

1 Imprint of eutrophication on methane-cycling microbes in freshwater sediment

2

3 Alice Bosco-Santos^{1*}, Eulalie Rose Beyala Bekono¹, Santona Khatun^{1,2}, Marie-Ève
4 Monchamp³, Joana Séneca^{4,5}, Petra Pjevac^{4,5}, Jasmine S. Berg¹

5 ¹Institute of Earth Surface Dynamics (IDYST), University of Lausanne, Lausanne, Switzerland

6 ² School of Architecture, Civil and Environmental Engineering, Environmental Engineering
7 Institute, Smart Environmental Sensing in Extreme Environments (SENSE), École
8 Polytechnique Fédérale de Lausanne (EPFL Valais Wallis), 1951 Sion, Switzerland

9 ³Biology Department, McGill University, Montreal, Canada

10 ⁴Division of Microbial Ecology, Centre for Microbiology and Environmental Systems Science,
11 University of Vienna, Vienna, Austria

12 ⁵Joint Microbiome Facility of the Medical University of Vienna and the University of Vienna,
13 Vienna, Austria

14 *(alice.boscosantos@unil.ch)

15

16

17

18

19

20

21

22

23

24

25

26

27

28

29

30

31

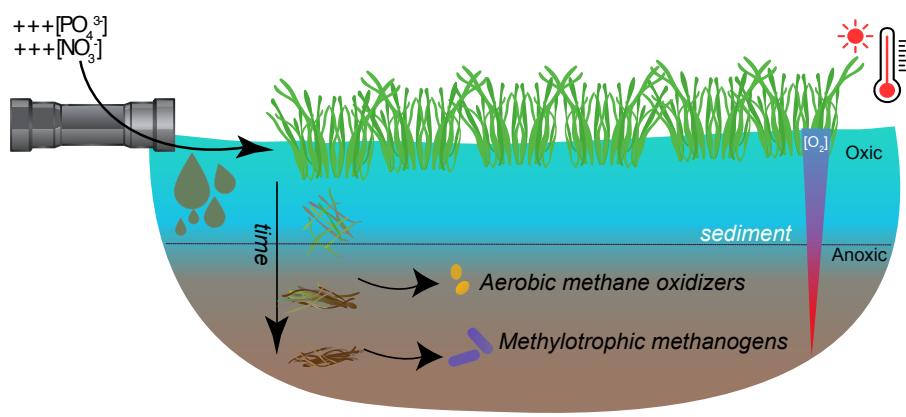
32

33

34

35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
61

Abstract. Eutrophication can alter methane (CH₄) cycling in lakes, yet its long-term effect on sediment microbial communities remains unclear. We analyzed a 400-year-old sediment record from the historically eutrophied Lake Joux, Switzerland, combining porewater and solid-phase geochemistry with 16S rRNA gene amplicon analyses to elucidate the effects of nutrient and carbon loading on methanogenic and methanotrophic sediment communities. Lithological and chemical stratification defined three intervals (deep eutrophic, middle carbonate, upper eutrophic) correlated with changes in organic matter sources. Methanogens were clearly depth-partitioned: methylotrophic Methanomassiliicoccales dominated deep eutrophic sediments, whereas hydrogenotrophic Methanomicrobiales and Methanobacteriales increased upward in shallower, more recent sediments with fresher organic matter. Paired isotopic data support this substrate-driven shift in CH₄ production. Although O₂ was not detected below ~0.4 cm, sequences of aerobic gammaproteobacterial methanotrophs (*Crenothrix* and *Methylobacter*) were abundant in surface sediments down to ~20 cm sediment depth, correlating with NO₃⁻ and PO₄³⁻ concentrations. The absence of anaerobic methanotrophs and C-isotopic evidence for ongoing methane oxidation suggest that these O₂-requiring, methane monooxygenase-utilizing Methylococcales constitute the dominant CH₄ sink in surface sediments. These findings reveal that eutrophication can cause a stratification of methane-cycling microbial communities, highlighting the role of sedimentary legacies in regulating benthic CH₄ emissions from freshwater ecosystems.



62
63
64
65
66
67
68

69 **1. Introduction**

70 Freshwater ecosystems are significant sources of the greenhouse gas methane (CH₄),
71 with natural lakes estimated to contribute more than 70% to freshwater CH₄ emissions (Sanches
72 et al., 2019). Despite their substantial contribution to atmospheric CH₄, the mechanisms
73 regulating CH₄ emissions from lakes at regional and global scales remain poorly understood
74 (Bastviken et al., 2011; Sanches et al., 2019). In freshwater sediments, CH₄ is abundantly
75 produced via anaerobic methanogenesis by archaea (Bastviken et al., 2004; Bastviken et al.,
76 2011; Conrad, 2020; Dean et al., 2018; Saunio et al., 2020; Tranvik et al., 2009). Methanogens
77 can respire different substrates produced during organic matter remineralization and are
78 classified according to three known pathways for CH₄ production: hydrogenotrophic (carbon
79 dioxide reduction using hydrogen), acetoclastic (splitting acetate), and methylotrophic (using
80 methylated compounds like methanol) (Garcia et al., 2000). Environmental factors such as
81 substrate concentration, temperature, salinity, and pH influence the predominance of these
82 pathways, with methylotrophic methanogenesis, for instance, being favored at higher salinity
83 and acidity (Bueno De Mesquita et al., 2023; Yvon-Durocher et al., 2014).

84 Much of the CH₄ produced in lake sediments is oxidized through both aerobic and
85 anaerobic microbial processes before it can reach the atmosphere (Bastviken et al., 2004;
86 Bastviken et al., 2008; Martinez-Cruz et al., 2017; Martinez-Cruz et al., 2018; Oswald et al.,
87 2016). Aerobic oxidation, predominantly performed by methane-oxidizing bacteria (MOB)
88 from the Gammaproteobacteria and Alphaproteobacteria classes, occurs at the sediment-water
89 interface and in the water column (Hanson and Hanson, 1996; Knief, 2015). All MOB known
90 to date rely on O₂-dependent methane monooxygenase enzymes and oxidize up to 90% of
91 sediment-derived CH₄, thus helping to mitigate greenhouse gas emissions from freshwater
92 ecosystems (Bastviken et al., 2004; Bastviken et al., 2008). Anaerobic oxidation of methane
93 (AOM) is performed by methanotrophic archaea (ANMEs), often in partnership with bacteria
94 that use electron acceptors other than oxygen (Knittel and Boetius, 2009; Milucka et al., 2012;

95 Wegener et al., 2015). In marine environments where sulfate concentrations are high, sulfate-
96 AOM is the dominant process (Jørgensen et al., 2001; Wegener and Boetius, 2009). In contrast,
97 the electron acceptors sulfate, nitrate/nitrite, humic substances, and diverse metal oxides
98 contribute in various degrees to AOM in freshwater sediments (Chen et al., 2023; Deutzmann
99 and Schink, 2011; Martinez-Cruz et al., 2018; Zhao et al., 2024).

100 Recently, it has been proposed that eutrophication induced by anthropogenic nutrient
101 inputs (e.g., nitrates and phosphates) into lake ecosystems influences methanogen and
102 methanotroph community structure by altering organic matter quality and quantity (Beaulieu
103 et al., 2019; Yang et al., 2021; Yang et al., 2019; Yang et al., 2020; Zhu et al., 2022). The influx
104 of organic carbon from phytoplankton blooms enhances organic matter mineralization in lake
105 bottom waters and sediments, depleting electron acceptors such as oxygen (O_2), nitrate (NO_3^-
106), sulfate (SO_4^{2-}), and metal oxides (Fe(III), Mn(IV)). The decomposition of phytoplankton
107 biomass also releases significant amounts of methyl-sulfur compounds, favoring
108 methylophilic CH_4 production (Penger et al., 2012; Tebbe et al., 2023; Tsola et al., 2021; Yan
109 et al., 2017; Zhou et al., 2022). Anaerobic conditions, combined with increased organic matter
110 availability, are expected to boost methanogenesis, resulting in elevated CH_4 release following
111 eutrophication (Fiskal et al., 2019; Sanches et al., 2019; Zhou et al., 2022).

112 Conversely, nutrient addition can stimulate microbial CH_4 oxidation (Yang et al., 2019).
113 Some aerobic MOB can oxidize CH_4 while respiring NO_3^- (denitrification), and a growing body
114 of evidence supports the widespread occurrence and activity of these bacteria in O_2 -limited
115 environments (Almog et al., 2024; Kits et al., 2015; Reis et al., 2024; Schorn et al., 2024).
116 Importantly, MOB exhibit niche partitioning along O_2 - CH_4 and nutrients gradients with
117 Gammaproteobacteria, Alphaproteobacteria, and nitrite-dependent taxa that produce O_2
118 intracellularly, such as *Candidatus Methylophilus*, occupying distinct layers (Mayr et al.,
119 2020; Reis et al., 2020). Gammaproteobacterial MOB are generally associated with fast-
120 growing life strategies in resource-rich conditions, whereas alphaproteobacterial MOB are
121 adapted to resource-limited or stable environments (Ho et al., 2013). Indeed, P and N
122 enrichment, for instance, can increase CH_4 oxidation rates and favor Gammaproteobacterial
123 over Alphaproteobacterial MOB (Nijman et al., 2022; Veraart et al., 2015). Together, these
124 findings highlight that nutrients modulate both CH_4 production and consumption, adding
125 complexity to how eutrophication shapes lacustrine CH_4 dynamics (Nijman et al., 2022; Reis
126 et al., 2020; Veraart et al., 2015; Wei et al., 2022).

127 Eutrophication of lakes in Switzerland reached critical levels during the mid-20th
128 century, particularly in the 1950-1970s, due to rapid industrialization, urbanization, and

129 agricultural intensification. Public outcry and scientific research prompted the introduction of
130 wastewater treatment plants and stricter regulations on phosphate detergents, leading to
131 significant improvements in water quality by the late 20th century. Nevertheless, sediments
132 retain a legacy of this eutrophication in the form of increased organic matter content (Fiskal et
133 al., 2019), which continues to shape microbial community structure (Han, 2020) long after lake
134 waters recovered. Some studies have reported clear vertical zonation of methanogenic and
135 methanotrophic communities in relation to trophic history and electron acceptor distributions
136 (Rissanen et al., 2023; Van Grinsven et al., 2022), while others found little to no stratification
137 (Meier et al., 2024). Consequently, the cascading impacts of anthropogenic nutrient inputs on
138 the balance of CH₄ production and oxidation in lake sediments remain poorly constrained.

139 Here, we test whether historical eutrophication has left a sedimentary legacy that
140 structures contemporary methane-cycling communities in Lake Joux (Vaud, Switzerland), a
141 site with a well-documented history of trophic regime shifts and phytoplankton bloom deposits
142 (Lavrieux et al., 2017; Monchamp et al., 2021). Using a ~400-year, 55-cm sediment archive,
143 we combine porewater and solid-phase geochemistry, 16S rRNA gene amplicon profiling, and
144 stable-carbon-isotope measurements to resolve depth zonation of methanogens and
145 methanotrophs, and link community shifts to organic-matter sources, lithology, and redox
146 conditions. By explicitly coupling the depositional record to present microbial community
147 structure and isotope compositions of different carbon pools, we provide process-level
148 constraints relevant for forecasting benthic CH₄ in eutrophying and recovering lakes.

149

150 **2. Materials and methods**

151 **2.1 Study Area**

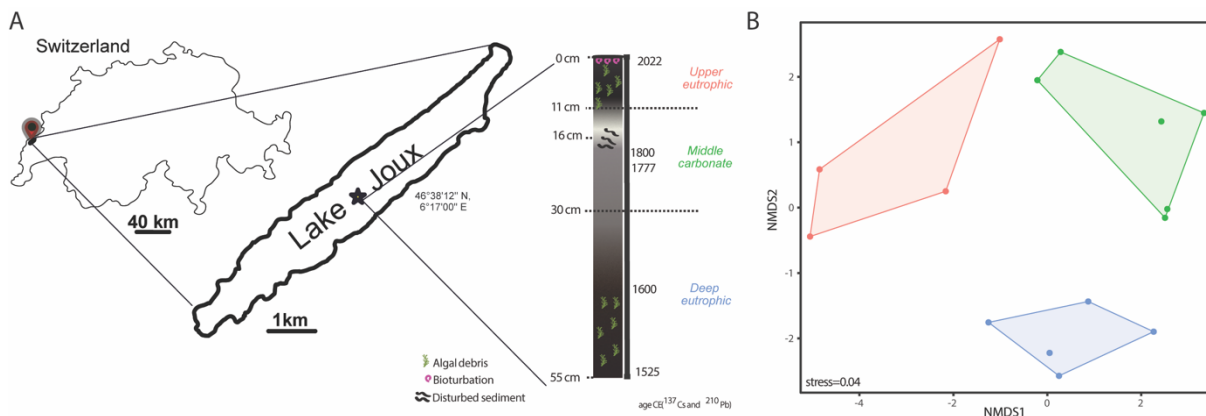
152 Lake Joux is a perialpine lake in the Joux Valley in the Swiss Jura Mountains (Fig. 1A).
153 The valley developed in a Jura syncline, marked by glacial erosion and Quaternary deposits,
154 and it lies mainly on Upper Jurassic and Tertiary limestones. The lake has an average depth of
155 32 m, a surface area of approximately 9 km² (maximum 9 km in length and 1 km in width), and
156 a watershed covering around 211 km². Situated at an altitude of 1,183 meters, the lake is subject
157 to intense seasonal variations and meteorological events, which drive the runoff of both natural
158 and anthropogenic materials.

159 Human activity in the watershed dates back over 6,850 years (Lavrieux et al., 2017;
160 Mitchell et al., 2001; Monchamp et al., 2021). By the 16th century, the area around Lake Joux
161 became more densely populated, leading to land drainage and deforestation for livestock

162 farming (Piguet, 1946). Frequent crop failures and food shortages during the late 17th century
163 spurred the growth of glassmaking and lapidary industries. Horology, introduced in the 18th
164 century, became the region's dominant economic activity by the 19th century. This period of
165 industrialization resulted in a transition from cultivated farmland to pastures and fallow fields
166 (Lavrieux et al., 2017).

167 Agricultural intensification and urban expansion during the 20th century significantly
168 increased nutrient inputs to Lake Joux, resulting in pronounced eutrophication. Phosphorus
169 levels peaked at 35 µg/L in 1979 (Lods-Crozet et al., 2006), triggering major ecological
170 changes, including rapid shifts in phytoplankton communities as eutrophication-adapted taxa
171 outcompeted the lake's original species (Monchamp et al., 2021). A re-oligotrophication phase
172 began in 1988–1989 following improved nutrient management and mitigation efforts.
173 However, despite these reductions in external nutrient loading, the lake has not returned to its
174 pre-eutrophication conditions. More than 70 years after the documented episode of
175 eutrophication, the water column remains altered, suggesting that the system has shifted to an
176 alternative stable state with a biological configuration resistant to reversal (Lods-Crozet et al.,
177 2006; Monchamp et al., 2021).

178



179

180 **Figure 1** – (A) Location of Lake Joux (Switzerland; sampling in 2023) and 55 cm core
181 schematic representation with the three stratigraphic intervals identified from lithology and age
182 markers: deep eutrophic, middle carbonate, and upper eutrophic. (B) Non-metric
183 multidimensional scaling (NMDS) of the porewater and solid-phase geochemical dataset,
184 showing separation of samples by stratigraphic interval (upper eutrophic = red, middle
185 carbonate = green, deep eutrophic = blue). Points are individual depth samples; colored
186 polygons outline the convex hull for each interval and symbols mark group centroids.
187 Ordination was performed on z-scored variables using Bray–Curtis dissimilarities.

188

189 2.2 Sampling

190 In May 2023, three gravity cores (45-55 cm long) were recovered from the lakebed of
191 Lake Joux using a Uwitec gravity corer. The cores were taken from one of the deepest parts of
192 the lake (46°38'12" N, 6°17'00" E; 28 m depth) and sealed with rubber caps. One core was pre-
193 drilled and taped at 3 cm intervals to facilitate rapid CH₄ sampling using cut-off 3 ml syringes
194 on shore. The other two cores, one for porewater and the other for sediment chemistry and
195 microbiome analyses, were processed in the laboratory within 24 h. Porewater was extracted
196 via N₂ flushed syringes attached to 0.2 µm Rhizons (Rhizosphere), inserted every 3 cm along
197 the core, stored at 4°C and analyzed within 48 h. The third sediment core was opened with a
198 handheld saw and sectioned every 3 cm. Each solid sample was split into an acid-cleaned vial
199 and a sterile vial, then frozen at -20°C.

200

201 2.3 Porewater chemistry

202 Porewater samples for dissolved anions (PO₄³⁻, NO₃⁻, NO₂⁻, SO₄²⁻) were transferred to
203 plastic vials while flushing with N₂, capped, and analyzed using an ion chromatograph (DX-
204 ICS-1000, DIONEX) equipped with an AS11-HC column. For dissolved inorganic carbon
205 (DIC) porewater samples were filled into 1.5 ml borosilicate vials and capped headspace-free
206 to prevent CO₂ degassing. DIC concentration (mmol L⁻¹) was obtained from the CO₂ yield after
207 acid conversion of aliquots transferred to helium-flushed Exetainers containing 200 µL of 99%
208 H₃PO₄, and the resulting CO₂ peak areas were quantified on a GasBench II (Thermo Fisher
209 Scientific). A response factor (µmol CO₂ per peak-area unit) was derived from identically
210 processed Carrara Marble (CM) standards and applied to the second CO₂ peak of each sample;
211 moles of CO₂ were converted to DIC using the injected sample volume. External uncertainty
212 on DIC concentration, based on CM reproducibility, was <7%. For δ¹³C_{DIC}, the headspace CO₂
213 was analyzed by GasBench II coupled to a Delta V Plus IRMS; each sample was measured six
214 times, and we report the mean with 1σ (typically <0.10‰). Values were normalized to the in-
215 house CM standard (δ¹³C = +2.05‰) calibrated against NBS-19 and NBS-18; external
216 reproducibility from CM replicates (n = 12) was <0.05‰ (1σ). Carbon isotopes are reported in
217 delta (δ) notation relative to the Vienna Pee Dee Belemnite (VPDB) standard, defined as δ =
218 $((R_{\text{sample}}/R_{\text{standard}}) - 1) \times 1000 \text{ ‰}$, with R the ¹³C/¹²C ratio; positive δ indicates enrichment in
219 ¹³C relative to Vienna Pee Dee Belemnite (VPDB) standard. For dissolved sulfide analysis,
220 porewater was fixed with 0.05 M Zn-acetate (Zn(CH₃COO)₂·2H₂O) solution at a 1:2 ratio
221 immediately after extraction, and dissolved sulfide was quantified photometrically using the
222 methylene blue method (Cline, 1969).

223

224 **2.4 Sediment description and chemistry**

225 Sediment from the opened core was visually assessed (using standard charts) for color
226 and granulometry based on observable differences in particle size, texture, and sorting within
227 the sediment layers. Lithological boundaries in our core were aligned to the dated Lake Joux
228 record of Lavrieux et al. (2017) using their carbonate sediment interval (whiter sediments) as
229 reference. Ages were transferred from their $^{210}\text{Pb}/^{137}\text{Cs}$ model; uncertainties are those reported
230 therein.

231 For total phosphorus (P), ~1 g of wet sediment was digested in 9 ml of 4:2 HNO_3 :HCl
232 using an Anton Paar microwave system, filtered (0.45 μm glass fiber), and analyzed by
233 inductive coupled plasma-optical emission spectrometry (ICP-OES, Agilent 5800). Calibration
234 used a multi-element standard, with certified reference materials yielding 85–102% recovery.

235 Elemental C, N, H, and S were measured on 1–3 mg of freeze-dried sediment using a
236 UNICUBE (Elementar®) at EPFL's ISIC-MSEAP. Total organic carbon (TOC) and total
237 inorganic carbon (TIC) were estimated by loss on ignition (500°C and 1200°C). $\delta^{13}\text{C}_{\text{org}}$ was
238 determined by Elemental Analyzer–Isotope Ratio Mass Spectrometry (EA-Isolink IRMS,
239 Thermo Fisher) after 48 h treatment with 6 N HCl to remove carbonates. Results are reported
240 in delta notation related to VPDB, as described above for $\delta^{13}\text{C}_{\text{DIC}}$, with a reproducibility better
241 than 0.2‰.

242 Acid-volatile sulfur (AVS) and chromium-reducible sulfur (CRS) were extracted from
243 1–2 g of frozen sediment as per Spangenberg and Bosco-Santos (2024). Sulfide in AVS and
244 CRS fractions was measured colorimetrically (Cline, 1969) and CRS sulfur isotopic
245 composition ($\delta^{34}\text{S}_{\text{CRS}}$) by IRMS (Spangenberg and Bosco-Santos, 2024). These measurements
246 help distinguish easily mobilized sulfide pools (AVS) from more stable sulfur forms (CRS) in
247 sediments.

248

249 **2.5 Dissolved oxygen and methane**

250 Oxygen concentrations were measured using a 200 μm -tip glass microsensor
251 (Unisense) after 2-point calibration in Na-dithionite and air-saturated water. Seven vertical
252 profiles from the same core were recorded at 250 μm steps with a motorized controller and
253 Field Multimeter (Unisense).

254 For CH_4 analysis, 3 cm^3 of sediment was transferred into 100 ml serum bottles with 5
255 ml of 10% NaOH, sealed, and homogenized. Dissolved CH_4 was extracted by headspace

256 displacement and quantified via gas chromatography (Joint Analytical Systems) equipped with
257 an FID at the Eawag (Khatun et al., 2024). $\delta^{13}\text{C}_{\text{CH}_4}$ was measured using Gas Chromatography–
258 Combustion–Isotope Ratio Mass Spectrometry (GCC-IRMS, Agilent 6890N with Thermo
259 Finnigan IRMS) and analyzed with IonVantage software (Khatun et al., 2024). Results are
260 reported in delta notation relative to VPDB with an analytical error <1.1‰.

261 Carbon isotopic fractionation factors (α) between organic carbon ($\delta^{13}\text{C}_{\text{org, substrate}}$)
262 and methane ($\delta^{13}\text{C}_{\text{CH}_4, \text{product}}$) were calculated as: $\alpha = (\delta^{13}\text{C}_{\text{org}} + 1000) / (\delta^{13}\text{C}_{\text{CH}_4} + 1000)$. The
263 corresponding isotopic fractionation (ϵ , ‰) was then determined by the relationship $\epsilon = (\alpha -$
264 $1) \times 1000$, allowing interpretation of trends in dominant methanogenic pathways.

265 In order to determine sediment zonation by environmental variables, we performed
266 non-metric multidimensional scaling (NMDS) on a Euclidean distance matrix of z-scored
267 environmental data for samples between 0.5 and 43.5 cm sediment depth, using the function
268 metaMDS() in the R package vegan. The NMDS stress value was 0.04. Differences among
269 depth-defined clusters based on environmental variables were tested using a permutational
270 multivariate analysis of variance (PERMANOVA) on smoothed Euclidean distance matrices.

271

272 **2.6 DNA extraction and 16S rRNA gene amplicon analysis**

273 DNA was extracted from Lake Joux sediments using the PowerSoil Pro Kit (Qiagen).
274 Extraction, sequencing, and raw data processing were conducted at the Joint Microbiome
275 Facility (Medical University of Vienna and University of Vienna; project ID JMF-2310-14).
276 The V4 hypervariable region of the 16S rRNA gene was amplified and sequenced to assess the
277 total microbial diversity in the collected samples. Amplification was performed with linker-
278 modified 515F and 806R (Apprill et al., 2015; Parada et al., 2016) primers, and amplicons were
279 barcoded, multiplexed, sequenced on an Illumina MiSeq (v3 chemistry, 2x 300 bp), and
280 extracted from the raw sequencing data as described in detail in Pjevac et al. (2021). Amplicon
281 Sequence Variants (ASVs) were inferred using the DADA2 R package v1.42 (Callahan et al.,
282 2016b), applying the recommended workflow (Callahan et al., 2016a). FASTQ reads 1 and 2
283 were trimmed at 220 nt and 150 nt with allowed expected errors of 2. ASV sequences were
284 subsequently classified using DADA2 and the SILVA database SSU Ref NR 99 release 138.1
285 (Quast et al., 2012; McLaren and Callahan, 2021) using a confidence threshold of 0.5. ASVs
286 without classification or classified as eukaryotes, mitochondria, or chloroplasts, as well as well-
287 known buffer contaminations, were removed. After filtering, only samples with at least 7000
288 read pairs were kept for further analyses, and relative abundances of ASVs grouped at higher

289 taxonomic levels were calculated in relation to all remaining data. The relative abundance of
290 chloroplast sequences, which were removed from the microbial community dataset, was
291 examined separately to assess phytoplankton debris abundance across the sediment profile.

292 Downstream analyses were performed using R v4.3.2 and Bioconductor v3.16
293 packages SummarizedExperiment v1.32, SingleCellExperiment v1.24,
294 TreeSummarizedExperiment v2.8 (Huang et al., 2021), mia v1.8
295 (<https://github.com/microbiome/mia>), LMDist (Hoops and Knights, 2023), vegan 2.6-8,
296 phyloseq v1.44 (Mcmurdie and Holmes, 2013) (Vegan R package; phyloseq R package),
297 microbiome v1.22 (<http://microbiome.github.io>), microViz v0.10.8 (Barnett et al., 2021), and
298 corrrplot (Wei, 2024). Microbial community alpha diversity indices were calculated on rarified
299 16S rRNA gene amplicon data using R packages vegan and mia. For community dissimilarity
300 analysis, microbial 16S rRNA gene amplicon sequence count data was centered log ratio (CLR)
301 transformed, a pairwise Aitchison distances matrix was computed, and oversaturated distances
302 in the dissimilarity matrix were corrected and smoothed using LMDist with default settings
303 prior to ordination using principal coordinates analysis (PCoA). Differences in 16S rRNA gene
304 amplicon community composition among three depth-defined clusters were tested using
305 permutational multivariate analysis of variance (PERMANOVA) on an Aitchison distance-
306 based dissimilarity matrix.

307 To identify the environmental variables that significantly contributed to the variation in
308 microbial community structure, correlations between microbial community composition and
309 environmental variables were assessed using Mantel tests, based on Euclidean distances
310 calculated from Z-score standardized environmental variables and LMDist corrected and
311 smoothed Aitchison distances of 16S rRNA gene amplicon sequencing data. Prior to
312 correlation analysis, five samples from the deep eutrophic layer without corresponding
313 environmental data were excluded. Mantel tests were performed using Spearman's rank
314 correlation as implemented in the R package vegan. The resulting p-values were adjusted for
315 multiple testing using the false discovery rate (FDR) method. Highly correlated environmental
316 variables (Spearman's $r > 0.8$, Figure S1), as assessed by the function `cor()` in the R package
317 `corrrplot`, were removed before the Mantel tests.

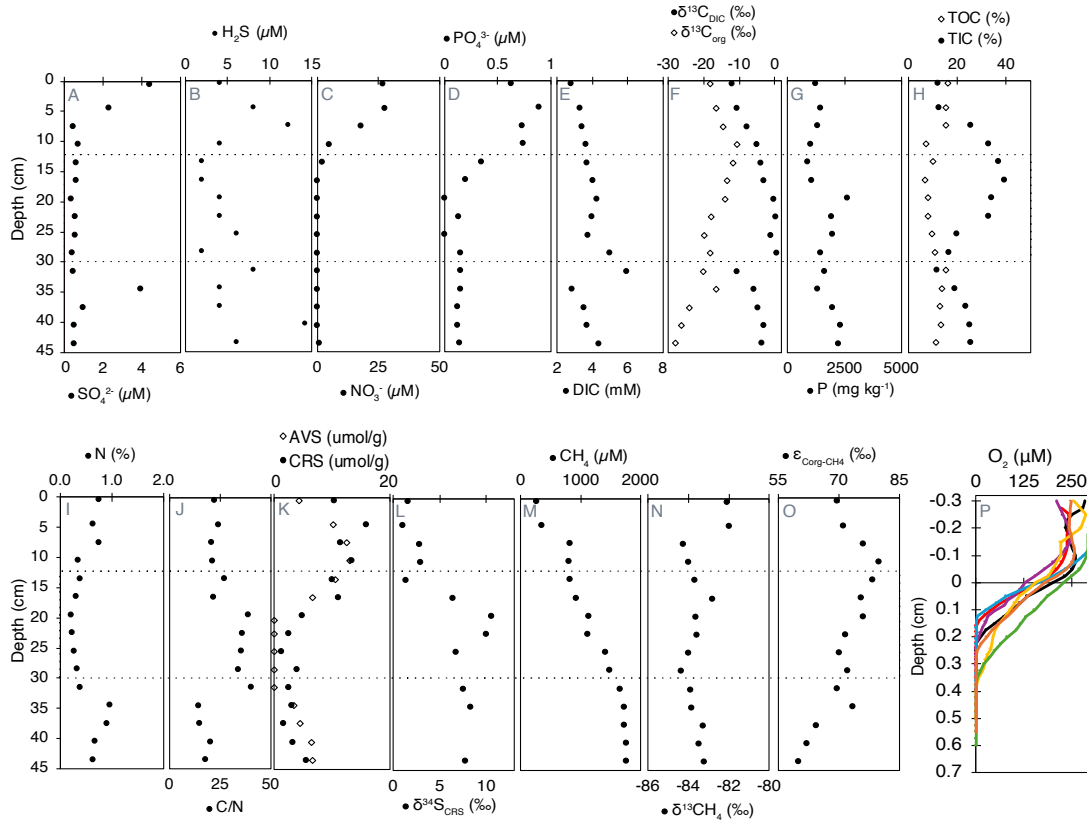
318

319 3. Results

320 3.1 Sediment description

321 The 55-cm deep sediment record of Lake Joux could be classified into three main
322 intervals based on distinct lithological and chemical features (Fig. 1 and Supplementary Fig.
323 1). The 'deep eutrophic' interval, from 55 cm to 30 cm, comprises black and silty sediments,
324 indicating a period of higher lake productivity and low oxygen conditions. Occasional fine sand
325 and organic fibers are also present. In the 'middle carbonate' interval, from 30 cm to 11 cm, the
326 sediments transition from a murky gray with heterogeneous brownish features, suggesting
327 changes in organic matter quality and oxidation states (Fig. 1) to whitish silty-sandy sediments,
328 with the contribution of shells above 13.5 cm, indicating the dominant deposition of carbonates
329 (Fig. 1). The 'upper eutrophic' interval, from 11 cm to 0 cm, contains intensely black sediment
330 with frequent plant debris, reflecting recent environmental changes.

331 To assign approximate ages to our sedimentary profile, we correlated our lithological
332 intervals to other Lake Joux sedimentary sequences previously published and dated using ^{237}Cs
333 and ^{210}Pb (Lavrieux et al., 2017; Magny et al., 2008) (Fig. 1). The middle carbonate unit (30–
334 11 cm) in our core aligns with their U3–U4 carbonate interval, including the distinctive pale
335 “white” boundary at ~16–11 cm. The overlying upper eutrophic black, organic-rich sediments
336 (11–0 cm) correspond to U5, which spans the 20th-century eutrophication phase and includes
337 the ^{137}Cs markers at ~1954 and ~1963 in the Lavrieux record. By transfer of their age–depth
338 model, the base of our deep eutrophic interval (below 30 cm) falls in the late 16th–early 17th
339 century. Reported sedimentation rates in Lavrieux (≈ 0.04 – 0.11 cm yr $^{-1}$ before the 18th century,
340 a short-lived peak ≈ 0.83 cm yr $^{-1}$ in the late 18th century, and ≈ 0.18 cm yr $^{-1}$ over recent decades)
341 are consistent with the thicknesses of our corresponding units (Lavrieux et al., 2017).



342

343 **Figure 2** - Geochemical profiles of porewater, solid-phase compounds, and dissolved gases in
 344 Lake Joux sediments. Dashed lines represent the transitions between the 'deep eutrophic'
 345 interval from 55 to 30.5 cm, the 'middle carbonate' interval from 28.5 to 11 cm, and the 'upper
 346 eutrophic' from 11 to 0 cm. All data are available in Table S1 (panels A to N) and Table S2
 347 (panel O).

348

349 3.2 Porewater chemistry

350 Sulfate (SO_4^{2-} , between 0.35 μM and 4.5 μM) and dissolved sulfide (H_2S , between 2 μM
 351 and 14.3 μM) were measurable throughout the entire sedimentary profile. In the upper
 352 eutrophic interval (0 – 11 cm), opposing gradients of SO_4^{2-} and H_2S from the surface to 7.5
 353 cm are evidence of sulfate reduction (Fig. 2A, B). Below this depth, sulfate is absent, but a
 354 broad H_2S maximum in the deep eutrophic sediments (~40 cm) could be associated with
 355 organic sulfur degradation.

356 Dissolved nitrate (NO_3^-) and phosphate (PO_4^{3-}) first appeared at 19.5 cm and 16.5 cm,
 357 respectively, with concentrations progressively increasing toward the surface, reaching
 358 maximum values of 27 μM for NO_3^- and 0.62 μM for PO_4^{3-} (Fig. 2C and 2D). Nitrite
 359 concentrations were close to the detection limit (0.03 μM) and uniformly low (range 0.038–
 360 0.087 μM , median ≈ 0.043 μM) with no systematic depth trend (Fig. S2).

361 Dissolved inorganic carbon (DIC) concentrations with isotopically heavier composition
362 ($\delta^{13}\text{C}_{\text{DIC}}$) were highest in the middle carbonate interval between 16.5 cm and 31.5 cm (Fig. 2E
363 and F). Above 7.5 cm depth, $\delta^{13}\text{C}_{\text{DIC}}$ values became progressively lighter toward the surface,
364 reaching a minimum of -12‰ (Fig. 2F).

365

366 3.3 Sediment chemistry

367 3.3.1 Phosphorous and organic matter characterization

368 Total phosphorus (P) content, ranging from 860 to 2612 mg kg⁻¹, was generally higher
369 in the deeper sediments and progressively decreased towards the surface, except for a sharp
370 peak at 19.5 cm depth (Fig. 2G). Organic carbon (TOC) exhibited higher concentrations in both
371 the deep eutrophic and upper eutrophic sediments, contrasting with TIC content, which peaked
372 in the middle carbonate interval (Fig. 2H). The $\delta^{13}\text{C}_{\text{org}}$ was lightest in the deep eutrophic
373 sediments (-28.22‰ at 43.5 cm) and heaviest in the upper eutrophic sediments (-10.76‰ at
374 10.5 cm) (Fig. 2F).

375 Nitrogen content followed the same pattern as TOC, with higher N in the deep and
376 upper eutrophic sediments compared to the middle carbonate region (Fig. 2I). The ratio
377 between C and N, a qualitative parameter of organic matter source (Meyers, 1994), exhibited
378 relatively lower values in the deep eutrophic sediments, increasing in the middle carbonate
379 sediments and decreasing again in the upper eutrophic sediments (Fig. 2J).

380

381 3.3.2 Solid-phase sulfides

382 Acid volatile sulfides (AVS) were measurable in the deep eutrophic sediments between
383 43.5 and 34.5 cm and within the upper eutrophic sediments above 19.5 cm depth. The
384 maximum concentrations of AVS in the upper eutrophic sediments (around 418 $\mu\text{g kg}^{-1}$ at 10.5
385 cm) were about twice as high as in deep eutrophic sediments (200 $\mu\text{g kg}^{-1}$ at 40.5 cm) (Fig.
386 2K). Chromium reducible sulfur (CRS) also exhibited higher concentrations in the shallower
387 sediments, becoming more prominent from 16.5 cm depth to the surface. CRS concentrations
388 were more variable than AVS, varying from 23 $\mu\text{g kg}^{-1}$ to 510 $\mu\text{g kg}^{-1}$ (Fig. 2K). The isotopic
389 composition $\delta^{34}\text{S}$ of CRS was positive throughout the profile, ranging from ~1‰ near the
390 surface to a maximum of 10.5‰ at 19.5 cm depth. Values remained elevated in the middle
391 carbonate and deep eutrophic zones (e.g., 8.3‰ at 34.5 cm and 7.7‰ at 43.5 cm), indicating
392 that the reduced sulfur pool is isotopically enriched in ³⁴S across the sediment column (Fig.
393 2L).

394

395 **3.3.3 Dissolved oxygen and methane**

396 Methane (CH₄) concentrations were highest in the deep eutrophic sediments, with a
397 maximum of approximately 1760 μM at 45 cm depth. From 31.5 cm depth, CH₄ exhibited a
398 clear decreasing trend, reaching the lowest concentration (253 μM) at the surface (Fig. 2M).
399 The most significant drop in CH₄ concentrations occurred between 7.5 and 4.5 cm depth, where
400 the concentrations decreased by half (Fig. 2M). The δ¹³C_{CH₄} exhibited minimal variation along
401 the profile, averaging -83.0 ± 0.7‰. The most pronounced isotopic shift (>2.2‰) towards
402 heavier values occurred at the same depth as the sharp decline in CH₄ concentration (Fig. 2N).

403 Fractionation factors (α) between C_{org} and CH₄ ranged from 1.069 to 1.080 across
404 sediment depths, corresponding to carbon isotope fractionations (ε) of 69‰ to 80‰ (Fig. 2O).
405 These values reflect the measurable discrimination between ¹³C and ¹²C during CH₄ production
406 from organic substrates, which arises from the enzymatic pathways and substrates utilized.
407 Lower ε values were consistently observed in deeper sediments compared to shallower layers.

408 Oxygen concentrations were measured across seven different profiles, and free O₂ was
409 detectable only in the uppermost sediments, between 0.165 cm and 0.365 cm depth (Fig. 2P).
410 Below 0.4 cm, sediments were consistently anoxic. The heterogeneous penetration of O₂ into
411 the sediments is attributed to bioturbation, which was confirmed by visual observations of
412 worm castings.

413

414 **3.3.4 Microbial community composition and chloroplast relative sequence abundances**

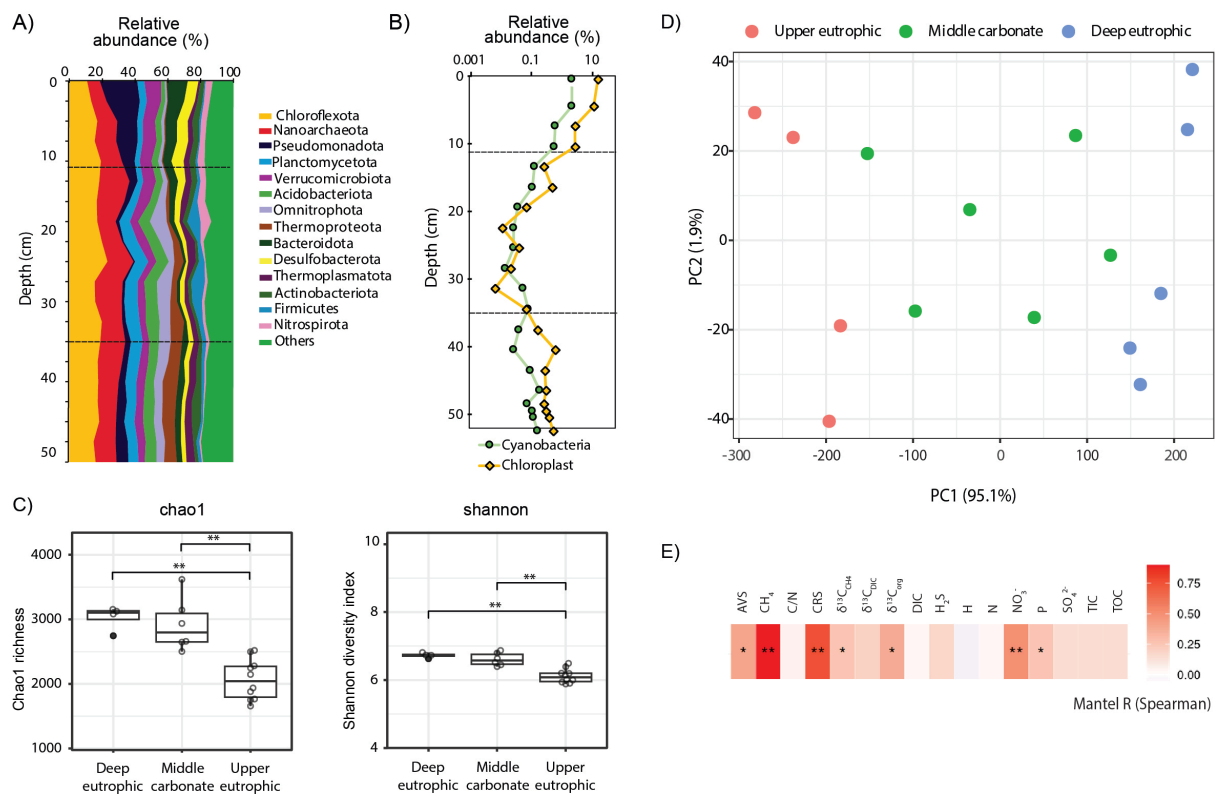
415 The microbial community in Lake Joux sediments was dominated by the phyla
416 Chloroflexota, Nanoarchaeota, and Pseudomonadota (Fig. 3A). In the upper eutrophic
417 sediments (below 30 cm), microbial species richness and evenness (Chao1 and Shannon alpha
418 diversity indices) were significantly lower than in overlying layers (Fig. 3C). In this zone,
419 Nanoarchaeota reached their highest relative sequence abundances (>10%), decreasing to ~7%
420 in the shallower sediments. These elevated abundances, also reported in freshwater (Chen et
421 al., 2023; Xie et al., 2024) and marine environments (Brick et al., 2025), likely reflect their
422 wide environmental tolerance and host associations (Jarett et al., 2018) (Fig. 3A).

423 In the middle carbonate-rich interval (30–11 cm), microbial diversity increased, and
424 Bacteroidota appeared, consistently representing >5% of the microbial community. Reduced
425 relative abundances of chloroplast sequences in this layer (Fig. 3B) also indicate limited input
426 from photosynthetic organisms during this depositional phase. Cyanobacteria-related ASVs

427 displayed similar depth trends to chloroplast sequences, but with lower overall abundance,
428 reaching a maximum of 2% at 4.5 cm depth (Fig. 3B). In the upper eutrophic sediments (11–0
429 cm), Pseudomonadota became more abundant (>20%) and chloroplast sequences markedly
430 increased, reflecting enhanced sedimentation of photosynthetic organisms.

431 Microbial community composition was more similar within sedimentary intervals than
432 between them (Fig. 3D). The separation of samples into three depth clusters based on sediment
433 biogeochemistry was statistically supported (PERMANOVA: $F = 25.05$, $R^2 = 0.81$, $p < 0.001$,
434 Figure 3D). Significant differences in microbial community composition between the three
435 depth clusters were also observed (PERMANOVA: methanogens - $F = 10.57$, $R^2 = 0.64$, $p <$
436 0.001 ; MOB - $F = 13.32$, $R^2 = 0.69$, $p < 0.001$; Cyanobacteria - $F = 6.57$, $R^2 = 0.52$, $p < 0.001$;
437 Chloroplasts - $F = 8.4$, $R^2 = 0.58$, $p < 0.001$; Fig. S4). Stratification was especially pronounced
438 for cyanobacterial and chloroplast sequences, which formed three distinct depth-specific
439 clusters corresponding to the eutrophic, carbonate, and deep eutrophic intervals (Fig. S4). The
440 methanotrophic community separated into two main groups, upper and deep eutrophic, while
441 samples from the carbonate layer did not form a distinct cluster (Fig. S4). Methanogens,
442 however, displayed clearer depth partitioning, with methylotrophic Methanomassiliicoccales
443 dominating in the deep eutrophic interval and hydrogenotrophic Methanobacteriales increasing
444 toward the surface (Fig. 4). Notably, depth patterns in methanogens and methanotrophs, as well
445 as cyanobacterial and chloroplast-related sequences, tracked the same environmental gradients,
446 with CH_4 , NO_3^- , and CRS showing the strongest correlations and AVS, $\delta^{13}\text{C}_{\text{org}}$, and
447 sedimentary P exhibiting secondary correlations (Fig. 3E; Fig. S3).

448



449

450 **Figure 3** - (A) Relative 16S rRNA gene amplicon sequence abundances of bacterial and
 451 archaeal phyla and (B) of cyanobacteria and chloroplasts (algae and plants) in the 55 cm
 452 sedimentary profile of Lake Joux; (C) Alpha diversity (chao1 richness and Shannon diversity)
 453 of methanogens and methanotrophs. (D) Principal component ordination of centered log ratio
 454 (CLR) transformed 16S rRNA gene amplicon data, based on an Aitchison distance for which
 455 oversaturated distances were corrected and smoothed using LMDist. (E) Mantel tests results
 456 (Spearman's rank correlation) of community dissimilarity (corrected and smoothed Aitchison
 457 distance) and environmental parameters (z-scored). P-values were adjusted for multiple testing
 458 using the false discovery rate (FDR) method. ** p <= 0.01; * p <= 0.05.

459

460 3.3.4.1 Methanogenic and methanotrophic microbial communities

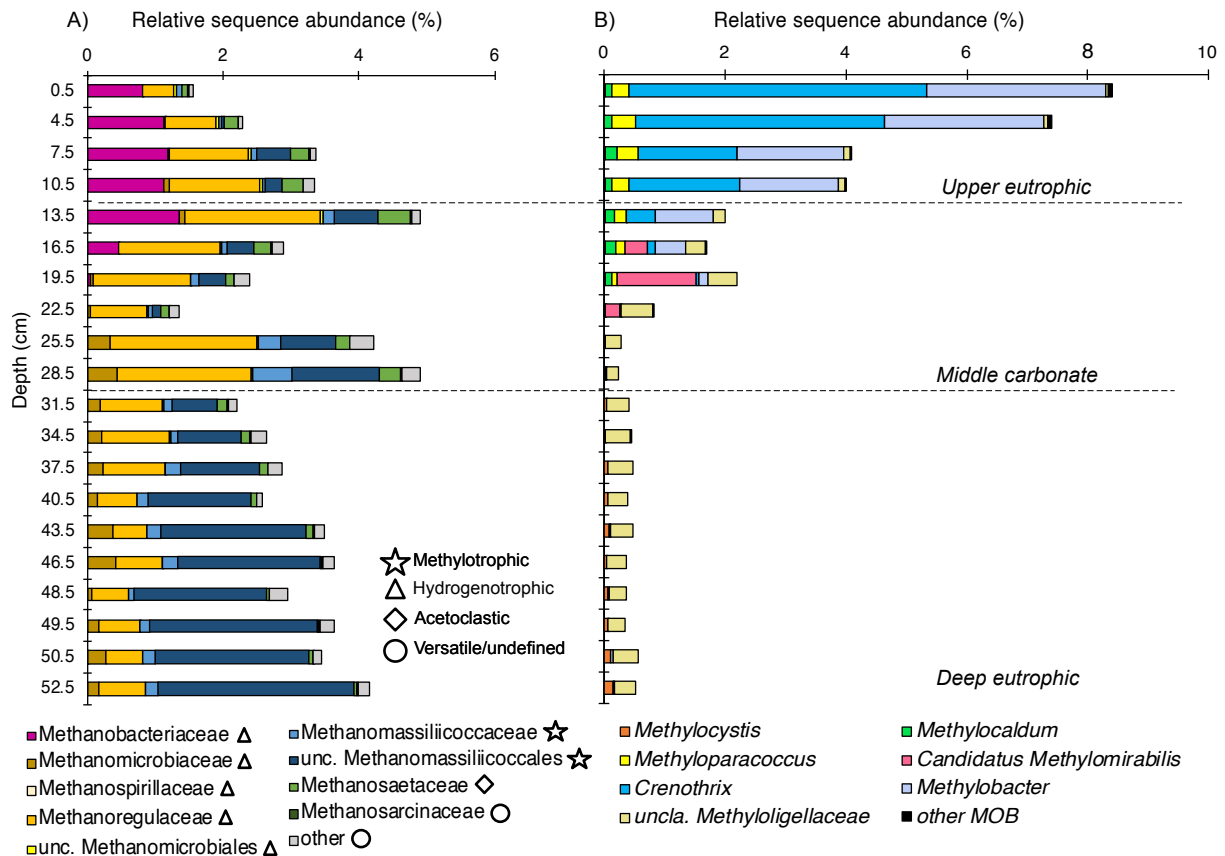
461

462 The relative sequence abundance of methanogens consistently accounted for more than
 463 1% of the microbial community across all sampled depths (Fig. 4A). Methanomassiliicoccales,
 464 which are H₂ dependent methylotrophs, were the dominant methanogenic group in the deep
 465 eutrophic sediments, accounting for 1.4% of the microbial community at a depth of 37.5 cm
 466 (Fig. 4A). In contrast, Methanomicrobiales (hydrogenotrophs) was the most abundant
 467 methanogen group in the middle carbonate interval, (reaching 2.1% of the microbial
 468 community) while Methanobacteriales (hydrogenotrophs) sequences were most abundant in
 469 the upper eutrophic sediments (11–0 cm, reaching 1.18% of the microbial community, Fig.
 470 4A). Sequences affiliated with Methanosarciniales (metabolic versatile) were rare throughout
 471 the profile (<0.01%).

472 Across samples, the relative sequence abundances of *Crenothrix* and *Methylobacter*
473 exhibited significant positive correlations with porewater PO_4^{3-} and NO_3^- (Spearman, $p < 0.05$;
474 Fig. 5). By contrast, methanogen families tracked depth-defined intervals rather than these
475 nutrients (Fig. 4). In the deep and middle sediments below 19.5 cm, Rhizobiales-affiliated
476 methylotrophs (e.g., *Methylocystis*, *Methylocapsa*, *Methyloligellaceae*) (Tamas et al., 2014;
477 Vekeman et al., 2016) were the dominant putative methanotrophs, although they represented a
478 modest portion of the community (max. 0.6%) (Fig. 4B). Anaerobic methanotrophs from
479 ANME archaeal groups could not be identified in the sedimentary profile of Lake Joux. Still,
480 between 23 and 16 cm, *Methylomirabilota* NC10 bacteria capable of nitrite-dependent methane
481 oxidation with intracellularly produced O_2 under anoxic conditions (Ettwig et al., 2010) were
482 detected at a relative abundance of 0.2–1.3%.

483 The 16S rRNA gene sequences of aerobic MOBs represented between 0.3% and 8.7% of
484 the microbial community throughout the sediment profile and were especially numerous (>1%)
485 above a depth of 19.5 cm (Fig. 3C). The most abundant methanotrophs from 19.5 cm depth to
486 the surface were members of the order Methylococcales, with two genera prevalent near the
487 surface: *Crenothrix* and *Methylobacter* (Fig. 4B). At the resolution available with V4-region
488 16S rRNA gene amplicon analyses, limited within-group diversity is detected, as four ASVs
489 affiliated with *Methylobacter*, 17 ASVs with *Crenothrix*, and a small number of ASVs assigned
490 to other Methylococcales genera, including a single abundant *Methylomonas* ASV (Figure S1),
491 were recovered. Within the MOB community, the fraction of the *Methylomonas* ASV increased
492 with depth ($\approx 30\%$ to $\approx 70\%$), whereas the fraction of the three most abundant *Crenothrix* ASVs
493 remained stable. The abundance of all individual MOB ASVs decreased with depth, relative to
494 the total community (Figure S1).

495



496

497

498

499

500

501

502

503

504

4. Discussion

505

4.1 Tracing historical land use, industrialization, and eutrophication

506

507

508

509

510

511

512

513

514

515

The intensely black sediments abundant in chloroplast-related sequences and elevated TOC content with low C:N ratios and light $\delta^{13}\text{C}_{\text{org}}$ in the deep eutrophic interval (55–30 cm, Fig. 1) denote predominantly autochthonous organic matter, derived from phytoplankton blooms (Lamb et al., 2006; Morales-Williams et al., 2017). Similar patterns recorded in other Lake Joux sediment profiles (Dubois, 2016; Lavrieux et al., 2017; Magny et al., 2008) correspond to a period of intensified deforestation and settlement expansion between 1525 and 1790 CE (Dubois, 2016; Lavrieux et al., 2017; Magny et al., 2008). While no official records confirm eutrophication during this period, these anthropogenic activities likely led to increased erosion and sediment/nutrient transport, stimulating phytoplankton productivity (Fig. 1). Notably, these sediments also exhibit high relative H_2S and AVS concentrations (Fig. 2B and 2K), which

516 are characteristic of late sediment diagenesis under eutrophic depositional conditions (Holmer
517 and Storkholm, 2001).

518 The middle carbonate layer (30–11 cm depth) reflects a shift towards more oligotrophic
519 conditions, likely linked to the abandonment of land-intensive activities and the switch to
520 manufacturing in the 18th century. In addition, the 1777 construction of a dike between Lake
521 Joux and Lake Brenet lowered the lake level by 3.6 m, mobilizing limestone-rich sediments
522 (high TIC) and terrestrial plant material with heavier $\delta^{13}\text{C}_{\text{org}}$ and higher C/N ratios (Lavrieux
523 et al., 2017; Magny et al., 2008; Monchamp et al., 2021) (Fig. 2). The sedimentological
524 transition that marks the beginning of this interval at 30 cm depth aligns with a shift from
525 methylophilic to hydrogenophilic methanogens (Fig. 4), likely responsible for the lighter
526 $\delta^{13}\text{C}_{\text{DIC}}$ values (Fig. 2F). Furthermore, the white-colored boundary of the middle carbonate
527 layer (16–11 cm) coincides with warmer post-Little Ice Age conditions, promoting calcium
528 carbonate precipitation and TIC enrichment (Lavrieux et al., 2017).

529 The upper eutrophic sedimentary interval (11–0 cm) consists of black sediments rich in
530 TOC, lighter $\delta^{13}\text{C}_{\text{org}}$ values, low C/N ratios, and abundant chloroplast- and cyanobacteria-
531 related sequences reflecting a well-documented 20th century eutrophication phase (Lavrieux et
532 al., 2017; Magny et al., 2008; Monchamp et al., 2021) (Fig. 2, Fig. 3B). Elevated nutrient levels
533 in this interval could result from external nutrient inputs trapped in porewater or from organic
534 matter remineralization. Porewaters are strongly reducing, reflected by elevated H_2S and CRS,
535 and the absence of O_2 below 0.5 cm. Downward diffusing SO_4^{2-} meets upward-diffusing CH_4 ,
536 and in the 7.5–0 cm horizon, CH_4 concentrations fall sharply while $\delta^{13}\text{C}_{\text{CH}_4}$ becomes heavier
537 and $\delta^{13}\text{C}_{\text{DIC}}$ lighter (Fig. 2A, B and K, M). Aerobic methanotrophy fractionates carbon,
538 preferentially consuming $^{12}\text{CH}_4$ while leaving behind heavier CH_4 (enriched in ^{13}C) and can
539 produce lighter (relatively less ^{13}C) co-localized DIC depending on mixing and available
540 electron acceptors. Together with the dominance of MOB and the absence of ANME-related
541 16S rRNA gene amplicon sequences, the paired isotopic shifts observed indicate
542 methanotrophy dominated by MOB as the main CH_4 sink in these anoxic, nutrient-replete
543 surface sediments.

544

545 **4.2 Methylophilic methanogens selected by past eutrophication**

546 Changes in organic matter sources to Lake Joux over the last four centuries appear
547 closely tied to shifts in dominant methanogenic groups within its sediments. Deep eutrophic
548 sediments, characterized by the highest CH_4 concentrations, are dominated by

549 Methanomassiliicoccales, which are hydrogen-dependent methylotrophic methanogens,
550 meaning that they use H₂ as the electron donor and methylated one-carbon compounds (e.g.,
551 methanol, methylamines, methylated S compounds) as electron acceptors, rather than reducing
552 CO₂. (Bueno De Mesquita et al., 2023; Ellenbogen et al., 2024; Söllinger and Urich, 2019; Sun
553 et al., 2019; Wang and Lee, 1994). The decomposition of algal and cyanobacterial biomass can
554 release methylated sulfur compounds (including DMS and dimethylsulfoxide) and methylated
555 amines, which have stimulated methylotrophic methanogenesis in laboratory experiments and
556 natural environments (Bose et al., 2008; Chistoserdova, 2011; Chistoserdova et al., 2009;
557 Huang et al., 2018; Singh et al., 2005; Tebbe et al., 2023; Whiticar, 1999; Zhou et al., 2022).

558 Indirect evidence for the presence of methylated sulfur compounds comes from
559 relatively higher concentrations of H₂S, AVS, and CRS at depth (Fig. 2B, 2K), indicating active
560 sulfur cycling despite limited SO₄²⁻ availability. Furthermore, δ³⁴S values measured in CRS
561 (primarily pyrite) consistently show positive isotopic signatures (7‰ to 10‰) in both the deep
562 eutrophic and middle carbonate zones. While microbial SO₄²⁻ reduction typically produces ³⁴S-
563 depleted sulfides (δ³⁴S < 0‰) under open-system or moderately sulfate-limited conditions
564 (Bradley et al., 2016; Canfield, 2001; Habicht and Canfield, 1997), the isotopic enrichment
565 observed here is more consistent with either the degradation of sulfurized organic matter or
566 methylated sulfur compounds (Phillips et al., 2022; Raven et al., 2019; Werne et al., 2004).
567 These could simultaneously fuel methylotrophic methanogenesis and pyrite formation. This
568 interpretation warrants confirmation through direct measurements of methylated sulfur species
569 in future studies. Alternatively, the ³⁴S enrichment could reflect near complete consumption of
570 a limited SO₄²⁻ pool so that ³⁴S sulfide reflects positive values of the original sulfate
571 (Bernasconi et al., 2017)(Fig. 2).

572 It is important to note that methylotroph distributions could also be influenced by
573 competition for H₂ with CO₂-reducing hydrogenotrophs. In sulfate-poor anoxic sediments, H₂
574 is typically buffered at low steady-state levels by continuous fermentative supply and rapid
575 consumption—reflecting thermodynamic control rather than chronic scarcity (Conrad, 1999;
576 Schütz et al., 1988; Kessler et al., 2019). Obligately methyl-reducing methanogens have very
577 low H₂ thresholds and are predicted to outcompete hydrogenotrophs for H₂ when methyl groups
578 are available. Thus, their activity is primarily considered to be limited by the availability of
579 methylated substrates (Borrel et al., 2023; Bueno De Mesquita et al., 2023; Feldewert et al.,
580 2020; Söllinger and Urich, 2019; Speth and Orphan, 2018). Given the dominance of
581 *Methanomassiliicoccales* at depth, we infer that methylated-substrate supply rather than H₂
582 limitation is the primary factor structuring the methanogenic community in the deep eutrophic

583 interval. This interpretation is consistent with isotope patterns, as we have recorded
584 comparatively heavier $\delta^{13}\text{C}_{\text{DIC}}$ in the deep eutrophic layer and a shift to lighter $\delta^{13}\text{C}_{\text{DIC}}$ above
585 ~30 cm where the relative abundance of CO_2 -reducing hydrogenotrophic methanogens
586 increased (Fig. 2F).

587 Methylotrophic methanogenesis is typically a minor pathway in freshwater sediments
588 because methylated substrates are scarce (Borrel et al., 2011; Bueno De Mesquita et al., 2023).
589 However, in the deep eutrophic layer, prolonged algal biomass degradation likely generated a
590 reservoir of recalcitrant methylated compounds (Achtnich et al., 1995; Rissanen et al., 2018),
591 favoring methylotrophic methanogens. In contrast, hydrogenotrophic (using $\text{CO}_2 + \text{H}_2$) and
592 acetoclastic (using acetate) methanogens primarily depend on fresh, labile organic matter,
593 which rapidly becomes limited with burial (Achtnich et al., 1995; Meier et al., 2024; Rissanen
594 et al., 2023; Rissanen et al., 2018). Thus, methylotrophs gain a selective advantage in these
595 older, more refractory sediments. Above ~28.5 cm, concurrent with a shift toward more
596 terrestrial organic matter, methylotrophic methanogens decline and hydrogenotrophs
597 progressively dominate (Fig. 4A). We interpret this pattern as consistent with a reduced supply
598 of methylated substrates typically derived from algal organic matter although these compounds
599 were not directly measured.

600 To further support the interpretation of distinct methanogenic pathways, we analyzed
601 the $\epsilon_{\text{C}_{\text{org}}-\text{CH}_4}$, reflecting the isotopic discrimination during CH_4 formation from C_{org} (Fig. 2O).
602 Methanogenesis discriminates against the heavier ^{13}C isotope (Conrad, 2005). In theory, when
603 CH_4 is produced from $\text{CO}_2 + \text{H}_2$ (hydrogenotrophy), microbes selectively withdraw ^{12}C from
604 the DIC pool, leaving residual DIC relatively ^{13}C -enriched (less negative $\delta^{13}\text{C}_{\text{DIC}}$); when
605 methylotrophy dominates, CH_4 carbon is drawn from methyl pools and $\delta^{13}\text{C}_{\text{DIC}}$ is affected less.
606 We observed lower ϵ values in deeper eutrophic sediments compared to shallower zones.
607 Although interpreting specific metabolic pathways from isotopic fractionation is challenging
608 in mixed microbial communities, the contrast in $\epsilon_{\text{C}_{\text{org}}-\text{CH}_4}$ (Fig. 2O) indicates distinct CH_4 -
609 producing processes dominate at different sediment depths.

610 Our results support the view that eutrophication leaves a distinct imprint on methanogen
611 stratification in sediments. In contrast to earlier studies that reported either weak vertical
612 structuring (Meier et al., 2024) or only subtle shifts in methanogen dominance (Rissanen et al.,
613 2023), we found clear zonation with Methanomassiliicoccales prevailing in the deepest
614 eutrophic interval, Methanomicrobiaceae in the carbonate-rich middle section, and
615 Methanobacteriaceae dominating the upper eutrophic sediments. Considered alongside these
616 previous observations in other lakes, our findings question the usefulness of broad

617 generalizations and suggest that methanogen communities are primarily shaped by habitat-
618 specific conditions—such as lithology, organic-matter quality, and redox context—rather than
619 exhibiting universal hydrogenotroph dominance. By comparison, a pronounced vertical
620 structuring of methane-oxidizing bacteria appears more consistent across systems (Mayr et al.,
621 2020; Rissanen et al., 2018; Van Grinsven et al., 2022).

622

623 **4.3 Aerobic methanotrophs are selected by nutrient availability**

624 Within anoxic lacustrine sediments, CH₄ is typically oxidized anaerobically (Borrel et
625 al., 2011; Martinez-Cruz et al., 2018). Interestingly, in the anoxic sediments of Lake Joux,
626 anaerobic methanotrophic archaea are not detectable. Sequences related to *Candidatus*
627 *Methylomirabilis*—capable of intracellular O₂ production to fuel methane monooxygenase
628 activity—occur in notable relative sequence abundances but are confined to 16–23 cm depth
629 within the middle carbonate interval, but are relatively scarce compared to their aerobic
630 counterparts. Namely, gammaproteobacterial MOB 16S rRNA gene sequences recovered from
631 Lake Joux sediments are highly abundant (1–9%) from 19.5 cm upward, despite prevailing
632 anoxic conditions (Fig. 4, 5). The MOB 16S rRNA gene sequences primarily affiliate with
633 Methylococcales, notably the genera *Crenothrix* and *Methylobacter* (Fig. 5). The dominance
634 of Methylococcales-associated MOB in the methane oxidation zone suggests that these taxa
635 serve as the dominant CH₄ sink in these nutrient-replete but anoxic surface sediments (Fig. 2N,
636 Fig. 5). Aerobic (and denitrifying) methanotrophy also fractionates carbon, preferentially
637 consuming ¹²CH₄. This depletes residual CH₄ in ¹²C while generating lighter (more ¹³C) co-
638 localized DIC depending on mixing and available electron acceptors. The paired heavier δ¹³C-
639 CH₄ and lighter δ¹³C-DIC in the 7.5–0 cm horizon thus support the occurrence of active
640 methane oxidation.

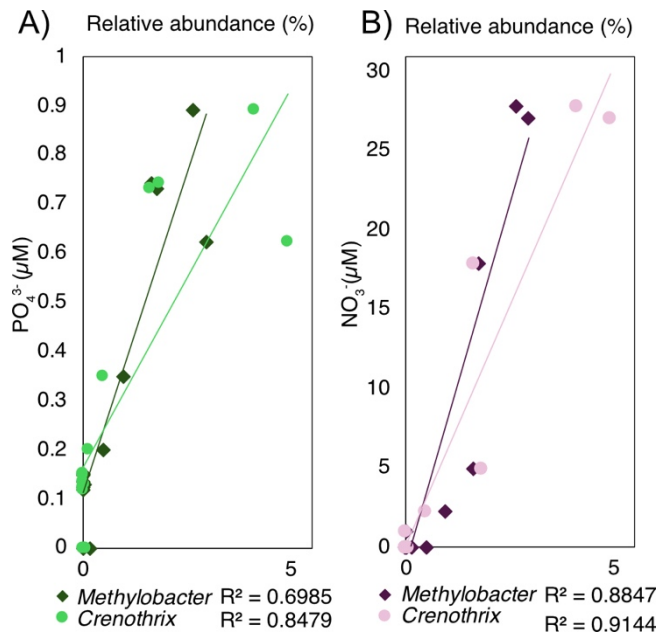
641 How these aerobic methanotrophs meet their O₂ demand below 0.4 cm—where no O₂
642 could be detected, remains unresolved. Nanomolar O₂ concentrations cannot be excluded, but
643 diffusive supply from the sediment–water interface to higher sediment depths is implausible.
644 While some Methylococcales respire alternative electron acceptors (e.g., nitrate, Fe(III)) at low
645 O₂ levels (Li et al., 2023; Van Grinsven et al., 2020; Yang et al., 2025), methane
646 monooxygenase remains O₂-dependent for the oxidation of CH₄ to methanol. Three microbial
647 mechanisms could generate microscale O₂ at depth within the sediment ("dark O₂"):
648 methanobactin-mediated water splitting (Dershwitz et al., 2021), chlorite (ClO₂⁻) dismutation
649 by (per)chlorate-respiring bacteria (Xu and Logan, 2003), and nitric-oxide dismutation as
650 described for NC10 bacteria (Ettwig et al., 2010).

651 Water lysis has been proposed as a potential mechanism for O₂ generation via
652 methanobactin-mediated metal reduction, particularly under metal-rich conditions. (Dershwitz
653 et al., 2021). This process has been primarily associated with Alphaproteobacterial
654 methanotrophs, which are not prevalent in Lake Joux. Chlorite dismutation, catalyzed by
655 chlorite dismutase found in over 60 genera across 13 phyla (Barnum and Coates, 2023), could
656 be a source of O₂, as Pseudomonadota and Actinobacteria are abundant in these sediments (Fig.
657 3A). However, environmental levels of (per)chlorate are likely too low to support this pathway
658 at significant levels (Lv et al., 2019; Miller et al., 2014; Wang et al., 2024).

659 One potential source of *in-situ* O₂ is nitric oxide dismutation catalyzed by the nitric oxide
660 dismutase (NOD) enzyme, which has been recently attributed to multiple bacterial lineages,
661 including several families within the phylum Bacteroidota (Ruff et al., 2024). In Lake Joux,
662 putatively NOD-containing Bacteroidota account for $\sim 0.54 \pm 0.2\%$ of the microbial community
663 in the upper eutrophic sediments, suggesting this pathway may contribute to localized O₂
664 production. However, as NOD is not encoded by all representatives of these taxa, we can not
665 perform further reliable abundance estimates of NOD based on the available 16S rRNA gene
666 amplicon data. The mechanism of O₂ production is nevertheless consistent with our
667 geochemical context: porewater NO₂⁻ remained near detection limit with no subsurface
668 maximum (Fig. S2), indicating rapid NO_x turnover typical of energy-limited sediments.
669 Independent work shows some gammaproteobacterial methanotrophs, including *Crenothrix*
670 and *Methylobacter*, possess genes for respiratory NO₃⁻ reduction (Almog et al., 2024; He et al.,
671 2022; Martinez-Cruz et al., 2017; Milucka et al., 2012; Schorn et al., 2024). Active NO₃⁻
672 respiration has only been demonstrated experimentally for *Methylomonas denitrificans*
673 cultures (Kits et al., 2015) and indirectly by denitrification gene expression by MOB in Lake
674 Zug (Schorn et al., 2024). The latter study revealed that *Crenothrix* and *Methylobacter* related
675 microorganisms continue CH₄ oxidation in hypoxic and anoxic regions of the water column by
676 performing denitrification or fermentation-based methanotrophy (Schorn et al., 2024). In Lake
677 Joux, these same MOB taxa dominate the highly reducing, upper eutrophic sediments (Fig.
678 4B).

679 The positive covariance of *Crenothrix* and *Methylobacter* with NO₃⁻ and PO₄³⁻ is also
680 consistent with nutrient-stimulated MOB activity and/or a shared response to favorable near-
681 surface conditions. While the source of surprisingly high NO₃⁻ concentrations cannot be
682 resolved here, possible mechanisms of NO₃⁻ generation include oxidation of NH₄⁺ by Mn(IV)
683 or Fe(III) oxides. Importantly, the MOB–nutrient correlations may also reflect a shared
684 response to favorable near-surface conditions (e.g., sustained inputs of labile organic matter or

685 higher porosity), rather than direct nutrient control. Nevertheless, it has been experimentally
 686 demonstrated that PO_4^{3-} , NO_3^- , and NH_4^+ additions can directly enhance CH_4 oxidation rates
 687 by MOB and, in particular, *Methylobacter* (Almog et al., 2024; Kits et al., 2015; Nijman et al.,
 688 2022; Xia et al., 2021; Yang et al., 2025). Taken together, these observations suggest that
 689 nutrient availability may play a direct role in shaping the structure and activity of MOB
 690 communities.



691
 692 **Figure 5** - (A) Correlation between PO_4^{3-} concentration and the relative sequence abundance
 693 of *Crenothrix* and *Methylobacter*. (B) Correlation between NO_3^- concentration and the relative
 694 sequence abundance of *Crenothrix* and *Methylobacter*.

695
 696 **5. Conclusion**

697 Our results show that historical eutrophication left a lasting sedimentary legacy that
 698 structures contemporary methane-cycling microbial communities, selecting methylophilic
 699 methanogens. In upper eutrophic, anoxic sediments, the surprisingly high relative sequence
 700 abundances of MOB (up to ~9%) specifically Methylococcales-affiliated
 701 Gammaproteobacteria, co-vary with elevated NO_3^- and PO_4^{3-} concentrations. This suggests that
 702 eutrophication can simultaneously stimulate CH_4 production and enhance its oxidation by
 703 shaping microbial assemblages.

704 As eutrophication continues to alter freshwater systems globally, understanding
 705 nutrient- and substrate-driven shifts in CH_4 -cycling communities becomes increasingly
 706 important. Future studies should focus on elucidating the in situ activity of aerobic
 707 methanotrophs and molecular mechanism of methane oxidation under anoxic conditions, as

708 presumably aerobic MOB have been widely reported in anoxic sediments (Almog et al., 2024;
709 Ruff et al., 2024; Schorn et al., 2024). Combining molecular, isotopic, and geochemical
710 approaches will be essential to better constrain methane fluxes in lakes undergoing or
711 recovering from eutrophication.

712

713 **Data availability**

714 All geochemical data are included in this published article and its supplementary information
715 files. The 16S rRNA gene amplicon sequencing data has been deposited at the Sequence Read
716 Archive under the BioProject accession PRJNA1207472.

717

718 **Author contribution**

719 ABS contributed to conceptualization, data curation, formal analysis, funding acquisition,
720 methodology, supervision, validation, visualization, writing original draft preparation, review,
721 and editing. ERBB contributed to data curation, formal analysis, and manuscript editing; SK
722 contributed to data curation, formal analysis, and manuscript editing; MEM contributed to
723 resources and manuscript editing; JS contributed to data curation, formal analysis,
724 methodology, validation, visualization, designing and implementing computer codes, and
725 writing original draft preparation, review, and editing. PP contributed to conceptualization,
726 formal analysis, funding acquisition, supervision, validation, visualization, writing original
727 draft preparation, review, and editing. JSB contributed to conceptualization, formal analysis,
728 funding acquisition, supervision, validation, visualization, writing original draft preparation,
729 review, and editing.

730

731 **Competing interests**

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

733

734 **Acknowledgments**

735 We would like to thank two anonymous reviewers whose comments helped improve the quality
736 of the manuscript. The authors would like to extend their gratitude to Floreana Marie Miesen
737 (UNIL) for her invaluable support with fieldwork and logistics; to Dr. Carsten Schubert
738 (EAWAG) for providing access to laboratory facilities for methane analyses; to Dr. Giulia
739 Ceriotti (UNIL) for analytical and logistical support with methane analyses; to Laetitia
740 Monbaron and Micaela Faria (UNIL) for their technical assistance in the laboratory; to Jorge

741 Spangenberg (UNIL) for his support in methodology development and analytical assistance; to
742 Frédéric Lardet for assistance with Fig. 1; and to Dr. William Leavitt (The University of Utah)
743 for his help during fieldwork. Special thanks are also due to the Fondation Agassiz for the
744 individual grant awarded to the first author, ABS, which made this study possible. The
745 amplicon sequencing section of this work has been achieved using the Life Science Compute
746 Cluster (LiSC) of the University of Vienna.

747

748 **Financial Support**

749 This study was funded by the 2022 Fondation Agassiz awarded to ABS.

750

751 **References**

752 Achtnich, C., Bak, F., and Conrad, R.: Competition for electron donors among nitrate reducers,
753 ferric iron reducers, sulfate reducers, and methanogens in anoxic paddy soil, *Biology and*
754 *fertility of soils*, 19, 65-72, 1995.

755 Almog, G., Rubin-Blum, M., Murrell, J. C., Vigderovich, H., Eckert, W., Larke-Mejía, N., and
756 Sivan, O.: Survival strategies of aerobic methanotrophs to hypoxia in methanogenic lake
757 sediments, 2024.

758 Apprill, A., McNally, S., Parsons, R., and Weber, L.: Minor revision to V4 region SSU rRNA
759 806R gene primer greatly increases detection of SAR11 bacterioplankton, *Aquatic Microbial*
760 *Ecology*, 75, 129-137, 2015.

761 Barnett, D. J., Arts, I. C., and Penders, J.: microViz: an R package for microbiome data
762 visualization and statistics, *Journal of Open Source Software*, 6, 3201, 2021.

763 Barnum, T. P. and Coates, J. D.: Chlorine redox chemistry is widespread in microbiology, *The*
764 *ISME journal*, 17, 70-83, 2023.

765 Bastviken, D., Cole, J., Pace, M., and Tranvik, L.: Methane emissions from lakes: Dependence
766 of lake characteristics, two regional assessments, and a global estimate, *Global biogeochemical*
767 *cycles*, 18, 2004.

768 Bastviken, D., Cole, J. J., Pace, M. L., and Van de Bogert, M. C.: Fates of methane from
769 different lake habitats: Connecting whole-lake budgets and CH₄ emissions, *Journal of*
770 *Geophysical Research: Biogeosciences*, 113, 2008.

771 Bastviken, D., Tranvik, L. J., Downing, J. A., Crill, P. M., and Enrich-Prast, A.: Freshwater
772 methane emissions offset the continental carbon sink, *Science*, 331, 50-50, 2011.

773 Beaulieu, J. J., DelSontro, T., and Downing, J. A.: Eutrophication will increase methane
774 emissions from lakes and impoundments during the 21st century, *Nature communications*, 10,
775 1375, 2019.

776 Bernasconi, S. M., Meier, I., Wohlwend, S., Brack, P., Hochuli, P. A., Bläsi, H., Wortmann,
777 U. G., and Ramseyer, K.: An evaporite-based high-resolution sulfur isotope record of Late
778 Permian and Triassic seawater sulfate, *Geochimica et Cosmochimica Acta*, 204, 331-349,
779 2017.

780 Borrel, G., Jézéquel, D., Biderre-Petit, C., Morel-Desrosiers, N., Morel, J.-P., Peyret, P., Fonty,
781 G., and Lehours, A.-C.: Production and consumption of methane in freshwater lake
782 ecosystems, *Research in microbiology*, 162, 832-847, 2011.

783 Borrel, G., Fadhlou, K., Ben Hania, W., Gaci, N., Pehau-Arnaudet, G., Chaudhary, P. P.,
784 Vandekerckove, P., Ballet, N., Alric, M., and O'toole, P. W.: *Methanomethylophilus alvi* gen.
785 nov., sp. nov., a Novel Hydrogenotrophic Methyl-Reducing Methanogenic Archaea of the

786 Order Methanomassiliicoccales Isolated from the Human Gut and Proposal of the Novel
787 Family Methanomethylophilaceae fam. nov, *Microorganisms*, 11, 2794, 2023.

788 Bose, A., Pritchett, M. A., and Metcalf, W. W.: Genetic analysis of the methanol-and
789 methylamine-specific methyltransferase 2 genes of *Methanosarcina acetivorans* C2A, *Journal*
790 *of bacteriology*, 190, 4017-4026, 2008.

791 Bradley, A., Leavitt, W., Schmidt, M., Knoll, A. H., Girguis, P. R., and Johnston, D. T.:
792 Patterns of sulfur isotope fractionation during microbial sulfate reduction, *Geobiology*, 14, 91-
793 101, 2016.

794 Brick, S., Niggemann, J., Reckhardt, A., Könneke, M., and Engelen, B.: Interstitial microbial
795 communities of coastal sediments are dominated by Nanoarchaeota, *Frontiers in Microbiology*,
796 16, 1532193, 2025.

797 Bueno de Mesquita, C. P., Wu, D., and Tringe, S. G.: Methyl-based methanogenesis: an
798 ecological and genomic review, *Microbiology and Molecular Biology Reviews*, 87, e00024-
799 00022, 2023.

800 Callahan, B. J., Sankaran, K., Fukuyama, J. A., McMurdie, P. J., and Holmes, S. P.:
801 Bioconductor workflow for microbiome data analysis: from raw reads to community analyses,
802 *F1000Research*, 5, 2016a.

803 Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., and Holmes, S.
804 P.: DADA2: High-resolution sample inference from Illumina amplicon data, *Nature methods*,
805 13, 581-583, 2016b.

806 Canfield, D. E.: Isotope fractionation by natural populations of sulfate-reducing bacteria,
807 *Geochimica et Cosmochimica Acta*, 65, 1117-1124, 2001.

808 Chen, M., Conroy, J. L., Sanford, R. A., Wyman-Feravich, D. A., Chee-Sanford, J. C., and
809 Connor, L. M.: Tropical lacustrine sediment microbial community response to an extreme El
810 Niño event, *Scientific reports*, 13, 6868, 2023.

811 Chistoserdova, L.: Modularity of methylotrophy, revisited, *Environmental microbiology*, 13,
812 2603-2622, 2011.

813 Chistoserdova, L., Kalyuzhnaya, M. G., and Lidstrom, M. E.: The expanding world of
814 methylotrophic metabolism, *Annual review of microbiology*, 63, 477-499, 2009.

815 Cline, J. D.: Spectrophotometric determination of hydrogen sulfide in natural waters,
816 *Limnology and Oceanography*, 14, 454-458, 1969.

817 Conrad, R.: Contribution of hydrogen to methane production and control of hydrogen
818 concentrations in methanogenic soils and sediments, *FEMS microbiology Ecology*, 28, 193-
819 202, 1999.

820 Conrad, R.: Quantification of methanogenic pathways using stable carbon isotopic signatures:
821 a review and a proposal, *Organic geochemistry*, 36, 739-752, 2005.

822 Conrad, R.: Importance of hydrogenotrophic, acetoclastic and methylotrophic methanogenesis
823 for methane production in terrestrial, aquatic and other anoxic environments: a mini review,
824 *Pedosphere*, 30, 25-39, 2020.

825 Dean, J. F., Middelburg, J. J., Röckmann, T., Aerts, R., Blauw, L. G., Egger, M., Jetten, M. S.,
826 de Jong, A. E., Meisel, O. H., and Rasigraf, O.: Methane feedbacks to the global climate system
827 in a warmer world, *Reviews of Geophysics*, 56, 207-250, 2018.

828 Dershwitz, P., Bandow, N. L., Yang, J., Semrau, J. D., McEllistrem, M. T., Heinze, R. A.,
829 Fonseca, M., Ledesma, J. C., Jennett, J. R., and DiSpirito, A. M.: Oxygen generation via water
830 splitting by a novel biogenic metal ion-binding compound, *Applied and Environmental*
831 *Microbiology*, 87, e00286-00221, 2021.

832 Deutzmann, J. S. and Schink, B.: Anaerobic oxidation of methane in sediments of Lake
833 Constance, an oligotrophic freshwater lake, *Applied and environmental microbiology*, 77,
834 4429-4436, 2011.

835 Dubois, N.: Traces of history in the sediments of Lake Joux, 2016.

836 Ellenbogen, J. B., Borton, M. A., McGivern, B. B., Cronin, D. R., Hoyt, D. W., Freire-Zapata,
837 V., McCalley, C. K., Varner, R. K., Crill, P. M., and Wehr, R. A.: Methylo-trophy in the Mire:
838 direct and indirect routes for methane production in thawing permafrost, *Msystems*, 9, e00698-
839 00623, 2024.

840 Ettwig, K. F., Butler, M. K., Le Paslier, D., Pelletier, E., Mangenot, S., Kuypers, M. M.,
841 Schreiber, F., Dutilh, B. E., Zedelius, J., and de Beer, D.: Nitrite-driven anaerobic methane
842 oxidation by oxygenic bacteria, *Nature*, 464, 543-548, 2010.

843 Feldewert, C., Lang, K., and Brune, A.: The hydrogen threshold of obligately methyl-reducing
844 methanogens, *FEMS Microbiology Letters*, 367, fnaa137, 2020.

845 Fiskal, A., Deng, L., Michel, A., Eickenbusch, P., Han, X., Lagostina, L., Zhu, R., Sander, M.,
846 Schroth, M. H., and Bernasconi, S. M.: Effects of eutrophication on sedimentary organic
847 carbon cycling in five temperate lakes, *Biogeosciences*, 16, 3725-3746, 2019.

848 Garcia, J.-L., Patel, B. K., and Ollivier, B.: Taxonomic, phylogenetic, and ecological diversity
849 of methanogenic Archaea, *Anaerobe*, 6, 205-226, 2000.

850 Habicht, K. S. and Canfield, D. E.: Sulfur isotope fractionation during bacterial sulfate
851 reduction in organic-rich sediments, *Geochimica et Cosmochimica Acta*, 61, 5351-5361, 1997.

852 Han, X.: Influence of eutrophication on microbial community structure, organic carbon
853 sources, and organic carbon degradation in lake sediments through time, ETH Zurich, 2020.

854 Hanson, R. S. and Hanson, T. E.: Methanotrophic bacteria, *Microbiological reviews*, 60, 439-
855 471, 1996.

856 He, R., Wang, J., Pohlman, J. W., Jia, Z., Chu, Y.-X., Wooller, M. J., and Leigh, M. B.:
857 Metabolic flexibility of aerobic methanotrophs under anoxic conditions in Arctic lake
858 sediments, *The ISME Journal*, 16, 78-90, 2022.

859 Ho, A., Kerckhof, F. M., Luke, C., Reim, A., Krause, S., Boon, N., and Bodelier, P. L.:
860 Conceptualizing functional traits and ecological characteristics of methane-oxidizing bacteria
861 as life strategies, *Environmental Microbiology Reports*, 5, 335-345, 2013.

862 Holmer, M. and Storkholm, P.: Sulphate reduction and sulphur cycling in lake sediments: a
863 review, *Freshwater Biology*, 46, 431-451, 2001.

864 Hoops, S. L. and Knights, D.: LMdist: Local Manifold distance accurately measures beta
865 diversity in ecological gradients, *Bioinformatics*, 39, btad727, 2023.

866 Huang, H., Xu, X., Shi, C., Liu, X., and Wang, G.: Response of taste and odor compounds to
867 elevated cyanobacteria biomass and temperature, *Bulletin of environmental contamination and
868 toxicology*, 101, 272-278, 2018.

869 Huang, R., Sonesson, C., Ernst, F. G., Rue-Albrecht, K. C., Yu, G., Hicks, S. C., and Robinson,
870 M. D.: TreeSummarizedExperiment: a S4 class for data with hierarchical structure,
871 *F1000Research*, 9, 1246, 2021.

872 Jarett, J. K., Nayfach, S., Podar, M., Inskeep, W., Ivanova, N. N., Munson-McGee, J., Schulz,
873 F., Young, M., Jay, Z. J., and Beam, J. P.: Single-cell genomics of co-sorted Nanoarchaeota
874 suggests novel putative host associations and diversification of proteins involved in symbiosis,
875 *Microbiome*, 6, 1-14, 2018.

876 Jørgensen, B. B., Weber, A., and Zopfi, J.: Sulfate reduction and anaerobic methane oxidation
877 in Black Sea sediments, *Deep Sea Research Part I: Oceanographic Research Papers*, 48, 2097-
878 2120, 2001.

879 Kessler, A. J., Chen, Y.-J., Waite, D. W., Hutchinson, T., Koh, S., Popa, M. E., Beardall, J.,
880 Hugenholtz, P., Cook, P. L., and Greening, C.: Bacterial fermentation and respiration processes
881 are uncoupled in anoxic permeable sediments, *Nature microbiology*, 4, 1014-1023, 2019.

882 Khatun, S., Berg, J. S., Jézéquel, D., Moiron, M., Escoffier, N., Schubert, C. J., Bouffard, D.,
883 and Perga, M. E.: Long-range transport of littoral methane explains the metalimnetic methane
884 peak in a large lake, *Limnology and Oceanography*, 69, 2095-2108, 2024.

885 Kits, K. D., Klotz, M. G., and Stein, L. Y.: Methane oxidation coupled to nitrate reduction
886 under hypoxia by the Gammaproteobacterium *Methylomonas denitrificans*, sp. nov. type strain
887 FJG1, Environmental microbiology, 17, 3219-3232, 2015.

888 Knief, C.: Diversity and habitat preferences of cultivated and uncultivated aerobic
889 methanotrophic bacteria evaluated based on *pmoA* as molecular marker, Frontiers in
890 microbiology, 6, 1346, 2015.

891 Knittel, K. and Boetius, A.: Anaerobic oxidation of methane: progress with an unknown
892 process, Annual review of microbiology, 63, 311-334, 2009.

893 Lamb, A. L., Wilson, G. P., and Leng, M. J.: A review of coastal palaeoclimate and relative
894 sea-level reconstructions using $\delta^{13}\text{C}$ and C/N ratios in organic material, Earth-Science
895 Reviews, 75, 29-57, 2006.

896 Lavrieux, M., Schubert, C. J., Hofstetter, T., Eglinton, T. I., Hajdas, I., Wacker, L., and Dubois,
897 N.: From medieval land clearing to industrial development: 800 years of human-impact history
898 in the Joux Valley (Swiss Jura), The Holocene, 27, 1443-1454, 2017.

899 Li, B., Tao, Y., Mao, Z., Gu, Q., Han, Y., Hu, B., Wang, H., Lai, A., Xing, P., and Wu, Q. L.:
900 Iron oxides act as an alternative electron acceptor for aerobic methanotrophs in anoxic lake
901 sediments, Water Research, 234, 119833, 2023.

902 Lods-Crozet, B., Reymond, O., and Strawczynski, A.: Evaluation de la qualité chimique et
903 biologique du lac de Joux (Jura Suisse) entre 1985 et 2004, Bull Soc Ne Sci Nat, 129, 29-47,
904 2006.

905 Lv, P.-L., Shi, L.-D., Wang, Z., Rittmann, B., and Zhao, H.-P.: Methane oxidation coupled to
906 perchlorate reduction in a membrane biofilm batch reactor, Science of the Total Environment,
907 667, 9-15, 2019.

908 Magny, M., Gauthier, E., Vanni re, B., and Peyron, O.: Palaeohydrological changes and
909 human-impact history over the last millennium recorded at Lake Joux in the Jura Mountains,
910 Switzerland, The Holocene, 18, 255-265, 2008.

911 Martinez-Cruz, K., Sepulveda-Jauregui, A., Casper, P., Anthony, K. W., Smemo, K. A., and
912 Thalasso, F.: Ubiquitous and significant anaerobic oxidation of methane in freshwater lake
913 sediments, Water Research, 144, 332-340, 2018.

914 Martinez-Cruz, K., Leewis, M.-C., Herriott, I. C., Sepulveda-Jauregui, A., Anthony, K. W.,
915 Thalasso, F., and Leigh, M. B.: Anaerobic oxidation of methane by aerobic methanotrophs in
916 sub-Arctic lake sediments, Science of the Total Environment, 607, 23-31, 2017.

917 Mayr, M. J., Zimmermann, M., Guggenheim, C., Brand, A., and B rgmann, H.: Niche
918 partitioning of methane-oxidizing bacteria along the oxygen–methane counter gradient of
919 stratified lakes, The ISME journal, 14, 274-287, 2020.

920 McLaren, M. R. and Callahan, B. J.: Silva 138.1 prokaryotic SSU taxonomic training data
921 formatted for DADA2, Zenodo, 2021.

922 McMurdie, P. J. and Holmes, S.: phyloseq: an R package for reproducible interactive analysis
923 and graphics of microbiome census data, PloS one, 8, e61217, 2013.

924 Meier, D., van Grinsven, S., Michel, A., Eickenbusch, P., Glombitza, C., Han, X., Fiskal, A.,
925 Bernasconi, S., Schubert, C. J., and Lever, M. A.: Hydrogen-independent CO₂ reduction
926 dominates methanogenesis in five temperate lakes that differ in trophic states, ISME
927 Communications, ycae089, 2024.

928 Meyers, P. A.: Preservation of elemental and isotopic source identification of sedimentary
929 organic matter, Chemical geology, 114, 289-302, 1994.

930 Miller, L. G., Baesman, S. M., Carlstr m, C. I., Coates, J. D., and Oremland, R. S.: Methane
931 oxidation linked to chlorite dismutation, Frontiers in Microbiology, 5, 275, 2014.

932 Milucka, J., Ferdelman, T. G., Polerecky, L., Franzke, D., Wegener, G., Schmid, M.,
933 Lieberwirth, I., Wagner, M., Widdel, F., and Kuypers, M. M.: Zero-valent sulphur is a key
934 intermediate in marine methane oxidation, Nature, 491, 541-546, 2012.

935 Mitchell, E., van der Knaap, W. O., van Leeuwen, J. F., Buttler, A., Warner, B. G., and Gobat,
936 J.-M.: The palaeoecological history of the Praz-Rodet bog (Swiss Jura) based on pollen, plant
937 macrofossils and testate amoebae (Protozoa), *The Holocene*, 11, 65-80, 2001.

938 Monchamp, M.-È., Bruel, R., Frossard, V., McGowan, S., Lavrieux, M., Muschick, M., Perga,
939 M.-É., and Dubois, N.: Paleocological evidence for a multi-trophic regime shift in a perialpine
940 lake (Lake Joux, Switzerland), *Anthropocene*, 35, 100301, 2021.

941 Morales-Williams, A. M., Wanamaker Jr, A. D., and Downing, J. A.: Cyanobacterial carbon
942 concentrating mechanisms facilitate sustained CO₂ depletion in eutrophic lakes,
943 *Biogeosciences*, 14, 2865-2875, 2017.

944 Nijman, T. P., Amado, A. M., Bodelier, P. L., and Veraart, A. J.: Relief of phosphate limitation
945 stimulates methane oxidation, *Frontiers in Environmental Science*, 10, 804512, 2022.

946 Oswald, K., Milucka, J., Brand, A., Hach, P., Littmann, S., Wehrli, B., Kuypers, M. M., and
947 Schubert, C. J.: Aerobic gammaproteobacterial methanotrophs mitigate methane emissions
948 from oxic and anoxic lake waters, *Limnology and Oceanography*, 61, S101-S118, 2016.

949 Parada, A. E., Needham, D. M., and Fuhrman, J. A.: Every base matters: assessing small
950 subunit rRNA primers for marine microbiomes with mock communities, time series and global
951 field samples, *Environmental microbiology*, 18, 1403-1414, 2016.

952 Penger, J., Conrad, R., and Blaser, M.: Stable carbon isotope fractionation by methylotrophic
953 methanogenic archaea, *Applied and environmental microbiology*, 78, 7596-7602, 2012.

954 Phillips, A. A., White, M. E., Seidel, M., Wu, F., Pavia, F. F., Kemeny, P. C., Ma, A. C.,
955 Aluwihare, L. I., Dittmar, T., and Sessions, A. L.: Novel sulfur isotope analyses constrain
956 sulfurized porewater fluxes as a minor component of marine dissolved organic matter,
957 *Proceedings of the National Academy of Sciences*, 119, e2209152119, 2022.

958 Piguet, A.: *Le territoire et la commune du Lieu jusqu'en 1536*, 1946.

959 Pjevac, P., Hausmann, B., Schwarz, J., Kohl, G., Herbold, C. W., Loy, A., and Berry, D.: An
960 economical and flexible dual barcoding, two-step PCR approach for highly multiplexed
961 amplicon sequencing, *Frontiers in microbiology*, 12, 669776, 2021.

962 Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., and Glöckner,
963 F. O.: The SILVA ribosomal RNA gene database project: improved data processing and web-
964 based tools, *Nucleic acids research*, 41, D590-D596, 2012.

965 Raven, M., Fike, D., Gomes, M., and Webb, S.: Chemical and isotopic evidence for organic
966 matter sulfurization in redox gradients around mangrove roots, *Frontiers in Earth Science*, 7,
967 98, 2019.

968 Reis, P. C., Thottathil, S. D., Ruiz-González, C., and Prairie, Y. T.: Niche separation within
969 aerobic methanotrophic bacteria across lakes and its link to methane oxidation rates,
970 *Environmental microbiology*, 22, 738-751, 2020.

971 Reis, P. C., Tsuji, J. M., Weiblen, C., Schiff, S. L., Scott, M., Stein, L. Y., and Neufeld, J. D.:
972 Enigmatic persistence of aerobic methanotrophs in oxygen-limiting freshwater habitats, *The*
973 *ISME Journal*, 18, wrac041, 2024.

974 Rissanen, A. J., Saarenheimo, J., Tirola, M., Peura, S., Aalto, S. L., Karvinen, A., and
975 Nykänen, H.: Gammaproteobacterial methanotrophs dominate methanotrophy in aerobic and
976 anaerobic layers of boreal lake waters, *Aquatic Microbial Ecology*, 81, 257-276, 2018.

977 Rissanen, A. J., Jilbert, T., Simojoki, A., Mangayil, R., Aalto, S. L., Khanongnuch, R., Peura,
978 S., and Jäntti, H.: Organic matter lability modifies the vertical structure of methane-related
979 microbial communities in lake sediments, *Microbiology spectrum*, 11, e01955-01923, 2023.

980 Ruff, S. E., Schwab, L., Vidal, E., Hemingway, J. D., Kraft, B., and Murali, R.: Widespread
981 occurrence of dissolved oxygen anomalies, aerobic microbes, and oxygen-producing metabolic
982 pathways in apparently anoxic environments, *FEMS Microbiology Ecology*, 100, fae132,
983 2024.

984 Sanches, L. F., Guenet, B., Marinho, C. C., Barros, N., and de Assis Esteves, F.: Global
985 regulation of methane emission from natural lakes, *Scientific Reports*, 9, 255, 2019.

986 Saunio, M., Stavert, A., Poulter, B., Bousquet, P., Canadell, J., Jackson, R., Raymond, P.,
987 Dlugokencky, E., Houweling, S., and Patra, P.: The Global Methane Budget 2000–2017, *Earth*
988 *Syst. Sci. Data*, 12, 1561–1623, 2020.

989 Schorn, S., Graf, J. S., Littmann, S., Hach, P. F., Lavik, G., Speth, D. R., Schubert, C. J.,
990 Kuypers, M. M., and Milucka, J.: Persistent activity of aerobic methane-oxidizing bacteria in
991 anoxic lake waters due to metabolic versatility, *Nature Communications*, 15, 5293, 2024.

992 Schütz, H., Conrad, R., Goodwin, S., and Seiler, W.: Emission of hydrogen from deep and
993 shallow freshwater environments, *Biogeochemistry*, 5, 295–311, 1988.

994 Singh, N., Kendall, M. M., Liu, Y., and Boone, D. R.: Isolation and characterization of
995 methylotrophic methanogens from anoxic marine sediments in Skan Bay, Alaska: description
996 of *Methanococcoides alaskense* sp. nov., and emended description of *Methanosarcina baltica*,
997 *International journal of systematic and evolutionary microbiology*, 55, 2531–2538, 2005.

998 Söllinger, A. and Urich, T.: Methylotrophic methanogens everywhere—physiology and
999 ecology of novel players in global methane cycling, *Biochemical Society Transactions*, 47,
1000 1895–1907, 2019.

1001 Spangenberg, J. E. and Bosco-Santos, A.: Sulfur isotope analyses using $3\times$ elemental
1002 analysis/isotope ratio mass spectrometry: Saving helium and energy while reducing analytical
1003 time and costs, *Rapid Communications in Mass Spectrometry*, 38, e9866, 2024.

1004 Speth, D. R. and Orphan, V. J.: Metabolic marker gene mining provides insight in global *mcrA*
1005 diversity and, coupled with targeted genome reconstruction, sheds further light on metabolic
1006 potential of the *Methanomassiliicoccales*, *PeerJ*, 6, e5614, 2018.

1007 Sun, J., Mausz, M. A., Chen, Y., and Giovannoni, S. J.: Microbial trimethylamine metabolism
1008 in marine environments, *Environmental Microbiology*, 21, 513–520, 2019.

1009 Tamas, I., Smirnova, A. V., He, Z., and Dunfield, P. F.: The (d) evolution of methanotrophy in
1010 the Beijerinckiaceae—a comparative genomics analysis, *The ISME journal*, 8, 369–382, 2014.

1011 Tebbe, D. A., Gruender, C., Dlugosch, L., Löhmus, K., Rolfes, S., Könneke, M., Chen, Y.,
1012 Engelen, B., and Schäfer, H.: Microbial drivers of DMSO reduction and DMS-dependent
1013 methanogenesis in saltmarsh sediments, *The ISME Journal*, 17, 2340–2351, 2023.

1014 Tranvik, L. J., Downing, J. A., Cotner, J. B., Loiselle, S. A., Striegl, R. G., Ballatore, T. J.,
1015 Dillon, P., Finlay, K., Fortino, K., and Knoll, L. B.: Lakes and reservoirs as regulators of carbon
1016 cycling and climate, *Limnology and oceanography*, 54, 2298–2314, 2009.

1017 Tsola, S. L., Zhu, Y., Ghurnee, O., Economou, C. K., Trimmer, M., and Eyice, Ö.: Diversity
1018 of dimethylsulfide-degrading methanogens and sulfate-reducing bacteria in anoxic sediments
1019 along the Medway Estuary, UK, *Environmental Microbiology*, 23, 4434–4449, 2021.

1020 van Grinsven, S., Meier, D. V., Michel, A., Han, X., Schubert, C. J., and Lever, M. A.: Redox
1021 zone and trophic state as drivers of methane-oxidizing bacterial abundance and community
1022 structure in lake sediments, *Frontiers in Environmental Science*, 10, 857358, 2022.

1023 van Grinsven, S., Sinninghe Damsté, J. S., Abdala Asbun, A., Engelmann, J. C., Harrison, J.,
1024 and Villanueva, L.: Methane oxidation in anoxic lake water stimulated by nitrate and sulfate
1025 addition, *Environmental microbiology*, 22, 766–782, 2020.

1026 Vekeman, B., Kerckhof, F. M., Cremers, G., De Vos, P., Vandamme, P., Boon, N., Op den
1027 Camp, H. J., and Heylen, K.: New *Methyloceanibacter* diversity from North Sea sediments
1028 includes methanotroph containing solely the soluble methane monooxygenase, *Environmental*
1029 *microbiology*, 18, 4523–4536, 2016.

1030 Veraart, A. J., Steenbergh, A. K., Ho, A., Kim, S. Y., and Bodelier, P. L.: Beyond nitrogen: the
1031 importance of phosphorus for CH_4 oxidation in soils and sediments, *Geoderma*, 259, 337–346,
1032 2015.

1033 Wang, X.-C. and Lee, C.: Sources and distribution of aliphatic amines in salt marsh sediment,
1034 *Organic Geochemistry*, 22, 1005-1021, 1994.

1035 Wang, Y., Liu, X., Wu, M., and Guo, J.: Methane-Driven Perchlorate Reduction by a Microbial
1036 Consortium, *Environmental Science & Technology*, 58, 13370-13379, 2024.

1037 Wegener, G. and Boetius, A.: An experimental study on short-term changes in the anaerobic
1038 oxidation of methane in response to varying methane and sulfate fluxes, *Biogeosciences*, 6,
1039 867-876, 2009.

1040 Wegener, G., Krukenberg, V., Riedel, D., Tegetmeyer, H. E., and Boetius, A.: Intercellular
1041 wiring enables electron transfer between methanotrophic archaea and bacteria, *Nature*, 526,
1042 587-590, 2015.

1043 Wei, H., Wang, M., Ya, M., and Xu, C.: The denitrifying anaerobic methane oxidation process
1044 and microorganisms in the environments: a review, *Frontiers in Marine Science*, 9, 1038400,
1045 2022.

1046 Wei, T.: i Simko V.(2021). R package'corrplot': Visualization of a Correlation Matrix.(Version
1047 0.92), Available at< Available at <https://github.com/taiyun/corrplot>>. Accessed on March, 20,
1048 2024.

1049 Werne, J. P., Hollander, D. J., Lyons, T. W., and Damsté, J. S. S.: Organic sulfur
1050 biogeochemistry: recent advances and future research directions, 2004.

1051 Whiticar, M. J.: Carbon and hydrogen isotope systematics of bacterial formation and oxidation
1052 of methane, *Chemical Geology*, 161, 291-314, 1999.

1053 Xia, F., Jiang, Q.-Y., Zhu, T., Zou, B., Liu, H., and Quan, Z.-X.: Ammonium promoting
1054 methane oxidation by stimulating the Type Ia methane-oxidizing bacteria in tidal flat sediments
1055 of the Yangtze River estuary, *Science of the Total Environment*, 793, 148470, 2021.

1056 Xie, Z., Li, W., Yang, K., Wang, X., Xiong, S., and Zhang, X.: Bacterial and Archaeal
1057 Communities in Erhai Lake Sediments: Abundance and Metabolic Insight into a Plateau Lake
1058 at the Edge of Eutrophication, *Microorganisms*, 12, 1617, 2024.

1059 Xu, J. and Logan, B. E.: Measurement of chlorite dismutase activities in perchlorate respiring
1060 bacteria, *Journal of microbiological methods*, 54, 239-247, 2003.

1061 Yan, X., Xu, X., Wang, M., Wang, G., Wu, S., Li, Z., Sun, H., Shi, A., and Yang, Y.: Climate
1062 warming and cyanobacteria blooms: Looks at their relationships from a new perspective, *Water
1063 Research*, 125, 449-457, 2017.

1064 Yang, R., Peng, C., Mo, Y., Kleindienst, S., Li, S., Wang, J., Kappler, A., Wang, Z., and Lu,
1065 L.: Electron acceptors modulate methane oxidation and active methanotrophic communities in
1066 anoxic urban wetland sediments, *Applied and Environmental Microbiology*, 91, e00386-
1067 00325, 2025.

1068 Yang, Y., Chen, J., Tong, T., Xie, S., and Liu, Y.: Influences of eutrophication on
1069 methanogenesis pathways and methanogenic microbial community structures in freshwater
1070 lakes, *Environmental Pollution*, 260, 114106, 2020.

1071 Yang, Y., Chen, J., Chen, X., Jiang, Q., Liu, Y., and Xie, S.: Cyanobacterial bloom induces
1072 structural and functional succession of microbial communities in eutrophic lake sediments,
1073 *Environmental Pollution*, 284, 117157, 2021.

1074 Yang, Y., Chen, J., Tong, T., Li, B., He, T., Liu, Y., and Xie, S.: Eutrophication influences
1075 methanotrophic activity, abundance and community structure in freshwater lakes, *Science of
1076 the Total Environment*, 662, 863-872, 2019.

1077 Yvon-Durocher, G., Allen, A. P., Bastviken, D., Conrad, R., Gudasz, C., St-Pierre, A., Thanh-
1078 Duc, N., and Del Giorgio, P. A.: Methane fluxes show consistent temperature dependence
1079 across microbial to ecosystem scales, *Nature*, 507, 488-491, 2014.

1080 Zhao, Y., Liu, Y., Cao, S., Hao, Q., Liu, C., and Li, Y.: Anaerobic oxidation of methane driven
1081 by different electron acceptors: A review, *Science of The Total Environment*, 174287, 2024.

1082 Zhou, C., Peng, Y., Yu, M., Deng, Y., Chen, L., Zhang, L., Xu, X., Zhang, S., Yan, Y., and
1083 Wang, G.: Severe cyanobacteria accumulation potentially induces methylotrophic methane
1084 producing pathway in eutrophic lakes, *Environmental Pollution*, 292, 118443, 2022.
1085 Zhu, Y., Chen, X., Yang, Y., and Xie, S.: Impacts of cyanobacterial biomass and nitrate
1086 nitrogen on methanogens in eutrophic lakes, *Science of The Total Environment*, 848, 157570,
1087 2022.
1088