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1 **Integrating isotopic, microbial, and modeling approaches to understand methane dynamics in**

2 **a frequently disturbed deep reservoir in Taiwan**

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45

Abstract

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

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

Background

Growing evidence indicates that the global methane budget is potentially influenced by methane release from freshwater systems (e.g., Bastviken et al. 2004; Ciais et al. 2013; Hamdan and Wickland 2016) and shallow coastal areas in marine systems (Borges et al. 2016; 2017), whereas open ocean (excluding areas with hydrates, especially in the arctic) is a minor contributor (Bates et al. 1996; Rhee et al. 2009). Freshwater studies, encompassing arctic (Kling et a. 1992, Laurion et al. 2010), boreal (Bastviken et al. 2004, Huttunen et al. 2003), and temperate (e.g., Michmerhuizen et al. 1996) systems, have led to an estimated emission rate of 103 Tg methane year^{-1} from lakes, reservoirs, and rivers (Bastviken et al. 2011). This estimate is equal to 0.65 Pg of C (expressed as CO_2 equivalent) and 25% of the estimated terrestrial greenhouse gas sink. The estimate would be even larger if more recent studies on rivers were to be considered (Borges et al. 2015a, b).

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

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

4

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% ± 40%) than in normal typhoon years (2006 and 2007: 12% ± 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

7

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

8

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 NO_3^- concentrations in the following summer.

211   Oxygen was depleted during the subsequent thermal stratification period, and this oxygen depletion

212   facilitated NO_3^- consumption by denitrifiers. Based on seasonal variations of the vertical profiles of

213   methane concentrations and stable isotope signal ($\delta^{13}C$) 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 δ^{13}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   δ^{13}C-methane values were consistently negative in the near-bottom layer, reflecting that large

239   isotope fractionation occurred during methanogenesis. The ^{13}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 $^{13}CH_4$ was due to methane oxidation because MOB

242   consume $^{12}CH_4$ slightly faster than $^{13}CH_4$. 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 Cadidatus *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 Beyesian mixing model demonstrated interesting

277 seasonal and interannual (2010–2013) variations in the contributions of MOB to the pelagic food

web (Ho et al. 2015). The MOB contribution tended to be highest in winter, consistent with patterns

in other climatic regions, including boreal (Taipale et al., 2011) and tropical lakes (Morana et al.,

2015). Interannual variations in winter are potentially influenced by two contrasting mechanisms.

On the one hand, deficiency of profundal DO in summer enhances methanogenesis and

accumulation of profundal methane toward winter, supplying more substrate to MOB and resulting

in a higher contribution of MOB in winter. On the other hand, oxygen supply for profundal waters

due to winter mixing enhances aerobic methane oxidation, resulting in a higher contribution of

MOB to the food web in winter. The former mechanism would be the case if MOB were more

limited by methane availability than by oxygen availability which is clearly the case in FTR.

Results of a vertically structured food web model using reaction-advection-diffusion

equations predicted that deeper disturbance during summer would suppress the contribution of

MOB in winter (Ho et al. 2016). Although winter mixing could have positive and negative effects

on the contribution of MOB over the year, stronger mixing in winter resulted in weaker deficiency

of profundal DO in the next summer, leading to lower MOB contribution in the following winter.

This result is consistent with the methane accumulation pattern (Itoh et al. 2015) and estimates from

the stable-isotope mixing model (Ho et al. 2016).

Molecular analyses of bacterial communities indicated that the taxonomic composition of

MOB (i.e., anaerobic MOB phylogenetically close to NC10 and aerobic Types I and II) changed

seasonally and vertically (Kojima et al. 2014; Kobayashi et al. 2016). However, due to the limited

availability of quantitative data, MOB functional activity in the food web model was parameterized

following an earlier experimental study (Harrits and Hanson 1980). MOB activity was assumed to

be suppressed by low or high oxygen availability and to be maximal at an oxygen level of around

200 mmol O_2 m^{-3}. This model is a black box approach to represent the diverse functionality of

MOB implicitly by assuming that the community is a mixture of aerobic and anaerobic MOB. If we

assumed much lower optimal DO levels (with predominance of anaerobic MOB), then the model

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_2, mainly occurs in the

313   anaerobic sediment. Productions of sedimentary methane (by methanogenesis) and CO_2 (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

13

implies *in situ* methane production in oxic waters (Bogard et al. 2014; Tang et al. 2014; Itoh et al.

2015; Yao et al. 2016). Therefore, subsurface rather than profundal methane production may

account for a portion of the methane emitted from the water surface. As frequently happens in

well-stratified tropical lakes (Verburg et al. 2003), cyanobacterial blooms occur during summer in

FTR. It may be that the subsurface cyanobacteria bloom in summer plays a neglected role in the

production of methane and the vertical distribution of oxygen and, thus, regulates anaerobic

methanogenesis in the bottom layer. Although interactions between cyanobacteria and

bacteria/archaea can result in methane production in oxic layers (Bogard et al. 2014), the

mechanism for this process is not fully understood (summarized in Tang et al. 2016). Another

controversy is whether methane produced in the oxic subsurface layers contributes much (Bogard et

al. 2014) or little (Encinas Fernández et al. 2016) to the amount of methane emitted from the lake

surface. Finally, the possible production of CH_4 under aerobic conditions (Karl et al. 2008; Damm

et al. 2008) in marine systems has been debated. Although this process could explain the very low

concentration (< 4 nM) of CH_4 in open and deep oceanic regions, it cannot explain the much larger

concentration of CH_4 (10–1000 nM) in shallow coastal areas, where CH_4 undoubtedly comes from

sediments (Borges et al. 2016; 2017).

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

From a food-web perspective, MOB are key players in a new mode of pelagic-benthic coupling in

lake ecosystems (Schindler and Scheuerell 2002). In the broadly accepted view of pelagic-benthic

coupling in deep lakes, sedimentation of organic matter produced by pelagic production is the basal

resource of benthic invertebrates and fishes, which act as alternative resources of pelagic mobile

predators such as zooplankton and fishes. In the new mode of pelagic-benthic coupling in deep

lakes, summer pelagic PP is transferred to the benthic layers, ultimately supporting the secondary

production of pelagic zooplankton mediated by methane-based food webs along the water column.

      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_2 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_2. Some of the CO_2

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_2 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_2 and MOB together with analysis of phospholipid fatty acids (e.g.,

15

382 Belova et al. 2011) will be necessary to elucidate the dominant reactions and estimate the timescale

383 of interactions between CO_2, 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

16

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

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Figure Legends

Fig. 1 DO saturation (%) in each sampling depth from June to September from 2004 to 2007 (strong typhoon years 2004 or 2005 and weak typhoon years 2006 or 2007). Numbers in parentheses indicate numbers of samples. Box plots show median (line), 25th to 75th percentiles (box), 10th to 90th percentiles (bars), and individual values $<10^{th}$ or $>90^{th}$ percentile (points). Differences were detected using one-way ANOVA and Tukey's multiple comparison tests. Different letters indicate a significant difference ($P < 0.05$).

Fig. 2 Schematic of methane dynamics in shallow lakes and coastal parts of deep lakes (modified from Bastviken et al. 2004) and pelagic parts of deep lakes (modified from Itoh et al. 2015) during the stratification period. Right panel shows vertical profiles of temperature, DO, methane concentration, methane carbon isotope ratio, and nitrate concentration at the end of stratification period (Dec 2013) in FTR (Itoh et al. 2015).

Fig. 3 Schematic diagram illustrating our new conceptual model of pelagic-benthic coupling mediated by vertically structured diverse MOB groups. Some arrows are omitted for simplicity (e.g., release of CO_2 from zooplankton). Distribution of microbes in benthic habitats (from microaerobic water column to anaerobic sediment) was not clearly separated in FTR project, although it is conceptually separable. Microbial members in sediment (fermenter and methanogen) were not targets of observation in this project. HB represents heterotrophic bacteria that utilize photosynthetic products (POM). Ref1: Belova et al. 2011; Im et al. 2011, Ref2: Morel et al. (2011), Ref3: Vecherskaya et al. (2009) and Kalyuzhnaya et al. (2013), Ref4: Ettwig et al. (2010) and Rasigraf et al. (2014).

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Figure 1

Figure 2

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Figure 3