



TITLE:

Community composition and methane oxidation activity of methanotrophs associated with duckweeds in a fresh water lake

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1 **Community composition and methane oxidation activity of**
2 **methanotrophs associated with duckweeds in a fresh water lake**

3

4 Short title: Methanotrophs associated with duckweeds

5

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25

26 **Abstract**

27 Methanotrophs are the only biological sink of the greenhouse gas methane. To
28 understand the ecological features of methanotrophs in association with plants in the
29 methane emitting environments, we investigated the community composition and
30 methane oxidation of methanotrophs associated with duckweeds in a fresh water lake.
31 Duckweeds collected from Lake Biwa, Japan over three summers showed methane
32 consumption activity between 0.0067 and 0.89 $\mu\text{mol h}^{-1} \text{g}^{-1}$ (wet weight), with the
33 highest values occurring from the end of July to August. The methanotrophic
34 community on duckweeds consisted primarily of γ -proteobacterial groups including the
35 genera *Methylomonas* and *Methylocaldum*. Further analysis of co-cultures of a
36 methanotroph isolate with sterilized duckweed revealed that the duckweed plant as well
37 as the duckweed spent culture supernatant exerted an enhancing effect on methane
38 oxidation. These results indicate that duckweeds not only provide a habitat for
39 methanotrophs but also stimulate methanotrophic growth.

40

41 Introduction

42 Methane is a greenhouse gas that is 21 times more heat-trapping than carbon dioxide,
43 and increases in its atmospheric concentration contribute to global warming. In natural
44 ecosystems, methane is biologically produced by methanogenic archaea that transform
45 carbon dioxide and hydrogen, formate, or acetate to methane in anoxic environments (1).
46 Plants emit methane generated by methanogens via their aerenchyma (2), and some
47 plants also produce methane originating from methyl ester groups of pectin coupled
48 with photosynthesis (3). A major portion (more than 80%) of the methane emitted into
49 the atmosphere is oxidized by hydroxyl radicals in the troposphere, and a portion of the
50 remaining methane is biologically oxidized by methane-oxidizing microorganisms,
51 methanotrophs (4). The balance between methane sources and sinks is important to
52 maintain the atmospheric methane concentration and the global climate. However,
53 human activities have raised methane emissions from paddy fields, livestock, landfills,
54 oil factories, and waste-treatment equipment. As a result, the atmospheric methane
55 concentration increased by a factor of 2.5 in the past 200 years (4). Therefore, social
56 demands are not only to decrease methane emission sources, but also to develop
57 technologies for the removal of methane at production sites.

58 Methanotrophs are the only biological sink of methane and they play an important
59 role in the global carbon cycle between two major green house gases, methane and
60 carbon dioxide, which is called the methane cycle (4). Methanotrophs utilize methane as
61 sole carbon and energy sources and represent a subset of methylotrophs, which utilize
62 one-carbon compounds (5). Methanotrophs consist of both bacteria and archaea, and the
63 bacterial groups are classified in three phyla: α -Proteobacteria, γ -Proteobacteria and
64 Verrucomicrobia (6). While methanotrophic archaea are anaerobes, methanotrophic
65 bacteria (MOB) are aerobic oxidizers of methane via the enzyme methane

66 monooxygenase. Methanotrophs (hereafter referred to as MOB) are distributed in a
67 wide range of environments such as soils, wetlands, lakes, and oceans.

68 Aquatic environments such as wetlands and lakes are major methane emission sites.
69 Global methane emission from lakes has been estimated to represent as much as 6-16%
70 of the total natural methane emission (7). Methanotrophs have been detected in lake
71 water and sediments, and the surface layers of sediments show high methane oxidation
72 activity (8-12). Previous studies also revealed that methanotrophs inhabit submerged
73 hydrophytes (13-16). It is noteworthy that hydrophytes have high methane oxidation
74 activity (17-21). Thus, hydrophytes are considered to be a niche for methanotrophs in
75 aquatic environments, and the ecosystem of methanotrophs and hydrophytes may
76 contribute significantly to the global carbon cycle.

77 Duckweeds are floating hydrophytes that are often found in calm waters or fresh
78 water lakes. The duckweed plant is recognized as an ideal biomass for biofuel
79 production and animal feed based on its characteristics of global distribution, high
80 starch content, and rapid growth (22). Techniques for cultivation under aseptic
81 conditions and genetic engineering have also been developed for duckweeds (23, 24).
82 Recent studies reported that bacteria-duckweed interactions are important for enhanced
83 biomass production as well as bioremediation; duckweed-growth promoting ability has
84 been demonstrated for several bacterial isolates from duckweeds, including
85 *Acinetobacter*, *Aquitalea*, *Pseudomonas*, and *Exiguobacterium* (25-27). In addition
86 *Acinetobacter* and *Exiguobacterium* were shown to degrade phenolic compounds and
87 remediate chromium toxicity, respectively (27, 28). Also, inoculation of the isolated
88 bacteria on the duckweed plant resulted in the formation of biofilms in the rhizosphere
89 of host plants (28). Furthermore, duckweeds inhabit the interface between water and air;
90 in other words, in oxic environments where methane oxidation occurs and anoxic

91 environments where methanogenesis occurs. They have air spaces that provide
92 buoyancy and can be a temporal reservoir for methane generated in anoxic water
93 environments. These characteristics suggest that duckweeds may be favorable sites for
94 methane oxidation by methanotrophs. However, whether methanotrophs inhabit and
95 exhibit methane oxidation activity on duckweeds is currently unknown.

96 In this study, we collected duckweed samples from Lake Biwa, a fresh water lake in
97 Japan over three summers, and analyzed the community composition and methane
98 oxidation activity of methanotrophs associated with duckweeds. Furthermore, we
99 describe the ecology of methanotrophs associated with duckweeds, and demonstrate that
100 positive interactions between methanotrophs and duckweeds enhance methane oxidation
101 activity.

102

103 **Materials and Methods**

104 **Sampling.** Duckweeds and lake water were sampled at the shore of Lake Biwa
105 (Shiga Prefecture, Japan) in the summers (July, August, and September) of 2011, 2012,
106 and 2013. The latitudes of the sampling sites were as follows: site A (35°7'52"N
107 135°93'91"E), site B (35°6'90"N 135°93'54"E) and, site C (35°3'15"N 135°91'29"E).
108 In each sampling site, duckweeds and the ambient lake water were collected from ca. 1
109 m² area and put together into a plastic container, and the samples were conveyed to the
110 laboratory. Methane consumption analysis of these samples was started within several
111 hours after sampling. For DNA extraction, the duckweed samples were stored at -80°C
112 until use.

113

114 **Analysis of methane consumption by environmental samples.** Environmental
115 samples, 3 g (wet weight) of duckweed or 3 mL of lake water, were placed in 25 mL
116 glass vials. After sealing the vials with butyl rubber caps and aluminium seals, 1 mL of
117 methane was added. Vial were incubated statically at 28°C in the dark. A duplicate or
118 triplicate experimental setup was prepared from the sample collected at each sampling
119 site. Methane concentrations in the headspace of the vials were determined using a
120 Shimadzu GC-14B gas chromatograph (Shimadzu, Kyoto, Japan) equipped with a flame
121 ionization detector and Porapak Q column (Shinwa Chemical Industries, Kyoto, Japan).
122 Nitrogen gas was used as the carrier. Analytical temperatures of the oven, injector and
123 detector were 100, 120 and 225°C, respectively. Methane consumption activity was
124 calculated from the linear portion of a graph of methane concentration against
125 incubation time (ca. 15-40 hours after the start of incubation).

126

127 **Clone library analysis of the *pmoA* and *mxnF* genes.** DNA was extracted from

128 0.5 g (wet weight) of duckweeds using ISOIL for Beads Beating (Nippon Gene, Tokyo,
129 Japan). The *pmoA* and *mxoF* genes were amplified from the extracted DNA with Ex Taq
130 DNA polymerase (TakaraBio, Shiga, Japan) using primer sets A189-mb661 and
131 *mxo1003-1561*, respectively (29). PCR products were cloned into pMD20 (TakaraBio)
132 using the TA cloning strategy, and the plasmid DNA was sequenced.

133

134 **Methane oxidation analysis by *Methylomonas* sp. BLU1.** The methanotrophic
135 strain *Methylomonas* sp. BLU1 was isolated from a duckweed sample from Lake Biwa
136 (site A, July 12, 2011). The isolation procedure was as follows: After enrichment with
137 methane in NMS liquid medium (ATCC 1306 medium), the serially diluted cultures
138 were spread on NMS agar plates, which were incubated under a methane/air atmosphere.
139 A single colony formed was transferred to NMS liquid medium. *Methylomonas* sp.
140 BLU1 was grown in NMS medium with methane at 28°C.

141 The duckweed *Spirodela polyrhiza* was sterilized by treatment with 5% bleach for 10
142 min, and washed with sterilized water. The sterilized duckweed was aseptically
143 cultivated in half-strength Hunter's medium (1/2H medium) under light/dark conditions
144 at 25°C in a plant growth chamber (Nippon Medical & Chemical Instruments, Osaka,
145 Japan). Hunter's medium includes only inorganic nutrients and has been used for
146 duckweed culture (30). Several colonies were inoculated in 30 ml of 1/2H medium in a
147 100-mL flask with a culture plug. After 1-month cultivation, duckweed colonies and the
148 culture medium (supernatant after centrifugation) were used for the following assays.

149 One mL of the methanotroph culture ($OD_{600}=0.1$, adjusted with NMS medium by
150 dilution), 1 mL of methane, and one of the following additives were placed into a 25 mL
151 glass vial; the additives were five individual samples of duckweed, 100 μ L of
152 filter-sterilized duckweed spent culture supernatant, or 100 μ L of 1/2H medium. The

153 methane concentration in the headspace of the vial, which was incubated statically at
154 28°C in the dark, was analyzed. Dead duckweeds were prepared by treating duckweeds
155 at 60°C for 30 min. Boiled duckweed culture supernatant was prepared by incubating
156 the duckweed spent culture supernatant in boiling water for 30 min. Either 1 mL of air,
157 carbon dioxide, or oxygen was added to the vial to test the effect of additional gases.

158

159 **Nucleotide sequence accession numbers.** Partial sequence of the 16S rRNA gene
160 of *Methylomonas* sp. BLU1 has been deposited in DDBJ under accession number
161 LC440492.

162

163 **Results**

164 **Methane consumption by duckweed samples from Lake Biwa.** To investigate
 165 the methane oxidation potential of methanotrophs associated with duckweeds, we
 166 sampled duckweeds growing on Lake Biwa over three summers. Duckweeds began to
 167 vigorously proliferate in July and disappeared in October at all sampling sites. Judging
 168 from the morphology of duckweeds, main portions of collected duckweeds were
 169 *Spirodela polyrhiza* and *Lemna aequinoctialis*. The methane consumption activity of
 170 duckweeds and the ambient lake water was measured under *in vitro* conditions as
 171 described in Materials and Methods. Fig. 1A shows the typical methane consumption of
 172 duckweeds and the lake water. The methane concentration in the vial with duckweeds
 173 collected from site A on July 19, 2012 significantly decreased with incubation time,
 174 while that with the lake water slightly decreased. All the tested duckweeds showed the
 175 methane consumption activity, and the values were varied between 0.0067 and 0.89
 176 $\mu\text{mol h}^{-1} \text{g}^{-1}$ (wet weight) by sampling site and period (Fig. 1B). The rate with
 177 duckweeds was more than one-order of magnitude higher than that with the lake water,
 178 and thus the methane consumption activity of the lake water attached to duckweeds
 179 could be considered as background level. These results indicate that duckweeds growing
 180 in natural environments have the potential for methane oxidation. At every site, the peak
 181 of the methane consumption activity was observed from the end of July to August.

182

183 **Analysis of the methanotrophic community on duckweeds.** Next, in order to
 184 analyze the methanotrophic community composition on duckweeds, the *pmoA* gene,
 185 which encodes a subunit of particulate methane monooxygenase (pMMO), was
 186 amplified by PCR from the DNA extracted from duckweed samples from site A, and
 187 ligated with a TA cloning vector to generate a clone library. Analysis of the *pmoA*

188 sequences revealed that the duckweeds predominantly harbored γ -proteobacterial
 189 methanotrophs, whereas *Methylocystis* was the only detected α -proteobacterial
 190 methanotroph (Fig. 2A). The composition of methanotrophs changed substantially
 191 during the first month (from July 12 to July 29 in 2011 and from July 19 to Aug 29 in
 192 2012) (Fig. 2A). The methanotrophic community was diverse, harboring three to seven
 193 genera, and members of the genera *Methylomonas* and *Methylocaldum* represented the
 194 core components throughout the sampling period. Additionally, the *mxoF* gene, which
 195 encodes the large subunit of methanol dehydrogenase, was sequenced to assess the
 196 methylotrophic community including methanotrophs and methanol utilizers (Fig. 2B).
 197 The ratio of methanotrophic members was relatively low among the methylotrophic
 198 community on duckweeds. *Hyphomicrobium* was present on all the tested duckweeds,
 199 and *Methyloversatilis* and *Methylobacterium* were also frequently found.

200

201 **Analysis of methane oxidation in methanotroph-duckweed co-cultures.** To
 202 investigate whether duckweeds only provide methanotrophs with a habitat in aquatic
 203 environments or have symbiotic interactions with methanotrophs, we examined the
 204 methane oxidation activity of methanotrophs in the presence and absence of duckweed.
 205 The methanotroph *Methylomonas* sp. BLU1, which was isolated from duckweeds in
 206 Lake Biwa, was subjected to cultivation tests with sterilized duckweed, *Spirodela*
 207 *polyrhiza*. A pure culture of *Methylomonas* sp. BLU1 grew well on NMS medium
 208 containing methane as the sole carbon source without the addition of any growth factors
 209 such as vitamins (data not shown), and methane was consumed along with cultivation
 210 time (Fig. 3). When *Methylomonas* sp. BLU1 was cultured with duckweeds, methane
 211 oxidation by *Methylomonas* sp. BLU1 was enhanced (Fig. 3). Addition of dead
 212 duckweeds that was prepared by treatment at 60°C also resulted in enhanced methane

213 oxidation at the same rate as co-cultures with living duckweeds (Fig. 3). These results
214 suggest that methane oxidation activity or growth of *Methylomonas* sp. BLU1 is
215 stimulated in the presence of duckweeds regardless of whether they were living or dead.
216

217 **Effect of duckweed culture supernatant on methane oxidation by *Methylomonas***
218 **sp. BLU1.** Because dead duckweeds enhanced the methane oxidation activity of
219 *Methylomonas* sp. BLU1 (Fig. 3), some metabolites of duckweeds seem to have
220 stimulatory effects. Next we tested whether duckweed culture supernatant enhances
221 methane oxidation by *Methylomonas* sp. BLU1. As shown in Fig. 4A, spent duckweed
222 culture supernatant also enhanced methane oxidation, whereas the 1/2H medium did not.
223 These results indicate that the stimulatory effect is mainly achieved by plant-derived
224 compounds. When boiled duckweed culture supernatant was added, the methane
225 oxidation rate was slightly enhanced (Fig. 4A), indicating that the major compounds
226 responsible for the stimulatory effect are heat-sensitive.

227 In general, methanotrophic growth and methane oxidation by methanotrophs are
228 affected by the concentrations of two kinds of gases, carbon dioxide and oxygen. Since
229 both gases can be generated by photosynthesis and respiration by duckweeds, we further
230 examined the effect of carbon dioxide or oxygen on methane oxidation by
231 *Methylomonas* sp. BLU1. As shown in Fig. 4B, addition of carbon dioxide but not
232 oxygen had an enhancing effect. Although carbon dioxide emitted by duckweeds may
233 affect the methane oxidation activity of methanotrophs, judging from the results with
234 dead duckweeds and boiled culture supernatant, metabolites generated by duckweeds
235 seem to be the major factors that enhance methane oxidation by methanotrophs.
236

237 **Discussion**

238 To reduce the increasing concentration of methane in the atmosphere, management of
239 both methane production and removal in natural and man-made environments is
240 required. In this study, we revealed that the ecosystem of methanotrophs and floating
241 hydrophytes, duckweeds, has high methane oxidation potential (Fig. 1B). Lake
242 sediments that generate methane are known to also abundantly consume methane (31),
243 and our results showed that methanotrophs associated with duckweeds have high
244 methane oxidation activity. Thus, duckweeds that expand over the water surface can
245 function as a biological filter of methane generated deep under water, and prevent
246 methane emission into the atmosphere.

247 The duckweeds examined here harbored a diverse methanotrophic community that
248 consisted of up to seven methanotrophic genera in addition to methanol utilizers (Fig. 2).
249 γ -Proteobacteria were the dominant population among the methanotrophic community
250 on duckweeds. This community composition is a common feature in lake water and
251 sediments (9, 10, 32, 33), indicating that duckweeds share a common methanotrophic
252 population with the ambient environment. Since the detected methanotrophic genera are
253 obligate methanotrophs with the exception of limited *Methylocystis* strain that have
254 been reported to be facultative methanotrophs, they are assumed to predominantly
255 utilize methane on duckweeds. It is known that terrestrial plants emit methanol (34);
256 however, this metabolic process has been not reported for duckweeds. The methanol
257 utilizers detected in this study represent facultative methylotrophs, which can utilize
258 methanol as well as complex carbon compounds secreted from duckweeds or present in
259 the lake water. There may be a trophic relationship in which methane metabolites such
260 as methanol, which are produced by methanotrophs are provided to methanol utilizers
261 (35).

262 Variations in the methane consumption activity are probably related to *in situ*
 263 methane concentrations from the sites where duckweeds were sampled. The highest
 264 methane oxidation potential by duckweeds was found from the end of July to August
 265 (Fig. 1B), during which the atmospheric temperature reached the highest of the year in
 266 this area. A possible explanation for this observation is that high temperature enhances
 267 biomass production including duckweed growth, biomass degradation, and
 268 methanogenic activity (36), which lead to an increase in methanotrophic growth. Based
 269 on our previous study, the high methane oxidation activity of duckweeds is likely to be
 270 due to the large population of associated methanotrophs (20). The methane oxidation
 271 potential of duckweeds reported here (0.0067 to $0.89 \mu\text{mol h}^{-1} \text{wet-g}^{-1}$) was lower than
 272 those in other studies with *Elodea canadensis* ($9.7 \mu\text{mol h}^{-1} \text{dry-g}^{-1}$) and *Egeria densa*
 273 ($22 \mu\text{mol h}^{-1} \text{dry-g}^{-1}$) (17, 20). This may be due to the structure of the duckweed plant,
 274 which has limited area for methanotrophs to inhabit, whereas duckweed plants have the
 275 advantage of growing to high density. Two reports showed that bacterial biofilm formed
 276 on duckweed roots (28) and higher methanotrophic activity occurred in submerged parts
 277 of plants (21), suggesting that methanotrophs constitute root biofilms on duckweeds
 278 with other bacteria rather than living on the frond body. Further analysis by fluorescent
 279 *in situ* hybridization with a methanotroph-specific probe could reveal the localization of
 280 methanotrophs on duckweeds.

281 We revealed that the duckweed plant has an enhanced effect on methane oxidation by
 282 methanotrophs. In microbe-plant interactions in soil, plant roots are considered to
 283 release nutritional compounds and oxygen (37). But in this study, oxygen addition did
 284 not affect methane oxidation by methanotrophs (Fig. 4B), and therefore oxygen does not
 285 appear to be the limiting factor in the surface water where duckweeds live. Since dead
 286 duckweeds as well as the spent culture supernatant of duckweeds enhanced methane

287 oxidation (Fig. 3 and 4A), metabolites produced by duckweeds appear to have a
288 stimulatory effect on methanotrophs and it seems that living duckweeds are not
289 necessary for the effect. The addition of carbon dioxide, which is a respiration product
290 of duckweeds, also enhanced methane oxidation (Fig. 4B). But carbon dioxide is
291 probably not the key compounds that enhanced methane oxidation, since both the dead
292 duckweeds and the boiled culture supernatant, which are not expected to generate
293 carbon dioxide, enhanced methane oxidation (Fig. 3 and 4A). It is well known that
294 carbon dioxide supports the carbon metabolism of γ -proteobacterial methanotrophs via
295 the Calvin cycle (38, 39).

296 The stimulatory compounds produced by duckweeds were characterized as heat
297 sensitive (at 100°C) (Fig. 4A), but their chemical identification remains for future study.
298 In general, photosynthetic products of plants, such as sugars and organic acids, can be
299 used as nutrients by plant-associated bacteria in natural environment where nutritious
300 substances are limited. By contrast, most of methanotrophs including *Methylomonas* sp.
301 BLU1 are obligate C1-utilizers that are only capable of growth on methane or methanol
302 as the sole carbon source. But several additional organic compounds such as organic
303 acids, yeast extract, and peptone have been reported to stimulate the growth of some
304 methanotrophs (40-42). Bacterial interactions can also stimulate methanotrophic growth.
305 Our previous study showed that vitamin B₁₂ produced by *Rhizobium* sp. stimulated the
306 growth of some methanotrophs (43). Stock et al. proposed that bacterial metabolites
307 such as quinones and vitamins B₆ and B₁₂ also stimulate methanotrophic growth (44).
308 Knowledge of the molecular mechanism of plant-methanotroph interactions is limited to
309 studies with *Sphagnum* mosses in which the interaction is mediated by carbon dioxide,
310 oxygen, and ammonium (45); no plant products that have stimulatory effects on
311 methanotrophs have been identified.

312 Duckweeds are useful biological materials for removing nitrogen, phosphorus, and
313 heavy metals, as well as organic pollutants from wastewater (28, 46). These removal
314 activities are attributed to utilization by duckweeds or degradation by root-associated
315 bacteria. However, treatment ponds, which are intended for pollutant removal through
316 activities of microorganisms or plants, are also a source of greenhouse gases including
317 methane. A study showed that the removal of duckweeds from a stormwater treatment
318 system increased methane flux and proposed a role for root-associated bacteria in
319 methane flux (47), although methanotrophic activity was not examined. We expect that
320 if methanotrophs can be stably colonized on duckweed roots together with other
321 functional bacteria that degrade pollutants, the duckweed ecosystem would add the
322 function of methane removal, mitigating methane emission into the atmosphere.

323 In conclusion, duckweeds living in fresh water lakes are inhabited by methanotrophs
324 and have high methane oxidation activity. Duckweeds can stimulate methanotrophic
325 growth, presumably by contributing some duckweed metabolites that are heat-sensitive.
326 Future studies analyzing the molecular mechanism of the duckweed-methanotroph
327 interaction will open the way to the application of methanotrophs for wastewater
328 treatment with duckweeds.

329

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336

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- 484
485

486 **Figure legends**

487

488 **FIG 1.** Methane consumption activity of duckweeds and ambient lake water. (A)
 489 Methane consumption of the duckweed sample (open circles) and lake water (filled
 490 circles) collected at site A on July 19, 2012. Duckweeds or lake water samples were
 491 enclosed in vials with methane, and the methane concentration in the headspace of the
 492 vials was analyzed over time. Data are provided as the means \pm standard deviations
 493 (N=3). (B) Methane consumption activity of duckweeds and lake water. Duckweeds or
 494 lake water samples from Lake Biwa collected at the indicated sites on the indicated date
 495 were enclosed in vials with methane, and the methane concentration in the headspace of
 496 the vials was analyzed over time. Methane consumption activity was determined as
 497 described in Materials and Methods. Data are provided as the means (N=1-3). N.T., not
 498 tested. D.T., under the detection limit.

499

500 **FIG 2.** Community composition of methanotrophs and methanol utilizers on
 501 duckweeds. The *pmoA* and *mxoF* genes were amplified by PCR with the DNA extracted
 502 from duckweed samples collected at site A on the indicated date, and plasmid clone
 503 libraries were constructed for sequencing. The methanotrophic community composition
 504 (A) was assessed by the analysis of *pmoA* gene sequences (N=17-23) and the
 505 methylotrophic community composition (B) was assessed by the analysis of *mxoF* gene
 506 sequences (N=17-21). α , β , and γ indicate subgroups of the Proteobacteria.

507

508 **FIG 3.** Effect of duckweeds on methane oxidation by *Methylomonas* sp. BLU1.
 509 Cells of *Methylomonas* sp. BLU1 were incubated with or without duckweeds in sealed
 510 vials filled with methane, and the methane concentration in the headspace of each vial

511 was analyzed over time. Data are provided as means (N=3) and error bars represent
512 standard deviations. Open circles, *Methylomonas* sp. BLU1; open triangles,
513 *Methylomonas* sp. BLU1 with living duckweeds; filled triangles, *Methylomonas* sp.
514 BLU1 with dead duckweeds.

515

516 **FIG 4.** Effect of duckweed culture or additional gases on methane oxidation by
517 *Methylomonas* sp. BLU1. Cells of *Methylomonas* sp. BLU1 were incubated with or
518 without duckweed culture supernatant (A) or additional gases (B) in sealed vials filled
519 with methane, and the methane concentration in the headspace of each vial was
520 analyzed over time. Data are provided as means (N=3) and error bars represent standard
521 deviations. (A) Open circles, *Methylomonas* sp. BLU1; open squares, *Methylomonas* sp.
522 BLU1 with 1/2H medium; open triangles, *Methylomonas* sp. BLU1 with duckweed
523 spent culture supernatant; filled triangles, *Methylomonas* sp. BLU1 with boiled
524 duckweed culture supernatant. (B) Open circles, *Methylomonas* sp. BLU1 with air; open
525 triangles, *Methylomonas* sp. BLU1 with carbon dioxide; open squares, *Methylomonas*
526 sp. BLU1 with oxygen.

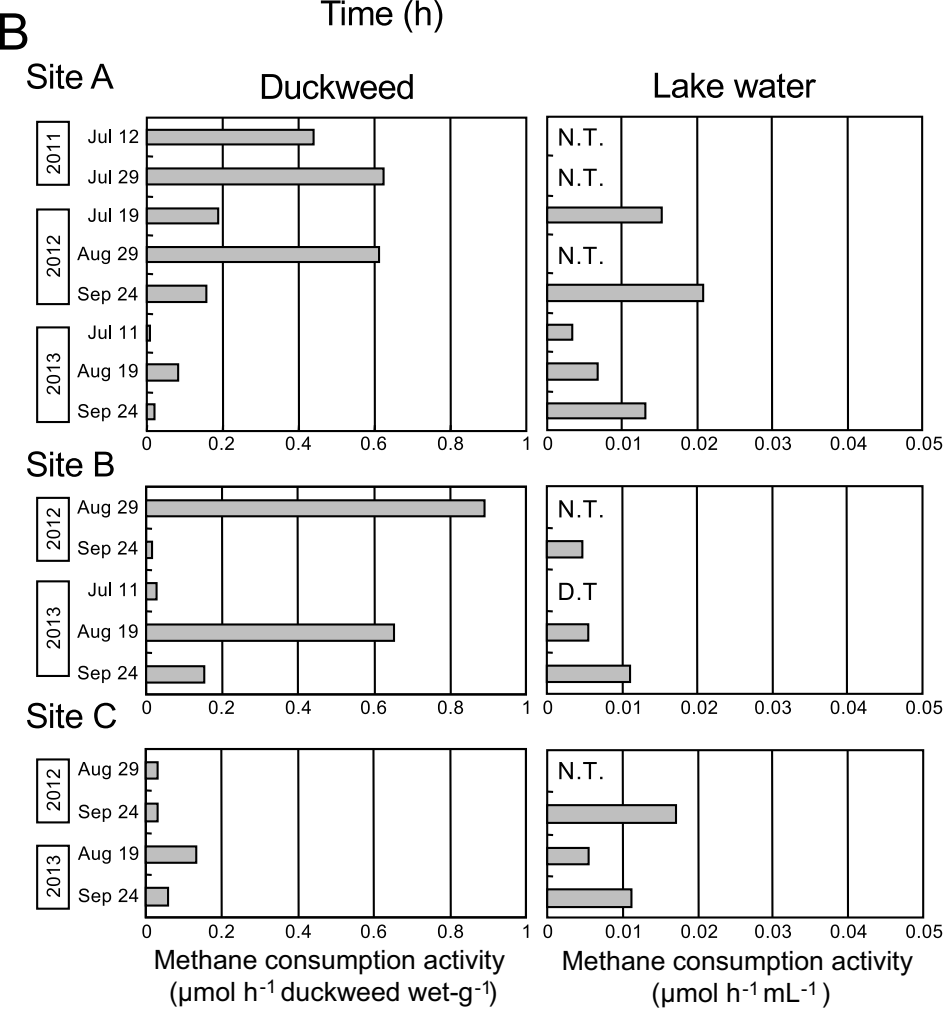
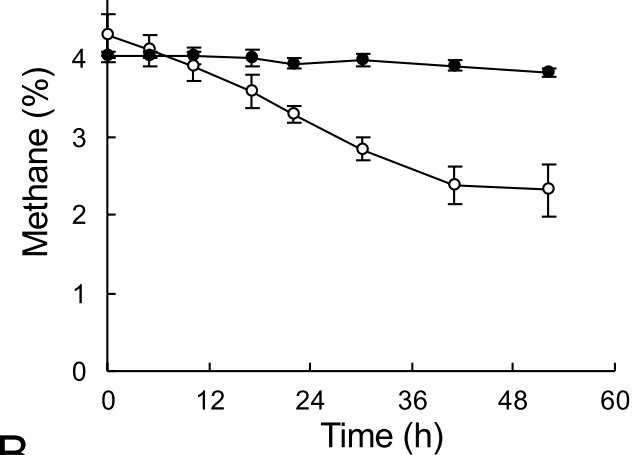


Figure 1.

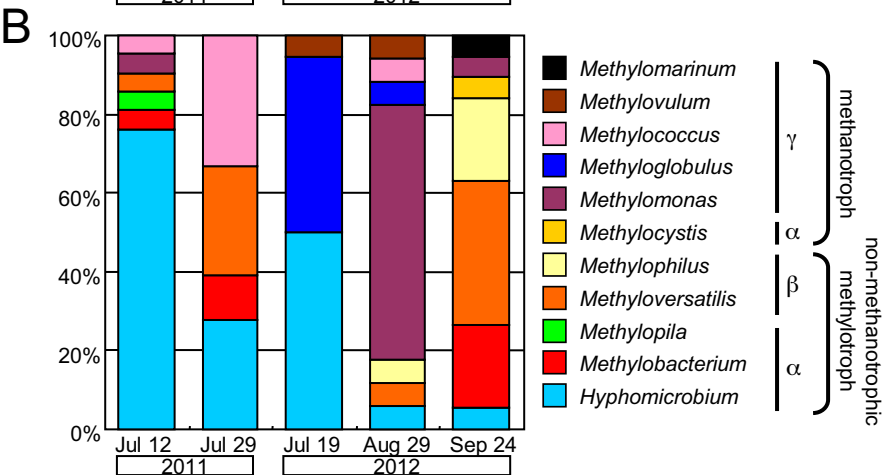
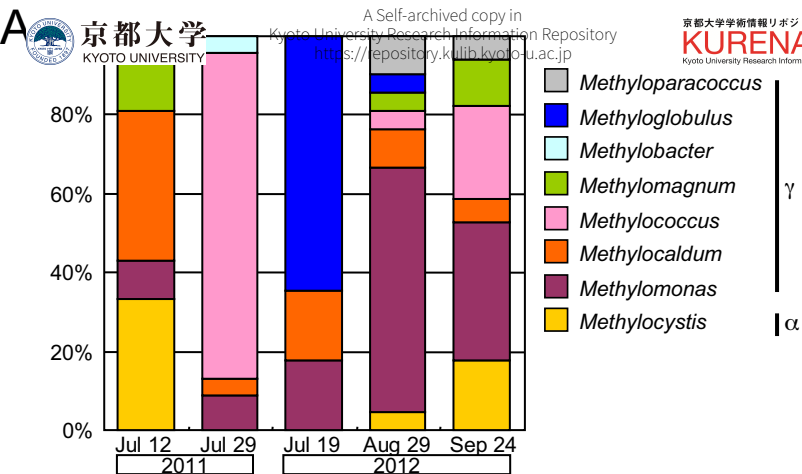


Figure 2.

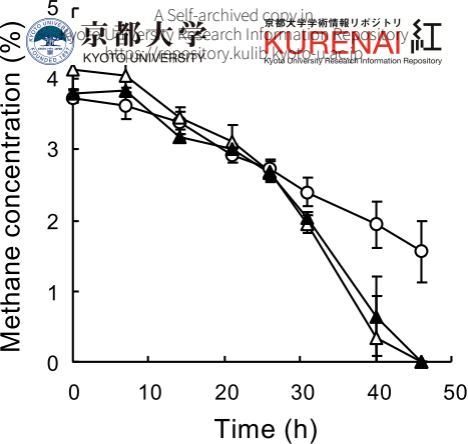


Figure 3.

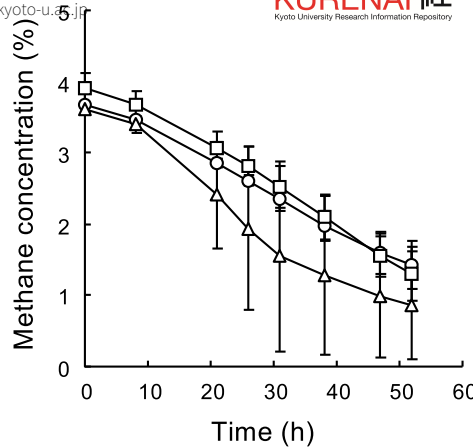
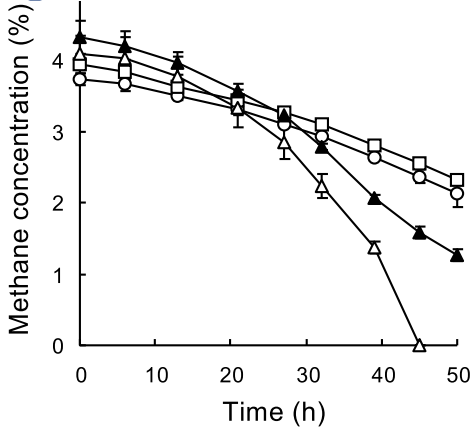


Figure 4.