

Methane oxidation in the waters of a humic-rich boreal lake stimulated by photosynthesis, nitrite, Fe(III) and humics

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1 **Abstract**

2 Small boreal lakes are known to contribute significantly to global CH₄ emissions. Lake Lovojärvi is a
3 eutrophic lake in Southern Finland with bottom water CH₄ concentrations up to 2 mM. However, the
4 surface water concentration, and thus the diffusive emission potential, was low (<0.5 μM). We studied
5 the biogeochemical processes involved in CH₄ removal by chemical profiling and through incubation
6 experiments. δ¹³C-CH₄ profiling of the water column revealed a methane-oxidation hotspot just below
7 the oxycline and zones of CH₄ oxidation within the anoxic water column. In incubation experiments
8 involving the addition of light and/or oxygen, CH₄ oxidation rates in the anoxic hypolimnion were
9 enhanced 3-fold, suggesting a major role for photosynthetically fueled aerobic CH₄ oxidation. We
10 observed a distinct peak in CH₄ concentration at the chlorophyll a maximum, caused by either in-situ
11 CH₄ production or other CH₄ inputs such as lateral transport from the littoral zone. In the dark anoxic
12 water column at 7 m depth, nitrite seemed to be the key electron acceptor involved in CH₄ oxidation,
13 yet additions of Fe(III), anthraquinone-2,6-disulfonate and humic substances also stimulated anoxic CH₄
14 oxidation. Surprisingly, nitrite seemed to inhibit CH₄ oxidation at all other depths. Overall, this study
15 shows that photosynthetically fueled CH₄ oxidation can be a key process in CH₄ removal in the water
16 column of humic, turbid lakes, thereby limiting diffusive CH₄ emissions from boreal lakes. Yet, it also
17 highlights the potential importance of a whole suite of alternative electron acceptors, including humics,
18 in these freshwater environments in the absence of light and oxygen.

19

20 **Introduction**

21 Lacustrine water bodies represent a substantial natural source of atmospheric methane (CH₄), a major
22 contributor to global warming. They may release up to ~72 Tg CH₄ a⁻¹ (12 % of total global emissions)
23 (Bastviken et al., 2011), despite covering a relatively small proportion of the land surface area (>3%,
24 Downing et al. 2006). In temperate and northern boreal regions, small lakes generally emit more CH₄
25 per unit area than larger systems (Juutinen et al., 2009; Kortelainen et al., 2000, 2004; Michmerhuizen
26 et al., 1996). Northern lakes alone are estimated to contribute 24.2±10.5 Tg CH₄ a⁻¹ to global CH₄
27 emissions (Walter et al., 2007).

28 The majority of lacustrine CH₄ is produced by anaerobic methanogenic archaea as the end product of
29 remineralization of organic matter in anoxic sediments (Bartlett and Harriss, 1993; Rudd, 1980). From
30 the sediments, CH₄ can diffuse into the water column and may be emitted to the atmosphere at the water-
31 air interface. Large fractions of this CH₄ may, however, be consumed by microbial CH₄ oxidation,
32 decreasing the CH₄ concentration and emissions. Research has shown that microbial CH₄ oxidation may
33 be the single most important control on CH₄ emissions from lakes and other ecosystems (Chistoserdova,
34 2015).

35 The vast majority of CH₄ consumption in limnic systems has been assigned to bacterial CH₄ oxidation
36 (Hanson and Hanson, 1996; King, 1992). This process is performed by methane-oxidizing bacteria
37 (MOB), affiliated with either gamma- or alphaproteobacteria. Typically, oxygen is used as the terminal
38 electron acceptor (TEA) in the respiratory chain. However, some aerobic gamma-MOB like
39 *Methylomonas denitrificans* (Kits et al., 2015a) and *Methylomicrobium album* (Kits et al., 2015b) can
40 switch to the use of nitrate (NO₃⁻) or nitrite (NO₂⁻) as their TEA. The hybrid metabolism of
41 *Methylomirabilis oxyfera* combines partial denitrification (NO₂⁻ to NO) and classical aerobic CH₄
42 oxidation, fueled by internal O₂ generation (splitting NO to N₂ and O₂) (Ettwig et al., 2010). While *M.*
43 *oxyfera* has similar metabolic traits as proteobacterial methanotrophs, it is associated with the novel
44 phylum NC10 (Holmes et al., 2001; Rappé and Giovannoni, 2003). Recently, methanotrophs of the
45 genera *Methylomonas* and *Methylosinus* have been shown to couple CH₄ oxidation to Fe(III) reduction
46 (Zheng et al., 2020). Bacterial methanotrophs require trace amounts of O₂ for the activation of their
47 enzymatic CH₄ oxidation pathway. Completely O₂-independent CH₄ consumption is assigned to three
48 distinct groups of anaerobic methanotrophic archaea (ANME-1, -2 and -3), which, at least in marine
49 settings, are often found in syntrophic relationship with sulfate-reducing bacteria (Boetius et al., 2000;
50 Michaelis et al., 2002; Orphan et al., 2001) and have been estimated to remove 90% of all produced CH₄
51 in marine systems (Hinrichs and Boetius, 2002; Reeburgh, 2007). Although rare, ANME can be present
52 in lake waters (Durisch-Kaiser et al., 2011; Eller et al., 2005; Oswald et al., 2016a) and sediments
53 (Schubert et al., 2011; Su et al., 2020). Interestingly, studies reporting CH₄ oxidation in anoxic zones of
54 lakes, in the absence of ANME and in the presence of MOB, are increasing (Biderre-Petit et al., 2011;
55 Blees et al., 2014; van Grinsven et al., 2020b; Oswald et al., 2016b; Schubert et al., 2010). While oxygen
56 supplied by episodic down-welling of cold O₂-laden water (Blees et al., 2014), or low-light
57 photosynthesis (Milucka et al., 2015; Oswald et al., 2015) may explain this phenomenon to some degree,
58 CH₄ oxidation may also be coupled to the reduction of electron acceptors other than O₂, such as NO_x
59 (Deutzmann et al., 2014; Graf et al., 2018; Oswald et al., 2016b), Fe(III) (Norði et al., 2013; Sivan et
60 al., 2011), Mn(IV) (Crowe et al., 2011; Oswald et al., 2016a) and humic substances (Valenzuela et al.,
61 2019).

62 The role of boreal lakes in worldwide greenhouse gas emissions is receiving increasing attention. Earlier
63 studies mainly highlighted the large role of aerobic CH₄ oxidation in the lake carbon cycle (Kankaala et
64 al., 2006). More recent studies have shown that boreal lakes can exhibit highly active CH₄ oxidizing
65 communities both in the oxic and anoxic parts of the water column (Taipale et al., 2011). A recent study
66 by Kallistova et al. (2019) showed a peak in CH₄ oxidation rates at the oxycline, but also in the
67 hypolimnion of boreal lake Svetloe. No terminal electron acceptor could, however, be identified in the
68 ferruginous hypolimnion. Rissanen et al. (2018) demonstrated enhanced CH₄ oxidation in the anoxic
69 zone by light and nitrate, but at the same time an inhibitory effect of sulfate and Fe(III). The
70 environmental controls on the modes of AOM in boreal lakes, and the TEAs involved, are therefore still
71 poorly understood. Here, we studied the microbial CH₄ turnover, in particular the oxidative side, in a

72 small lake rich in humic substances in southern Finland (Lake Lovojärvi). Sedimentation regime,
73 stratigraphy and phytoplankton community have been studied intensively in this lake (Keskitalo, 1977;
74 Saarnisto et al., 1977; Simola et al., 1990). A recent study by Rissanen et al. (2020) provided insight in
75 the genomic potential of methanotrophic species living in the Lake Lovojärvi water column, revealing
76 microbial community variation along the oxygen gradient that suggests adaptation and specialization of
77 specific MOB types. To further reveal the methanotrophic potential in the water column of Lake
78 Lovojärvi, and to gain an increased understanding of the biogeochemical controls on its biological CH₄
79 consumption, we combined physical and chemical water column profiling with incubation experiments
80 with different electron acceptors and light/dark conditions. Furthermore, we performed 16S rRNA gene
81 sequencing to characterize the key microbial players involved.

82

83 **Materials and Methods**

84 **Study site**

85 Lake Lovojärvi is a small (5.4 ha) eutrophic lake near the town of Lammi in southern Finland. It is part
86 of a glaciofluvial esker deposit (Simola, 1979), which gives the lake its elongated shape (600 m long,
87 130 m wide) and shields it from strong winds (Hakala, 2004). Lake Lovojärvi is shallow, with an average
88 depth of 7.7 m (Ilmavirta et al., 1974) and a maximum depth of 17.5 m in the southeastern part (Simola,
89 1979). Due to the sheltered location and basin morphology, the lake undergoes strong thermal
90 stratification and has a permanently anoxic hypolimnion (Saarnisto et al., 1977). The catchment of Lake
91 Lovojärvi is 7.2 km² and drains water from predominantly agricultural and swampy areas (Simola, 1979).
92 It has been suggested that anthropogenic pollution of Lake Lovojärvi started as early as the Iron Age,
93 by the soaking of hemp and flax (Tolonen et al., 1976). Hydrologically connected to marsh/wetlands
94 (Limminjärvi), the lake receives high inputs of humic substances and dissolved ions (Hakala, 2004). To
95 our knowledge, no information on groundwater inflow is available.

96 **In situ profiling and sample collection**

97 Profiling and sample collection were carried out in September 2015, at the deepest part of the lake (61°
98 04.584'N, 25°02.116'E). A custom-made profiling device equipped with various probes and sensors
99 was used to measure the following parameters in situ: conductivity, turbidity, temperature, depth
100 (pressure) and pH (XRX 620, RBR); photosynthetically active radiation (PAR; LI-193 Spherical
101 Underwater Quantum Sensor, LI-COR); chlorophyll a (ECO-FL, Wetlands, EX/EM= 470/695); and
102 dissolved O₂ (micro-optodes PSt1 and TOS7, PreSens). The detection limits of the two O₂ optodes were
103 125 and 20 nM, respectively.

104 Samples for the analysis of all other parameters were pumped to the surface with a peristaltic pump
105 (Zimmermann AG Elektromaschinen, Horw, Switzerland) connected to gas tight tubing (PVC Solaflex,
106 Maagtechnik) attached to the profiler. To guarantee that water was taken from the correct depth, a
107 custom-built inlet system was used (designed after Miracle et al., 1992) and water was pumped for 2
108 minutes (time necessary to replace the entire tube volume) prior to filling 60 mL syringes directly from
109 the tube outlet avoiding air contact. Water from the syringes was then sub-sampled into different vials
110 for further processing: For total sulfide analysis ($\text{HS}^- + \text{H}_2\text{S}$) zinc acetate was added (1.3% final
111 concentration). To quantify dissolved ($<0.45 \mu\text{m}$) and total fractions of metals, iron(II)/(III) and organic
112 carbon, samples were acidified immediately to a final concentration of 0.1 M (Suprapur HNO_3 , Merck),
113 0.5 M (HCl) and 0.02 M (HCl), respectively. Aliquots were sterile filtered ($<0.22 \mu\text{m}$) to analyze
114 concentrations of dissolved nitrogen species (NO_3^- , NO_2^- and NH_4^+), sulfate (SO_4^{2-}), phosphate (PO_4^{3-})
115 and dissolved inorganic carbon (DIC). DIC samples were filled into gas-tight 12 mL Exetainers (Labco
116 Ltd) without a headspace, and stored upside down. Water samples intended for hybridization techniques
117 were fixed immediately with formaldehyde (2 % [v/v] final concentration), and stored in the dark at 4°C.
118 All other samples requiring larger water volumes were taken directly from the tube outlet anoxically
119 (without headspace or bubbles and by letting water overflow 2-3 volumes). For CH_4 concentration and
120 isotopic measurements, 120 mL serum bottles were filled prior to adding Cu(I)Cl (~ 0.15 % [w/v] final
121 concentration) and sealing the bottles with butyl stoppers (Geo-Microbial Technologies, Inc.) and
122 aluminum crimp caps. Similarly, sterile 160 mL serum bottles or 1 L Schott bottles served to store water
123 for incubation experiments and DNA analysis. These were sealed with butyl stoppers and crimp or screw
124 caps, and were kept in the dark at 4 °C.

125 **Carbon and isotopic parameters**

126 A headspace was created by exchanging 20 mL lake water with 20 mL N_2 gas. The bottles were then
127 left for at least 24 hours to equilibrate the gas content between the gas and water phase. Afterwards,
128 headspace gas samples were used to measure the CH_4 concentration by gas chromatography (GC;
129 Agilent 6890N, Agilent Technologies) using a Carboxen 1010 column (30 m x 0.53 mm, Supelco), a
130 flame ionization detector and an auto-sampler (Valco Instruments Co. Inc.). Resulting headspace
131 concentrations were converted to dissolved water-phase CH_4 by applying calculated Bunsen solubility
132 coefficients (Wiesenburg and Guinasso, 1979). Stable carbon isotopes of CH_4 were analyzed in the same
133 headspace by isotope ratio mass spectrometry (IRMS; GV Instruments, Isoprime). For this, injected gas
134 samples first passed through a trace gas unit (T/GAS PRECON, Micromass UK Ltd) for purification,
135 concentration, and combustion to CO_2 (for details see Oswald et al., 2016a, 2016b). Isotopic ratios of
136 $^{13}\text{C}/^{12}\text{C}$ are presented in the standard $\delta^{13}\text{C}$ -notation (relative to the Vienna Pee Dee Belemnite (VPDB)
137 reference) with a precision of ~ 1.2 ‰.

138 Total organic carbon (TOC), dissolved organic carbon (DOC) and DIC were quantified with a total
139 carbon analyzer (TOC-L, Shimadzu) equipped with a nondispersive infrared detector (NDIR). TOC
140 was measured as CO₂ after combustion (680 °C) of the untreated sample. For DOC determination, the
141 samples were acidified before combustion. For DIC analysis, unacidified samples were injected and
142 DIC was volatilized to CO₂ (internal addition of HCl, pH <3, in a CO₂-free closed reaction chamber)
143 and quantified subsequently. For carbon isotope analysis, 1 mL of the remaining liquid was then
144 transferred to a He-flushed 3.7 mL exetainer and acidified (100 µl 85 % H₃PO₄). The δ¹³C-DIC of the
145 released CO₂ (overnight equilibration) was measured with a gas-bench system (MultiFlow, Isoprime)
146 connected to an IRMS (Micromass, Isoprime). Isotopic ratios of the DIC are also expressed in the δ¹³C-
147 notation (VPDB reference) with a precision of ~0.15 ‰.

148 **Nutrients and metals**

149 Nitrite, ammonium, sulfide and iron(II)/(III) concentrations were measured on the same day as sampled
150 using photometric protocols according to Griess (1879), Krom (1980), Cline (1969) and Stookey (1970),
151 respectively. High background concentrations of organic carbon in the deep water column (9 – 17 m)
152 may have affected the nitrite concentration measurements, along with possible oxidation of small
153 amounts of ammonium during sample processing. Fe(III) concentrations were determined as the
154 difference between total iron, after reduction with hydroxylamine hydrochloride, and Fe(II), which was
155 measured directly (Viollier et al., 2000). Concentrations of nitrate and phosphate were quantified by
156 flow injection analysis (SAN++, Skalar), and sulfate concentrations were determined by ion
157 chromatography (882 Compact IC plus, Metrohm). Total and dissolved Mn concentrations were
158 analyzed by inductively coupled plasma-mass spectrometry (ICP-MS; Element2, Thermo-Fisher).

159 **Catalyzed reporter deposition – fluorescence in situ hybridization (CARD-FISH)**

160 Formaldehyde-fixed lake water samples (15 mL, incubated for ~12 h at 4 °C) were filtered onto 0.2 µM
161 polycarbonate filters (GTTP, Millipore) and rinsed 2x with 1x phosphate buffered saline. Filters were
162 stored at -20 °C until standard CARD-FISH (Pernthaler et al., 2002) was carried out using specific
163 oligonucleotide probes with horseradish peroxidase labels (purchased from Biomers) An overview of
164 the probes and percentage formamide used is supplied in Table S1. Probes EUB338 I-III and
165 Mgamma84+705 were applied as a mix of equal proportions. Background signals were assessed with
166 probe NON338. Permabilization of cell walls, inactivation of endogenous peroxidase activity,
167 hybridization, amplification (Oregon Green 488, Thermo-Fischer Scientific), counter staining (4',6'-
168 diamidino-2-phenylindole, DAPI) and embedding of the filter pieces was carried out as described in
169 detail previously (Oswald et al., 2016b). Total cell numbers (DAPI-stained cells) and cells belonging to
170 the different targeted groups (CARD-FISH signals) were enumerated in 20 randomly selected fields of
171 view using the grid ocular of the Axioskop 2 (Zeiss) epifluorescence microscope. Proportions of the

172 microbial groups are based on total DAPI cell counts (260 - 550 cells counted per sample, distributed
173 over 20 randomly chosen fields of view).

174 **DNA extraction and 16S rRNA gene amplicon sequencing**

175 Microbial biomass from different depths of the water column was collected on 0.2 µm polycarbonate
176 membrane filters (Cyclopore, Whatman) and kept frozen (-20 °C) until DNA extraction using the
177 FastDNA SPIN Kit for Soil (MP Biomedicals). A two-step PCR approach (Monchamp et al., 2016) was
178 applied in order to prepare the library for Illumina sequencing at the Genomics Facility Basel. Briefly,
179 10 ng of extracted DNA were used, and a first PCR of 25 cycles was performed using universal primers
180 515F-Y (5'-GTGYCAGCMGCCGCGGTAA) and 926R (5'-CCGYCAATTYMTTTRAGTTT-3')
181 targeting the V4 and V5 regions of the 16S rRNA gene (Parada et al., 2016). The primers of this first
182 PCR were composed of the target region and an Illumina Nextera XT specific adapter sequence. Four
183 sets of forward and reverse primers, which contained 0-3 additional and ambiguous bases after adapter
184 sequence, were used in order to introduce frame shifts to increase complexity (details described in Su et
185 al, bioarxiv, 2021). Sample indices and Illumina adaptors were added in a second PCR of 8 cycles.
186 Purified, indexed amplicons were finally pooled at equimolar concentration, denatured, spiked with 10 %
187 PhiX, and sequenced on an Illumina MiSeq platform using the 2×300 bp paired-end protocol (V3-Kit).
188 The initial sequence treatment was done at the Genetic Diversity Center (ETH Zurich) where FastQC
189 (v 1.2.11; Babraham Bioinformatics) was used to check the quality of the raw reads and FLASH (Magoč
190 and Salzberg, 2011) to merge forward and reverse reads into amplicons of about 374 bp length. The
191 procedure allowed a minimum overlap of 15 nucleotides and a mismatch density of 0.25. Full-length
192 primer regions were trimmed using USEARCH (v10.0.240), allowing a maximum of one mismatch.
193 Merged and primer-trimmed amplicons were quality-filtered (size range: 250-550, no ambiguous
194 nucleotides, minimum average quality score of 20) using PRINSEQ (Schmieder and Edwards, 2011).
195 OTU (operational taxonomic unit) clustering with a 97 % identity threshold was performed using the
196 UPARSE-OTU algorithm in USEARCH v10.0.240 (Edgar, 2010, 2013). Taxonomic assignment of
197 OTUs was done using SINTAX (Edgar, 2016) and the SILVA 16S rRNA reference database v128
198 (Quast et al., 2013). Downstream sequence analyses were done in R v3.5.1 using Phyloseq v1.25.2
199 (McMurdie and Holmes, 2013). Raw sequences have been deposited at NCBI under the Bioproject
200 number PRJNA717665 with the accession numbers SAMN18500068 to SAMN18500079.

201 **CH₄ oxidation incubation experiments**

202 To determine the CH₄ oxidation potential and possible stimulation by potential electron acceptors,
203 incubation experiments were setup with water from 3, 4, 5, 7 and 9 m depth no later than 2 h after
204 sampling. These depths were selected based on their expected relevance for CH₄ turnover: previous
205 research has repeatedly shown the highest CH₄ oxidation rates to occur around the oxycline (Blees et
206 al., 2014; Mayr et al., 2020; Milucka et al., 2015; Oswald et al., 2015; Panganiban et al., 1979; Sundh

207 et al., 2005). The followed approach is described in detail by Oswald et al. (2016b), and is based on
208 adapted protocols for ^{15}N incubations (Holtappels et al., 2011). Briefly, water collected in 160 mL serum
209 bottles was first degassed (10 – 15 min with He) and then individually amended with the different
210 electron acceptors tested, except for the dark and light setups (Table S2). After this, 5 mL of a saturated
211 $^{13}\text{CH}_4$ (99 atom%, Campro Scientific) solution was injected under anoxic and sterile conditions into
212 each bottle to a final concentration of $\sim 50 \mu\text{M CH}_4$. Finally, water was dispensed into 12 mL exetainers
213 without headspace, and incubated at $\sim 8^\circ\text{C}$ (average lake temperature between 3 – 9 m) under dark or
214 light ($\sim 5 \mu\text{E m}^{-2} \text{ s}^{-1}$) conditions. At selected time points ($\sim 0, 6, 12, 24$ and 48 h), ZnCl_2 (200 μl , 50 %
215 [w/v] solution) was used to stop microbial activity in one exetainer per setup to analyze $\delta^{13}\text{C-DIC}$ by
216 GC-IRMS (see above). CH_4 oxidation rates were estimated by linear regression of the change of $^{13}\text{C-}$
217 DIC over the experimental interval, under consideration of the in situ DIC concentration at the different
218 incubation depths (1 – 1.2 mM) (for details see Oswald et al., 2015, 2016a). For comparison between
219 all setups and depths, the CH_4 oxidation potential was always determined over the initial 24 h time
220 interval, as the production of $^{13}\text{C-DIC}$ remained linear during this time period in all setups.

221

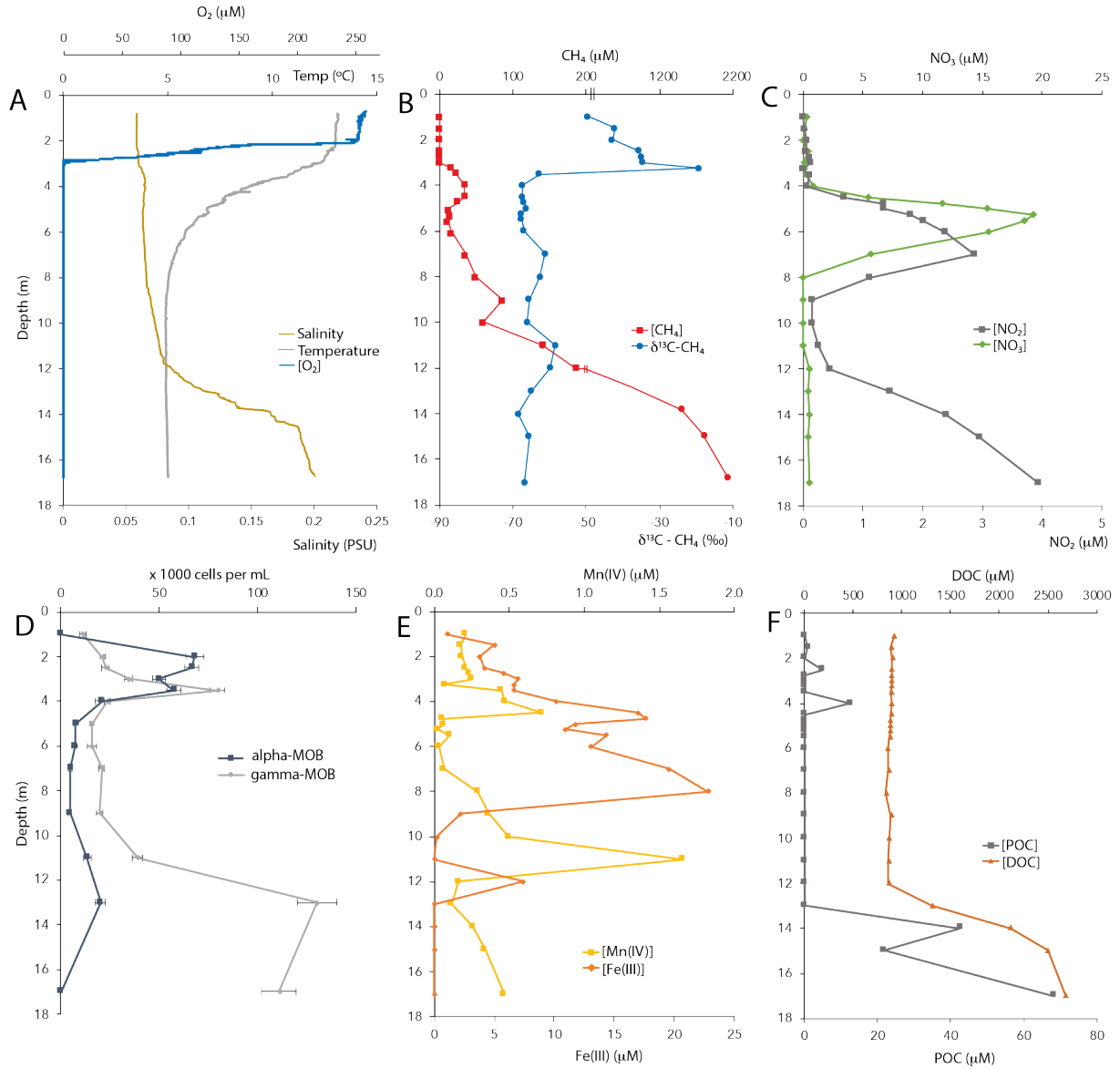
222 **Results**

223 **Physicochemical conditions in the water column**

224 Oxygen concentrations were around 250 μM in the top 2 m of the Lake Lovojärvi water column (Fig.
225 1A). Below, the O_2 profile displayed a sharp gradient between 2 – 3 m depth, and complete oxygen
226 depletion was observed already below 3.1 m. A small peak in the O_2 concentration was observed
227 between 3 and 3.1 m depth (Fig. 2). The thermo- and pycnoclines were evidenced by gradients in
228 temperature between 3 – 5 m (surface temperature 13°C , bottom 5°C) and in salinity between 12 – 14
229 m, respectively (Fig. 1A). Compared to the total radiation at the surface, PAR decreased from 27% (80
230 $\mu\text{E m}^{-2} \text{ s}^{-1}$) at 0.6 m to 1% ($3 \mu\text{E m}^{-2} \text{ s}^{-1}$) at 2.2 m (Fig. 2). Light diminished between 5 and 6.6 m (0.05
231 $- 0.01 \mu\text{E m}^{-2} \text{ s}^{-1}$; Fig. 2). Nitrate concentrations peaked between 4 – 7 m, with the highest concentrations
232 of 19 μM at 5.25 m (Fig. 1C). Above and below the nitrate peak, NO_3^- concentrations averaged at 0.3
233 μM . A nitrite peak was visible at similar depths, but with the maximum concentration found at 7 m (3
234 μM , Fig. 1C). Below 12 m, NO_2^- increased to 4 μM (Fig. 1C). Sulfate concentrations in the top were
235 relatively invariant around 150 μM , and declined sharply to $\sim 12 \mu\text{M}$ at 12 m depth, whereas total sulfide
236 was $< 1 \mu\text{M}$ down to 9 m, from where it increased steadily to $\sim 14 \mu\text{M}$ at 14 m (Fig. S1). Fe(III) showed
237 a peak at 4–9 m depth, with a maximum of 23 μM at 8 m (Fig. 1E). Dissolved Fe(II) increased from 8
238 m downwards to reach a concentration of 830 μM at 17 m (Fig. S1). Manganese concentrations were
239 much lower than those of iron, with particulate Mn(IV) ranging around 0.3 μM showing subtle peaks at
240 4.5 m (0.7 μM) and 11 m (1.7 μM ; Fig. 1E). Dissolved Mn(II) was nearly undetectable in the top 3 m

241 of the water column (100 nM average), yet reached rather constant values of $\sim 2 \mu\text{M}$ below (3 – 11 m),
242 before increasing towards the sediment ($16 \mu\text{M}$ at 17 m, Fig. S1).

243 **Fig. 1.** Physicochemical characteristics and CH_4 oxidizing bacterial (MOB) abundance in the Lake
244 Lovojärvi water column in September 2015. POC – Particulate organic carbon. DOC – Dissolved
245 organic carbon. Note the break at the $[\text{CH}_4]$ axis in panel B. The oxygen profile combines data
246 obtained by two different oxygen sensors, for low and high concentrations (see Methods).



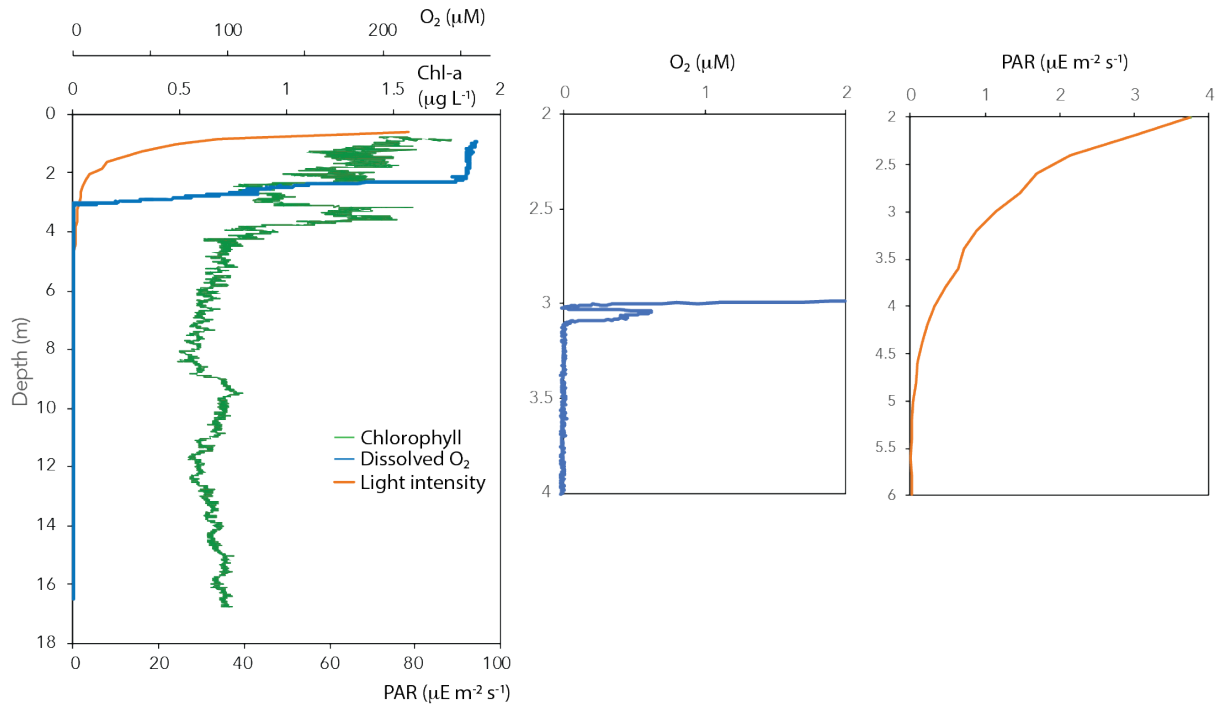
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249 **Fig. 2.** Chlorophyll, light intensity (PAR) and dissolved oxygen in the water column of Lake
250 Lovojärvi.

251

252



253 **CH₄ and other carbon compounds**

254 CH₄ was present throughout the water column of Lake Lovojärvi, yet increased by more than four orders
255 of magnitude from the surface (0.3 μM) to the sediment (~2 mM; Fig. 1B). The profile exposed four
256 'zones': i) low (≤0.3 μM) concentrations in the epilimnion, ii) a distinct peak in [CH₄] below the
257 oxycline, from 3 – 5 m (max concentration 33 μM), iii) a zone of gradual increase, from 11 μM at 5.5
258 m to 140 μM at 11 m, and iv) a zone of rapid increase, from 190 μM at 12 m to 1990 μM at 17 m (Fig.
259 1B). The δ¹³C-CH₄ profile showed values of -50 ‰ to -35 ‰ in the epilimnion and of -58 to -69 ‰ in
260 the hypolimnion, with a trend towards heavier values directly at the oxycline: the δ¹³C-CH₄ increased
261 from -63 ‰ (3.5 m) to -19 ‰ (3.25 m), to decline to -35 ‰ at 3 m (Fig. 1B).

262 The majority of organic carbon was present in its dissolved form, with DOC concentrations being 100x
263 higher than POC concentrations (Fig. 1F). Both DOC and POC profiles showed a constant concentration
264 from the surface to the chemocline at 12 m depth, where both DOC and POC concentration profiles
265 indicated a strong increase towards the sediment surface.

266 The DIC concentration profile followed that of CH₄ closely. Concentrations of DIC also increased by
267 an order of magnitude from the surface (700 μM) to the sediment (5.6 mM), with a peak just below the
268 oxycline (Fig. S2). δ¹³C-DIC values decreased from the surface waters (-11.5 ‰) to the oxycline (-
269 18 ‰), remained relatively constant until 12 m depth, and then increased strongly towards the sediment
270 (-4 ‰ at 17m; Fig. S2), a trend that could not be linked to that of δ¹³C-CH₄ (Fig. 1B).

271 **Microbial community and chlorophyll a distribution**

272 Cell counts showed that both gamma- (probes Mgamma84+705) and alpha-MOB (probe Ma450)
273 abundances showed a distinct peak near the oxycline (Fig. 1D). Gamma-MOB were present at all
274 sampled depths, with peaks at 3.5 m (8.0·10⁴ cells mL⁻¹; 1.8% of DAPI counts), and in the hypolimnion
275 at 13 m (1.3·10⁵ cells mL⁻¹; 3.5% of DAPI counts). Alpha-MOB were most numerous near the oxycline
276 at 2 – 3.5 m, where they comprised a relatively large proportion of the total community (6.8·10⁴ cells
277 mL⁻¹; 3.6 % of DAPI counts). A second, smaller peak was observed at 13 m (2.0·10⁴ cells mL⁻¹, 0.5 %
278 of DAPI counts). Both types of MOB were least abundant between 4-9 m depth. Known representatives
279 of ANME-1 (probe ANME-1-350) and ANME-2 (probe ANME-2-538) did not exceed 0.4 % of total
280 DAPI counts at any depth of the water column (data not shown).

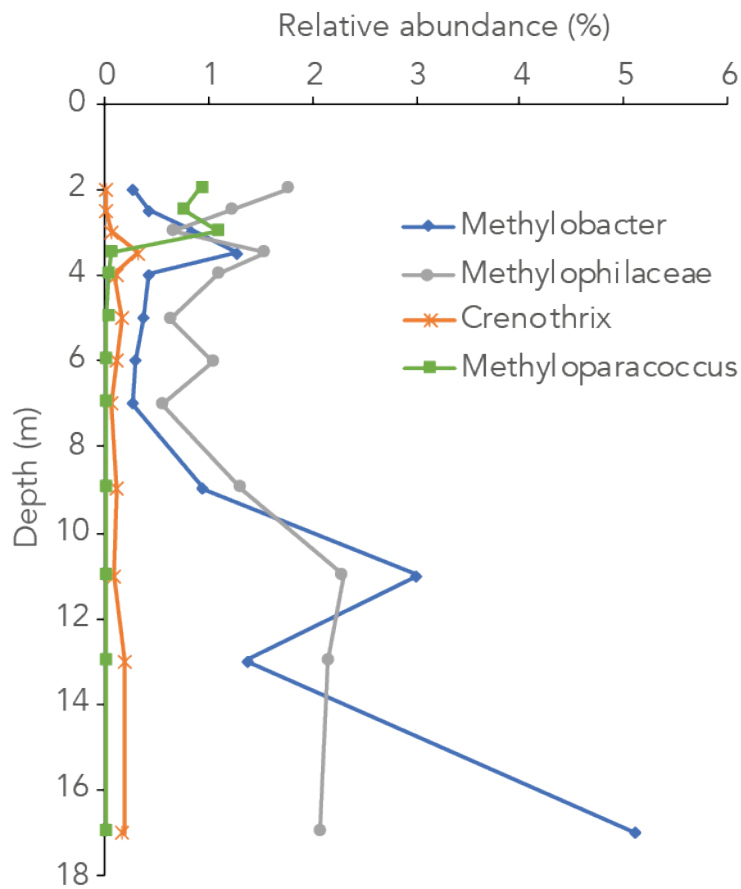
281 16S rRNA gene sequencing data showed that the archaeal relative abundance was below 0.5 %
282 throughout the upper- and middle water column. Only between 11 and 17 m depth, the archaeal
283 abundance was higher than 0.5 % (0.7, 1.0 and 4.0 % of all reads at 11, 13 and 17 m, respectively). The
284 only known archaeal methanogens present belonged to the genus *Methanoregula* and were detected at
285 9, 11 and 17 m depth (0.1, 0.1 and 0.3 %; at all other depths <0.05 % and thus considered insignificant).

286 Gammaproteobacterial methane-oxidizing bacteria reads were detected throughout the water column,
287 and were dominantly assigned to the genus *Methylobacter* (0.3 – 5 % of total 16S rRNA reads) and to a
288 lesser extent to the genus *Crenothrix* (0 – 0.3 %; Fig 3). *Methyloparacoccus* dominated the oxic
289 epilimnion (0.9 – 1.1 %; Fig. 3), but was undetectable below 3.5 m depth. At 3.5, 13 and 17 m,
290 respectively 0.3, 0.1 and 0.3 % of ‘other Methylococcaceae’, specified as 16S rRNA sequence assigned
291 to the family Methylococcaceae but not to the above-mentioned genera, were found.
292 Alphaproteobacteria were highly abundant in the oxic water column (14 – 15 %), but only 0.1 – 0.3 %
293 of these reads were assigned to the genus *Methylocystaceae*. 30 – 35 % of the Alphaproteobacterial
294 reads at 2 – 3 m depth were, however, assigned to unknown bacteria of the Rhizobiales order, the order
295 to which the alpha-MOB belong (Fig. S3). Possibly, part of these unknown Rhizobiales-assigned
296 sequences belongs to methane-oxidizing bacteria. Bacteria of the family Methylophilaceae were present
297 throughout the water column (0.6 – 2.3 %, Fig. 3). Sequence reads of *Candidatus Methylophilus* sp.,
298 belonging to the NC10 phylum, were detected only at one single depth (13 m) but at a comparatively
299 high relative abundance (2.3 %).

300 Chlorophyll a was present throughout the water column (Fig. 2). Yet, concentrations were highest in the
301 surface waters ($1.8 \mu\text{g L}^{-1}$), from where they decreased towards 2 m depth. A second peak in chlorophyll
302 a was visible at 3 – 4 m depth ($1.6 \mu\text{g L}^{-1}$; Fig. 2).

303

304 **Fig. 3.** Relative abundance of 16S rRNA gene sequences annotated to the methanotrophic genera
 305 *Methylobacter*, *Methyloparacoccus* and *Crenothrix*, and the methylotrophic family *Methylophilaceae*
 306 in the water column of Lake Lovojärvi.



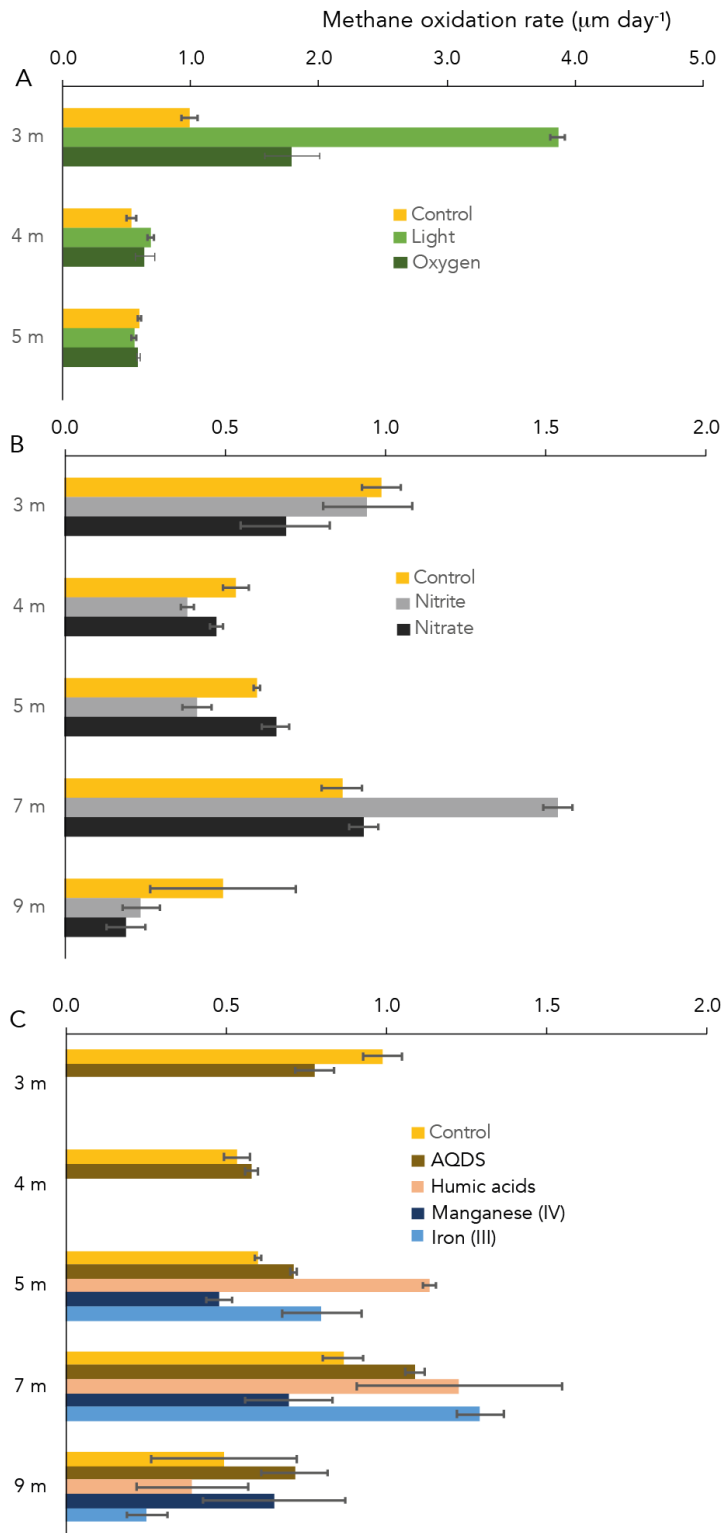
307

308 Potential CH₄ oxidation rates

309 CH₄ oxidation under “control” conditions (dark, starting concentration ~50 μM CH₄ after ¹³CH₄ addition)
 310 peaked at the oxycline (3 m) and at 7 m depth (1.0 and 0.9 μM d⁻¹, respectively; Fig. 4). At 3 and 4 m
 311 depth, of all dark incubations with substrate additions (overview in Table S2), only the addition of
 312 oxygen enhanced the CH₄ oxidation rate (from 1.0 in the control to 1.8 μM d⁻¹ with oxygen at 3 m; Fig.
 313 4). Even more pronounced was the effect of light on the potential CH₄ oxidate rate at 3 m depth, which
 314 accelerated the CH₄ oxidation rate to 3.9 μM d⁻¹ (Fig. 2). At 4 m, the effects of light and oxygen addition
 315 were minor (0.5, 0.7 and 0.6 μM d⁻¹ in the control, light and O₂ incubations, respectively; Fig. 2). At 5
 316 m depth, neither light nor oxygen increased CH₄ oxidation rates (Fig. 2). Additions of anthraquinone-
 317 2,6-disulfonate (AQDS), humic substances, and Fe(III) increased the CH₄ oxidation rate at 5 and 7 m
 318 depth (Fig. 4). Mn(IV) and nitrite increased the CH₄ oxidation rate only at one specific depth (9 m and
 319 7 m, respectively; Fig. 1). Nitrate did not enhance CH₄ oxidation at any of the depths (Fig. 4).

320

321 Fig. 4. CH₄ oxidation rates in control and amended incubations, at different water depths. Note
 322 the different x-axis in panel A versus B + C.



323

324 Discussion

325 Despite extremely high CH₄ concentrations in the bottom waters of Lake Lovojärvi (up to 2000 µM),
 326 the surface water CH₄ concentration, and thus the diffusive emission potential, remained relatively low

327 (<0.5 μM). The pycnocline and thermocline seem to act as physical barrier, hindering diffusive transport
328 and containing dissolved CH_4 in certain water layers, where the process of CH_4 oxidation can consume
329 CH_4 and diminish the CH_4 concentration. Lake Lovojärvi incubation experiments and the natural
330 abundance $\delta^{13}\text{C}$ - CH_4 signal in the water column suggest that natural CH_4 oxidation rates are highest at
331 3 and 7 m depth (Fig. 1 and 4).

332 **Aerobic and photosynthesis-fueled CH_4 oxidation**

333 Oxygen was detected down to a depth of 3.1 m (oxycline) within Lake Lovojärvi (Fig. 1A and 2).
334 Immediately below this depth, $\delta^{13}\text{C}$ - CH_4 showed a pronounced shift to high values from -63‰ at 3.5 m
335 to -19‰ at 3.25 m (Fig. 1B). As methanotrophs fractionate carbon isotopes (just like many other
336 biological reactions breaking carbon bonds), and preferentially oxidize the light carbon ^{12}C isotopes, the
337 residual pool of CH_4 becomes enriched in the heavier ^{13}C isotopes with fractional CH_4 turnover. Hence,
338 the distinct change in $\delta^{13}\text{C}$ at 3 – 3.5 m pinpoints a hotspot of CH_4 oxidation (Barker and Fritz, 1981).
339 The relatively high abundance of both types of aerobic methanotrophs (i.e. gamma- and alpha-MOB;
340 Fig. 1D) supports the existence of a CH_4 oxidation hotspot at the oxycline depth. Furthermore, CH_4
341 oxidation rates were highest directly at the oxycline ($\sim 1 \mu\text{M d}^{-1}$ at 3 m; Fig. 4), confirming that aerobic
342 methanotrophs are most active at the oxic-anoxic transition, where both substrates (CH_4 and O_2) overlap
343 and conditions are most favorable for aerobic CH_4 oxidation (Rudd et al., 1976, Blumenberg et al., 2007;
344 Fenchel and Blackburn, 1979). These findings correspond well with previous studies in stratified lakes,
345 where highest CH_4 turnover was also shown to occur in the vicinity of the oxycline (Blees et al., 2014;
346 Mayr et al., 2020; Milucka et al., 2015; Oswald et al., 2015; Panganiban et al., 1979; Sundh et al., 2005).

347 The oxygen availability at 3 m depth is likely rate-limiting for CH_4 oxidation, given the in situ
348 concentration of 0.5 μM (Fig. 2) and the enhanced CH_4 oxidation rate upon the addition of oxygen (Fig.
349 2). Oxygen availability below the oxycline of stratified lakes is often limited due to the low speed of
350 diffusive oxygen transport across the oxycline (Kreling et al., 2014). In shallow Lake Lovojärvi, another
351 source of oxygen besides diffusive supply is likely enhancing oxygen availability to methanotrophs,
352 stimulating CH_4 removal rates. A strong peak in chlorophyll a concentration was observed at 3 – 4 m
353 depth, where the light intensity was 0.3 – 1.14 $\mu\text{E m}^{-2} \text{s}^{-1}$ (Fig. 2), still exceeding the threshold for
354 photosynthesis (0.09 $\mu\text{E m}^{-2} \text{s}^{-1}$, Gibson, 1985). At that same depth, a small peak in the O_2 concentration
355 is observed (Fig. 2), indicating in situ oxygen production. Milucka et al. (2015) and Oswald et al. (2015,
356 2016b) showed that photosynthetic oxygen production can fuel aerobic CH_4 oxidation deep within the
357 anoxic water column, where CH_4 is often replete. Produced oxygen is immediately consumed by the
358 oxygen-limited aerobic methanotrophs, keeping the dissolved oxygen concentrations in the water
359 column low. Our experimental results indicate that photosynthetically fueled CH_4 oxidation is also a
360 key process in CH_4 removal in the water column of this humic, turbid lake. The photosynthesis effect
361 on methanotrophy is most pronounced at 3 m depth, where the CH_4 oxidation rates increased

362 significantly from $0.99 \pm 0.06 \mu\text{M d}^{-1}$ under dark conditions to $3.9 \pm 0.06 \mu\text{M d}^{-1}$ under light conditions.
363 Why light stimulates the CH_4 oxidation rate at 3 m much stronger than the addition of O_2 directly (1.8
364 $\pm 0.2 \mu\text{M d}^{-1}$) remains unclear. Perhaps the oxygen availability and consumption are better balanced in
365 the case of light stimulation, with a direct linkage between the production by phytoplankton and the
366 consumption by methanotrophs, possibly even via a physical interaction, allowing the produced O_2 to
367 be more efficiently, and exclusively, used for CH_4 oxidation. In the case of an O_2 pulse, as in the oxygen
368 addition experiment, part of the O_2 may be used for non- CH_4 -oxidation related processes (including e.g.
369 dark respiration by phototrophs). It is also possible that the methanotrophs were partly inhibited by the
370 higher O_2 concentrations, as methanotrophs have been suggested to be microaerophiles (Van Bodegom
371 et al., 2001; Rudd and Hamilton, 1975; Thottathil et al., 2019).

372 In incubations with water from 4 m depth, there was only a minor observable effect of O_2 addition and
373 light on the CH_4 oxidation rate (0.5 , 0.7 and $0.6 \mu\text{M d}^{-1}$ for control, light and O_2 , respectively; Fig. 2).
374 Oxygen availability may not be the rate-limiting factor here. The dark incubation experiments indicate
375 that natural CH_4 oxidation rates are lower at 4 m than at 3 m (Fig. 4). The addition of nitrate, nitrite and
376 AQDS did not enhance CH_4 oxidation at 4 m either (Fig. 4). Hence, what the dominant terminal electron
377 acceptor(s) involved in CH_4 oxidation at 4 m depth is/are, and why oxidation rates and methanotroph
378 abundance were lower at 4 m than at 3 m, despite the elevated CH_4 concentrations, remains uncertain.

379 **Water column CH_4 production**

380 The major part of CH_4 production in Lake Lovojärvi takes place in the sediment, where high amounts
381 of the CH_4 diffuse up into the water column ($\sim 2 \text{ mM}$ at 17 m; Fig. 1B). The carbon isotopic signature
382 ($\delta^{13}\text{C}$ of -66% , Fig. 1B) is indicative of a biogenic origin, the production by methanogens (Whiticar,
383 1999). The concentration declines rapidly by an order of magnitude ($\sim 200 \mu\text{M}$ at 12 m) upwards through
384 the pycnocline (Fig. 1B), further decreases from 12 to 6 m depth, but then shows another maximum at
385 3 – 5 m depth. The observed peak in the CH_4 concentration at this depth, just below the oxycline,
386 suggests in situ CH_4 production (Fig. 1B). CH_4 is generally produced by methanogens, anaerobic archaea
387 that do not tolerate oxygen (Kiener and Leisinger, 1983). It would therefore be remarkable that a zone
388 of CH_4 production is observed just below the oxycline, where traces of oxygen are still present, and
389 where oxygen is likely produced by the highly abundant phototrophs (Fig. 2). These phototrophs may,
390 however, not only play a role in enabling aerobic methanotrophy, but also in CH_4 production. Recent
391 research has suggested that cyanobacteria are capable of forming CH_4 as a by-product of photosynthesis
392 (Bižić et al., 2020), and that this might contribute to CH_4 emissions from oxic waters (Günthel et al.,
393 2020). As the zone of CH_4 production in Lake Lovojärvi coincides with the chlorophyll peak (Fig. 1 and
394 2), phytoplankton-mediated CH_4 production may be responsible for the observed CH_4 production near
395 the oxycline. CH_4 production under oxic conditions is, however, still highly debated. Another reasonable
396 explanation for the observed CH_4 peak could be lateral transport of CH_4 produced in sediments in the

397 littoral zone (Peeters et al., 2019). Archaeal methanogens of the genus *Methanoregula* were detected in
398 the water column, but only at 9, 11 and 17 m depth (0.1, 0.1 and 0.3 %).

399 **CH₄ oxidation in the anoxic water column**

400 Besides the peak in CH₄ oxidation at 3 m depth, high CH₄ oxidation rates were also detected at 7 m,
401 within the anoxic part of the water column (Fig. 4). Both the incubation experiments and the $\delta^{13}\text{C-CH}_4$
402 profile, which showed a slight increase in the $\delta^{13}\text{C-CH}_4$ values, suggest active CH₄ oxidation within the
403 anoxic hypolimnion (4 – 9 m). The $\delta^{13}\text{C-CH}_4$ and methanotroph-abundance profiles also suggest a zone
404 of active CH₄ oxidation between 11 and 13 m depth (Fig. 1; 3). Earlier studies have demonstrated high
405 CH₄ oxidation rates in the anoxic water column of lakes, which exceeded oxic CH₄ oxidation rates in
406 some cases (Blees et al., 2014; van Grinsven et al., 2020b). In the anoxic water column of Lake Lovojärvi,
407 nitrate, nitrite, sulfate, Fe(III) and organic matter are all present, in varying concentrations with water
408 column depth (Fig. 1; Fig. S1). These compounds have all been recognized as electron acceptors
409 potentially involved in lacustrine CH₄ oxidation (Ettwig et al., 2010; Kits et al., 2015a; Saxton et al.,
410 2016; Schubert et al., 2011). Lake Lovojärvi incubation experiments showed that nitrite, AQDS, humic
411 substances and Fe(III) all enhanced CH₄ oxidation at 7 m (Fig. 4). This stands in contrast to a study by
412 Rissanen et al. (2018) in a nearby lake, where nitrate stimulated CH₄ oxidation, but Fe(III) inhibited
413 CH₄ oxidation instead. Although each of the aforementioned substances may have stimulated CH₄
414 oxidation directly, as terminal electron acceptor for CH₄ oxidation, they may also have stimulated the
415 internal cycling of other redox components instead, fostering CH₄ oxidation indirectly. For example, Su
416 et al. (2020) showed Mn and Fe oxides can support sulfate-dependent AOM. The stimulating effect of
417 nitrite on the CH₄ oxidation rate was the strongest among all substrates tested ($1.5 \pm 0.1 \mu\text{M d}^{-1}$ with
418 nitrate, $0.9 \pm 0.1 \mu\text{M d}^{-1}$ in the control experiment; Fig. 4). As CH₄ oxidation coupled to the reduction
419 of nitrite yields the largest Gibbs free energy ($\Delta G^\circ = -1007 \text{ kJ mol}^{-1} \text{ CH}_4$), this form of CH₄ oxidation
420 may outcompete CH₄ oxidation coupled to the reduction of Fe(III) ($\Delta G^\circ = -571 \text{ kJ mol}^{-1} \text{ CH}_4$) or AQDS
421 ($\Delta G^\circ = -41 \text{ kJ mol}^{-1} \text{ CH}_4$, Reed et al. 2017). Nitrite was present in the water column of Lake Lovojärvi
422 at relatively high concentrations ($3 \mu\text{M}$) at 7 m and below 12m (Fig. 1C), supporting the hypothesis that
423 nitrite could serve as an electron acceptor involved in natural CH₄ oxidation in the Lake Lovojärvi water
424 column. Nitrite has been found to support CH₄ oxidation by Candidatus *Methylomirabilis oxyfera* and
425 *Methylomicrobium album* (Ettwig et al., 2010; Kits et al., 2015b), but is also known to inhibit CH₄
426 oxidation at higher concentrations (Dunfield and Knowles, 1995; Hütsch, 1998). Surprisingly, nitrite
427 stimulated CH₄ oxidation at 7 m but seemed to inhibit CH₄ oxidation at all other depths (Fig. 4). As the
428 same amounts of nitrite were added at all depths, it is unclear why an inhibitory effect would occur at
429 all depths but 7 m. It may be reasonable to assume that the overall microbial community is involved in
430 the (de)toxification of compounds inhibitory for methanotrophs, or that the differential response is
431 caused by the presence of diverse methanotrophic communities, with different tolerance levels. The

432 methanotrophic community composition is, however, similar at 7 m compared to the other depths (Fig.
433 3).

434 Organic material is present throughout the water column of Lake Lovojärvi (Fig. 1F). Potential
435 involvement of organic molecules in CH₄ oxidation is generally tested with the humic acids analogue
436 AQDS (Saxton et al., 2016; Scheller et al., 2016) or a standard mixture of humic substances provided
437 by commercial companies or the International Humic Substances Society (van Grinsven et al., 2020a;
438 Valenzuela et al., 2019). In this study, both AQDS and leonardite humic acids were used as potential
439 electron acceptors in the incubation experiments (Fig. 1F). A difference in the effect of these two humic
440 substrates was observed, with the humic substances providing a stronger stimulating effect on the CH₄
441 oxidation rates than the AQDS at both 5 and 7 m (Fig. 4). As organic matter in natural systems is highly
442 diverse and complex in composition, it is difficult to assess how similar the added material is to the
443 natural organic material present in the water column, and what causes the observed difference between
444 the two organic materials used in this study. Independent of the exact mechanisms/controls with regards
445 to the role of humics in CH₄ oxidation, our results show, however, that a whole spectrum of organic
446 substrates maybe able to support AOM.

447 **CH₄ oxidizing community**

448 Both alpha- and gammaproteobacterial CH₄ oxidizing bacteria are present throughout the water column
449 according to our cell-count data (Fig. 1D). Although concentrations of CH₄ were very low above the
450 oxycline (~300 nM), alpha-MOB still make up several percent of microbial community here (3.5% of
451 DAPI counts at 2 m). Possibly, these methanotrophs are supported by CH₄ that reaches the upper water
452 column via ebullition, in contrast to the continuous CH₄ supply by diffusion to MOB in the lower water
453 layers. CH₄ is a gas with a low solubility and can therefore form bubbles at high sedimentary
454 concentrations, which are then released into the water column at instability events (Joyce and Jewell,
455 2003). These bubbles exchange gas with the water during their travel upwards through the water column
456 (Delsontro et al., 2010). Possibly, pulses of CH₄ are regularly delivered to the surface water via ebullition,
457 feeding the epilimnetic methanotrophic community. Another possibility is the influx of CH₄ from the
458 littoral zone, via lateral transport. Alpha-MOB are known to predominantly occur at higher O₂ levels,
459 whereas gamma-MOB tend to prefer high CH₄ levels (Amaral and Knowles, 1995; Crevecoeur et al.,
460 2017). This zonation is visible in the Lake Lovojärvi water column, with alpha-MOB abundance peaking
461 at 2 m ($6.8 \cdot 10^4$ cells mL⁻¹, Fig. 1D). The gamma-MOB abundance peaks just below the oxycline ($8.0 \cdot 10^4$
462 cells mL⁻¹, Fig. 1D), at the same depth where the peaks in $\delta^{13}\text{C-CH}_4$ and CH₄ oxidation rate were
463 observed. A second peak in gamma-MOB abundance was observed in the deep water column, at 13 m
464 ($13 \cdot 10^4$ cells mL⁻¹, Fig. 1D). These patterns are in line with a recent 16S rRNA gene and metagenomic
465 sequencing study in Lake Lovojärvi (Rissanen et al., 2021), which also showed the presence of nitrite-
466 reduction genes in *Methylococcales* metagenome assemblies of the water column, as well as genes

467 related to extracellular electron transfer. Our 16S rRNA gene sequencing data suggests that
468 *Methylobacter* sp. represent the dominant methanotrophs in the water column (Fig. 3), both at the
469 oxycline and in the deep water column. This is in line with previous findings, suggesting that
470 *Methylobacter* sp. is a versatile methanotroph that can use both oxygen and other substrates, such as
471 nitrate and nitrite, for CH₄ oxidation (van Grinsven et al., 2020b; Martinez-Cruz et al., 2017; Smith et
472 al., 2018). Methanotrophs belonging to the genus *Methyloparacoccus* dominate the oxic epilimnion, but
473 they are absent in the zone with the highest chlorophyll a concentrations (3 – 4 m; Fig. 3). Bacteria of
474 the family Methylophilaceae were also found throughout the water column, with the highest abundances
475 at depths where CH₄ oxidation occurred (Fig. 1, 3 and 4). Methylophilaceae are methylotrophs that do
476 not possess genes encoding for CH₄ monooxygenases (pMMO nor sMMO), and are therefore incapable
477 of methanotrophy. They are known to oxidize methanol and methylamine (Jenkins et al., 1987), which
478 can be released by methanotrophs (Oshkin et al., 2014; Tavormina et al., 2017; Wei et al., 2016). These
479 may be consumed by methylotrophs belonging to the Methylophilaceae (van Grinsven et al., 2020c),
480 explaining the spatial co-occurrence of the two groups in the lake water column. *Candidatus*
481 *Methylomirabilis* sp. were only detected at 13 m depth, but at a relatively large abundance (2.3 % of
482 16S rRNA reads).

483 Similar CH₄ oxidation rates were measured at 3 and 7 m depth (1.0 ± 0.1 and 0.9 ± 0.1 $\mu\text{M d}^{-1}$,
484 respectively; Fig. 4), despite a large difference in methanotroph abundance (8.5 and $2.6 \cdot 10^4$ cells mL⁻¹,
485 respectively; Fig. 1D). Water column CH₄ oxidation rates therefore seem not necessarily coupled to
486 methanotroph cell numbers, but rather to cell activity rates instead.

487 **Conclusions**

488 Lake Lovojärvi is a productive humic lake. Despite the extremely high CH₄ concentrations in its bottom
489 waters, it is likely not a major source of CH₄ to the atmosphere due to effective CH₄ consumption in the
490 water column, combined with limited gas diffusion from the deep water layers. Nitrite seems to serve
491 as the main TEA for CH₄ oxidation at the most active anoxic CH₄ oxidation hotspot, yet a number of
492 other potential organic and inorganic electron acceptors for CH₄ oxidation are present in the water
493 column and were demonstrated to stimulate AOM, demonstrating the high versatility of aerobic and
494 anaerobic methanotrophic communities in freshwater environments. Near the oxycline, aerobic
495 methanotrophy is supported by oxygen, via diffusion from above and by local production by phototrophs,
496 and by a local input of CH₄, either provided by in situ production of CH₄ by the phototrophic community
497 or by lateral transport. Overall, our study in Lake Lovojärvi shows that even in shallow lakes, CH₄
498 oxidation in the water column can form an efficient two-step (anaerobic/aerobic) biological CH₄
499 removal process, limiting CH₄ emissions from highly productive systems.

500 **Author contributions**

501 KO, CJ and CS were involved in designing the study, sampling campaign and experimental setups while
502 CS and BW developed the overall project. KO and CJ conducted the field sampling and experiments as
503 well as the subsequent laboratory analyses. Amplicon sequence analyses were done by SG and JZ. SG
504 and KO wrote the original draft. SG adapted successive versions of the manuscript that led to the final
505 version. CS, BW, MFL, and JZ reviewed and commented on the manuscript.

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

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514 **References**

515 Amaral, J. A. and Knowles, R.: Growth of methanotrophs in methane and oxygen counter
516 gradients, *FEMS Microbiol. Lett.*, 126(3), 215–220, doi:10.1111/j.1574-6968.1995.tb07421.x,
517 1995.

518 Barker, J. F. and Fritz, P.: Carbon isotope fractionation during microbial methane oxidation,
519 *Nature*, 293(5830), 289–291, doi:10.1038/293289a0, 1981.

520 Bartlett, K. B. and Harriss, R. C.: Review and assessment of methane emissions from wetlands,
521 *Chemosphere*, 26(1–4), 261–320, doi:10.1016/0045-6535(93)90427-7, 1993.

522 Bastviken, D., Tranvik, L. J., Downing, J. A., Crill, P. M. and Enrich-Prast, A.: Freshwater
523 Methane Emissions Offset the Continental Carbon Sink, *Science*, 331, 50,
524 doi:10.1126/science.1196808, 2011.

525 Biderre-Petit, C., Jézéquel, D., Dugat-Bony, E., Lopes, F., Kuever, J., Borrel, G., Viollier, E.,
526 Fonty, G. and Peyret, P.: Identification of microbial communities involved in the methane cycle
527 of a freshwater meromictic lake, *FEMS Microbiol. Ecol.*, 77(3), 533–545, doi:10.1111/j.1574-
528 6941.2011.01134.x, 2011.

529 Bižić, M., Klintzsch, T., Ionescu, D., Hindiyeh, M. Y., Günthel, M., Muro-Pastor, A. M., Eckert,

530 W., Urich, T., Keppler, F. and Grossart, H. P.: Aquatic and terrestrial cyanobacteria produce
531 methane, *Sci. Adv.*, 6(3), 1–10, doi:10.1126/sciadv.aax5343, 2020.

532 Brees, J., Niemann, H., Wenk, C. B., Zopfi, J., Schubert, C. J., Kirf, M. K., Veronesi, M. L.,
533 Hitz, C. and Lehmann, M. F.: Micro-aerobic bacterial methane oxidation in the chemocline and
534 anoxic water column of deep south-Alpine Lake Lugano (Switzerland), *Limnol. Oceanogr.*,
535 59(2), 311–324, doi:10.4319/lo.2014.59.2.0311, 2014.

536 Blumenberg, M., Seifert, R. and Michaelis, W.: Aerobic methanotrophy in the oxic–anoxic
537 transition zone of the Black Sea water column, *Org. Geochem.*, 38(1), 84–91,
538 doi:10.1016/J.ORGGEOCHEM.2006.08.011, 2007.

539 Van Bodegom, P., Stams, F., Mollema, L., Boeke, S. and Leffelaar, P.: Methane Oxidation and
540 the Competition for Oxygen in the Rice Rhizosphere, *Appl. Environ. Microbiol.*, 67(8), 3586–
541 3597, doi:10.1128/AEM.67.8.3586-3597.2001, 2001.

542 Boetius, A., Ravensschlag, K., Schubert, C. J., Rickert, D., Widdel, F., Gieseke, A., Amann, R.,
543 Jürgensen, B. B., Witte, U. and Pfannkuche, O.: A marine microbial consortium apparently
544 mediating anaerobic oxidation of methane, *Nature*, 407(6804), 623–626,
545 doi:10.1038/nrmicro2944 10.1073/ 10.1111/mmi.12082, 2000.

546 Chistoserdova, L.: Methylophiles in natural habitats: current insights through metagenomics,
547 *Appl. Microbiol. Biotechnol.*, 99(14), 5763–5779, doi:10.1007/s00253-015-6713-z, 2015.

548 Cline, J. D.: Spectrophotometric determination of hydrogen sulfide in natural waters, *Limnol.*
549 *Oceanogr.*, 14(3), 454–458, doi:10.4319/lo.1969.14.3.0454, 1969.

550 Crevecoeur, S., Vincent, W. F., Comte, J., Matveev, A. and Lovejoy, C.: Diversity and potential
551 activity of methanotrophs in high methane-emitting permafrost thaw ponds, edited by Z. Zhou,
552 *PLoS One*, 12(11), e0188223, doi:10.1371/journal.pone.0188223, 2017.

553 Crowe, S. A., Katsev, S., Leslie, K., Sturm, A., Magen, C., Nomosatryo, S., Pack, M. A.,
554 Kessler, J. D., Reeburgh, W. S., Roberts, J. A., Gonzalez, L., Douglas Haffner, G., Mucci, A.,
555 Sundby, B. and Fowle, D. A.: The methane cycle in ferruginous Lake Matano, *Geobiology*,
556 9(1), 61–78, doi:10.1111/j.1472-4669.2010.00257.x, 2011.

557 Delontro, T., McGinnis, D. F., Sobek, S., Ostrovsky, I. and Wehrli, B.: Extreme methane
558 emissions from a swiss hydropower Reservoir: Contribution from bubbling sediments, *Environ.*

559 Sci. Technol., 44(7), 2419–2425, doi:10.1021/es9031369, 2010.

560 Deutzmann, J. S., Stief, P., Brandes, J. and Schink, B.: Anaerobic methane oxidation coupled
561 to denitrification is the dominant methane sink in a deep lake, Proc. Natl. Acad. Sci. U. S. A.,
562 111(51), 18273–18278, doi:10.1073/pnas.1411617111, 2014.

563 Downing, J. A., Prairie, Y. T., Cole, J. J., Duarte, C. M., Tranvik, L. J., Striegl, R. G., McDowell,
564 W. H., Kortelainen, P., Caraco, N. F., Melack, J. M. and Middelburg, J. J.: The global
565 abundance and size distribution of lakes, ponds, and impoundments, Limnol. Oceanogr., 51(5),
566 2388–2397, doi:10.4319/lo.2006.51.5.2388, 2006.

567 Dunfield, P. and Knowles, R.: Kinetics of inhibition of methane oxidation by nitrate, nitrite,
568 and ammonium in a humisol, Appl. Environ. Microbiol., 61, 3129–3135, 1995.

569 Durisch-Kaiser, E., Schmid, M., Peeters, F., Kipfer, R., Dinkel, C., Diem, T., Schubert, C. J.
570 and Wehrli, B.: What prevents outgassing of methane to the atmosphere in Lake Tanganyika?,
571 J. Geophys. Res., 116(G2), G02022, doi:10.1029/2010JG001323, 2011.

572 Edgar, R.: SINTAX: a simple non-Bayesian taxonomy classifier for 16S and ITS sequences,
573 bioRxiv, doi:10.1101/074161, 2016.

574 Edgar, R. C.: Search and clustering orders of magnitude faster than BLAST, Bioinformatics,
575 26(19), 2460–2461, doi:10.1093/bioinformatics/btq461, 2010.

576 Edgar, R. C.: UPARSE: Highly accurate OTU sequences from microbial amplicon reads, Nat.
577 Methods, 10(10), 996–998, doi:10.1038/nmeth.2604, 2013.

578 Eller, G., Känel, L., Krüger, M., Ka, L. and Kru, M.: Cooccurrence of Aerobic and Anaerobic
579 Methane Oxidation in the Water Column of Lake Plußsee, Appl. Environ. Microbiol., 71(12),
580 8925–8928, doi:10.1128/AEM.71.12.8925, 2005.

581 Ettwig, K. F., Butler, M. K., Le Paslier, D., Pelletier, E., Mangenot, S., Kuypers, M. M. M.,
582 Schreiber, F., Dutilh, B. E., Zedelius, J., de Beer, D., Gloerich, J., Wessels, H. J. C. T., van
583 Alen, T., Luesken, F., Wu, M. L., van de Pas-Schoonen, K. T., Op den Camp, H. J. M., Janssen-
584 Megens, E. M., Francoijs, K.-J., Stunnenberg, H., Weissenbach, J., Jetten, M. S. M. and Strous,
585 M.: Nitrite-driven anaerobic methane oxidation by oxygenic bacteria., Nature, 464(7288), 543–
586 548, doi:10.1038/nature08883, 2010.

587 Fenchel, T. and Blackburn, T. H.: Bacteria and mineral cycling., Academic Press, Inc. (London)

588 Ltd., 1979.

589 Gibson, C. E.: Growth rate, maintenance energy and pigmentation of planktonic Cyanophyta
590 during one-hour light: Dark cycles, Br. Phycol. J., 20(2), 155–161,
591 doi:10.1080/00071618500650161, 1985.

592 Graf, J. S., Mayr, M. J., Marchant, H. K., Tienken, D., Hach, P. F., Brand, A., Schubert, C. J.,
593 Kuypers, M. M. M. and Milucka, J.: Bloom of a denitrifying methanotroph, ‘*Candidatus*
594 *Methylomirabilis limnetica*’, in a deep stratified lake, Environ. Microbiol., 20(7), 2598–2614,
595 doi:10.1111/1462-2920.14285, 2018.

596 Griess, P.: „Über einige Azoverbindungen”, Berichte der Dtsch. Chem. Gesellschaft, 12(1),
597 426–428, doi:10.1002/cber.187901201117, 1879.

598 van Grinsven, S., Sinninghe Damsté, J. S. and Villanueva, L.: Assessing the effect of humic
599 substances and Fe(III) as potential electron acceptors for anaerobic methane oxidation in a
600 marine anoxic system, Microorganisms, 8(9), 1–15, doi:10.3390/microorganisms8091288,
601 2020a.

602 van Grinsven, S., Sinninghe Damsté, J. S., Abdala Asbun, A., Engelmann, J. C., Harrison, J.
603 and Villanueva, L.: Methane oxidation in anoxic lake water stimulated by nitrate and sulfate
604 addition, Environ. Microbiol., 22(2), 766–782, doi:10.1111/1462-2920.14886, 2020b.

605 van Grinsven, S., Sinninghe Damsté, J. S., Harrison, J., Polerecky, L. and Villanueva, L.:
606 Nitrate promotes the transfer of methane-derived carbon from the methanotroph *Methylobacter*
607 sp. to the methylotroph *Methylotenera* sp. in eutrophic lake water, Limnol. Oceanogr., 2, 1–14,
608 doi:10.1002/lno.11648, 2020c.

609 Günthel, M., Klawonn, I., Woodhouse, J., Bižić, M., Ionescu, D., Ganzert, L., Kümmel, S.,
610 Nijenhuis, I., Zoccarato, L., Grossart, H. P. and Tang, K. W.: Photosynthesis-driven methane
611 production in oxic lake water as an important contributor to methane emission, Limnol.
612 Oceanogr., 1–13, doi:10.1002/lno.11557, 2020.

613 Hakala, A.: Meromixis as a part of lake evolution; observations and a revised classification of
614 true meromictic lakes in Finland, Boreal Environ. Res., 9(1), 37–53, 2004.

615 Hanson, R. S. and Hanson, T. E.: Methanotrophic bacteria., Microbiol. Rev., 60(2), 439–471,
616 1996.

617 Hinrichs, K.-U. and Boetius, A.: The Anaerobic Oxidation of Methane: New Insights in
618 Microbial Ecology and Biogeochemistry, in *Ocean Margin Systems*, pp. 457–477, Springer -
619 Verlag., 2002.

620 Holmes, A. J., Tujula, N. A., Holley, M., Contos, A., James, J. M., Rogers, P. and Gillings, M.
621 R.: Phylogenetic structure of unusual aquatic microbial formations in Nullarbor caves, Australia,
622 *Environ. Microbiol.*, 3(4), 256–264, doi:10.1046/j.1462-2920.2001.00187.x, 2001.

623 Holtappels, M., Lavik, G., Jensen, M. M. and Kuypers, M. M. M.: ¹⁵N-Labeling Experiments
624 to Dissect the Contributions of Heterotrophic Denitrification and Anammox to Nitrogen
625 Removal in the OMZ Waters of the Ocean, *Methods Enzymol.*, 486, 223–251,
626 doi:10.1016/B978-0-12-381294-0.00010-9, 2011.

627 Hütsch, B. W.: Methane oxidation in arable soil as inhibited by ammonium, nitrite, and organic
628 manure with respect to soil pH, *Biol. Fertil. Soils*, 28(1), 27–35, doi:10.1007/s003740050459,
629 1998.

630 Ilmavirta, V., Ilmavirta, K. and Kotimaa, A.-L.: Phytoplanktonic primary production during the
631 summer stagnation in the eutrophicated lakes Lovojärvi and Ormajärvi, southern Finland, *Ann.*
632 *Bot. Fenn.*, 11, 121–132, doi:10.2307/23725044, 1974.

633 Jenkins, O., Byrom, D. and Jones, D.: *Methylophilus*: A New Genus of Methanol-Utilizing
634 Bacteria, *Int. J. Syst. Bacteriol.*, doi:10.1099/00207713-37-4-446, 1987.

635 Joyce, J. and Jewell, P. W.: Physical controls on methane ebullition from reservoirs and lakes,
636 *Environ. Eng. Geosci.*, 9(2), 167–178, doi:10.2113/9.2.167, 2003.

637 Juutinen, S., Rantakari, M., Kortelainen, P., Huttunen, J. T., Larmola, T., Alm, J., Silvola, J.
638 and Martikainen, P. J.: Methane dynamics in different boreal lake types, *Biogeosciences*, 6(2),
639 209–223, doi:10.5194/bg-6-209-2009, 2009.

640 Kallistova, A., Kadnikov, V., Rusanov, I., Kokryatskaya, N., Beletsky, A., Mardanov, A.,
641 Savvichev, A., Ravin, N. and Pimenov, N.: Microbial communities involved in aerobic and
642 anaerobic methane cycling in a meromictic ferruginous subarctic lake, *Aquat. Microb. Ecol.*,
643 82(1), 1–18, doi:10.3354/ame01878, 2019.

644 Kankaala, P., Huotari, J., Peltomaa, E., Saloranta, T. and Ojala, A.: Methanotrophic activity in
645 relation to methane efflux and total heterotrophic bacterial production in a stratified, humic,

646 boreal lake, *Limnol. Oceanogr.*, 51(2), 1195–1204, doi:10.4319/lo.2006.51.2.1195, 2006.

647 Keskitalo, J.: The species composition and biomass of phytoplankton in the eutrophic Lake
648 Lovojärvi, southern Finland, *Ann. Bot. Fenn.*, 14, 71–81, doi:10.2307/43922123, 1977.

649 Kiener, A. and Leisinger, T.: Oxygen Sensitivity of Methanogenic Bacteria, *Syst. Appl.*
650 *Microbiol.*, doi:10.1016/S0723-2020(83)80017-4, 1983.

651 King, G.: Ecological aspects of methane oxidation, a key determinant of global methane
652 dynamics, *Adv. Microb. Ecol.*, 431–468, 1992.

653 Kits, D. K., Campbell, D. J., Rosana, A. R. and Stein, L. Y.: Diverse electron sources support
654 denitrification under hypoxia in the obligate methanotroph *Methylomicrobium album* strain
655 BG8, *Front. Microbiol.*, 6(OCT), 1–11, doi:10.3389/fmicb.2015.01072, 2015a.

656 Kits, D. K., Klotz, M. G. and Stein, L. Y.: Methane oxidation coupled to nitrate reduction under
657 hypoxia by the Gammaproteobacterium *Methylomonas denitrificans*, sp. nov. type strain FJG1,
658 *Environ. Microbiol.*, 17, 3219–3232, doi:10.1111/1462-2920.12772, 2015b.

659 Kortelainen, P., Huttunen, J. T., Väisänen, T., Mattsson, T., Karjalainen, P. and Martikainen, P.
660 J.: CH₄, CO₂ and N₂O supersaturation in 12 Finnish lakes before and after ice-melt, *SIL*
661 *Proceedings*, 1922-2010, 27(3), 1410–1414, doi:10.1080/03680770.1998.11901468, 2000.

662 Kortelainen, P., Pajunen, H., Rantakari, M. and Saarnisto, M.: A large carbon pool and small
663 sink in boreal Holocene lake sediments, *Glob. Chang. Biol.*, 10(10), 1648–1653,
664 doi:10.1111/j.1365-2486.2004.00848.x, 2004.

665 Kreling, J., Bravidor, J., McGinnis, D. F., Koschorreck, M. and Lorke, A.: Physical controls of
666 oxygen fluxes at pelagic and benthic oxyclines in a lake, *Limnol. Oceanogr.*, 59(5), 1637–1650,
667 doi:10.4319/lo.2014.59.5.1637, 2014.

668 Krom, M. D.: Spectrophotometric determination of ammonia: a study of a modified Berthelot
669 reaction using salicylate and dichloroisocyanurate, *Analyst*, 105(1249), 305,
670 doi:10.1039/an9800500305, 1980.

671 Magoč, T. and Salzberg, S. L.: FLASH: Fast length adjustment of short reads to improve
672 genome assemblies, *Bioinformatics*, 27(21), 2957–2963, doi:10.1093/bioinformatics/btr507,
673 2011.

674 Martinez-Cruz, K., Leewis, M. C., Herriott, I. C., Sepulveda-Jauregui, A., Anthony, K. W.,
675 Thalasso, F. and Leigh, M. B.: Anaerobic oxidation of methane by aerobic methanotrophs in
676 sub-Arctic lake sediments, *Sci. Total Environ.*, 607–608, 23–31,
677 doi:10.1016/j.scitotenv.2017.06.187, 2017.

678 Mayr, M. J., Zimmermann, M., Dey, J., Brand, A., Wehrli, B. and Bürgmann, H.: Growth and
679 rapid succession of methanotrophs effectively limit methane release during lake overturn,
680 *Commun. Biol.*, doi:10.1038/s42003-020-0838-z, 2020.

681 McMurdie, P. J. and Holmes, S.: Phyloseq: An R Package for Reproducible Interactive Analysis
682 and Graphics of Microbiome Census Data, *PLoS One*, 8(4), 1–11,
683 doi:10.1371/journal.pone.0061217, 2013.

684 Michaelis, W., Seifert, R., Nauhaus, K., Treude, T. and Thiel, V.: Microbial Reefs in the Black
685 Sea fueled by anaerobic Oxidation of Methane, *Science*, 297(August), 1013–1015,
686 doi:10.1126/science.1072502, 2002.

687 Michmerhuizen, C. M., Striegl, R. G. and McDonald, M. E.: Potential methane emission from
688 north-temperate lakes following ice melt, *Limnol. Oceanogr.*, 41(5), 985–991,
689 doi:10.4319/lo.1996.41.5.0985, 1996.

690 Milucka, J., Kirf, M., Lu, L., Krupke, A., Lam, P., Littmann, S., Kuypers, M. M. M. and
691 Schubert, C. J.: Methane oxidation coupled to oxygenic photosynthesis in anoxic waters, *ISME*
692 *J.*, 9(9), 1991–2002, doi:10.1038/ismej.2015.12, 2015.

693 Miracle, M., Vicente, E. and Pedrós-Alió, C.: Biological studies of Spanish meromictic and
694 stratified karstic lakes, *Limnetica*, 8, 59–77, 1992.

695 Monchamp, M. E., Walser, J. C., Pomati, F. and Spaak, P.: Sedimentary DNA reveals
696 cyanobacterial community diversity over 200 years in two perialpine lakes, *Appl. Environ.*
697 *Microbiol.*, 82(21), 6472–6482, doi:10.1128/AEM.02174-16, 2016.

698 Norði, K. à., Thamdrup, B. and Schubert, C. J.: Anaerobic oxidation of methane in an iron-rich
699 Danish freshwater lake sediment, *Limnol. Oceanogr.*, 58(2), 546–554,
700 doi:10.4319/lo.2013.58.2.0546, 2013.

701 Orphan, V. J., House, C. H. and Hinrichs, K.: Methane-consuming archaea revealed by directly
702 coupled isotopic and phylogenetic analysis, *Science*, 293(5529), 484–488,

703 doi:10.1126/science.1061338, 2001.

704 Oshkin, I. Y., Beck, D. A., Lamb, A. E., Tchesnokova, V., Benuska, G., McTaggart, T. L.,
705 Kalyuzhnaya, M. G., Dedysh, S. N., Lidstrom, M. E. and Chistoserdova, L.: Methane-fed
706 microbial microcosms show differential community dynamics and pinpoint taxa involved in
707 communal response., *ISME J.*, 9(5), 1–11, doi:10.1038/ismej.2014.203, 2014.

708 Oswald, K., Milucka, J., Brand, A., Littmann, S., Wehrli, B., Kuypers, M. M. M. and Schubert,
709 C. J.: Light-dependent aerobic methane oxidation reduces methane emissions from seasonally
710 stratified lakes, *PLoS One*, 10(7), e0132574, doi:10.1371/journal.pone.0132574, 2015.

711 Oswald, K., Milucka, J., Brand, A., Hach, P., Littmann, S., Wehrli, B., Kuypers, M. M. M. and
712 Schubert, C. J.: Aerobic gammaproteobacterial methanotrophs mitigate methane emissions
713 from oxic and anoxic lake waters, *Limnol. Oceanogr.*, 61, doi:10.1002/lno.10312, 2016a.

714 Oswald, K., Jegge, C., Tischer, J., Berg, J., Brand, A., Miracle, M. R., Soria, X., Vicente, E.,
715 Lehmann, M. F., Zopfi, J. and Schubert, C. J.: Methanotrophy under versatile conditions in the
716 water column of the ferruginous meromictic Lake La Cruz (Spain), *Front. Microbiol.*, 7(Nov),
717 1–16, doi:10.3389/fmicb.2016.01762, 2016b.

718 Panganiban, A. T., Patt, T. E., Hart, W. and Hanson, R. S.: Oxidation of methane in the absence
719 of oxygen in lake water samples., *Appl. Environ. Microbiol.*, 37(2), 303–309,
720 doi:10.1128/aem.37.2.303-309.1979, 1979.

721 Parada, A. E., Needham, D. M. and Fuhrman, J. A.: Every base matters: Assessing small subunit
722 rRNA primers for marine microbiomes with mock communities, time series and global field
723 samples, *Environ. Microbiol.*, 18(5), 1403–1414, doi:10.1111/1462-2920.13023, 2016.

724 Peeters, F., Encinas Fernandez, J. and Hofmann, H.: Sediment fluxes rather than oxic
725 methanogenesis explain diffusive CH₄ emissions from lakes and reservoirs, *Sci. Rep.*, 9(1), 1–
726 10, doi:10.1038/s41598-018-36530-w, 2019.

727 Pernthaler, A., Pernthaler, J. and Amann, R.: Fluorescence in situ hybridization and catalyzed
728 reporter deposition for the identification of marine bacteria, *Appl. Environ. Microbiol.*, 68(6),
729 3094–3101, doi:10.1128/AEM.68.6.3094-3101.2002, 2002.

730 Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J. and Glöckner,
731 F. O.: The SILVA ribosomal RNA gene database project: improved data processing and web-

- 732 based tools., *Nucleic Acids Res.*, 41(Database issue), D590-6, doi:10.1093/nar/gks1219, 2013.
- 733 Rappé, M. S. and Giovannoni, S. J.: The Uncultured Microbial Majority, *Annu. Rev. Microbiol.*,
734 57(1), 369–394, doi:10.1146/annurev.micro.57.030502.090759, 2003.
- 735 Reeburgh, W. S.: Oceanic Methane Biogeochemistry, *Chem. Rev.*, 107(2), 486–513,
736 doi:10.1021/cr050362v, 2007.
- 737 Reed, D. C., Deemer, B. R., van Grinsven, S. and Harrison, J. A.: Are elusive anaerobic
738 pathways key methane sinks in eutrophic lakes and reservoirs?, *Biogeochemistry*, 134(1–2),
739 29–39, doi:10.1007/s10533-017-0356-3, 2017.
- 740 Rissanen, A. J., Saarenheimo, J., Tirola, M., Peura, S., Aalto, S. L., Karvinen, A. and Nykänen,
741 H.: Gammaproteobacterial methanotrophs dominate methanotrophy in aerobic and anaerobic
742 layers of boreal lake waters, *Aquat. Microb. Ecol.*, 81(3), 257–276, doi:10.3354/ame01874,
743 2018.
- 744 Rissanen, A. J., Saarela, T., Jäntti, H., Buck, M., Peura, S., Aalto, S. L., Ojala, A., Pumpanen,
745 J., Tirola, M., Elvert, M. and Nykänen, H.: Vertical stratification patterns of methanotrophs
746 and their genetic controllers in water columns of oxygen-stratified boreal lakes, *FEMS*
747 *Microbiol. Ecol.*, 97(2), 1–16, doi:10.1093/femsec/fiaa252, 2021.
- 748 Rudd, J. W. M.: Methane cycling in aquatic environments, *Adv. Aquat. Microbiol.*, 1, 77–150,
749 1980.
- 750 Rudd, J. W. M. and Hamilton, R. D.: Factors controlling rates of methane oxidation and the
751 distribution of the methane oxidizers in a small stratified lake, *Arch. Hydrobiol.*, 75, 522–538,
752 doi:10.1126/science.aad7154, 1975.
- 753 Rudd, J. W. M., Furutani, A., Flett, R. J. and Hamilton, R. D.: Factors controlling methane
754 oxidation in shield lakes: The role of nitrogen fixation and oxygen concentration, *Limnol.*
755 *Oceanogr.*, 21(3), 357–364, doi:10.4319/lo.1976.21.3.0357, 1976.
- 756 Saarnisto, M., Huttunen, P. and Tolonen, K.: Annual lamination of sediments in Lake Lovojärvi,
757 southern Finland, during the past 600 years, *Ann. Bot. Fenn.*, 14, 35–45, doi:10.2307/23726048,
758 1977.
- 759 Saxton, M. A., Samarkin, V. A., Schutte, C. A., Bowles, M. W., Madigan, M. T., Cadieux, S.
760 B., Pratt, L. M. and Joye, S. B.: Biogeochemical and 16S rRNA gene sequence evidence

761 supports a novel mode of anaerobic methanotrophy in permanently ice-covered Lake Fryxell,
762 Antarctica, *Limnol. Oceanogr.*, 61, S119–S130, doi:10.1002/lno.10320, 2016.

763 Scheller, S., Yu, H., Chadwick, G. L. and Mcglynn, S. E.: Artificial electron acceptors decouple
764 archaeal methane oxidation from sulfate reduction, *Science*, 351(6274), 703–707, 2016.

765 Schmieder, R. and Edwards, R.: Quality control and preprocessing of metagenomic datasets,
766 *Bioinformatics*, 27(6), 863–864, doi:10.1093/bioinformatics/btr026, 2011.

767 Schubert, C. J., Lucas, F. S., Durisch-Kaiser, E., Stierli, R., Diem, T., Scheidegger, O., Vazquez,
768 F. and Muller, B.: Oxidation and emission of methane in a monomictic lake (Rotsee,
769 Switzerland), *Aquat. Sci.*, 72(4), 455–466, doi:10.1007/s00027-010-0148-5, 2010.

770 Schubert, C. J., Vazquez, F., Loesekann-Behrens, T., Knittel, K., Tonolla, M. and Boetius, A.:
771 Evidence for anaerobic oxidation of methane in sediments of a freshwater system (Lago di
772 Cadagno), *FEMS Microbiol. Ecol.*, 76(1), 26–38, doi:10.1111/j.1574-6941.2010.01036.x, 2011.

773 Simola, H.: Micro-stratigraphy of sediment laminations deposited in a chemically stratifying
774 eutrophic lake during the years 1913-1976, *Ecography (Cop.)*, 2(3), 160–168,
775 doi:10.1111/j.1600-0587.1979.tb00696.x, 1979.

776 Simola, H., Hanski, I. and Liukkonen, M.: Stratigraphy, species richness and seasonal dynamics
777 of plankton diatoms during 418 years in Lake Lovojärvi, South Finland, *Ann. Bot. Fenn.*, 27,
778 241–259, doi:10.2307/23725364, 1990.

779 Sivan, O., Adler, M., Pearson, A., Gelman, F., Bar-Or, I., John, S. G. and Eckert, W.:
780 Geochemical evidence for iron-mediated anaerobic oxidation of methane, *Limnol. Oceanogr.*,
781 56(4), 1536–1544, doi:10.4319/lo.2011.56.4.1536, 2011.

782 Smith, G. J., Angle, J. C., Solden, L. M., Daly, R. A., Johnston, M. D., Borton, M. A., Wolfe,
783 R., Stefanik, K. C., Morin, T. H., Gil, B. and Wrighton, K. C.: Members of the Genus
784 *Methylobacter* Are Inferred To Account for the Majority of Aerobic Methane Oxidation in Oxidic
785 Soils from a Freshwater Wetland, *MBio*, 9(6), 1–17, doi:10.1128/mbio.00815-18, 2018.

786 Stookey, L. L.: Ferrozine - a new spectrophotometric reagent for iron, *Anal. Chem.*, 42(7), 779–
787 781, doi:10.1021/ac60289a016, 1970.

788 Su, G., Zopfi, J., Yao, H., Steinle, L., Niemann, H. and Lehmann, M. F.: Manganese/iron-
789 supported sulfate-dependent anaerobic oxidation of methane by archaea in lake sediments,

790 Limnol. Oceanogr., 65(4), 863–875, doi:10.1002/lno.11354, 2020.

791 Sundh, I., Bastviken, D. and Tranvik, L. J.: Abundance, activity, and community structure of
792 pelagic methane-oxidizing bacteria in temperate lakes, *Appl. Environ. Microbiol.*, 71(11),
793 6746–6752, doi:10.1128/AEM.71.11.6746-6752.2005, 2005.

794 Taipale, S., Kankaala, P., Hahn, M., Jones, R. and Tirola, M.: Methane-oxidizing and
795 photoautotrophic bacteria are major producers in a humic lake with a large anoxic hypolimnion,
796 *Aquat. Microb. Ecol.*, 64(1), 81–95, doi:10.3354/ame01512, 2011.

797 Tavormina, P. L., Kellermann, M. Y., Antony, C. P., Tocheva, E. I., Dalleska, N. F., Jensen, A.
798 J., Valentine, D. L., Hinrichs, K. U., Jensen, G. J., Dubilier, N. and Orphan, V. J.: Starvation
799 and recovery in the deep-sea methanotroph *Methyloprofundus* sedimenti, *Mol. Microbiol.*,
800 103.2, 242–252, doi:10.1111/mmi.13553, 2017.

801 Thottathil, S. D., Reis, P. C. J. and Prairie, Y. T.: Methane oxidation kinetics in northern
802 freshwater lakes, *Biogeochemistry*, 143(1), 105–116, doi:10.1007/s10533-019-00552-x, 2019.

803 Tolonen, K., Tolonen, M., Honkasalo, L., Lehtovaara, A., Sorsa, K. and Sundberg, K.: The
804 influence of of prehistoric and historic land use on Lake Lampellonjärvi, South Finland.,
805 *Luonnon Tutkija*, 80, 1–15, 1976.

806 Valenzuela, E. I., Avendaño, K. A., Balagurusamy, N., Arriaga, S., Nieto-Delgado, C., Thalasso,
807 F. and Cervantes, F. J.: Electron shuttling mediated by humic substances fuels anaerobic
808 methane oxidation and carbon burial in wetland sediments, *Sci. Total Environ.*, 650, 2674–
809 2684, doi:10.1016/J.SCITOTENV.2018.09.388, 2019.

810 Viollier, E., Inglett, P. ., Hunter, K., Roychoudhury, A. . and Van Cappellen, P.: The ferrozine
811 method revisited: Fe(II)/Fe(III) determination in natural waters, *Appl. Geochemistry*, 15(6),
812 785–790, doi:10.1016/S0883-2927(99)00097-9, 2000.

813 Walter, K. M., Smith, L. C. and Chapin, S. F.: Methane bubbling from northern lakes: Present
814 and future contributions to the global methane budget, *Philos. Trans. R. Soc. A Math. Phys.*
815 *Eng. Sci.*, 365(1856), 1657–1676, doi:10.1098/rsta.2007.2036, 2007.

816 Wei, X. M., He, R., Chen, M., Su, Y. and Ma, R. C.: Conversion of methane-derived carbon
817 and microbial community in enrichment cultures in response to O₂-availability, *Environ. Sci.*
818 *Pollut. Res.*, 23(8), 7517–7528, doi:10.1007/s11356-015-6017-y, 2016.

819 Whiticar, M. J.: Carbon and hydrogen isotope systematics of bacterial formation and oxidation
820 of methane, *Chem. Geol.*, 161(1–3), 291–314, doi:10.1016/S0009-2541(99)00092-3, 1999.

821 Wiesenburg, D. A. and Guinasso, N. L.: Equilibrium solubilities of methane, carbon monoxide,
822 and hydrogen in water and sea water, *J. Chem. Eng. Data*, 24(4), 356–360,
823 doi:10.1021/je60083a006, 1979.

824 Zheng, Y., Wang, H., Liu, Y., Zhu, B., Li, J., Yang, Y., Qin, W., Chen, L., Wu, X.,
825 Chistoserdova, L. and Zhao, F.: Methane-Dependent Mineral Reduction by Aerobic
826 Methanotrophs under Hypoxia, *Environ. Sci. Technol. Lett.*, 7(8), 606–612,
827 doi:10.1021/acs.estlett.0c00436, 2020.

828