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Abundance and δ^{13}C values of fatty acids in lacustrine surface sediments:

Relationships with in-lake methane concentrations

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Highlights

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30   * Fatty acid abundances in lake sediments were compared with methane concentrations

31   * Three ^{13}C-depleted FA groups were correlated with CH_4 concentration in the lakes

32   * The results support earlier interpretations that MOB increase with CH_4 concentrations

33   * Further studies with lipids specific to MOB are needed to corroborate our results

34

35

Abstract

Proxy-indicators in lake sediments provide the only approach by which the dynamics of in-lake methane cycling can be examined on multi-decadal to centennial time scales. This information is necessary to constrain how lacustrine methane production, oxidation and emissions are expected to respond to global change drivers. Several of the available proxies for reconstructing methane cycle changes of lakes rely on interpreting past changes in the abundance or relevance of methane oxidizing bacteria (MOB), either directly (e.g. via analysis of bacterial lipids) or indirectly (e.g. via reconstructions of the past relevance of MOB in invertebrate diet). However, only limited information is available about the extent to which, at the ecosystem scale, variations in abundance and availability of MOB reflect past changes in in-lake methane concentrations. We present a study examining the abundances of fatty acids (FAs), particularly of ^{13}C-depleted FAs known to be produced by MOB, relative to methane concentrations in 29 small European lakes. 39 surface sediment samples were obtained from these lakes and FA abundances were compared with methane concentrations measured at the lake surface, 10 cm above the sediments and 10 cm within the sediments. Three of the FAs in the surface sediment samples, $C_{16:1\omega 7c}$, $C_{16:1\omega 5c/t}$, and $C_{18:1\omega 7c}$ were characterized by lower $\delta^{13}C$ values than the remaining FAs. We show that abundances of these FAs, relative to other short-chain FAs produced in lake ecosystems, are related with sedimentary MOB concentrations assessed by quantitative polymerase chain reaction (qPCR). We observed positive relationships between methane concentrations and relative abundances of $C_{16:1\omega 7c}$, $C_{16:1\omega 5c/t}$, and $C_{18:1\omega 7c}$ and the sum of these FAs. For the full dataset these relationships were relatively weak (Spearman's rank correlation (r_s) of 0.34 to 0.43) and not significant if corrected for multiple testing. However, noticeably stronger and statistically significant relationships were observed when sediments from near-shore and deep-water oxic environments (r_s = 0.57 to 0.62) and those from anoxic deep-water environment (r_s = 0.55 to

3

62 0.65) were examined separately. Our results confirm that robust relationships exist between

63 in-lake CH_4 concentrations and ^{13}C-depleted groups of FAs in the examined sediments,

64 agreeing with earlier suggestions that the availability of MOB-derived, ^{13}C-depleted organic

65 matter for aquatic invertebrates increases with increasing methane concentrations. However,

66 we also show that these relationships are complex, with different relationships observed for

67 oxic and anoxic sediments and highest values measured in sediments deposited in oxic

68 environments overlain with relatively methane-rich water. Furthermore, allthough all three

69 ^{13}C-depleted FA groups identified in our survey are known to be produced by MOB, they also

70 receive contributions by other organism groups, and this will have influenced their

71 distribution in our dataset.

72

73   1. Introduction

74

75   Methane (CH_4) is a major greenhouse gas and lakes are an important natural source of CH_4 to

76   the atmosphere (Bastviken et al. 2011; Bridgham et al. 2013). CH_4 concentrations in lakes are

77   determined by the rate of biogenic CH_4 production, a process in which methanogens produce

78   CH_4 using the hydrogenotrophic, acetoclastic, or methylotrophic pathway (Borrel et al. 2011),

79   and by the rate of CH_4 diffusion and ebullition into the water column and further into the

80   atmosphere. Another process that can remove CH_4 from lakes is methanotrophy, a microbially

81   mediated process that oxidizes CH_4 with O_2 or other electron acceptors, like nitrate (Hanson

82   and Hanson 1996; Blees et al. 2014, Oswald et al. 2016). Most of the CH_4 consumption in

83   freshwaters seems to be performed by CH_4-oxidizing bacteria (MOB) under oxic conditions,

84   which have been estimated to oxidize 30 to 99 % of the CH_4 produced in lakes (Bastviken et

85   al. 2008). Biogenic CH_4 has a distinctly ^{13}C-depleted stable carbon isotopic composition, with

86    $\delta^{13}C$ values typically between -80 and -50 ‰ (vs. VPDB) (Whiticar et al. 1986; Jedrysek 2005).

87   MOB, which use CH_4 as their energy and carbon source, incorporate CH_4-derived carbon into

88   their biomass. Therefore they are characterized by very negative $\delta^{13}C$ values as well, which can

89   be even lower than those of the original CH_4 due to isotopic fractionation during CH_4 uptake

90   (Summons et al 1994). Since CH_4 is significantly more depleted in ^{13}C than other carbon

91   sources, $\delta^{13}C$ is a good tracer for CH_4-related processes in lakes. For example, modern and

92   palaeoecological food web studies have used $\delta^{13}C$ analysis to assess the importance of MOB as

93   a food source for aquatic invertebrates in lakes (e.g. Sanseverino et al. 2012; Schilder et al.

94   2015; Grey 2016).

95   Systematic field surveys constraining CH_4 production, abundance and emissions in lakes have

96   only been developed very recently (e.g. Rasilo et al. 2015; Yang et al. 2015; Rinta et al. 2017).

97   Since long instrumental time series are lacking it is challenging to predict how and at which

98   time scales lacustrine CH_4 production and emission will respond to future environmental

99 pressures such as global warming and widespread eutrophication and reoligotrophication of

100 inland waters. Proxy-based reconstructions of past changes in lacustrine carbon cycling have

101 been explored as alternative approaches for constraining how the carbon cycle of lakes, and

102 particularly CH_4 production, oxidation, and uptake in the food web, respond to environmental

103 change. Such studies are particularly relevant for assessing how these processes react on multi-

104 decadal to centennial timescales to global change drivers such as increasing air and water

105 temperatures and anthropogenic nutrient release, since these timescales are not covered by

106 instrumental measurements.

107 Proxy-based approaches used to constrain past changes in CH_4 availability, production and

108 oxidation in lakes have been based either on geochemical measurements on specific organic

109 microfossil groups (e.g. Wooller et al. 2012; Belle et al. 2014; Rinta et al., 2016; Schilder et al.

110 2017) or lipid groups (e.g. Bechtel and Schubert, 2009; Hollander and Smith, 2001; Naeher et

111 al., 2014; Davies et al. 2016) that are expected to be related either in their carbon isotopic

112 composition or abundance (or both) with MOB or CH_4-producing microorganisms in lakes.

113 Furthermore, ancient DNA (aDNA) analyses of lake sediments have recently been used to

114 constrain past changes in MOB (Belle et al. 2014). If absolute or relative abundances of these

115 microorganisms are in turn systematically related to CH_4 concentrations in lakes, these and

116 similar approaches may even allow quantitative statements about past changes in CH_4

117 abundance in lakes based on lacustrine proxy records (e.g. van Hardenbroek et al. 2013;

118 Schilder et al. 2015a; Elvert et al. 2016). Recent studies have demonstrated that $\delta^{13}C$ analyses

119 of chitinous remains of aquatic invertebrates may have considerable potential in this respect.

120 Several aquatic invertebrate groups can feed on MOB or other microorganisms such as ciliates

121 feeding on them (e.g. Kankaala et al. 2006; Deines et al., 2007; Deines and Fink, 2011; Jones

122 and Grey, 2011), which leads to strongly ^{13}C-depleted isotope signatures in their chitinous

123 remains (e.g. resting egg sheaths, exoskeleton parts) preserved in lake sediments (Wooller et

124 al., 2012; Belle et al. 2014; Schilder et al. 2015a). Reconstructions of changes in $\delta^{13}C$ values of

125 these invertebrate fossils have been used to reconstruct past changes in the relevance of CH_4-

126 derived carbon for different parts of lacustrine food webs. Furthermore, several studies have

127 revealed quantitative relationships between $\delta^{13}C$ values of these remains and measurements of

128 CH_4 abundances, suggesting that estimates of past variations in in-lake CH_4 abundance may be

129 possible based on $\delta^{13}C$ analyses of some of these invertebrate groups. However, the

130 mechanisms that lead from high CH_4 availability in the examined lakes to a higher proportion

131 of CH_4-derived C in invertebrate biomass (and their microfossils) are still poorly constrained.

132 One potential explanation is that CH_4-rich ecosystems are characterized by higher abundances

133 of MOB and therefore also a higher availability of CH_4-derived carbon for filter-feeding and

134 deposit feeding aquatic invertebrates.

135 Here we present a survey of fatty acid (FA) concentrations and $\delta^{13}C$ values in the surface

136 sediments of 29 lakes from Finland, the Netherlands, Sweden and Switzerland. Concentrations

137 of ^{13}C-depleted FAs are compared with CH_4 concentration estimates in the open water column,

138 above the sediments and in deeper sediment layers (10 cm below the sediment surface). We

139 focus on ^{13}C-depleted FA groups that are produced by MOB but also receive contributions from

140 other organism groups. If there is a higher contribution of MOB to particulated organic matter

141 in the water column and sedimentary organic matter in surface sediments in CH_4-rich systems,

142 as speculated in earlier studies (e.g. van Hardenbroek et al. 2013, Schilder et al. 2015), we

143 expect to see positive relationships between relative abundances of these FAs and CH_4

144 concentrations in our dataset. Since the ^{13}C-depleted FA groups in our dataset are not strictly

145 limited to MOB we also assess the abundance of MOB in the examined sediments using

146 quantitative polymerase chain reaction (qPCR) to support our interpretations.

147

148

149 2. Study sites and study setup

150

151 Surface sediments (0 to 2 cm) were collected from the deepest part of 29 lakes across Europe,

152 including 2 Dutch (NL), 6 Finnish (FI), 11 Swedish (SE) and 10 Swiss (CH) lakes (Fig. 1). The

153 lakes were sampled in a multi-year campaign and sites were selected to cover a range of small

154 lake types on different bedrocks and with variable water chemistry conditions (e.g. in respect

155 to transparency, pH, conductivity, deepwater oxygen concentrations) in Northern Europe

156 (Finland, Sweden), Western Europe (The Netherlands) and Central Europe (Switzerland). The

157 aim of the survey was to assess whether quantitative relationships existed between invertebrate

158 δ^{13}C values and variables relevant for determining the overall CH_4 production, abundance and

159 emissions of these lakes (e.g. Schilder et al. 2015; Rinta et al. 2015). Therefore, CH_4

160 concentrations where measured in the open water column, just above the coring site, and deeper

161 within the sediments (i.e. at 10 cm depth) rather than within the sediment samples that were

162 analysed for invertebrate δ^{13}C values and that are available for the FA analyses presented in

163 this study. Most of the lakes were thermally stratified and developed anoxic conditions during

164 the summer months. To expand the number of sediment samples accumulated under oxic

165 conditions, near-shore surface sediments from the littoral zone were also analysed for 11

166 Swedish lakes (lake abbreviations in small letters). All of these samples were located above the

167 oxycline. The lakes are all relatively small (average surface area 0.32 km^2), shallower than

168 32 m, and characterized by variable nutrient concentrations and mixing conditions (Suppl.

169 Table 1). The sampling of the lakes took place during late summer stratification (August-

170 September) in 2010 in Sweden and 2011 in Finland, the Netherlands, and Switzerland. More

171 details on the study lakes and the environmental variables measured during fieldwork are

172 available in Rinta et al. (2015).

173

174 3. Methods

175

176 **3.1 Water chemistry**

178 The lakes were analysed for basic limnological variables including temperature and oxygen

179 profiles, pH and conductivity as well as total nitrogen, total phosphorus and dissolved inorganic

180 carbon (DIC) concentrations in the surface and/or bottom water (presented in detail in Rinta et

181 al. 2015). CH_4 concentrations were measured both in the lake centre and near the shore for

182 surface water (5 cm below water surface), and 10 cm above the sediment-water interface. In

183 addition, CH_4 concentrations were measured for each coring site 10 cm below the sediment-

184 water boundary to provide an estimate about CH_4-richness of the sediments of the different

185 lakes. Samples 10 cm below the sediment surface rather than the 0-2 cm surface sediment

186 sample were collected, since these reflect CH_4 levels in sediments deeper than the surficial zone

187 of oxidation. Furthermore, these sediments were already consolidated and could be transferred

188 to a container for expulsing and subsampling the CH_4 without major loss of CH_4.

189 Surface water CH_4 concentrations were calculated using the headspace equilibration method

190 (McAuliffe 1971) by applying Henry's law describing gas-water portioning (Stumm and

191 Morgan 1996, see methods in Bastviken et al. 2010). The water was sampled with 60 ml

192 syringes 5 cm below the surface in the deep and littoral part of the lakes. 40 ml of water was

193 equilibrated with 20 ml ambient air in the syringes (60 s shaking). A sample of the equilibrated

194 headspace was injected into a glass vial pre-filled with saturated brine solution. Ambient air

195 was collected to correct for background air CH_4 concentrations. Water 10 cm above the

196 sediment surface was sampled from gravity cores with an intact sediment surface both in the

197 lake centre and near-shore environments using a small tube connected to a syringe with a three-

198 way luer-lock valve (Rinta et al. 2015). After rinsing the tube several times and removing any

199 gas bubbles, 60 ml of water were collected above the sediment in the core and injected into 118

200 ml N_2-filled glass vials holding 0.2 ml phosphoric acid for CH_4 measurements. A standard

201 volume of sediment from 10 cm depth in the cores was collected and rapidly transferred to an

202 airtight 130 ml flask and 45 ml lake water, equilibrated with ambient air in terms of CH_4

203   concentrations, was added with a syringe attached to a valve in the cap (Rinta et al. 2015). After

204   shaking to force the CH_4 from the sediment into the headspace, 45 ml of the headspace was

205   sampled through the valve and injected into a 50 ml glass vial pre-filled with saturated brine

206   solution, using a second needle to partly drain the brine solution. CH_4 concentrations were

207   measured by gas chromatography with flame ionization detector (GC-FID, Agilent 6890 N,

208   Plot Q capillary column for water samples from Finnish lakes and Shimadzu GC-8, Poropak N

209   column for the others) with a repeatability error below 1.4 % for measurements around

210   100 ppm, 1.7 % around 500 ppm and 0.4 % around 1000 ppm (Rinta et al. 2015, 2017).

211   Conversions to µmol/l units were made using Henry's law and the common gas law as

212   explained in Bastviken et al. (2008). Concentrations were expressed per unit volume water for

213   samples taken in the water column and per unit volume of wet sediment for sediment samples.

214

215   **3.2 FA analysis**

216

217   Sediments for FA analysis were frozen in the field, and stored frozen in the dark until they were

218   freeze-dried in the laboratory. Ca. 1 g freeze-dried sediment was extracted with

219   dichloromethane/methanol (MeOH) in a micro-wave (Anton Paar) and by ultrasonication.

220   Traces of water were removed by running the extract over a Na_2SO_4 column and sulphur was

221   removed using a Cu column. The extract was saponified with 6 % KOH in MeOH for 3 h at

222   80 °C. The acid fraction was extracted from the aqueous phase after the addition of HCl until a

223   pH below 2. After removing water with a Na_2SO_4 column again, the FAs were methylated with

224   10 % BF_3/MeOH to produce methyl esters (FAMEs) (2 h at 100 °C). Lipid concentrations were

225   examined using gas chromatography with flame ionization (GC FID, Schimadzu GC-2010 Plus

226   with Inert Caps 5MS/NP column). Individual compounds were identified with gas

227   chromatography-mass spectrometry (GC-MS, Schimadzu GCMS-QP 2010 Ultra with

228   phenomenex Zebron phase ZB-5MSi column), based on the retention time and comparison to

229 published mass spectra. Compound specific $\delta^{13}C$ values (‰VPDB) were obtained with GC-

230 IRMS (Agilent Technologies 6890 N with Restek RXi 5ms column and IsoPrime micromass

231 IRMS) with an analytical error below 1.1 ‰ (*n*-C25 alkane standard). $\delta^{13}C$ values of FAs were

232 corrected for methylation by examining the methylation-influence on a standard FA (lauric

233 acid). For identification of multiple bond positions in FAs, an aliquot was derivatised to form

234 2-alkenyl-4,4-dimethoxyloxazline (DMOX) derivatives. These DMOX derivatives were

235 measured with GC-MS (Schimadzu GCMS-QP 2010 Ultra with phenomenex Zebron phase

236 ZB-5MSi column) and double bond position identified by comparing the results with published

237 mass spectra and following the rule of Spitzer (1997).

238

239 **3.3 Molecular analysis**

240 **3.3.1 DNA extraction**

241 DNA was extracted using a modification of the method described by Pan et al. (2010) based on

242 the FastDNA spin kit for soil (MP Biomedicals, LLC, Solon, OH, USA). Freeze-dried sediment

243 (0.1-0.2 g) and 780 µl lysis buffer [200 mmol l^{-1} NaPO4, pH 7.0; 1% CTAB; 1.5 mol l^{-1} NaCl;

244 2% Polyvinylpyrrolidone K30; 5 mg ml^{-1} lysozyme (added right before use)] was added into a

245 multimix FastPrep tube and incubated at 37 °C for 30 min. MT buffer (122 µl) was added and

246 tubes were shaken in the FastPrep instrument (MP, Biomedicals, LLC, Solon, OH, USA) for

247 30 s at 5.5 m s^{-1}. Subsequently, samples were centrifuged for 15 min at 10000 rpm and 700 µl

248 supernatant was collected. The pellet was re-extracted by adding lysis buffer (500 µl) and 50 µl

249 MT buffer to the FastPrep tubes, shaken in the FastPrep instrument for 30 seconds at 5.5 m s^{-1}

250 again followed by the transfer of the second 700 µl of supernatant into separate Eppendorf

251 tubes. At this step, 2×700 µl supernatant was obtained from each sample. 5 µl of 10 mg ml^{-1}

252 freshly made proteinase K was added to each tube. Tubes were incubated at 65°C for 30 min.

253 Samples were extracted with phenol-chloroform-isoamyl alcohol (25:24:1), followed by a

254 chloroform-isoamyl alcohol (24:1) extraction. 125 µl of 7.5 mol l^{-1} potassium acetate was

255   added, samples were incubated on ice for 5 min and then centrifuged at 10000 rpm for 10 min.

256   Supernatants (2×700 µl per sediment sample) were transferred to new tubes, 700 µl Binding

257   Matrix was added and tubes were mixed for 5 min on a rotator. Binding Matrix, with bound

258   DNA, was pelleted by 1 min centrifugation at 10000 rpm. The supernatant was discarded and

259   the pellet was resuspended in 500 µl wash buffer. The resulting suspension was added into a

260   Spinfilter, and centrifuged for 1 min at 10000 rpm. The eluate was discarded and the pellet was

261   washed again in 500 µl wash buffer. After discarding the second eluate, the Spinfilter was

262   centrifuged for another 10 s to dry the pellet. The filter was taken into a new tube and 50 µl of

263   TE pH 8.0 was added. The filter was incubated at room temperature for 1 min and centrifuged

264   for 1 min. The filter was re-eluted in the same way with 50 µl of TE pH 8.0.

265

266   **3.3.2 Quantitative PCR (qPCR)**

267   Three methanotrophic sub-groups (Ia, Ib, and II) were quantified by pmoA (particulate

268   methanemonooxygenase)-based quantitative PCR based on the assays described by Kolb et al.

269   (2003). The type Ia and II assays were carried out as described by Bodelier et al. (2009b). For

270   the type Ib assay, DNA standards were prepared by dilution of a known amount of PCR product

271   amplified from a reference clone (clone WPBN2, accession KF395333) by using the 189-

272   Mc468 primer set (Kolb et al., 2003). PCRs were carried out in 25 µl reactions containing 12.5

273   µl 2 ×SYBR green mix (AB gene, Epsom, UK), 2.5 µl of diluted DNA template and 0.8 mmol

274   l^{-1} of each primer. The samples were diluted accurately to 1 ng $µl^{-1}$. The thermal cycle started

275   with an initial denaturation at 95 °C for 15 min, followed by 45 cycles of denaturation at 95 °C

276   for 20 s, annealing at 64 °C for 20 s, and extension at 72 °C for 45 s. Fluorescence was recorded

277   at 84 °C and DNA melting curve analysis was performed at temperatures ranging from 70 °C

278   to 99 °C. All of three assays were performed with a Rotor Gene 6000 thermal cycling system

279   (Corbett Research, Eight Mile Plains, Qld, Australia), where samples were added to aliquots of

280   the master mixture using a CAS-1200 (Corbett Robotics Eight Mile Plains, Qld, Australia)

281   liquid handling system. Every sample was performed in duplicate. Quantification analysis was

282   performed by the RotorGene software. Copy numbers were calculated assuming a PCR product

283   length of 412 bp, 279 bp and 423 bp for type Ia, type Ib and type II methanotrophs, respectively.

284

285   **3.4 Data treatment and analysis**

286   The examined sediments varied in respect to composition (e.g. carbonate and organic content),

287   texture (fine grained versus coarse, consolidated versus unconsolidated) as well as expected

288   sedimentation rates, with Fennoscandian lakes usually characterized by much lower

289   sedimentation rates than Swiss hardwater lakes (e.g., Lotter et al. 1997; van Hardenbroek et al.

290   2014; Rinta et al. 2016). Since these factors can strongly affect absolute abundances of sediment

291   components, FAs were expressed as proportion (relative abundances) relative to the sum of

292   other FAs identified by GC-FID/GC-MS.

293   Variations in the composition of different FA samples were initially examined by principal

294   component analysis (PCA). Variables were centred and analysed using the program Canoco for

295   Windows 4.5 (Ter Braak and Smilauer 2002). This initial analysis revealed systematic

296   differences in the importance of FAs originating from terrestrial environments between samples

297   from different study regions (see Section 4.1). Since terrestrial input can strongly affect relative

298   abundance data of lacustrine sediment components, and the main interest of this study was on

299   the aquatic components, FA abundances were expressed relative to the sum of short-chain FAs

300   (C_{14} to C_{22}) for all further analyses, excluding the longer chained FAs (C_{24} to C_{28}) which

301   originate from terrestrial organic matter (Meyers 2003).

302   GC-IRMS data were screened to identify FA groups with ^{13}C-depleted values typical for

303   organic compounds produced by MOB. All of the ^{13}C-depleted FA groups found in our survey

304   are known to be produced by MOB, but also by other organism groups (see Sections 4 and 5).

305   To confirm that in our dataset they originated to a significant extent from MOB we compared

306   the abundances of these FAs with the number of DNA copies (*pmoA* gene) per g organic matter

307　(org C) measured for different MOB types using Spearman's rank correlation (r_s) and associated

308　p values. DNA copies were expressed relative to total organic carbon content (TOC) to reduce

309　the effects of variable proportions of inorganic sediment components (e.g. clay, silt, sand or

310　autochthonous carbonates) on the results. Organic matter content was measured using loss on

311　ignition at 550°C (Heiri et al. 2001).

312　The relationship between relative abundances of ^{13}C-depleted FA groups and CH_4

313　concentrations was again assessed by calculating r_s and associated p values. r_s values were

314　calculated for the entire dataset but also separately for two categories of sediments: sediments

315　deposited in anoxic deepwater sections of the lake basins (referred to as anoxic sediments

316　samples, $O_2 < 1$ mg l^{-1}, 0.5 m above sediment surface), and oxic deepwater sediments together

317　with near-shore water samples (referred to as oxic sediment samples; see Suppl. Table 1).

318　Correlations (r_s values) were calculated with the program PAST (Hammer et al. 2001). The

319　results were corrected for multiple testing using the False Discovery Rate method (FDR;

320　Benjamini and Hochberg 1995) as described in Garcia (2004).

321

322　4. Results

323

324　**4.1 Biomarker composition**

325

326　　　The FA composition showed a strong even over odd predominance and maxima at n-

327　C_{16} in most of the samples. There were clear differences in the FA composition between the

328　study areas. The Fennoscandian lakes showed a higher proportion of longer chain FAs (C_{24} to

329　C_{28}), but the overall composition was similar to samples from Western and Central Europe and

330　n-C_{16} remained the dominant FA. These interregional differences are confirmed by the PCA

331　analysis based on relative FA abundances (Fig. 2). Longer chain FAs (C_{24}, C_{26}, C_{28}) were all

332　characterized by positive axis 1 scores and negative axis 2 scores, in agreement with positive

axis 1 scores for most of the Fennoscandian samples. C_{20} and C_{22} also followed a similar

distribution in our dataset as the longer chain FAs. $C_{22:1}$ and C_{18} were characterized by high

axis 1 and 2 scores, indicating that these compounds had a different distribution in the lake

sediments than the rest of the FAs. FAs with negative axis 1 scores, e.g. C_{15}, C_{16}, or $C_{18:2}$ form

another, more heterogeneous group. The observation that most of the Swiss lake sediment

samples were also characterized by negative axis 1 scores indicates higher relative abundances

of these compounds in the Swiss sediment samples. The littoral sample of Stora Vänstern (stv)

was different in its FA composition from all other samples (Fig. 2). However, this sample was

already identified as potentially contaminated by older sediments and sediment redeposition in

the field and characterized by sandy material. We therefore excluded this sample from further

analyses. The deep-water sample of Glimmingen (GLI), which is plotted in a similar area of the

PCA biplot as stv, contained also sandy material, although to a lesser extent. Although GLI

showed a different FA composition than the other lakes, the sample was not apparent as an

outlier in the relationships between FAs and CH_4 concentrations and therefore retained in

further analyses. Since the aim of the study was to assess whether with higher CH_4

concentrations ^{13}C-depleted FA groups become more abundant relative to other FAs typically

produced by aquatic organisms (see Sections 1 and 2) we eliminated longer chain FAs (C_{24},

C_{26}, C_{28}) originating from terrestrial environments from further analyses.

Most of the individual FAs showed a similar range of $\delta^{13}C$ values (Fig. 3). However,

$C_{16:1\omega7c}$, $C_{16:1\omega5c/t}$, and $C_{18:1\omega7c}$ were more depleted in ^{13}C values (Fig. 3), with median $\delta^{13}C$

values of -45.5 ‰, -59.6 ‰, and -41.5 ‰, respectively. The rest of the analysed FAs were less

^{13}C-depleted with median $\delta^{13}C$ values between -36.6 and -30.3 ‰. All of these ^{13}C-depleted

FAs are known to be produced by MOB, although not exclusively (see Section 5). Comparison

of FA abundances with in-lake CH_4 concentrations therefore focused on these ^{13}C-depleted

FAs. $C_{16:1\omega8c}$ and $C_{18:1\omega8c}$, known to be produced by MOB type I and II only (Bodelier et al.

358   2009a), were not found in the examined lake sediments or the peak areas of these specific FAs

359   were too low to be quantified and analysed reliably.

360   The qPCR analyses successfully quantified the number of DNA copies of total MOB in

361   34 of the examined sediment samples, MOB type Ia in 34, MOB type Ib in 30 and MOB type

362   II in 25 (Supplementary Table S2). However, nested PCR revealed that MOB were present in

363   all but one of the 30 sediment samples. Comparison of the relative abundances of $C_{16:1\omega7c}$,

364    $C_{16:1\omega5c/t}$, and $C_{18:1\omega7c}$ with DNA copies of MOB in the sediments (Table 1) confirmed that

365    $C_{16:1\omega7c}$ was significantly correlated with the abundance of MOB Ib and $C_{18:1\omega7c}$ with MOB Ia,

366   MOB Ib, MOB II and the sum of MOB DNA copies. The sum of the ^{13}C-depleted FAs

367   correlated with MOB type Ia, MOB type Ib and the total number of DNA copies of MOB.

368    $C_{16:1\omega5c/t}$ was not correlated with the overall abundance of any MOB type.

369   Over the entire dataset, relative abundances of $C_{16:1\omega7c}$, $C_{18:1\omega7c}$, and the sum of ^{13}C-

370   depleted FAs showed weak positive correlations (r_s = 0.34-0.40) with surface water CH_4

371   concentrations (Table 2). $C_{16:1\omega7c}$ and the sum of ^{13}C-depleted FAs were furthermore correlated

372   with CH_4 concentrations in the sediments (r_s = 0.36-0.43). However, if the results were

373   corrected for multiple testing none of these relationships remained significant.

374   If only oxic sediments were examined, r_s values were distinctly higher (0.57-0.62). Both

375   surface water and sedimentary CH_4 concentrations were correlated with $C_{16:1\omega7c}$. Furthermore,

376   the sum of the ^{13}C-depleted FAs was correlated with CH_4 concentrations in the surface water,

377   10 cm above and 10 cm below the sediment surface. $C_{16:1\omega5c/t}$ was also correlated with CH_4

378   concentrations in the sediments, but this relationship was no longer significant after correction

379   for multiple testing (Table 2).

380   For anoxic sediment samples correlations with the abundances of ^{13}C-depleted FAs were

381   also stronger (r_s = 0.46-0.65) than observed for the entire dataset. For this group of samples,

382    $C_{18:1\omega7c}$ was correlated with surface water CH_4 concentrations (r_s = 0.55) and $C_{16:1\omega7c}$ and the

383   sum of ^{13}C-depleted FAs were correlated with CH_4 concentrations in the lake water 10 cm

384 above the sediments, although the latter two relationships were no longer significant after

385 correcting for multiple testing. The strongest relationships for anoxic sediments were apparent

386 between CH_4 concentrations 10 cm below the sediment surface and $C_{16:1\omega7c}$ and the sum of ^{13}C-

387 depleted FAs ($r_s = 0.61$-0.65).

388

389 5. Discussion

390

391 **5.1 FA composition of sediments**

392

393 The high content of short-chain FAs, with a classical even over odd predominance, and maxima

394 at n-C_{16} in the FA composition of the sediment samples, indicates a predominantly

395 autochthonous organic matter production (Stefanova and Disnar 2000; Woszczyk et al. 2011).

396 The longer-chain FAs, with high axis 1 values in the PCA analysis (Fig. 2), are reported to be

397 derived from terrestrial sources, for example C_{24}-C_{30} from waxy coatings of land plants (Meyers

398 2003). $C_{22:1}$, which together with C_{18} shows a different distribution than the rest of the FAs, is

399 known to be produced by zooplankton, copepods and higher plants (Pearson et al. 2007). C_{18}

400 may originate from many sources, mainly freshwater algae (Meyers 2003). The more

401 heterogeneous group of FAs with negative axis 1 values in the PCA are known to have multiple,

402 mainly autochthonous sources (Pearson et al. 2007; Woszczyk et al. 2011). PCA analysis

403 indicates that FA assemblages from Swiss lakes are generally more strongly influenced by FAs

404 from autochthonous sources, while the Fennoscandian lakes and especially some littoral

405 samples show high relative abundances of terrestrial FAs. As mentioned above (Section 3.4)

406 we therefore eliminated longer-chain FAs (C_{24}, C_{26}, C_{28}) originating from terrestrial

407 environments from further analyses of FA abundances to reduce the effects of varying terrestrial

408 influences on our comparisons between relative abundances of FAs and CH_4 concentrations in

409 lakes.

410   The relatively lower $\delta^{13}C$ values of $C_{16:1\omega7c}$, $C_{16:1\omega5c/t}$, and $C_{18:1\omega7c}$ (Fig. 3) indicate that

411   these FAs are, at least partly, produced by organisms incorporating isotopically light carbon,

412   such as MOB. Literature sources confirm that they are produced by MOB, though not

413   exclusively. $C_{16:1\omega7c}$, for example, was found to be associated with MOB type I, and also some

414   MOB type II (Boschker et al. 1998; Bodelier et al. 2009a, 2012). However, this FA has also

415   been reported for phytoplankton, zooplankton, fungi, mycobacteria and higher plants (Volkman

416   et al. 1980; Woszczyk et al. 2011). $C_{16:1\omega5c/t}$ was also found to be associated with MOB type I

417   (Bodelier et al. 2012), and $C_{18:1\omega7c}$ with MOB type II (Bowman et al. 1993; Deines et al. 2007;

418   Bodelier et al. 2009a) and some MOB type Ia (Bodelier et al. 2009a). $C_{18:1\omega7c}$ was also reported

419   to be present in some other bacteria (Zegouagh et al. 2000). As we were mainly interested in

420   relationships between MOB-derived FAs and CH_4 concentrations, we focused our numerical

421   analyses on these compounds, which were more depleted in ^{13}C and were characterized by a

422    $\delta^{13}C$ signature typical for organisms incorporating CH_4-derived carbon. Other chemotrophs,

423   possibly abundant in stratified lakes at the chemocline, have also been reported to produce

424   isotopically light lipids (Enrich-Prast et al. 2009; Zemskaya et al. 2012). However, the highest

425   abundances of $C_{16:1\omega7c}$, $C_{16:1\omega5c/t}$, and $C_{18:1\omega7c}$ were observed in oxic sediment samples and

426   therefore, above the oxycline (and above any existing chemocline) in the lakes (Fig 5). Also,

427   the correlations of these FAs with MOB concentrations of the sediments (Table 1), and the

428   correlations of the relative abundances of these FAs with in-lake CH_4 concentrations in our

429   dataset (Table 2) support that MOB are a relevant source of these FAs in our study lakes.

430

431

432   **5.2 Relationships between FA and MOB abundances**

433

434   The distribution of MOB types I and II across the entire set of analysed lakes differs from analyses

435   of terrestrial soils where type II usually dominates (Pan et al., 2010; Bodelier et al., 2013).

436   This difference has been observed in freshwater sediments before (Borrel et al., 2011), where

437   MOB type II are less dominant and sometimes even absent (Sundh et al. 2005; Schubert et al.,

438   2010). Some of the genetic clusters belonging to the abundant type Ib MOB have been

439   designated as typical freshwater MOB, only being found in these habitats (Lüke and Frenzel,

440   2011; Knief 2015).

441   Relatively strong correlations ($r_s = 0.42$-0.69) are apparent between relative abundances of ^{13}C -

442   depleted FAs and all three MOB groups (Ia, Ib, II), as well as with the sum of MOB. These

443   relationships are apparent even though different confounding processes potentially affect the

444   relative abundance data of FAs (e.g. in-lake production rates of short chain FAs by organisms

445   other than MOB) and the absolute abundances of MOB DNA expressed relative to the organic

446   content of the sediments (e.g. sediment homogeneity, variable accumulation rates, dilution by

447   terrestrial organic matter). The observed relationships confirm that in our dataset abundances of

448   ^{13}C -depleted FAs are positively related with abundances of MOB as quantified by qPCR.

449   The strongest and most consistent correlations were observed between MOB abundances and

450      $C_{18:1\omega7c}$. However, the abundances of the different MOB types were strongly inter-correlated (r_s

451      $= 0.67$-0.83). Therefore, this finding does not necessarily indicate that $C_{18:1\omega7c}$ is produced by all

452   three MOB types in our study lakes, since, in principle, the apparent correlation of $C_{18:1\omega7c}$ to all

453   MOB types could also result from production by one MOB type only. $C_{16:1\omega7c}$ is only related

454   significantly to MOB type Ib. This suggests that MOB type Ib may be one of the main producers

455   of $C_{16:1\omega7c}$ in the studied ecosystems. However, significant amounts of this FA have been

456   demonstrated to be produced also by MOB type Ia as well as type II (Bowman et al., 1993;

457   Bodelier et al., 2009a).

458   The relative abundance of $C_{16:1\omega5c/t}$ was not clearly related to MOB abundance, although the

459   depletion in ^{13}C supports that the compound contains CH_4-derived carbon. In general, $C_{16:1\omega5c/t}$

460   was not very abundant in the analysed sediments. $C_{16:1\omega5}$ is not abundant in *Methylobacter*,

461   which is the type Ia genus mostly dominating freshwater sediments (Borrel et al., 2011) and this

462   could explain the lack of relationship of $C_{16:1\omega5c/t}$ with MOB abundance.

463   The sum of $C_{16:1\omega7c}$, $C_{16:1\omega5c/t}$, and $C_{18:1\omega7c}$ correlated most closely with MOB type Ib. This

464   relationship is possibly driven by $C_{16:1\omega7c}$, since in most sediments this was the most abundant

465   of the three more ^{13}C-depleted FAs. However, the sum of $C_{16:1\omega7c}$, $C_{16:1\omega5c/t}$, and $C_{18:1\omega7c}$ is more

466   robustly correlated with MOB type Ib than $C_{16:1\omega7c}$, suggesting that the less abundant ^{13}C-

467   depleted FAs, particularly $C_{18:1\omega7c}$, reinforced this relationship.

468

469

470   **5.3 Relationships between FA abundances and CH$_4$ concentrations**

471

472   As expected we observed positive relationships between the abundance of ^{13}C-depleted FAs

473   and CH$_4$ concentrations in our dataset. However, only relatively weak relationships were

474   observed when all sediment samples were examined together, with $C_{16:1\omega7c}$ and the sum of ^{13}C-

475   depleted FAs correlating with CH$_4$ concentrations in the surface water and the sediments and

476    $C_{18:1\omega7c}$ with CH$_4$ concentrations in the surface water. However, none of these relationships

477   remained significant after correction for multiple testing (Table 2). Relationships between FA

478   abundances and CH$_4$ concentrations were noticeably stronger and statistically significant when

479   oxic and anoxic sediments were examined separately. The highest correlations between FA

480   concentrations (for $C_{16:1\omega7c}$ and the sum of ^{13}C-depleted FAs) and lake-water CH$_4$

481   concentrations were apparent for oxic sediments ($r_s = 0.56$-0.62), whereas observed r_s values

482   were slightly lower (for $C_{18:1\omega7c}$, $C_{16:1\omega7c}$ and the sum of ^{13}C-depleted FAs) for anoxic sediments

483   ($r_s = 0.46$-0.55). These findings agree with the interpretation that the proportion of organic

484   carbon originating from MOB increases in lakes and in lake sediments with higher CH$_4$

485   concentrations, as has been suggested to explain observed relationships between estimates of

486   in-lake CH$_4$ abundance and δ^{13}C values of aquatic invertebrates that can feed on MOB (van

487   Hardenbroek et al. 2013; Schilder et al. 2015). However, our results also suggest that this

488   proportion increases to a different extent in sediments deposited in oxic and anoxic

489   environments. The highest abundances of ^{13}C-depleted FAs were observed in oxic deep-water

490   and littoral sediments (Fig 4). CH_4 can be oxidized in both oxic and anoxic conditions, but

491   aerobic CH_4 oxidation is considered to be the dominant process, oxidizing up to 99 % of CH_4

492   produced in lakes (Bastviken et al. 2008; Blees et al. 2014). The growth of aerobic MOB is not

493   only limited by CH_4 but also by the availability of electron acceptors, mainly oxygen (Amaral

494   and Knowles 1995; Hanson and Hanson 1996). In oxic deep-water and littoral environments

495   oxygen is generally abundant and MOB can profit most from increasing CH_4 availability,

496   explaining the highest abundances of ^{13}C-depleted FA groups observed for sediments deposited

497   under oxic condition. In the lakes where anoxic conditions develop, the CH_4 concentrations in

498   the deep water layers are high during summer stratification, when our samples were taken.

499   However, the absence of oxygen or other electron acceptors can limit CH_4 oxidation (Rudd et

500   al. 1976; Amaral and Knowles 1995) at the sediment-water interface and in stratified, anoxic

501   lakes CH_4 oxidation is usually most extensive at the oxycline in the open water (Bastviken et

502   al. 2008, Schubert et al. 2010, Milucka et al. 2015). Our results could be explained if MOB

503   occur at higher concentrations where the sediment-water interface coincides with the oxycline

504   than in the open water, since here the sediments would provide a stable substrate for these

505   microorganisms to grow on, and/or if FAs originating from MOB in the open water would be

506   partly decomposed during sedimentation and contribute to a lower extent to FAs measured at

507   the sediment-water interface than MOB growing in the surfical sediment layers. In these

508   situations, a high contribution of MOB-derived FAs would be expected for sediment samples

509   located at or just below the oxycline, as confirmed by the highest abundance of ^{13}C-depleted

510   FAs observed for our dataset in oxic samples overlain by relatively CH_4-rich water.

511   The abundances of $C_{16:1\omega7c}$ and of the sum of ^{13}C-depleted FAs in the surficial sediment layers

512   were robustly correlated with CH_4 concentrations of the deeper sediment layers 10 cm below

513   the sediment surface, both for the anoxic and oxic sediment samples (r_s = 0.57-0.59 and 0.61-

514   0.65, respectively; Table 2; Fig. 4). This suggests that the overall CH_4 richness of sediments,

515   and supply of CH_4 to the uppermost sediment layers, also promote higher abundances of CH_4-

516   derived carbon in sedimentary organic carbon in the surface sediment samples.

517

518   6. Conclusions and **implications for palaeoenvironmental reconstructions**

519

520   We show that in 29 small lakes across Europe the abundance of ^{13}C-depleted FA groups

521   relative to other FAs produced in freshwater ecosystems increases with increasing CH_4

522   concentrations, at least when relationships are examined separately for sediments deposited in

523   oxic and anoxic environments. This is expected if the relative contribution of CH_4-derived

524   carbon in lacustrine sedimentary organic matter, originating from MOB, increases with

525   increasing in-lake CH_4 concentrations. This interpretation is supported by the analysis of the

526   number of DNA copies of MOB in the examined sediments, which in our dataset is clearly

527   correlated with the abundance of ^{13}C-depleted FAs. However, our analyses also indicate that

528   the proportion of ^{13}C-depleted FA groups was highest in oxic sediment samples deposited in

529   environments with relatively high CH_4 concentrations, suggesting that the proportion of CH_4-

530   derived sedimentary organic carbon may also be elevated in these sediments. In contrast, lower

531   proportions of these ^{13}C-depleted FAs were observed in sediments deposited in anoxic sections

532   of the study lakes. Our analyses also show that different relationships between the relative

533   abundance of ^{13}C-depleted FA groups and in-lake CH_4 concentrations are observed for

534   sediments deposited in oxic and anoxic sediments. This implies that relationships between the

535   proportion of CH_4-derived carbon in aquatic organic matter and CH_4 concentrations may also

536   differ between these two depositional environments, at least for sedimentary organic matter

537   deposited in small temperate lakes such as the ones we examined in our study.

The robust correlations observed between CH_4 concentrations of the sediments and the abundance of ^{13}C-depleted FAs suggest that these relationships are not just with CH_4 abundances at or above the sediments. Instead, CH_4-rich lakes, characterized by high CH_4 production and concentrations in the sediments, seem to be generally characterized by higher abundances of ^{13}C-depleted FA groups in the surface sediment, although we again observed different relationships between the abundance of these FAs and CH_4 concentrations in the sediments for oxic and anoxic environments.

Our findings have two potential implications for approaches reconstructing past changes in CH_4 availability in lakes based on geochemical analyses of lake sediments. First, they confirm that higher abundances of ^{13}C-depleted FAs, from FA groups that are known to be produced by MOB, can be found in the sediments of small lakes under higher CH_4 concentrations. This agrees with earlier interpretations that the observed relationships between $\delta^{13}C$ values of unspecific deposit- or filter-feeding invertebrate groups and estimates of in-lake CH_4 abundance (van Hardenbroek et al. 2013; Schilder et al. 2015) can be explained by higher MOB abundances under higher in-lake CH_4 concentrations. The results therefore support that reconstructions of $\delta^{13}C$ values of CH_4-sensitive invertebrate groups, such as Chironomini and *Daphnia*, may provide information on past changes in in-lake CH_4 concentrations in small temperate lakes, as has been suggested in earlier studies (e.g. van Hardenbroek et al. 2013; Schilder et al. 2015), since the $\delta^{13}C$ values of these organism groups can be expected to be strongly driven by the availability of ^{13}C-depleted lacustrine organic matter, which, as our results suggest, is related to CH_4 concentrations. Second, our results support that lipids originating from MOB may be more abundant in environments with high CH_4 concentrations in the water or the sediments, and that lipid records may provide insights into past changes in CH_4 concentrations in lakes. Although FAs have been reported to decay rapidly in lake sediments (e.g. Muri and Wakeham 2006) other lipid groups produced by MOB or methanogens, such as bacteriohopanepolyols, and glycerol dialkyl glycerol tetraethers

564   (GDGTs) (Coolen et al., 2008; Naeher et al., 2012; Sinninghe Damsté et al., 2012) have been

565   analysed in downcore records and related to past variations in CH_4 cycling of lakes. However,

566   our results also suggest that the relationship between the abundance of lipids originating from

567   MOB and CH_4 concentrations may be complex and strongly influenced by oxygen availability

568   at the sediment water-interface and by a different preservation of lipids produced in the open

569   water column compared with those produced in sediments.

570   A major constraint of our study is that other organisms than MOB can contribute to the

571   three ^{13}C-depleted FA groups detected at our study sites and that FA groups strictly limited to

572   MOB were not detected in our survey. Although the different lines of evidence (FA analyses,

573   qPCR analyses, correlations with CH_4 concentrations) are all consistent with the interpretation

574   that the contribution of MOB-derived organic matter in lake sediments increases with

575   increasing in-lake CH_4 concentrations, it is unclear how the observed relationships were

576   influenced by the production of $C_{16:1\omega7c}$, $C_{16:1\omega5c/t}$, and $C_{18:1\omega7c}$ FAs by other organisms than

577   MOB. Our results should therefore be corroborated by similar surveys focusing on lipid groups

578   specific to MOB such as more specific FAs or specific bacteriohopanepolyols (e.g.

579   methylcarbamate lipids related to C-35 amino-bacteriohopanepolyols, Rush et al. 2016), or

580   alternative approaches which, e.g., assess the abundance of MOB-derived DNA relative to other

581   bacterial and algal DNA in lake sediments. An additional uncertainty associated with our

582   approach is the extent to which catchment carbon cycling reinforced or masked the relationship

583   between FAs and CH_4 concentrations. For example, relationships are noticeably stronger in our

584   dataset when analyses focus on shorter chain FAs only, since longer chain FAs originating from

585   terrestrial plants apparently influence the relative concentrations of FAs in the analysed

586   sediments. Similar effects may affect the relative abundances of short-chain FAs in our data as

587   well. Future studies would therefore ideally aim to more fully quantify varying terrestrial

588   organic matter input between and across lake basins to assess the extent that this organic matter

589   source influences FA abundance analyses in surficial lake sediment samples.

590

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592

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Figures and Tables
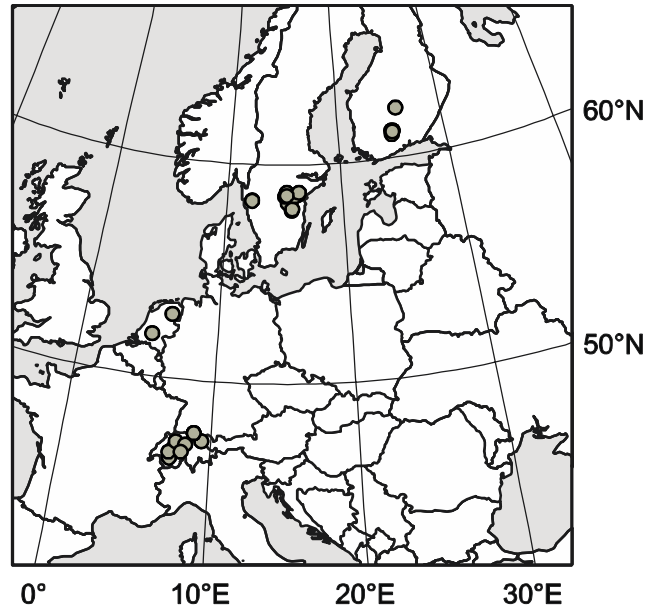
Fig. 1. Study sites located in Finland (6 lakes), the Netherlands (2), Sweden (11)

and Switzerland (10).
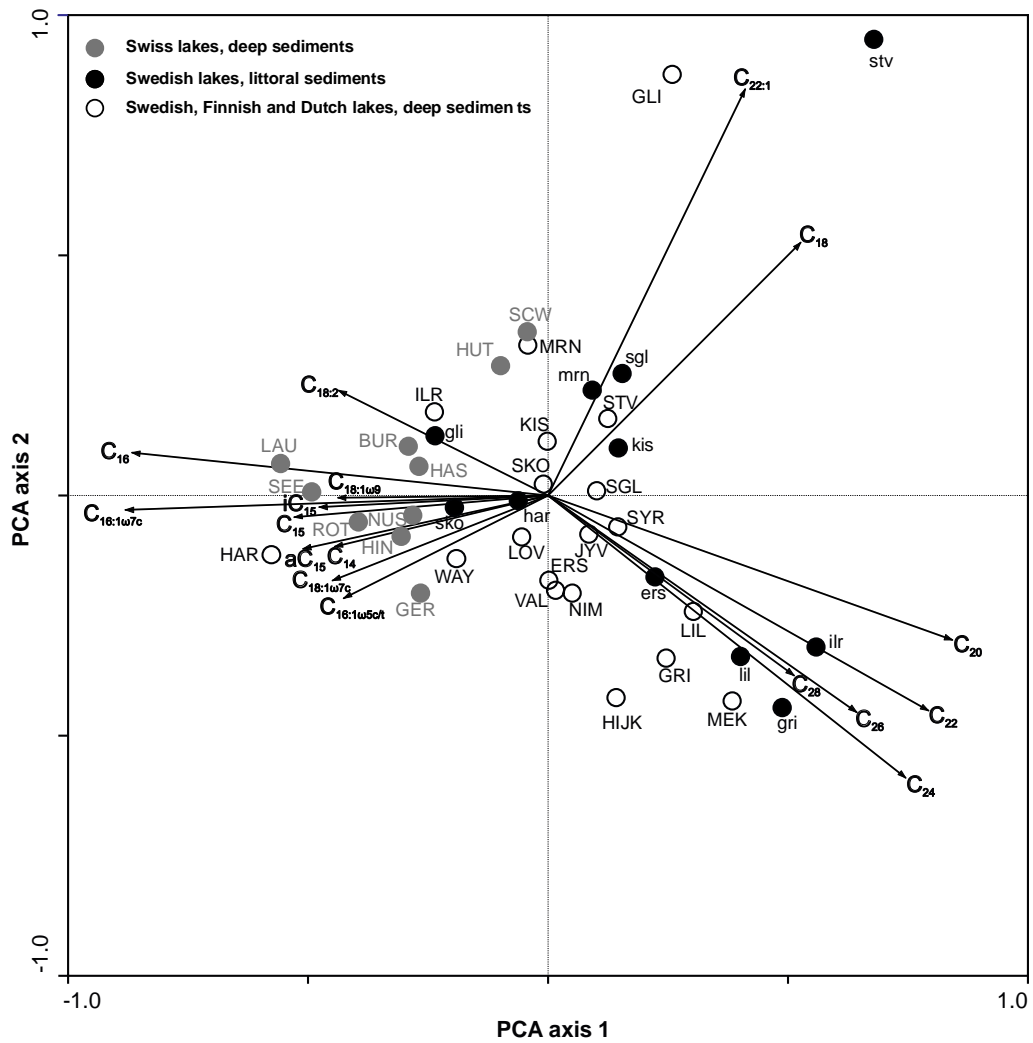
Fig. 2. Principal components analysis (PCA) summarizing variations in relative abundances of FAs in the surface sediment samples. Site names in capital and lowercase letters refer to samples taken in the deepest parts of lakes and littoral samples, respectively.
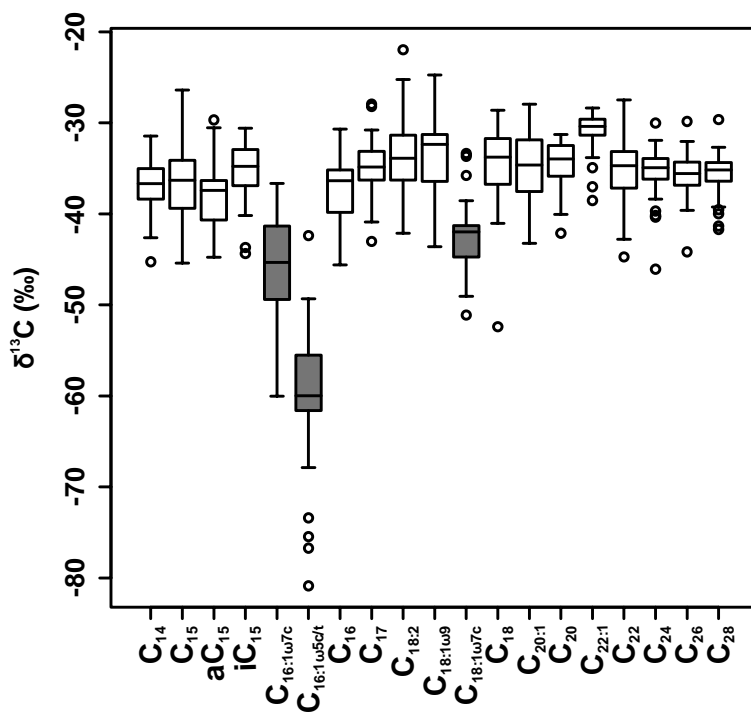
Fig. 3. Boxplots indicating the distribution of FA δ^{13}C values in deep water sediment samples from the 29 examined lakes. Central horizontal lines indicate median values and the bottom and top of boxes the 25th and 75th percentiles, respectively. Circles indicate samples more than 1.5 times the interquartile range above the third and below the first quartile, respectively. Boxplots for the ^{13}C-depleted FAs that are discussed in detail in the text are marked in grey.
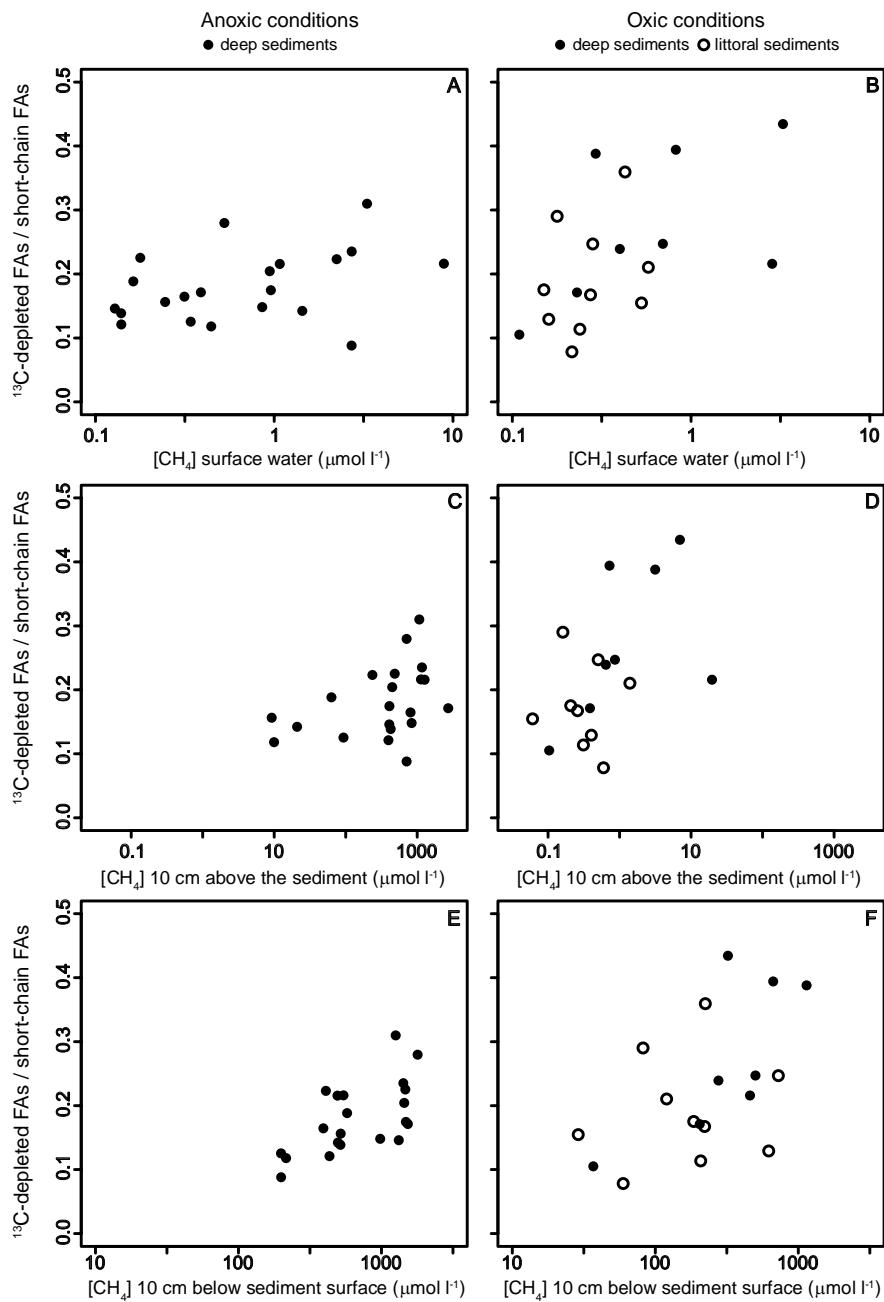
Fig. 4. Total abundance of ^{13}C-depleted FAs ($C_{16:1\omega7c}$, $C_{16:1\omega5c/t}$, and $C_{18:1\omega7c}$) in the sediment samples compared with CH_4 concentrations ($[CH_4]$) in the surface waters (A-B), 10 cm above the sediment (C-D), and 10 cm below the sediment surface (E-F) (note

the logarithmic scale). FAs are expressed as abundances relative to the sum of short-chain FAs (C_{14} to C_{22}). Samples obtained from the deepest point of lakes with anoxic bottom waters (A, C, E) are plotted separately from samples obtained in the centre of lakes with oxic bottom waters (filled circles) and in shallower sections of lakes (open circles; B, D, F). Concentrations are expressed per volume of water (A-D) or wet sediment (E-F).

Table 1 Spearman rank correlation (r_s) values for relationships between relative abundances of FAs in the sediments and DNA copies (*pmoA* gene) of MOB Ia, MOB Ib, MOB II and all MOB. *,** and *** mark p-values of below 0.05, 0.005 and 0.0005, respectively, and values in brackets indicate relationships that are no longer significant after False Discovery Rate (FDR) correction for multiple testing (Garcia 2004). The r_s values are provided for $C_{16:1\omega7c}$, $C_{16:1\omega5c/t}$, $C_{18:1\omega7c}$, and the sum of these ^{13}C-depleted FAs.

	$C_{16:1\omega7c}$	$C_{16:1\omega5c/t}$	$C_{18:1\omega7c}$	**Sum**
MOB Ia	-	-	0.50**	0.42*
MOB Ib	0.42*	-	0.63***	0.53**
MOB II	-	-	0.62**	-
Total MOB	-	-	0.69***	0.45*

Table 2 Spearman rank correlation (r_s) values for relationships between relative abundances of FAs in the sediments and CH_4 concentrations. Symbols *, ** and *** mark p-values below 0.05, 0.005 and 0.0005, respectively, and values in brackets indicate relationships that are no longer significant after FDR correction for multiple testing (within each group; Garcia 2004). Relationships with CH_4 concentrations are calculated for relative abundances of $C_{16:1\omega7c}$, $C_{16:1\omega5c/t}$, and $C_{18:1\omega7c}$, the sum of these ^{13}C-depleted FAs.

All lakes	$C_{16:1\omega7c}$	$C_{16:1\omega5c/t}$	$C_{18:1\omega7c}$	Sum
CH_4 surface water	(0.34*)	-	(0.34*)	(0.40*)
CH_4 +10cm	-	-	-	-
CH_4-10cm	(0.43*)		-	(0.36*)

Oxic sediments	$C_{16:1\omega7c}$	$C_{16:1\omega5c/t}$	$C_{18:1\omega7c}$	Sum
CH_4 surface water	0.57*	-	-	0.62*
CH_4 +10cm	-	-	-	0.56*
CH_4 -10cm	0.57 *	(0.51*)	-	0.59*

Anoxic sediments	$C_{16:1\omega7c}$	$C_{16:1\omega5c/t}$	$C_{18:1\omega7c}$	Sum
CH_4 surface water	-	-	0.55*	-
CH_4 +10cm	(0.46*)	-	-	(0.46*)
CH_4-10cm	0.65**	-	-	0.61**

Supplementary material

Table S1 Geographical location and limnological characteristics of the 29 examined lakes. See Rinta et al. (2015) for more details

Lake name	Abbre-viation	Country	Lon decimal °E	Lat decimal °N	Altitude m a.s.l.	Lake area (km²)	Max. depth (m)	O$_2$ bottom (mg l^{-1})	Core depth (m)	Organic matter C/N	CH$_4$ -10 cm (μmol l^{-1})
Erssjön	ERS	SE	12.16	58.37	75	0.063	5.0	7.0	5.0	13.6	459.7
Glimmingen	GLI	SE	15.57	57.93	145	1.672	31.5	7.4	14.0	11.6	36.9
Grissjön	GRI	SE	15.14	58.77	139	0.227	16.0	1.9	12.2	20.1	204.6
Hargsjön	HAR	SE	15.24	58.27	108	0.994	6.2	5.9	6.2	9.4	1142.7
Illersjön	ILR	SE	14.99	58.58	96	0.069	12.1	0.2	12.1	11.4	1469.6
Kisasjön north	KIS	SE	15.65	58.01	99	0.958	8.5	0.2	8.5	10.2	575.1
Lillsjön	LIL	SE	16.14	58.66	84	0.026	7.7	0.1	7.7	16.4	432.8
Mårn	MRN	SE	15.87	58.59	27	0.617	15.3	0.2	15.3	10.2	1322.5
Skärgölen	SGL	SE	16.23	58.76	72	0.156	13.0	0.1	12.6	13.3	215.4
Skottenesjön	SKO	SE	12.14	58.35	51	0.263	6.0	7.5	2.5	12.9	276.7
Stora Vänstern	STV	SE	15.15	58.62	102	1.135	21.4	0.7	21.4	12.8	516.2
Hijkermeer	HIJK	NL	6.49	52.89	14	0.023	2.0	5.6	1.2	14.3	502.0

De Waay	WAY	NL	5.15	51.93	0	0.041	14.5	0.0	14.5	10.5	1443.2
Jyväsjärvi	JYV	FI	25.77	62.24	78	3.032	25.0	0.0	24.1	11.1	518.8
Lovojärvi	LOV	FI	25.03	61.08	108	0.050	18.1	0.0	17.7	12.2	1529.8
Mekkojärvi	MEK	FI	25.14	61.23	136	0.003	4.0	0.1	4.0	18.8	198.8
Nimetön	NIM	FI	25.19	61.23	152	0.004	12.7	0.0	12.4	14.3	392.0
Syrjänalunen	SYR	FI	25.14	61.19	138	0.009	9.2	0.1	9.1	14.2	408.5
Valkea-Kotinen	VAL	FI	25.06	61.24	156	0.041	8.3	0.0	5.8	14.3	198.5
Burgäschisee	BUR	CH	7.67	47.17	434	0.204	30.0	0.1	30.0	9.0	1482.0
Gerzensee	GER	CH	7.55	46.83	603	0.240	10.7	0.1	9.3	10.5	1257.9
Hasensee east	HAS	CH	8.83	47.61	434	0.067	5.8	0.3	5.5	9.8	543.0
Hinterburgsee	HIN	CH	8.07	46.72	1516	0.046	11.4	0.1	11.0	8.9	1421.6
Hüttwilersee	HUT	CH	8.84	47.61	434	0.344	15.5	0.1	15.4	8.9	980.1
Lauenensee	LAU	CH	7.33	46.40	1381	0.087	3.5	6.8	3.0	8.2	321.7
Nussbaumersee	NUS	CH	8.82	47.62	434	0.253	8.0	0.1	8.0	9.3	492.9
Rotsee	ROT	CH	8.31	47.07	403	0.443	16.0	0.1	14.6	9.4	1791.6
Schwarzsee	SCW	CH	7.28	46.67	1046	0.446	9.1	0.1	8.8	10.0	495.5

Seealpsee	SEE	CH	9.40	47.27	1141	0.135	14.5	10.1	13.9	11.0	667.1
Erssjön littoral	ers	SE	12.16	58.37	75	0.063	5.0	7.0	2.0	13.9	119.9
Glimmingen littoral	gli	SE	15.57	57.93	145	1.672	31.5	7.4	4.2	10.7	82.0
Grissjön littoral	gri	SE	15.14	58.77	139	0.227	16.0	1.9	3.1	17.7	207.8
Hargsjön littoral	har	SE	15.24	58.27	108	0.994	6.2	5.9	2.9	11.4	725.4
Illersjön littoral	ilr	SE	14.99	58.58	96	0.069	12.1	0.2	2.6	20.7	59.6
Kisasjön north littoral	kis	SE	15.65	58.01	99	0.958	8.5	0.2	4.0	11.1	221.2
Lillsjön littoral	lil	SE	16.14	58.66	84	0.026	7.7	0.1	2.3	18.8	620.5

Mårn littoral	mrn	SE	15.87	58.59	27	0.617	15.3	0.2	8.0	11.0	185.7
Skärgölen littoral	sgl	SE	16.23	58.76	72	0.156	13.0	0.1	4.0	13.1	28.9
Skottenesjön littoral	sko	SE	12.14	58.35	51	0.263	6.0	7.5	1.6	11.5	223.9
Stora Vänstern littoral	stv	SE	15.15	58.62	102	1.135	21.4	0.7	5.1	12.1	6.1

Table S2. Number of DNA copies (*pmoA* gene) per g organic matter (org C) of different MOB types and methanogens.

Lake	Site	MOB Ia copies per g org C	MOB Ib copies per g org C	MOB II copies per g org C	Total MOB copies per g org C	Presence of MOB Nested PCR	Methanogens copies per g org C
BUR	centre	1.19E+09			1.19E+09	+	3.89E+08
ers	littoral	5.15E+07	1.24E+08	6.94E+08	8.70E+08	+	1.56E+09
ERS	centre	1.83E+07	5.15E+07	7.49E+07	1.45E+08	+	4.23E+08
GER	centre	1.36E+10			1.36E+10	+	2.60E+10
gli	littoral	5.55E+07	4.69E+08	6.32E+08	1.16E+09	+	6.28E+08
GLI	centre	1.02E+08	1.35E+08	4.91E+08	7.29E+08	+	9.26E+06
gri	littoral	5.52E+07	5.28E+07	1.02E+09	1.13E+09	+	1.23E+08
GRI	centre	4.14E+06	4.40E+06	5.50E+07	6.35E+07	+	3.13E+06
har	littoral	9.33E+07	4.81E+08	5.90E+07	6.33E+08	+	1.41E+08
HAR	centre	5.95E+07	1.86E+08	3.35E+07	2.79E+08	+	1.05E+08
HAS	centre	2.12E+08	3.93E+09		4.14E+09	+	7.08E+07
HIJK	centre	2.10E+09	9.05E+09	2.85E+09	1.40E+10	+	6.76E+08
HIN	centre					+	3.04E+08
HUT	centre	3.28E+08	2.18E+08		5.46E+08	+	5.57E+08
ilr	littoral	1.45E+06	2.69E+06	6.95E+06	1.11E+07	+	5.71E+06
ILR	centre	5.06E+06	1.22E+07	2.16E+07	3.88E+07	+	3.33E+07
JYV	centre					+	1.80E+08
kis	littoral	4.12E+08	1.73E+08	1.95E+08	7.80E+08	+	3.32E+08
KIS	centre	1.92E+08	2.29E+07	3.00E+07	2.45E+08	+	1.03E+08
LAU	centre					+	1.44E+07
lil	littoral	4.12E+07	4.26E+07	1.93E+08	2.76E+08	+	7.02E+07
LIL	centre	4.78E+07	4.58E+07	1.79E+08	2.73E+08	+	
LOV	centre	1.24E+07			1.24E+07	+	3.07E+07
MEK	centre	1.45E+07	1.35E+07		2.81E+07	+	3.32E+06
mrn	littoral	4.52E+07	4.99E+06	8.10E+07	1.31E+08	+	
MRN	centre	6.50E+06	2.38E+06	1.64E+07	2.53E+07	+	
NIM	centre	1.55E+08			1.55E+08	+	1.27E+09
NUS	centre	1.50E+08	4.95E+09		5.10E+09	+	5.97E+07
ROT	centre					+	1.83E+08
SCW	centre					-	6.99E+05
SEE	centre					+	9.05E+07
sgl	littoral	1.54E+08	9.09E+07	8.94E+08	1.14E+09	+	
SGL	centre	3.76E+06	9.99E+06	2.11E+07	3.49E+07	+	
sko	littoral	2.56E+08	5.85E+08	4.45E+08	1.29E+09	+	4.45E+08
SKO	centre	5.68E+07	1.66E+08	2.76E+08	4.99E+08	+	1.45E+08
stv	littoral	1.48E+07	9.24E+07	8.40E+07	1.91E+08	+	5.19E+06
STV	centre	5.46E+06	7.24E+06	3.29E+07	4.56E+07	+	
SYR	centre	6.66E+08	7.63E+08	6.03E+08	2.03E+09	+	9.66E+08
VAL	centre	1.70E+08	5.12E+08	1.15E+09	1.83E+09	+	3.95E+08
WAY	centre	5.11E+07	7.11E+06		5.82E+07	+	4.19E+07