater lake sediment based on*
430 *mcrA gene*
431 Based on *mcrA* gene clone library analysis, *Methanomicrobiales* was found to be the
432 dominant methanogen group in sediments of many freshwater lakes (Biderre-Petit et
433 al., 2011; Youngblut et al., 2014), which consisted with the result found in this current
434 study. *Methanoregula* and *Methanolinea*, affiliated within *Methanomicrobiales*, were
435 among the most frequently detected archaeal genera in freshwater lake (Borrel et al.,
436 2011). So far, the change pattern of methanogen community at genus level along the
437 layer depth gradient has not been addressed. In the current study, the proportion of
438 *Methanoregula*-like *mcrA* sequences tended to decrease with increasing sediment
439 layer depth, while the proportion of *methanolinea*-like *mcrA* sequences did not show a
440 clear change pattern. *Methanoplasmatales* -like methanogens were related to

Biogeosciences Discuss., doi:10.5194/bg-2016-321, 2016
Manuscript under review for journal Biogeosciences
Published: 9 August 2016
© Author(s) 2016. CC-BY 3.0 License.

441 *Thermoplasmatales* archaeons. It was usually present in termite guts and high-salinity
442 environments (e.g., marine sediment), and was regarded as the seventh order of
443 methanogens (Paul et al., 2012). Only several previous studies reported their existence
444 in freshewater lake sediment (Conrad et al., 2014; Liu et al., 2013; Webster et al.,
445 2014). In this study, *Methanoplasmatales* -like methanogens were detected in both
446 Dianchi Lake and Erhai Lake, but the distribution pattern along sediment layer depth
447 in these two lakes was different. *Methanosarcinales* and *Methanobacteriale*s were
448 also detected in different sediment layers in both lakes. However, their change
449 patterns were not clear because of their low relative abundance. In addition, compared
450 with other freshwater lake sediments (Borrel et al., 2012; Zhu et al., 2012), sediments
451 in Dianchi Lake and Erhai Lake showed much higher *Methanomicrobiales* proportion
452 but lower *Methanosarcinales* proportion.
453
454 The phylogeny of *mcrA* gene was congruent with that of 16S rRNA gene (Luton et al.,
455 2002; Springer et al., 1995), but in this study, the results based on *mcrA* gene clone
456 library were not always consistent with that based on16S rRNA Illumina MiSeq
457 sequencing. For an example, *Methanosarcinales* organisms accounted for 7–20% of
458 the total methanogens according to 16S rRNA sequencing, but showed a very low
459 proportion in *mcrA* clone library (less than 3% in 11 out of 12 samples).
460 *Methanobacteriales* was abundant in uppermost layer sediments based on 16S rRNA
461 sequencing, but was a minor group in *mcrA* clone library.
462

© Author(s) 2016. CC-BY 3.0 License.

463 Methanogen communities in surface sediment were usually found to be lake-
464 dependent (Milferstedt et al., 2010; Youngblut et al., 2014), whereas in this study,
465 UniFrac-based cluster analysis indicated that methanogen communities in sediments
466 of Dianchi Lake and Erhai Lake were not phylogenetically separated. Sediment layer
467 depth was found to shape methanogen community structure.
468

469 **5. Conclusions**

470 The MPP and abundance of sediment methanogens differed greatly in Dianchi Lake
471 and Erhai Lake, while these two lakes had the similar methanogen community
472 structure, with the dominance of *Methanomicrobiales* and *Methanobacteriales*.
473 Hydrogenotrophic methanogenesis was the major methane production pathway in
474 sediments of both lakes. The layer depth-related changes of methanogenesis potential,
475 and the abundance and community structure of methanogens were observed in either
476 Dianchi Lake or Erhai Lake. Sediment methanogen community and activity might be
477 influenced by lake tropic status.
478

479 **Acknowledgments**

480 This work was financially supported by National Natural Science Foundation of
481 China (No. 41571444) and National Basic Research Program of China
482 (2015CB458900).
483
484

Biogeosciences Discuss., doi:10.5194/bg-2016-321, 2016
Manuscript under review for journal Biogeosciences
Published: 9 August 2016
© Author(s) 2016. CC-BY 3.0 License.

Biogeosciences Discussions
Open Access EGU

485 **Competing interests**

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

487

488 **References**

489 Bastviken, D., Cole, J., Pace, M., and Tranvik, L.: Methane emissions from lakes:
490 Dependence of lake characteristics, two regional assessments, and a global
491 estimate, Global Biogeochem. Cy., 18, GB4009, 2004.
492 Bastviken, D., Cole, J. J., Pace, M. L., and Van de Bogert, M. C.: Fates of methane
493 from different lake habitats: Connecting whole-lake budgets and CH4 emissions,
494 J. Geophys. Res., 2008.
495 Biderre-Petit, C., Jézéquel, D., Dugat-Bony, E., Lopes, F., Kuever, J., Borrel, G.,
496 Viollier, E., Fonty, G., and Peyret, P.: Identification of microbial communities
497 involved in the methane cycle of a freshwater meromictic lake, FEMS Microbiol.
498 Ecol., 77, 533-545, 2011.
499 Bolger, A. M., Lohse, M., and Usadel, B.: Trimmomatic: a flexible trimmer for
500 Illumina sequence data, Bioinformatics, 30, 2114-2120, 2014.
501 Borrel, G., Jezequel, D., Biderre-Petit, C., Morel-Desrosiers, N., Morel, J. P., Peyret,
502 P., Fonty, G., and Lehours, A. C.: Production and consumption of methane in
503 freshwater lake ecosystems, Res. Microbiol., 162, 832-847, 2011.
504 Borrel, G., Lehours, A.-C., Crouzet, O., Je ́quel D, and Rockne K: Stratification of
505 Archaea in the Deep Sediments of a Freshwater Meromictic Lake: Vertical Shift

Biogeosciences Discuss., doi:10.5194/bg-2016-321, 2016
Manuscript under review for journal Biogeosciences
Published: 9 August 2016
© Author(s) 2016. CC-BY 3.0 License.

Biogeosciences Discussions Open Access EGU

506 from Methanogenic to Uncultured Archaeal Lineages, PLoS ONE, 7, e3346,
507 2012.
508 Casamayor, E., Massana, R., Benlloch, S., Øvreås, L., Díez, B., Goddard, V., Gasol,
509 J., Joint, I., Rodríguez-Valera, F., and Pedrós-Alió, C.: Changes in archaeal,
510 bacterial and eukaryal assemblages along a salinity gradient by comparison of
511 genetic fingerprinting methods in a multipond solar saltern, Environ. Microbiol.,
512 4, 338-348, 2002.
513 Chan, O. C., Claus, P., Casper, P., Ulrich, A., Lueders, T., and Conrad, R.: Vertical
514 distribution of structure and function of the methanogenic archaeal community in
515 Lake Dagow sediment, Environ. Microbiol., 7, 1139-1149, 2005.
516 Conrad, R.: Contribution of hydrogen to methane production and control of hydrogen
517 concentrations in methanogenic soils and sediments, FEMS Microbiol. Ecol., 28,
518 193-202, 1999.
519 Conrad, R.: Quantification of methanogenic pathways using stable carbon isotopic
520 signatures: a review and a proposal, Org. Geochem., 36, 739-752, 2005.
521 Conrad, R., Chan, O.-C., Claus, P., and Casper, P.: Characterization of methanogenic
522 Archaea and stable isotope fractionation during methane production in the
523 profundal sediment of an oligotrophic lake (Lake Stechlin, Germany), Limnol.
524 Oceanogr., 53, 1393-1406, 2007.
525 Conrad, R., Klose, M., Claus, P., and Enrich-Prast, A.: Methanogenic pathway, C-13
526 isotope fractionation, and archaeal community composition in the sediment of
527 two clear-water lakes of Amazonia, Limnol. Oceanogr., 55, 689-702, 2010.

© Author(s) 2016. CC-BY 3.0 License.

528 Conrad, R., Ji, Y., Noll, M., Klose, M., Claus, P., and Enrich-Prast, A.: Response of
529 the methanogenic microbial communities in Amazonian oxbow lake sediments to
530 desiccation stress, Environ. Microbiol., 16, 2014.
531 Dai, Y., Yang, Y., Wu, Z., Feng, Q., Xie, S., and Liu, Y.: Spatiotemporal variation of
532 planktonic and sediment bacterial assemblages in two plateau freshwater lakes at
533 different trophic status, Appl. Microbiol. Biot., 100, 4161-4175, 2016.
534 Dan, J., Kumai, T., Sugimoto, A., and Murase, J.: Biotic and abiotic methane releases
535 from Lake Biwa sediment slurry, Limnology, 5, 149-154, 2004.
536 Duc, N. T., Crill, P., and Bastviken, D.: Implications of temperature and sediment
537 characteristics on methane formation and oxidation in lake sediments,
538 Biogeochemistry, 100, 185-196, 2010.
539 Earl, J., Hall, G., Pickup, R., Ritchie, D., and Edwards, C.: Analysis of methanogen
540 diversity in a hypereutrophic lake using PCR-RFLP analysis of *mcr* sequences,
541 Microbiol. Ecol., 46, 270-278, 2003.
542 Garcia, J. L.: Taxonomy and ecology of methanogens, FEMS Microbiol. Rev., 87,
543 297-308, 1990.
544 Gies, E. A., Konwar, K. M., Beatty, J. T., and Hallam, S. J.: Illuminating Microbial
545 Dark Matter in Meromictic Sakinaw Lake, Appl. Environ. Microbiol., 80, 6807-
546 6818, 2014.
547 Gruca-Rokosz, R., and Tomaszek, J. A.: Methane and Carbon Dioxide in the
548 Sediment of a Eutrophic Reservoir: Production Pathways and Diffusion Fluxes at
549 the Sediment–Water Interface, Water Air Soil Poll., 226, 16, 2015.

Biogeosciences Discuss., doi:10.5194/bg-2016-321, 2016
Manuscript under review for journal Biogeosciences
Published: 9 August 2016
© Author(s) 2016. CC-BY 3.0 License.

550 He, T., Guan, W., Luan, Z., Xie, S.: Spatiotemporal variation of bacterial and archaeal
551 communities in a pilot-scale constructed wetland for surface water treatment.
552 Appl. Microbiol. Biot., 100, 1479-1488, 2016.
553 Herfort, L., Kim, J. H., Coolen, M. J. L., Abbas, B., Schouten, S., Herndl, G. J.,
554 Damste, J. S. S.: Diversity of *Archaea* and detection of crenarchaeotal *amoA*
555 genes in the rivers, Rhine and Tet, Aquat. Microb. Ecol., 55, 189-201, 2009.
556 Koizumi, Y., Takii, S., and Fukui, M.: Depth-related change in archaeal community
557 structure in a freshwater lake sediment as determined with denaturing gradient
558 gel electrophoresis of amplified 16S rRNA genes and reversely transcribed rRNA
559 fragments, FEMS Microbiol. Ecol., 48, 285-292, 2004.
560 Lim, J., Woodward, J., Tulaczyk, S., Christoffersen, P., and Cummings, S. P.: Analysis
561 of the microbial community and geochemistry of a sediment core from Great
562 Slave Lake, Canada, Anton. Leeuw., 99, 423-430, 2011.
563 Liu, Y., Yao, T., Gleixner, G., Claus, P., and Conrad, R.: Methanogenic pathways, ^{13}C
564 isotope fractionation, and archaeal community composition in lake sediments
565 and wetland soils on the Tibetan Plateau, J. Geophys. Res. Biogeo., 118, 650-
566 664, 2013.
567 Liu, Y., Conrad, R., Yao, T., Gleixner, G., and Claus, P.: Change of methane
568 production pathway with sediment depth in a lake on the Tibetan plateau,
569 Palaeogeogr. Palaeoecol., doi: 10.1016/j.palaeo.2016.06.021, 2016.

© Author(s) 2016. CC-BY 3.0 License.

570 Lofton, D. D., Whalen, S. C., and Hershey, A. E.: Vertical sediment distribution of
571 methanogenic pathways in two shallow Arctic Alaskan lakes, Polar Biol., 38,
572 815-827, 2015.
573 Long, Y., Zhang, Z. , Pan, X., Li, B., Xie, S. , Guo, Q.: Substrate influence on
574 archaeal and bacterial assemblages in constructed wetland microcosm, Ecol.
575 Eng., 94, 437-442, 2016.
576 Luton, P. E., Wayne, J. M., Sharp, R. J., and Riley, P. W.: The mcrA gene as an
577 alternative to 16S rRNA in the phylogenetic analysis of methanogen populations
578 in landfill, Microbiology, 148, 3521-3530, 2002.
579 Magoc, T., and Salzberg, S.: FLASH Fast Length Adjustment of Short Reads to
580 Improve Genome, Bioinformatics, 27, 2957-2963, 2011.
581 Milferstedt, K., Youngblut, N. D., and Whitaker, R. J.: Spatial structure and
582 persistence of methanogen populations in humic bog lakes, ISME J., 4, 764-776,
583 2010.
584 Nüsslein, B., Chin, K.-J., Eckert, W., and Conrad, R.: Evidence for anaerobic
585 syntrophic acetate oxidation during methane production in the profundal
586 sediment of subtropical Lake Kinneret (Israel), Environ. Microbiol., 3, 460-470,
587 2001.
588 Nam, Y. D., Sung, Y., Chang, H. W., Roh, S. W., Kim, K. H., Rhee, S. K., Kim, J. C.,
589 Kim, J. Y., Yoon, J. H., and Bae, J. W.: Characterization of the depth-related
590 changes in the microbial communities in Lake Hovsgol sediment by 16S rRNA
591 gene-based approaches, J. Microbiol., 46, 125-136, 2008.

© Author(s) 2016. CC-BY 3.0 License.

592 Nozhevnikova, A. N., Nekrasova, V., Ammann, A., Zehnder, A. J. B., Wehrli, B., and
593 Holliger, C.: Influence of temperature and high acetate concentrations on
594 methanogenensis in lake sediment slurries, FEMS Microbiol. Ecol., 62, 336-344,
595 2007.
596 Orphan, V. J., Jahnke, L. L., Embaye, T., Turk, K. A., Pernthaler, A., Summons, R. E.,
597 and DJ, D. E. S. M.: Characterization and spatial distribution of methanogens
598 and methanogenic biosignatures in hypersaline microbial mats of Baja
599 California, Geobiology, 6, 376-393, 2008.
600 Paul, K., Nonoh, J. O., Mikulski, L., and Brune, A.: "*Methanoplasmatales*,"
601 *Thermoplasmatales*-related archaea in termite guts and other environments, are
602 the seventh order of methanogens, Appl. Environ. Microbiol., 78, 8245-8253,
603 2012.
604 Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., and
605 Glockner, F. O.: The SILVA ribosomal RNA gene database project: improved
606 data processing and web-based tools, Nucleic Acids Res., 41, D590-596, 2013.
607 Rinta, P., Bastviken, D., van Hardenbroek, M., Kankaala, P., Leuenberger, M.,
608 Schilder, J., Stötter, T., and Heiri, O.: An inter-regional assessment of
609 concentrations and $\delta^{13}C$ values of methane and dissolved inorganic carbon in
610 small European lakes, Aquat. Sci., 77, 667-680, 2015.
611 Sakai, S., Imachi, H., Hanada, S., Ohashi, A., Harada, H., and Kamagata, Y.:
612 *Methanocella paludicola* gen. nov., sp. nov., a methane-producing archaeon, the

613 first isolate of the lineage 'Rice Cluster I', and proposal of the new archaeal order
614 *Methanocellales* ord. nov, Int. J. Evol. Syst. Micr., 58, 929-936, 2008.
615 Schloss, P. D., Gevers, D., and Westcott, S. L.: Reducing the Effects of PCR
616 Amplification and Sequencing Artifacts on 16S rRNA-Based Studies, PLoS
617 ONE, 6, e27310, 2011.
618 Schloss, P. D.,Westcott, S. L., Ryabin, T., Hall, J. R., Hartmann, M., Hollister, E. B.,
619 Lesniewski, R. A., Oakley, B. B., Parks, D. H., Robinson, C. J., Sahl, J. W.,
620 Stres, B., Thallinger, G. G., Van Horn, D. J., Weber, C. F.: Introducing
621 mothur, open-source, platform-independent, community-supported software
622 for describing and comparing microbial communities. Appl. Environ.
623 Microbiol., 75, 7537-7541, 2009.
624 Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular
625 evolutionary genetics analysis version 6.0. Mol. Biol. Evol., 30, 2725-2729,
626 2013.
627 Springer, E., Sacks, M., Woese, C., and Boone, D.: Partial Gene Sequences for the A
628 Subunit of Methyl-Coenzyme M Reductase (*mcrI*) as a Phylogenetic Tool for the
629 Family *Methanosarcinaceae*, Int. J. Syst. Evol. Micr., 45, 554-559, 1995.
630 Tamura, K., Stecher, G., Peterson, D., Filipski, A., and Kumar, S.: MEGA6:
631 Molecular Evolutionary Genetics Analysis version 6.0, Mol. Biol. Evol., 30,
632 2725-2729, 2013.
633 Wang, S., Zheng, B., Chen, C., Dohmann, M., and Kolditz, O.: Thematic issue: water
634 of the Erhai and Dianchi Lakes, Environ. Earth. Sci., 74, 3685-3688, 2015.

Biogeosciences Discuss., doi:10.5194/bg-2016-321, 2016
Manuscript under review for journal Biogeosciences
Published: 9 August 2016
© Author(s) 2016. CC-BY 3.0 License.

635 Webster, G., O'Sullivan, L. A., Meng, Y., Williams, A. S., Sass, A. M., Watkins, A. J.,
636 Parkes, R. J., and Weightman, A. J.: Archaeal community diversity and
637 abundance changes along a natural salinity gradient in estuarine sediments,
638 FEMS Microbiol. Ecol., 91, 1-18, 2014.
639 Yang, Y., Dai, Y., Wu, Z., Xie, S., and Liu, Y.: Temporal and Spatial Dynamics of
640 Archaeal Communities in Two Freshwater Lakes at Different Trophic Status,
641 Front. Microbiol., 7, 451, 2016.
642 Ye, W., Liu, X., Lin, S., Tan, J., Pan, J., Li, D., and Yang, H.: The vertical distribution
643 of bacterial and archaeal communities in the water and sediment of Lake Taihu,
644 FEMS Microbiol. Ecol., 70, 107-120, 2009.
645 Youngblut, N. D., Dell'Aringa, M., and Whitaker, R. J.: Differentiation between
646 sediment and hypolimnion methanogen communities in humic lakes, Environ.
647 Microbiol., 16, 1411-1423, 2014.
648 Zhu, D.-L., Sun, C., and He, H.: Detection Methanogens in Newly Settled Sediments
649 from Xuanwu Lake in Nanjing, China, Curr. Microbiol., 64, 539-544, 2012.
650
651

© Author(s) 2016. CC-BY 3.0 License.

652

653 **Table 1** Diversity and library coverage of archaeal 16S rRNA and *mcrA* genes. Samples
654 D1–D6 and E1–E6 were retrieved from Dianchi Lake and Erhai Lake, respectively.
655 Digits "1" – "6" were referred to sediment depth 0–5, 5–8, 8–11, 11–14, 14–17 and 17–
656 20 cm, respectively.

657

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

658

Biogeosciences Discuss., doi:10.5194/bg-2016-321, 2016
Manuscript under review for journal Biogeosciences
Published: 9 August 2016
© Author(s) 2016. CC-BY 3.0 License.

Biogeosciences
Discussions
Open Access
EGU

Figure captions

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

Figure 2 Abundance of archaeal 16S rRNA (a) and *mcrA* (b) genes in different layer 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 bold number in parentheses represents the numbers of the total sequences in the library. Numbers at the nodes indicate the levels of bootstrap support based on neighbor-joining analysis of 1,000 resampled datasets. The values less than 50 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. Samples D1–D6 and E1–E6 were retrieved

Biogeosciences Discuss., doi:10.5194/bg-2016-321, 2016
Manuscript under review for journal Biogeosciences
Published: 9 August 2016
© Author(s) 2016. CC-BY 3.0 License.

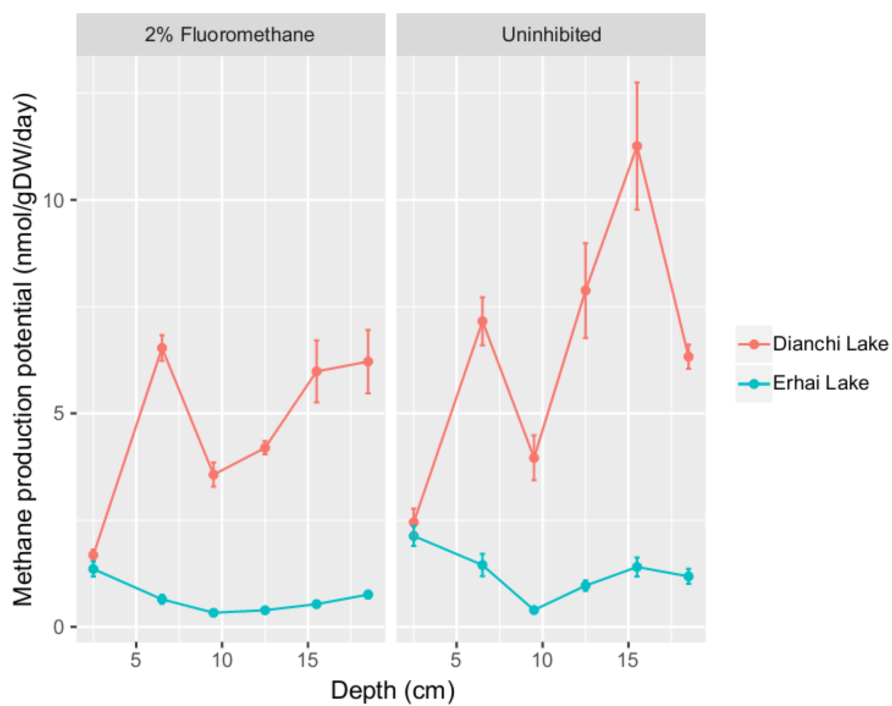
Biogeosciences
Discussions
Open Access
EGU

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.

Biogeosciences Discuss., doi:10.5194/bg-2016-321, 2016
Manuscript under review for journal Biogeosciences
Published: 9 August 2016
© Author(s) 2016. CC-BY 3.0 License.

Biogeosciences
Discussions
Open Access
EGU

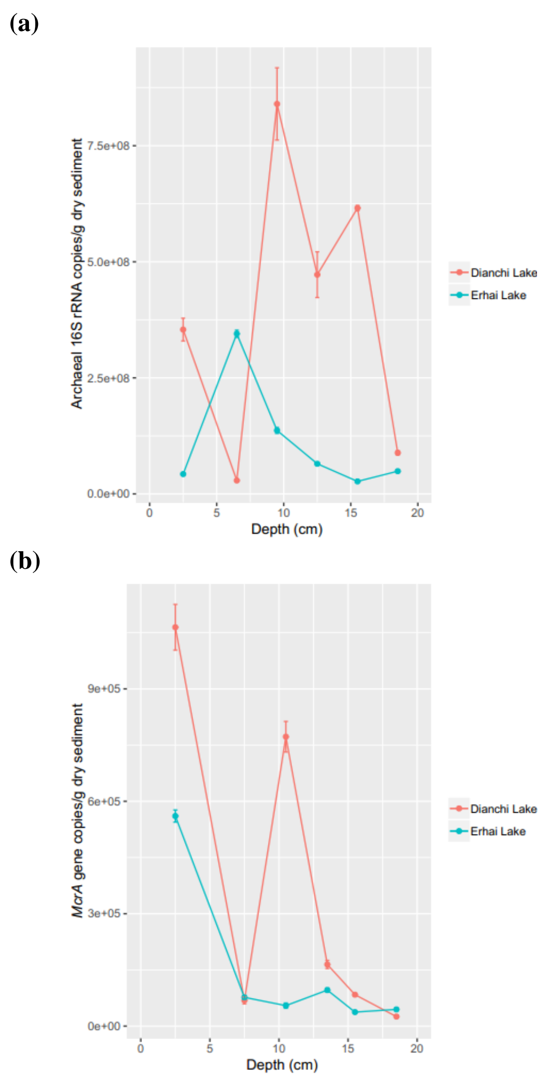
Figure 1



Biogeosciences Discuss., doi:10.5194/bg-2016-321, 2016
Manuscript under review for journal Biogeosciences
Published: 9 August 2016
© Author(s) 2016. CC-BY 3.0 License.

Biogeosciences
Discussions
Open Access
EGU

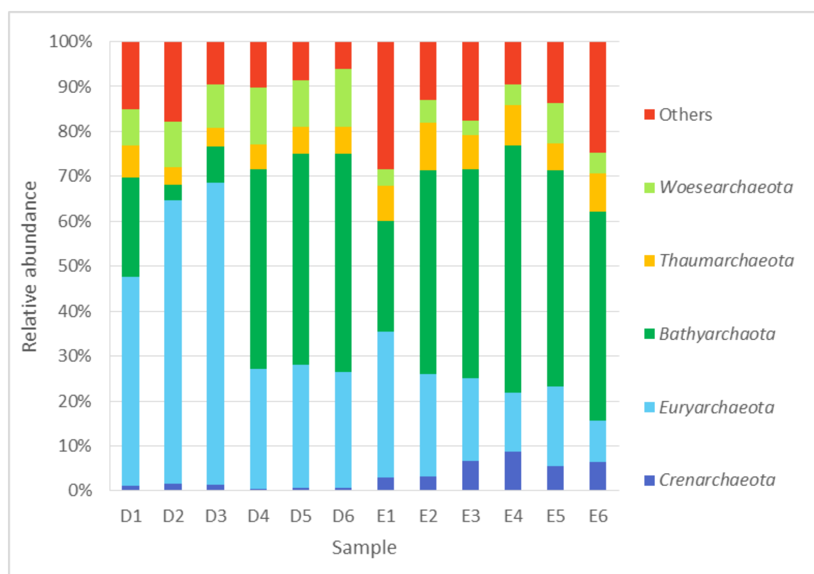
Figure 2



Biogeosciences Discuss., doi:10.5194/bg-2016-321, 2016
Manuscript under review for journal Biogeosciences
Published: 9 August 2016
© Author(s) 2016. CC-BY 3.0 License.

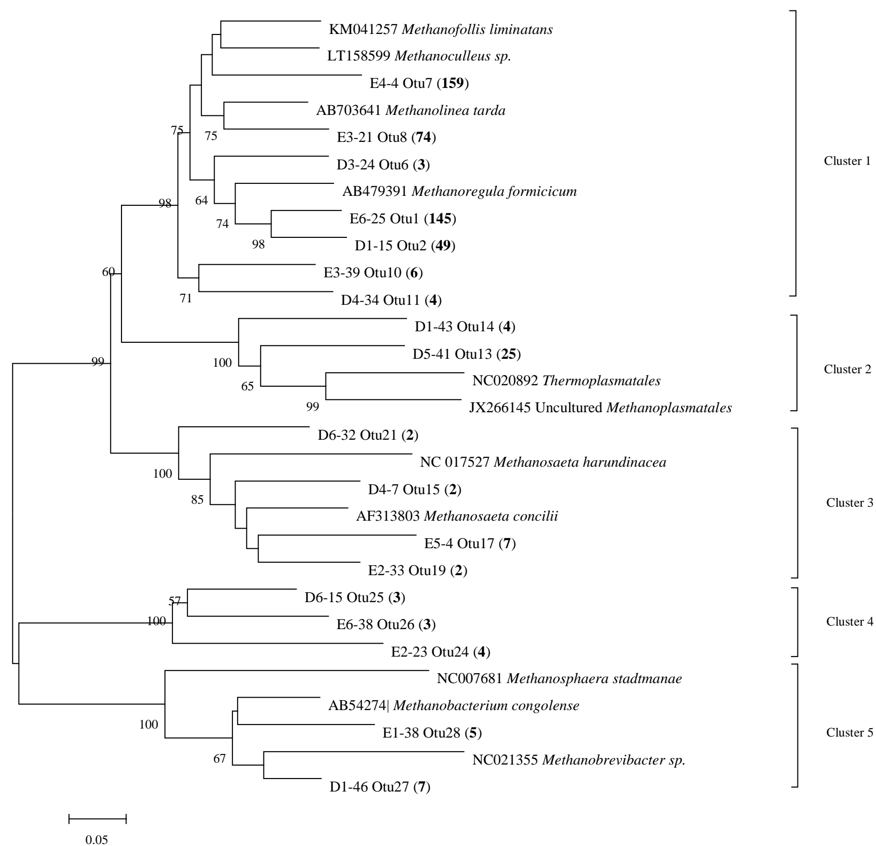
Biogeosciences Discussions Open Access EGU

Figure 3



Biogeosciences Discuss., doi:10.5194/bg-2016-321, 2016
Manuscript under review for journal Biogeosciences
Published: 9 August 2016
© Author(s) 2016. CC-BY 3.0 License.

Figure 4

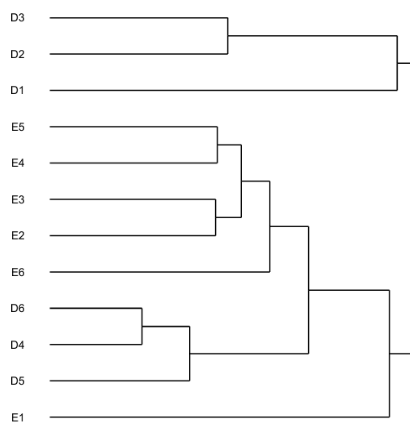


Biogeosciences Discuss., doi:10.5194/bg-2016-321, 2016
Manuscript under review for journal Biogeosciences
Published: 9 August 2016
© Author(s) 2016. CC-BY 3.0 License.

Biogeosciences Discussions
Open Access
EGU

Figure 5

(a)



(b)