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1 **Integrating isotopic, microbial, and modeling approaches to understand methane dynamics in**  
2 **a frequently disturbed deep reservoir in Taiwan**

3 **Authors**

4 **Masayuki Itoh<sup>1</sup>, Hisaya Kojima<sup>2</sup>, Pei-Chi Ho<sup>3,4</sup>, Chun-Wei Chang<sup>3,4</sup>, Tzong-Yueh Chen<sup>5</sup>,**  
5 **Silver Sung-Yun Hsiao<sup>6</sup>, Yuki Kobayashi<sup>7</sup>, Megumi Fujibayashi<sup>8</sup>, Shuh-Ji Kao<sup>9</sup>, Chih-hao**  
6 **Hsieh<sup>10,11,12,13</sup>, Manabu Fukui<sup>2</sup>, Noboru Okuda<sup>14</sup>, Takeshi Miki<sup>10,12\*</sup>, Fuh-Kwo Shiah<sup>12\*</sup>**

7

8 **Affiliations:**

- 9 1. Center for Southeast Asian Studies, Kyoto University, 46 Shimoadachi-cho, Yoshida Sakyo-ku,  
10 Kyoto, 606-8501 Japan.
- 11 2. The Institute of Low Temperature Science, Hokkaido University, Kita-19, Nishi-8, Kita-ku,  
12 Sapporo 060-0819, Japan
- 13 3. Earth System Sciences Program, Taiwan International Graduate Program, Academia Sinica, 128  
14 Academia Road, Section 2, Nankang, Taipei 11529, Taiwan
- 15 4. Earth System Sciences Program, Taiwan International Graduate Program, National Central  
16 University, No. 300, Zhongda Rd., Zhongli District, Taoyuan 32001, Taiwan
- 17 5. Institute of Marine Environment and Ecology, National Taiwan Ocean University, No. 2,  
18 Beining Road, Zhongzheng District, Keelung, 202. Taiwan
- 19 6. Institute of Earth Science, Academia Sinica, 128 Academia Road, Section 2, Nankang, Taipei  
20 11529, Taiwan
- 21 7. Faculty of Medicine and Health Sciences, Yamaguchi University, 1-1 Minamikogushi, Ube,  
22 755-8505, Japan
- 23 8. Department of Biological Environment, Akita Prefectural University, 84-4 Aza Ebinokuchi  
24 Tsuchiya, Yurihonjo City 015-0055 Japan
- 25 9. State Key Laboratory of Marine Environmental Science, Xiamen University, 422 Siming S Rd,  
26 Siming Qu, Xiamen Shi, Fujian Sheng, 361005, PROC

27 10. Institute of Oceanography, National Taiwan University, No. 1 Sec. 4 Roosevelt Rd, Taipei,  
28 10617, Taiwan

29 11. Institute of Ecology and Evolutionary Biology, Department of Life Science, National Taiwan  
30 University, No. 1 Sec. 4 Roosevelt Rd, Taipei, 10617, Taiwan

31 12. Research Center for Environmental Changes, Academia Sinica, 128 Academia Road, Section 2,  
32 Nankang, Taipei 11529, Taiwan

33 13. National Center for Theoretical Sciences , No. 1 Sec. 4 Roosevelt Rd, Taipei, 10617, Taiwan

34 14. Research Institute for Humanity and Nature, 457-4 Motoyama, Kamigamo, Kita-ku, Kyoto,  
35 603-8047 Japan

36

37 \*Corresponding Author

38 **Takeshi Miki**

39 [tksmiki@ntu.edu.tw](mailto:tksmiki@ntu.edu.tw),

40 tel/fax: +886-(0)2-33669903

41 **Fuh-Kwo Shiah**

42 [fkshiah@rcec.sinica.edu.tw](mailto:fkshiah@rcec.sinica.edu.tw),

43 tel +886-(0)2-2653-9885 (#271), fax: +886-(0)2-2789-3234

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45

46 **Abstract**

47 It has been estimated that more than 48% of global methane emissions from lakes and reservoirs  
48 occur at low latitudes (<24°). To improve this estimate, knowledge from underexplored ecosystems,  
49 particularly deep lakes and reservoirs in Asian monsoon regions, is needed, because the magnitude  
50 of methane emissions is influenced by lake bathymetry and climatic conditions. We conducted  
51 long-term studies beginning in 2004 at Feitsui Reservoir (FTR) in Taiwan, a subtropical  
52 monomictic system with a maximal depth of 120 m. Our aim was to monitor seasonal and  
53 interannual variations of three key characteristics and to understand the mechanisms underlying  
54 these variations. Key characteristics were as follows: 1) the balance of primary production and  
55 heterotrophic respiration as a determinant of vertical oxygen distribution, 2) methane production at  
56 the bottom of the reservoir, oxidation in the water column, and emission from the lake surface, and  
57 3) contribution of methane-originated carbon to the pelagic food web through methane-oxidizing  
58 bacteria (MOB). This review highlights major achievements from FTR studies integrating isotopic,  
59 microbial, and modeling approaches. Based on our findings, we proposed two conceptual models:  
60 1) a model of methane dynamics, which addresses the difference in methane emission mechanisms  
61 between deep and shallow lakes, and 2) a spatially explicit model linking benthic methane  
62 production to the pelagic food web, which addresses the diversity of MOB metabolisms and their  
63 dependence on oxygen availability. Finally, we address why long-term studies on subtropical lakes  
64 and reservoirs are important for better understanding the effects of climate on low- to mid-latitude  
65 ecosystems.

66

67 **Keywords:** methane production; methane oxidizing bacteria; food web model; isotope ecology;  
68 environmental microbiology

69

70 **Background**

71 Growing evidence indicates that the global methane budget is potentially influenced by methane  
72 release from freshwater systems (e.g., Bastviken et al. 2004; Ciais et al. 2013; Hamdan and  
73 Wickland 2016) and shallow coastal areas in marine systems (Borges et al. 2016; 2017), whereas  
74 open ocean (excluding areas with hydrates, especially in the arctic) is a minor contributor (Bates et  
75 al. 1996; Rhee et al. 2009). Freshwater studies, encompassing arctic (Kling et al. 1992, Laurion et al.  
76 2010), boreal (Bastviken et al. 2004, Huttunen et al. 2003), and temperate (e.g., Michmerhuizen et  
77 al. 1996) systems, have led to an estimated emission rate of 103 Tg methane year<sup>-1</sup> from lakes,  
78 reservoirs, and rivers (Bastviken et al. 2011). This estimate is equal to 0.65 Pg of C (expressed as  
79 CO<sub>2</sub> equivalent) and 25% of the estimated terrestrial greenhouse gas sink. The estimate would be  
80 even larger if more recent studies on rivers were to be considered (Borges et al. 2015a, b).

81 The magnitude of methane emission from the lake water surface is largely influenced by  
82 climate and lake bathymetry (i.e., depth and area) (Bastviken 2004), which critically determine the  
83 vertical distribution of oxygen. For example, the duration of the thermal stratification period and the  
84 lake bathymetry control the depth and stratification intensity of the mixed layer (Wilhelm and  
85 Adrian, 2008), which, in turn, determine the balance of primary production (PP) and aerobic  
86 respiration (Ostrom et al. 2005), especially in the surface layers. This balance affects the degree of  
87 oxygen depletion in deeper layers, which controls the production and oxidation of methane because  
88 these processes are regulated by oxygen availability (Murase et al. 2005). For these reasons,  
89 regional variations in freshwater methane emission are important considerations for a reliable global  
90 estimate (Bastviken et al. 2004; Tranvik et al. 2009; Pacheco et al. 2013).

91 According to estimates based on several studies, more than 48% of global methane  
92 emissions from lakes and reservoirs are due to methane release at lower latitudes (<24°) (calculated  
93 from Table 1 in Bastviken et al. 2011). However, most studies of methane emissions from lakes and  
94 reservoirs at lower latitudes concerned shallow lakes (i.e., Amazon floodplains; Bastviken et al.  
95 2010), only five of which were located in Asia. Although this distribution is partly reasonable

96 because most lakes are shallow (Wetzel 1990), more studies from deep lakes and reservoirs in Asian  
97 monsoon regions will contribute to improve the accuracy of the estimate.

98 Multiple factors influence methane biogeochemistry. Key determinants of methane  
99 oxidation are oxygen availability, oxygen-to-substrate ratio (Morana et al. 2015), and temperature  
100 (Lofton et al. 2014). The main determinants of anaerobic methane production are oxygen deficiency  
101 and substrate availability, factors that are also influenced by lake bathymetry (Bastviken et al. 2004;  
102 2008). Anaerobic methane oxidation is believed to be coupled with denitrification, which is affected  
103 by nitrogen availability (Deutzmann et al. 2014). The phylogeny of archaea and bacterial groups  
104 indicates their specific roles in methane production and oxidation (Borrel et al. 2011).

105 In addition to their role in biogeochemical cycling, methane-oxidizing bacteria (MOB), also  
106 known as methanotrophs, represent alternative carbon resources at higher trophic levels in benthic  
107 and pelagic food webs (Kiyashko et al. 2001; Deines and Fink 2011; Jones and Grey 2011). The  
108 contribution of methane-derived carbon via MOB to the pelagic food web changes with season in  
109 temperate regions (Taipale et al. 2009). However, this topic remains underexplored for lakes and  
110 reservoirs in subtropical and tropical regions (hereinafter referred to as “lower-latitude regions”).

111 Due to high water temperature and meromixis, tropical lakes have high potential for  
112 methane production in anoxic deep waters and sediment (e.g., Abril et al. 2005; Pasche et al. 2011),  
113 resulting in characteristic methane accumulation near the bottom under reducing conditions. In  
114 contrast, monomictic subtropical lakes can recover from hypoxia in deep water by vertical mixing  
115 in winter or extreme weather events such as typhoons (e.g., Tanaka and Tsuda, 1996; Yoshimizu et  
116 al., 2010) and hurricanes. This effect may decrease the potential for methane production by  
117 methanogens in the sediment but can facilitate methane oxidation by MOB in the water column.  
118 Therefore, an understanding of these processes in subtropical deep lakes will provide insights into  
119 the mechanisms underlying carbon budget, methane emission, and MOB roles in food webs of lakes  
120 and reservoirs at lower latitudes, and will aid in improving the estimate of the global budget of  
121 carbon and methane release.

122 The aim of this paper is to provide information for better understanding the mechanisms of  
123 methane dynamics at lower latitudes. First, we highlight the research questions, approaches, and  
124 some achievements from a long-term study of a subtropical deep reservoir. Second, we propose  
125 some perspectives, including a revised conceptual model for methane dynamics in lakes and  
126 reservoirs at lower latitudes and new research directions.

127

## 128 **Research Questions and Approaches**

129 With the aim of improving understanding of methane dynamics at lower latitudes, we specifically  
130 focused on three questions. (1) How do physical structure and seasonal disturbances alter the  
131 balance between PP and bacterial heterotrophy? (2) Under what conditions would methane  
132 production and oxidation be enhanced in lower-latitude lakes? (3) Under what conditions would the  
133 contribution of MOB to the food web increase? An answer to the first question would help address  
134 the subsequent two questions because these two counter biological processes (PP and bacterial  
135 heterotrophy) control the redox conditions that, in turn, affect methane dynamics.

136 To address our research questions, we carried out a multiyear survey in which we observed  
137 the PP, aerobic respiration, and dynamics of methane in response to environmental changes. This  
138 study was motivated by the notion that a small lake system is particularly sensitive to different  
139 environmental conditions with interannual variations in climate.

140 Beginning in November 2004, we conducted field sampling at Feitsui Reservoir (FTR)  
141 (120.34E, 24.54N; maximal depth: 120 m) in northern Taiwan. FTR is a good model system for  
142 deep monomictic lakes in subtropical regions because: (1) it is well-protected from anthropogenic  
143 pollution and, thus, habitat destruction, and its nutrient status is oligotrophic to mesotrophic (Chang  
144 and Wen 1997); and (2) the region has substantial interannual variations in winter mixing intensity,  
145 degree of summer stratification, and thickness of hypoxic hypolimnion, depending on weather  
146 conditions (Itoh et al. 2015; Ho et al. 2016). In addition, typhoons are a major disturbance of  
147 summer stratification in this region (e.g., Fan and Kao 2008). Extreme weather events like typhoons,

148 which typically occur at lower latitudes, affect water and material cycling in lakes. The strength and  
149 frequency of typhoons passing on or near FTR change interannually; thus, we can focus on and  
150 observe the ecosystem responses to hydrodynamical changes.

151

## 152 **Results of Research on FTR**

### 153 *R1. Disproportionate enhancement of bacterial over algal activity induced by typhoons*

154 Using time-series data from 2004 to 2007, Tseng et al. (2010) showed that the ratio of bacterial  
155 production (BP) to PP (hereafter; BP/PP) in FTR was higher in strong typhoon years (2004 and  
156 2005:  $27\% \pm 40\%$ ) than in normal typhoon years (2006 and 2007:  $12\% \pm 9\%$ ). This result indicates  
157 a disproportionate increase of BP relative to PP after typhoons. In FTR, BP was two-fold greater,  
158 but PP was only 20% greater, in strong than in normal/weak typhoon years. Such disproportionate  
159 enhancement of heterotrophic bacterial activity by typhoons has seldom been described in  
160 freshwater ecosystems. Previous studies focused on the effect of typhoons on autotrophic activity  
161 (PP) only (Ko et al. 2015, 2017; and citations therein), whereas only a few studies quantified both  
162 PP and BP (Shiah et al. 2000; Tsuchiya et al. 2015).

163 The increased BP/PP with typhoons in FTR can be explained by the relative extent of the  
164 phosphorus (P) limitation in PP vs. BP. Whereas bioassay experiments demonstrated that  
165 autotrophic and heterotrophic activities are limited by P but not carbon or nitrogen (Tseng et al.  
166 2010), heterotrophic bacteria exhibited a faster response than algae did to phosphate enrichment  
167 (Fig. 7 in Tseng et al. 2010). This experiment suggests that, in the field, the P pulse introduced by  
168 typhoons might relieve bacteria more than phytoplankton from P limitation. This result fits well  
169 with past empirical studies and theories indicating that bacteria are responsible for the major uptake  
170 of P due to their superior competition capacity in oligotrophic ecosystems (Currie and Kalff 1984,  
171 Thingstad et al. 1997, Vadstein 2000).

172 Strong typhoons may affect plankton activities in both euphotic and aphotic zones. Heavy  
173 rains caused by typhoons resulted in supply of P by hyperpycnal flow. This P entered aphotic zones

174 and enhanced bacterial production in aphotic and euphotic zones. Decoupling of BP and PP (i.e.,  
175 increased BP/PP) in euphotic zones and increased BP in aphotic zones resulted in enhanced  
176 consumption and reduced concentration of dissolved organic carbon (DOC) in euphotic and deeper  
177 zones (Tseng et al. 2010).

178

179 *R2. Distinct interannual variations of dissolved oxygen (DO) vertical profile between summer and*  
180 *winter*

181 The vertical profile of DO is the key controlling factor for aerobic and anaerobic respiration. Itoh et  
182 al. (2015) found that the interannual DO patterns in summer were different from those in winter in  
183 FTR, implying the presence of season-specific controlling mechanisms. In a typical monomictic  
184 lake, DO levels in the deep layer should be highest after vertical mixing of the water column in the  
185 coolest part of the year and lowest at the end of the stratification period. DO levels at the bottom  
186 during the coolest period negatively correlated with surface water temperature in 2005–2014 (Itoh  
187 et al. 2015). Higher surface water temperature led to weaker winter mixing and deficiency of DO at  
188 the bottom, which could last until the next stratification period.

189 In the summer period (June to September), when rainfall peaks with summer monsoon  
190 fronts and typhoons, interannual variation of DO levels (evaluated by saturation level to normalize  
191 temperature dependence) was complex and tended to depend on depth. This result was attributed to  
192 an increase in lateral water flow from upstream rivers and hillslopes with intensive rainfall and its  
193 effects on microbial activities. In mid-depth layers (20–30 m), DO levels tended to be lower in  
194 strong typhoon years (2004 or 2005) than in weak typhoon years (2006 or 2007) (Fig. 1). There was  
195 no difference in DO levels at 0, 10, and 50 m between years in the summer. In the summer of 2004,  
196 typhoons might induce lateral turbid flow from upstream, with movement of eroded soils or  
197 suspended sediments into mid-depth layers (20–50 m) (Fan and Kao 2008). Particles suspended in  
198 turbid lateral flow would contribute to oxygen consumption in mid-depth layers. These results  
199 imply that DO levels at mid-depth layers might be affected by typhoons and subsequent

200 disproportionate increases in bacterial activity over algal growth. At the same time, however, the  
201 vertical profile of DO in strong typhoon years (2004 and 2005) indicated that the turbid lateral flow  
202 provided external DO to deeper layers (90 m; Fig. 1). DO levels at the bottom would not only result  
203 from effects of typhoons, but also would be influenced by the legacy of winter mixing in the  
204 previous year (Itoh et al. 2015).

205

### 206 *R3. Role of winter mixing in determining anaerobic methane production in lake sediment*

207 Observations in FTR indicated that: (1) stratification can be maintained even during winter when  
208 mixing is weak due to high surface water temperature, and (2) lower surface water temperature in  
209 winter leads to more intense mixing. Reducing conditions in the bottom layer due to incomplete  
210 vertical mixing in winter decreased profundal DO and  $\text{NO}_3^-$  concentrations in the following summer.  
211 Oxygen was depleted during the subsequent thermal stratification period, and this oxygen depletion  
212 facilitated  $\text{NO}_3^-$  consumption by denitrifiers. Based on seasonal variations of the vertical profiles of  
213 methane concentrations and stable isotope signal ( $\delta^{13}\text{C}$ ) values from 2012 to 2014, weak winter  
214 mixing can increase sedimentary methane production and, thus, profundal methane storage through  
215 hypoxia during the thermal stratification period (Itoh et al. 2015). Unfortunately, we did not observe  
216 methane dynamics in 2004–2007 and, therefore, were unable to investigate directly the effects of  
217 typhoons on methane production (see section above). Overall, this situation is analogous to the  
218 study of Marotta et al., (2014) of an Amazonian tropical lake in which winter mixing is not  
219 expected. Results demonstrated that anaerobic biological methane production in the sediments  
220 increased exponentially in response to increased temperature.

221

### 222 *R4. Consequences of methane oxidation in the water column on methane release from the surface*

223 Although a long, strong stratification period increased the amount of methane that was produced  
224 and accumulated in the bottom layer, this process did not directly enhance the amount of methane  
225 emitted from the surface. With a maximal depth of 120 m, FTR is sufficiently deep that most

226 methane produced in the profundal layer is consumed and oxidized by MOB (Itoh et al. 2015). Such  
227 decoupling of methane accumulation in the bottom layer from methane emission at the surface was  
228 observed in strongly stratified tropical meromictic lakes (e.g., Borges et al. 2011). Methane  
229 oxidation in the water column was evidenced by a decrease in methane concentration with  
230 increasing distance from sediment (mostly within 20–30 m above the sediment) in tropical (Rudd  
231 1980; Guerin and Abril 2007; Borges et al. 2011), subtropical, and temperate lakes (Eckert and  
232 Conrad 2007; Bastviken et al. 2008; Chanudet et al. 2011; Roland et al. 2017). Other studies  
233 directly demonstrated methane oxidation in the water column by water incubation experiments (e.g.,  
234 Utsumi et al. 1998).

235 In FTR, substantial methane oxidation in the water column was initially revealed by  
236 studying the  $\delta^{13}\text{C}$ -methane profile in water samples with low methane concentrations (Itoh et al.  
237 2015). The same method was used in the tropical Lake Kivu (Morana et al. 2015). The  
238  $\delta^{13}\text{C}$ -methane values were consistently negative in the near-bottom layer, reflecting that large  
239 isotope fractionation occurred during methanogenesis. The  $^{13}\text{C}$ -methane values were higher in the  
240 oxic/anoxic boundary layer (especially up to 30 m above the sediment surface). This result suggests  
241 that much of the enrichment of dissolved  $^{13}\text{CH}_4$  was due to methane oxidation because MOB  
242 consume  $^{12}\text{CH}_4$  slightly faster than  $^{13}\text{CH}_4$ . Anaerobic and aerobic methane oxidation would be  
243 involved in methane consumption. Even during the stratified period, MOB were the predominant  
244 component of the whole bacterial community near the bottom of the water column, where oxygen  
245 was almost depleted, as shown by Kojima et al. (2014) using catalyzed reporter deposition  
246 fluorescence in situ hybridization (CARD-FISH) analysis.

247

#### 248 *R5. Major types of methanotrophs in FTR*

249 Molecular analysis of bacterial communities in FTR revealed eight species-level operational  
250 taxonomic units (OTUs) of Type I MOB (gammaproteobacteria, commonly found in temperate  
251 lakes), one OTU of Type II MOB (alphaproteobacterial, commonly found in tropical lakes), and one

252 Methylomirabilis-like OTU belonging to candidate phylum NC10 (Kojima et al. 2014).

253 *Methylomirabilis oxyfera* is a nitrite-dependent methane oxidizer (Ettwig et al. 2010).

254 Vertically, analysis of 16S rRNA gene-based clone libraries demonstrated that Types I and II  
255 MOB were distributed in the hypoxic layer at 90 m, even in the summer stratification period, and in  
256 the oxic surface layer at 10 m (Kojima et al. 2014). Clone libraries of pmoA genes encoding  
257 particulate methane monooxygenase confirmed their presence at 90 m in the winter. This result is  
258 inconsistent with the conventional hypothesis that Types I and II MOB are aerobic. The number of  
259 16S rRNA gene clone libraries analyzed was not enough to permit discussion of the seasonal or  
260 interannual variations in relative abundances of Types I and II MOB. However, CARD-FISH  
261 analysis of bacteria in the 90-m layer in winter (December 2013) demonstrated the dominance of  
262 Methylomirabilis-like OTUs (Kojima et al. 2014). These records represent the first evidence of  
263 anaerobic methane oxidizers in the water column of lake ecosystems, although many studies have  
264 reported the presence of anaerobic methane oxidizers coupled with denitrification in freshwater  
265 sediment (Raghoebarsing et al. 2006; Ettwig et al. 2009, 2010; Deutzmann et al. 2014; Norði and  
266 Thamdrup 2014).

267 These results have two implications. First, spatial distributions of Types I and II MOB imply  
268 that they are involved in the carbon flow under both oxic and hypoxic conditions, relying on distinct  
269 biochemical pathways (Vecherskaya et al. 2009; Kits et al. 2015). Second, the presence of the NC10  
270 OTU close to the anaerobic nitrite reducer Candidate *M. oxyfera* implies that methane oxidation  
271 would be coupled with nitrogen cycling in the water column of FTR. Further study of anaerobic  
272 methane oxidizers in lower-latitude lakes will be needed for a thorough understanding of MOB  
273 activities in lake ecosystems.

274

275 *R6. Interannual variations in the MOB contribution to the pelagic food web*

276 Results of isotope analyses based on the MixSIR Bayesian mixing model demonstrated interesting  
277 seasonal and interannual (2010–2013) variations in the contributions of MOB to the pelagic food

278 web (Ho et al. 2015). The MOB contribution tended to be highest in winter, consistent with patterns  
279 in other climatic regions, including boreal (Taipale et al., 2011) and tropical lakes (Morana et al.,  
280 2015). Interannual variations in winter are potentially influenced by two contrasting mechanisms.  
281 On the one hand, deficiency of profundal DO in summer enhances methanogenesis and  
282 accumulation of profundal methane toward winter, supplying more substrate to MOB and resulting  
283 in a higher contribution of MOB in winter. On the other hand, oxygen supply for profundal waters  
284 due to winter mixing enhances aerobic methane oxidation, resulting in a higher contribution of  
285 MOB to the food web in winter. The former mechanism would be the case if MOB were more  
286 limited by methane availability than by oxygen availability which is clearly the case in FTR.

287         Results of a vertically structured food web model using reaction-advection-diffusion  
288 equations predicted that deeper disturbance during summer would suppress the contribution of  
289 MOB in winter (Ho et al. 2016). Although winter mixing could have positive and negative effects  
290 on the contribution of MOB over the year, stronger mixing in winter resulted in weaker deficiency  
291 of profundal DO in the next summer, leading to lower MOB contribution in the following winter.  
292 This result is consistent with the methane accumulation pattern (Itoh et al. 2015) and estimates from  
293 the stable-isotope mixing model (Ho et al. 2016).

294         Molecular analyses of bacterial communities indicated that the taxonomic composition of  
295 MOB (i.e., anaerobic MOB phylogenetically close to NC10 and aerobic Types I and II) changed  
296 seasonally and vertically (Kojima et al. 2014; Kobayashi et al. 2016). However, due to the limited  
297 availability of quantitative data, MOB functional activity in the food web model was parameterized  
298 following an earlier experimental study (Harrits and Hanson 1980). MOB activity was assumed to  
299 be suppressed by low or high oxygen availability and to be maximal at an oxygen level of around  
300 200 mmol O<sub>2</sub> m<sup>-3</sup>. This model is a black box approach to represent the diverse functionality of  
301 MOB implicitly by assuming that the community is a mixture of aerobic and anaerobic MOB. If we  
302 assumed much lower optimal DO levels (with predominance of anaerobic MOB), then the model  
303 would be unable to explain the observed higher contribution of MOB in winter than summer when

304 oxygen availability is high due to vertical water mixing. Therefore, we argue that both aerobic and  
305 anaerobic reactions are responsible for sustaining the food web productivity, especially in winter.

306

## 307 **Perspectives**

### 308 *Perspective 1: Vertical distribution of methane in deep lake/reservoir at lower latitudes*

309 Here, we propose a conceptual model for the vertical patterns of methane and its related elements in  
310 a deep lake during the stratification period (Fig. 2). This model includes stable isotope signatures  
311 for methane compared to the shallow lake illustration modified from Bastviken et al. (2004).

312 Methane production, originating from organic matter and CO<sub>2</sub>, mainly occurs in the  
313 anaerobic sediment. Productions of sedimentary methane (by methanogenesis) and CO<sub>2</sub> (by  
314 heterotrophic respiration) are controlled by profundal DO. The vertical distribution of DO depends  
315 on the difference in the intensities of stratification and mixing, which are affected by the climate  
316 condition. Despite the high profundal methane concentration in the stratified period, most of the  
317 dissolved methane can be oxidized within 20 to 30 m above the sediment layer (see R4) in deep  
318 lakes. This fact suggests that sedimentary methane production is not a main source of methane  
319 emission from lakes with sufficient depth (right diagram in Fig. 2). By contrast, in shallow lakes,  
320 methane produced in sediment affects methane flux at the water surface as both ebullition and  
321 diffusion flux with being oxidized incompletely (left diagram in Fig. 2). This phenomenon is also  
322 true for methane produced in sediment of the shallow part of a deep lake. For example, findings in  
323 German lakes showed that the ratio of the surface area of the shallow water zone to the entire lake  
324 area was a better predictor of surface methane concentration than the total surface area (Encinas  
325 Fernández et al. 2016). Nevertheless, studies of the distinct methane dynamics in shallow vs. deep  
326 parts of lakes at lower latitudes are needed to confirm the robustness of Encinas Fernández's  
327 conclusion.

328 Methane emissions from deep lakes could potentially be explained by subsurface methane  
329 production. The maximum amount of subsurface methane reported in some oceans and lakes

330 implies *in situ* methane production in oxic waters (Bogard et al. 2014; Tang et al. 2014; Itoh et al.  
331 2015; Yao et al. 2016). Therefore, subsurface rather than profundal methane production may  
332 account for a portion of the methane emitted from the water surface. As frequently happens in  
333 well-stratified tropical lakes (Verburg et al. 2003), cyanobacterial blooms occur during summer in  
334 FTR. It may be that the subsurface cyanobacteria bloom in summer plays a neglected role in the  
335 production of methane and the vertical distribution of oxygen and, thus, regulates anaerobic  
336 methanogenesis in the bottom layer. Although interactions between cyanobacteria and  
337 bacteria/archaea can result in methane production in oxic layers (Bogard et al. 2014), the  
338 mechanism for this process is not fully understood (summarized in Tang et al. 2016). Another  
339 controversy is whether methane produced in the oxic subsurface layers contributes much (Bogard et  
340 al. 2014) or little (Encinas Fernández et al. 2016) to the amount of methane emitted from the lake  
341 surface. Finally, the possible production of CH<sub>4</sub> under aerobic conditions (Karl et al. 2008; Damm  
342 et al. 2008) in marine systems has been debated. Although this process could explain the very low  
343 concentration (< 4 nM) of CH<sub>4</sub> in open and deep oceanic regions, it cannot explain the much larger  
344 concentration of CH<sub>4</sub> (10–1000 nM) in shallow coastal areas, where CH<sub>4</sub> undoubtedly comes from  
345 sediments (Borges et al. 2016; 2017).

346

#### 347 *Perspective 2: Roles of diverse methanotrophs (MOB) in food web dynamics*

348 From a food-web perspective, MOB are key players in a new mode of pelagic-benthic coupling in  
349 lake ecosystems (Schindler and Scheuerell 2002). In the broadly accepted view of pelagic-benthic  
350 coupling in deep lakes, sedimentation of organic matter produced by pelagic production is the basal  
351 resource of benthic invertebrates and fishes, which act as alternative resources of pelagic mobile  
352 predators such as zooplankton and fishes. In the new mode of pelagic-benthic coupling in deep  
353 lakes, summer pelagic PP is transferred to the benthic layers, ultimately supporting the secondary  
354 production of pelagic zooplankton mediated by methane-based food webs along the water column.

355 Two aspects of this new mode of pelagic-benthic coupling need to be addressed. First, the

356 contribution from the benthic (methanotrophs) to pelagic (zooplankton) habitats mainly occurs  
357 during winter. The coupling effect has a time delay, due to the time required for sedimentation of  
358 particulate organic matter from the pelagic to benthic and for subsequent biogeochemical processes  
359 in benthic habitats (sediment plus deep water column), which supply resources to zooplankton  
360 through methane-based food webs (Ho et al. 2016). Second, our microbial ecology studies (Kojima  
361 et al. 2014; Kobayashi et al. 2016) and other existing evidence (refs. in Fig. 3) indicate that the  
362 sources of biomass carbon from MOB to the microbial food web are more diverse than previously  
363 thought (Fig. 3). Methane is not always *directly* integrated into the microbial food web via MOB  
364 assimilating methane through aerobic methane oxidation. For example, carbon biomass of *M.*  
365 *oxyfera* (NC10) is assimilated by fixation of CO<sub>2</sub> but not directly by carbon from methane (Rasigraf  
366 et al. 2014). Therefore, dominance of the *M. oxyfera*-like phylotype in anoxic layers of FTR  
367 (Kojima et al. 2014) implies that methane is completely respired as CO<sub>2</sub>. Some of the CO<sub>2</sub>  
368 assimilated into the bacterial biomass could have originated from methane oxidation; thus, carbon  
369 from methanogenesis is only *indirectly* incorporated into MOB biomass and the microbial food web.  
370 In addition, the presence of Types I and II MOB (Methylocystis) in the deep layers (anaerobic or  
371 microaerobic condition) implies that fermentative reactions support their activities, which are  
372 coupled with the release of organic acids such as acetate (Vecherskaya et al. 2009; Kalyuzhnaya et  
373 al. 2013). These organic acids are substrates for the growth of some bacteria, including *Methlocystis*  
374 (Belova et al. 2011; Im et al. 2011), and are finally incorporated into the microbial food web (i.e.,  
375 *indirect* incorporation of methane-originated carbon).

376         These diverse types of MOB and metabolic pathways from methane should be further  
377 explored to understand better the importance and mechanisms of methane-based food web  
378 dynamics. The next version of the dynamical model coupling methane processes and food web  
379 dynamics should incorporate these diverse processes. In addition, CO<sub>2</sub> would be repeatedly recycled  
380 within the anaerobic food chain in the sediment and sediment-water column boundary (Fig. 3).  
381 Therefore, isotope analysis of CO<sub>2</sub> and MOB together with analysis of phospholipid fatty acids (e.g.,

382 Belova et al. 2011) will be necessary to elucidate the dominant reactions and estimate the timescale  
383 of interactions between CO<sub>2</sub>, methane, and MOB and, thus, the new pelagic-benthic coupling.

384

### 385 *Perspective 3: Importance of studying underexplored tropical/subtropical lakes*

386 Lakes in subtropical regions are highly dynamic in terms of their interannual climate variations and  
387 strength/frequency of disturbances. These factors are determinants of the vertical, seasonal, and  
388 interannual variations of microbes and biogeochemical processes. Such variations control the 1)  
389 balance of PP and aerobic respiration, 2) production and oxidation of methane, and 3) incorporation  
390 of methane-originated carbon into the pelagic food web (Figs. 1–3). Our multiple approaches to  
391 understand methane dynamics targeted a subtropical reservoir (FTR) with an essentially  
392 monomictic pattern. As is the case in some reported subtropical lakes, FTR occasionally  
393 experiences not only incomplete vertical mixing in winter but also stronger and longer thermal  
394 stratification periods, resulting in profundal hypoxia (e.g., Sahoo and Schladow 2008; Yoshimizu et  
395 al. 2010). In contrast, intensive winter mixing can be observed in cold winters. These findings  
396 indicate that long-term studies of deep monomictic lakes and reservoirs at lower latitudes can reveal  
397 aspects of both meromictic and monomictic lakes.

398 Our study sheds new light on other important controlling factors of biogeochemical cycles,  
399 such as the disruption of stratification by heavy-rain events. Lower-latitude areas experience a  
400 higher frequency of heavy precipitation than mid- or high-latitude regions (Dai 2012). Under recent  
401 warming conditions, the frequency of heavy precipitation and the temperature of mid-latitude  
402 regions have been increasing and are predicted to increase further (e.g., Meehl et al. 2005).  
403 Knowledge of the response of methane dynamics in lower-latitude lakes to climate variations will  
404 make it possible to predict the future condition of mid-latitude lakes. Our case study showed that  
405 the effects of typhoons in summer on the ratio of PP to aerobic respiration and, thus, DO levels were  
406 depth-specific. Long-term comparative studies of other mero/monomictic lakes at the  
407 tropical/subtropical boundary (e.g., Okuda et al. 2017) will provide more comprehensive

408 understanding of mechanisms in lakes at a wide latitudinal scale in a changing world.

409

410

411

412 **Figure Legends**

413 **Fig. 1 DO saturation (%) in each sampling depth from June to September from 2004 to 2007**

414 **(strong typhoon years 2004 or 2005 and weak typhoon years 2006 or 2007).** Numbers in

415 parentheses indicate numbers of samples. Box plots show median (line), 25<sup>th</sup> to 75<sup>th</sup> percentiles

416 (box), 10<sup>th</sup> to 90<sup>th</sup> percentiles (bars), and individual values <10<sup>th</sup> or >90<sup>th</sup> percentile (points).

417 Differences were detected using one-way ANOVA and Tukey's multiple comparison tests. Different

418 letters indicate a significant difference ( $P < 0.05$ ).

419

420 **Fig. 2 Schematic of methane dynamics in shallow lakes and coastal parts of deep lakes**

421 **(modified from Bastviken et al. 2004) and pelagic parts of deep lakes (modified from Itoh et al.**

422 **2015) during the stratification period.** Right panel shows vertical profiles of temperature, DO,

423 methane concentration, methane carbon isotope ratio, and nitrate concentration at the end of

424 stratification period (Dec 2013) in FTR (Itoh et al. 2015).

425

426 **Fig. 3 Schematic diagram illustrating our new conceptual model of pelagic-benthic coupling**  
427 **mediated by vertically structured diverse MOB groups.** Some arrows are omitted for simplicity

428 (e.g., release of CO<sub>2</sub> from zooplankton). Distribution of microbes in benthic habitats (from

429 microaerobic water column to anaerobic sediment) was not clearly separated in FTR project,

430 although it is conceptually separable. Microbial members in sediment (fermenter and methanogen)

431 were not targets of observation in this project. HB represents heterotrophic bacteria that utilize

432 photosynthetic products (POM). Ref1: Belova et al. 2011; Im et al. 2011, Ref2: Morel et al. (2011),

433 Ref3: Vecherskaya et al. (2009) and Kalyuzhnaya et al. (2013), Ref4: Ettwig et al. (2010) and

434 Rasigraf et al. (2014).

435

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656

Figure 1

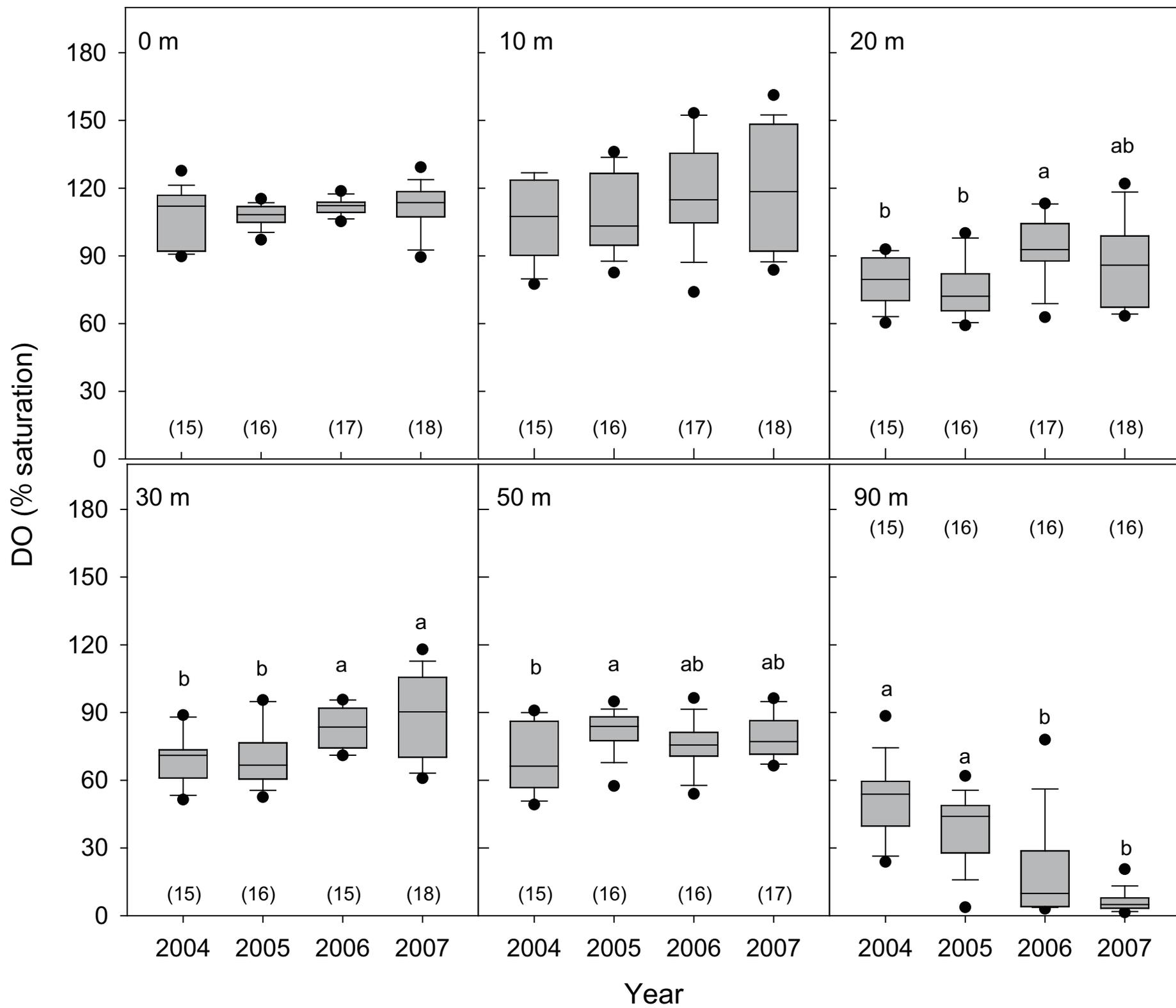


Figure 2

[Click here to download Figure1Fig2.eps](#)

Shallow lakes  
or  
littoral zone  
(shallow part)  
in deeplakes

Pelagic region  
(deep part)

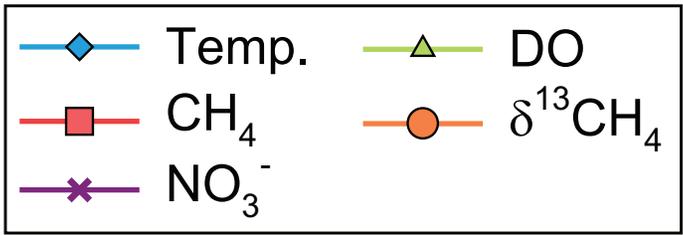
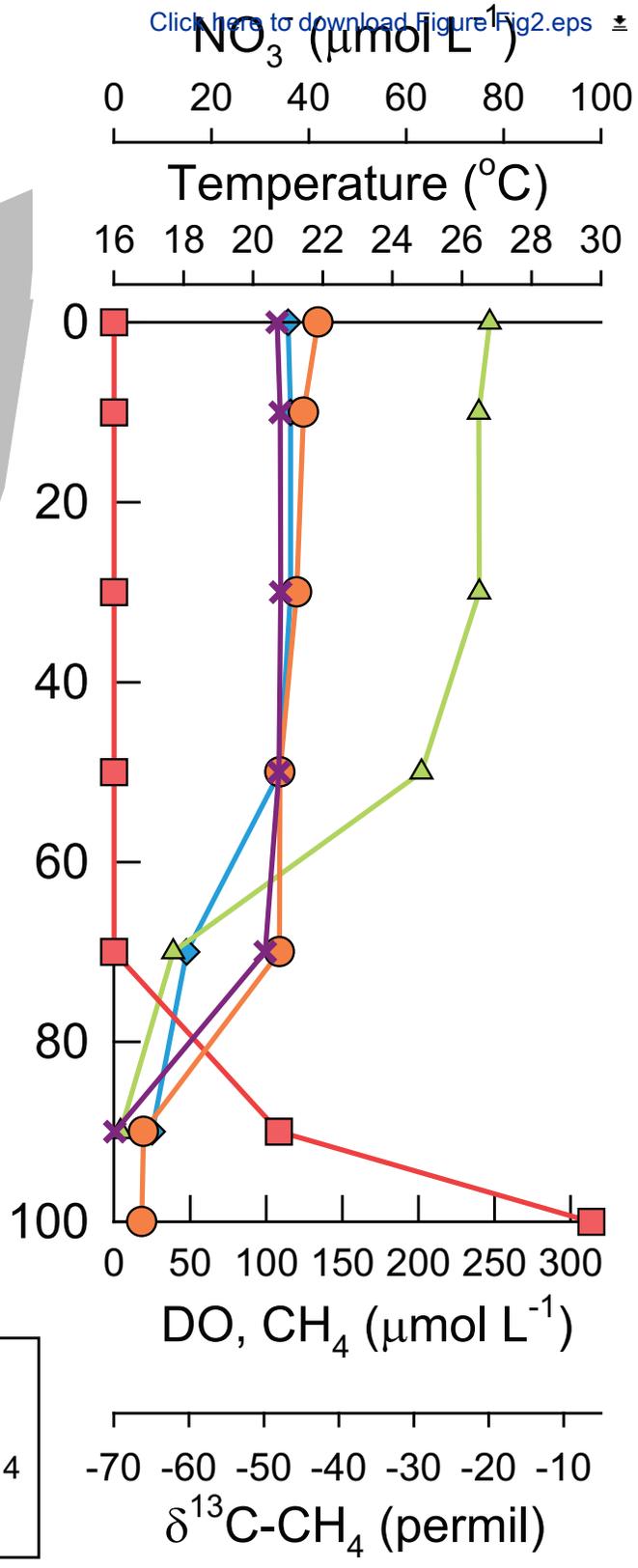
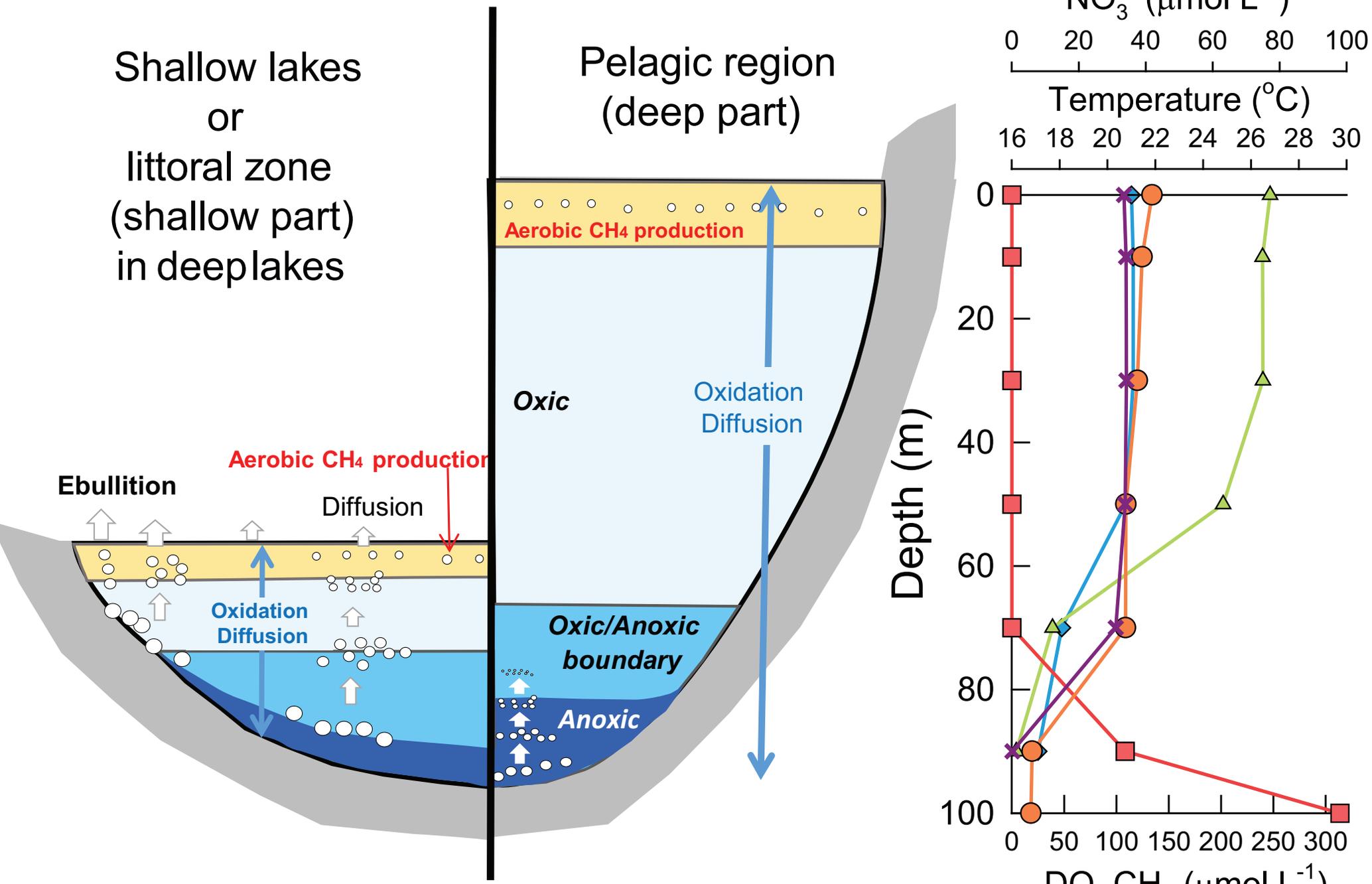


Figure 3

