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**Author(s):** Stötter, Tabea; Bastviken, David; Bodelier, Paul L.E.; van Hardenbroek, Maarten; Rinta, Päivi; Schilder, Johannes; Schubert, Carsten J.; Heiri, Oliver

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## **Abundance and $\delta^{13}\text{C}$ values of fatty acids in lacustrine surface sediments: Relationships with in-lake methane concentrations**

Tabea Stötter<sup>a,\*</sup>, David Bastviken<sup>b</sup>, Paul L. E. Bodelier<sup>c</sup>, Maarten van Hardenbroek<sup>a,d</sup>, Päivi Rinta<sup>a</sup>, Jos Schilder<sup>a, e</sup>, Carsten J. Schubert<sup>f</sup>, Oliver Heiri<sup>a</sup>

<sup>a</sup> Institute of Plant Sciences and Oeschger Centre for Climate Change Research, University of Bern, Switzerland

<sup>b</sup> Department of Thematic Studies – Water and Environmental Studies, Linköping University, Linköping, Sweden

<sup>c</sup> Department of Microbial Ecology, Netherlands Institute of Ecology (NIOO-KNAW), Wageningen, The Netherlands

<sup>d</sup> School of Geography, Politics and Sociology, Newcastle University, Newcastle, UK

<sup>e</sup> Department of Biological and Environmental Science, University of Jyväskylä, Jyväskylä, Finland

<sup>f</sup> EAWAG, Surface Waters – Research and Management, Kastanienbaum, Switzerland

\*Corresponding author: [tabea.stoetter@gmail.com](mailto:tabea.stoetter@gmail.com)

**Keywords:** Methane, Fatty acids, Methane oxidizing bacteria, Stable carbon isotopes, aquatic invertebrates, Lakes, Sediment

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28 Highlights

29

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

31 \* Three  $^{13}\text{C}$ -depleted FA groups were correlated with  $\text{CH}_4$  concentration in the lakes

32 \* The results support earlier interpretations that MOB increase with  $\text{CH}_4$  concentrations

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

34

35

36 **Abstract**

37

38 Proxy-indicators in lake sediments provide the only approach by which the dynamics of in-  
39 lake methane cycling can be examined on multi-decadal to centennial time scales. This  
40 information is necessary to constrain how lacustrine methane production, oxidation and  
41 emissions are expected to respond to global change drivers. Several of the available proxies  
42 for reconstructing methane cycle changes of lakes rely on interpreting past changes in the  
43 abundance or relevance of methane oxidizing bacteria (MOB), either directly (e.g. via  
44 analysis of bacterial lipids) or indirectly (e.g. via reconstructions of the past relevance of  
45 MOB in invertebrate diet). However, only limited information is available about the extent to  
46 which, at the ecosystem scale, variations in abundance and availability of MOB reflect past  
47 changes in in-lake methane concentrations. We present a study examining the abundances of  
48 fatty acids (FAs), particularly of  $^{13}\text{C}$ -depleted FAs known to be produced by MOB, relative to  
49 methane concentrations in 29 small European lakes. 39 surface sediment samples were  
50 obtained from these lakes and FA abundances were compared with methane concentrations  
51 measured at the lake surface, 10 cm above the sediments and 10 cm within the sediments.  
52 Three of the FAs in the surface sediment samples,  $\text{C}_{16:1\omega7c}$ ,  $\text{C}_{16:1\omega5c/t}$ , and  $\text{C}_{18:1\omega7c}$  were  
53 characterized by lower  $\delta^{13}\text{C}$  values than the remaining FAs. We show that abundances of  
54 these FAs, relative to other short-chain FAs produced in lake ecosystems, are related with  
55 sedimentary MOB concentrations assessed by quantitative polymerase chain reaction (qPCR).  
56 We observed positive relationships between methane concentrations and relative abundances  
57 of  $\text{C}_{16:1\omega7c}$ ,  $\text{C}_{16:1\omega5c/t}$ , and  $\text{C}_{18:1\omega7c}$  and the sum of these FAs. For the full dataset these  
58 relationships were relatively weak (Spearman's rank correlation ( $r_s$ ) of 0.34 to 0.43) and not  
59 significant if corrected for multiple testing. However, noticeably stronger and statistically  
60 significant relationships were observed when sediments from near-shore and deep-water oxic  
61 environments ( $r_s = 0.57$  to  $0.62$ ) and those from anoxic deep-water environment ( $r_s = 0.55$  to

62 0.65) were examined separately. Our results confirm that robust relationships exist between  
63 in-lake CH<sub>4</sub> concentrations and <sup>13</sup>C-depleted groups of FAs in the examined sediments,  
64 agreeing with earlier suggestions that the availability of MOB-derived, <sup>13</sup>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, although all three  
69 <sup>13</sup>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<sub>4</sub>) is a major greenhouse gas and lakes are an important natural source of CH<sub>4</sub> to  
76 the atmosphere (Bastviken et al. 2011; Bridgman et al. 2013). CH<sub>4</sub> concentrations in lakes are  
77 determined by the rate of biogenic CH<sub>4</sub> production, a process in which methanogens produce  
78 CH<sub>4</sub> using the hydrogenotrophic, acetoclastic, or methylotrophic pathway (Borrel et al. 2011),  
79 and by the rate of CH<sub>4</sub> diffusion and ebullition into the water column and further into the  
80 atmosphere. Another process that can remove CH<sub>4</sub> from lakes is methanotrophy, a microbially  
81 mediated process that oxidizes CH<sub>4</sub> with O<sub>2</sub> or other electron acceptors, like nitrate (Hanson  
82 and Hanson 1996; Brees et al. 2014, Oswald et al. 2016). Most of the CH<sub>4</sub> consumption in  
83 freshwaters seems to be performed by CH<sub>4</sub>-oxidizing bacteria (MOB) under oxic conditions,  
84 which have been estimated to oxidize 30 to 99 % of the CH<sub>4</sub> produced in lakes (Bastviken et  
85 al. 2008). Biogenic CH<sub>4</sub> has a distinctly <sup>13</sup>C-depleted stable carbon isotopic composition, with  
86  $\delta^{13}\text{C}$  values typically between -80 and -50 ‰ (vs. VPDB) (Whiticar et al. 1986; Jedrysek 2005).  
87 MOB, which use CH<sub>4</sub> as their energy and carbon source, incorporate CH<sub>4</sub>-derived carbon into  
88 their biomass. Therefore they are characterized by very negative  $\delta^{13}\text{C}$  values as well, which can  
89 be even lower than those of the original CH<sub>4</sub> due to isotopic fractionation during CH<sub>4</sub> uptake  
90 (Summons et al 1994). Since CH<sub>4</sub> is significantly more depleted in <sup>13</sup>C than other carbon  
91 sources,  $\delta^{13}\text{C}$  is a good tracer for CH<sub>4</sub>-related processes in lakes. For example, modern and  
92 palaeoecological food web studies have used  $\delta^{13}\text{C}$  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<sub>4</sub> 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<sub>4</sub> 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<sub>4</sub> 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<sub>4</sub> 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<sub>4</sub>-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<sub>4</sub> concentrations in lakes, these and  
116 similar approaches may even allow quantitative statements about past changes in CH<sub>4</sub>  
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  $\delta^{13}\text{C}$  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 <sup>13</sup>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  $\delta^{13}\text{C}$  values of

125 these invertebrate fossils have been used to reconstruct past changes in the relevance of CH<sub>4</sub>-  
126 derived carbon for different parts of lacustrine food webs. Furthermore, several studies have  
127 revealed quantitative relationships between  $\delta^{13}\text{C}$  values of these remains and measurements of  
128 CH<sub>4</sub> abundances, suggesting that estimates of past variations in in-lake CH<sub>4</sub> abundance may be  
129 possible based on  $\delta^{13}\text{C}$  analyses of some of these invertebrate groups. However, the  
130 mechanisms that lead from high CH<sub>4</sub> availability in the examined lakes to a higher proportion  
131 of CH<sub>4</sub>-derived C in invertebrate biomass (and their microfossils) are still poorly constrained.  
132 One potential explanation is that CH<sub>4</sub>-rich ecosystems are characterized by higher abundances  
133 of MOB and therefore also a higher availability of CH<sub>4</sub>-derived carbon for filter-feeding and  
134 deposit feeding aquatic invertebrates.

135 Here we present a survey of fatty acid (FA) concentrations and  $\delta^{13}\text{C}$  values in the surface  
136 sediments of 29 lakes from Finland, the Netherlands, Sweden and Switzerland. Concentrations  
137 of <sup>13</sup>C-depleted FAs are compared with CH<sub>4</sub> 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 <sup>13</sup>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<sub>4</sub>-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<sub>4</sub>  
144 concentrations in our dataset. Since the <sup>13</sup>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.

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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  $\delta^{13}\text{C}$  values and variables relevant for determining the overall  $\text{CH}_4$  production, abundance and  
159 emissions of these lakes (e.g. Schilder et al. 2015; Rinta et al. 2015). Therefore,  $\text{CH}_4$   
160 concentrations were 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  $\delta^{13}\text{C}$  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  $0.32 \text{ km}^2$ ), 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

177

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<sub>4</sub> 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<sub>4</sub> concentrations were measured for each coring site 10 cm below the sediment-  
184 water boundary to provide an estimate about CH<sub>4</sub>-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<sub>4</sub> 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<sub>4</sub> without major loss of CH<sub>4</sub>.

189 Surface water CH<sub>4</sub> concentrations were calculated using the headspace equilibration method  
190 (McAuliffe 1971) by applying Henry's law describing gas-water partitioning (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<sub>4</sub> 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<sub>2</sub>-filled glass vials holding 0.2 ml phosphoric acid for CH<sub>4</sub> 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<sub>4</sub>

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<sub>4</sub> 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<sub>4</sub> 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  $\mu\text{mol/l}$  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<sub>2</sub>SO<sub>4</sub> 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<sub>2</sub>SO<sub>4</sub> column again, the FAs were methylated with  
224 10 % BF<sub>3</sub>/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  $\delta^{13}\text{C}$  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).  $\delta^{13}\text{C}$  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  $\mu\text{l}$  lysis buffer [200  $\text{mmol l}^{-1}$   $\text{NaPO}_4$ , pH 7.0; 1% CTAB; 1.5  $\text{mol l}^{-1}$   $\text{NaCl}$ ;  
244 2% Polyvinylpyrrolidone K30; 5  $\text{mg ml}^{-1}$  lysozyme (added right before use)] was added into a  
245 multimix FastPrep tube and incubated at 37 °C for 30 min. MT buffer (122  $\mu\text{l}$ ) was added and  
246 tubes were shaken in the FastPrep instrument (MP, Biomedicals, LLC, Solon, OH, USA) for  
247 30 s at 5.5  $\text{m s}^{-1}$ . Subsequently, samples were centrifuged for 15 min at 10000 rpm and 700  $\mu\text{l}$   
248 supernatant was collected. The pellet was re-extracted by adding lysis buffer (500  $\mu\text{l}$ ) and 50  $\mu\text{l}$   
249 MT buffer to the FastPrep tubes, shaken in the FastPrep instrument for 30 seconds at 5.5  $\text{m s}^{-1}$   
250 again followed by the transfer of the second 700  $\mu\text{l}$  of supernatant into separate Eppendorf  
251 tubes. At this step, 2  $\times$  700  $\mu\text{l}$  supernatant was obtained from each sample. 5  $\mu\text{l}$  of 10  $\text{mg ml}^{-1}$   
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  $\mu\text{l}$  of 7.5  $\text{mol l}^{-1}$  potassium acetate was

255 added, samples were incubated on ice for 5 min and then centrifuged at 10000 rpm for 10 min.  
256 Supernatants ( $2 \times 700 \mu\text{l}$  per sediment sample) were transferred to new tubes,  $700 \mu\text{l}$  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  $500 \mu\text{l}$  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  $500 \mu\text{l}$  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  $50 \mu\text{l}$  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  $50 \mu\text{l}$  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 methanomonooxygenase)-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  $25 \mu\text{l}$  reactions containing  $12.5$   
273  $\mu\text{l}$   $2 \times$  SYBR green mix (AB gene, Epsom, UK),  $2.5 \mu\text{l}$  of diluted DNA template and  $0.8 \text{ mmol}$   
274  $\text{l}^{-1}$  of each primer. The samples were diluted accurately to  $1 \text{ ng } \mu\text{l}^{-1}$ . The thermal cycle started  
275 with an initial denaturation at  $95 \text{ }^\circ\text{C}$  for 15 min, followed by 45 cycles of denaturation at  $95 \text{ }^\circ\text{C}$   
276 for 20 s, annealing at  $64 \text{ }^\circ\text{C}$  for 20 s, and extension at  $72 \text{ }^\circ\text{C}$  for 45 s. Fluorescence was recorded  
277 at  $84 \text{ }^\circ\text{C}$  and DNA melting curve analysis was performed at temperatures ranging from  $70 \text{ }^\circ\text{C}$   
278 to  $99 \text{ }^\circ\text{C}$ . 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<sub>14</sub> to C<sub>22</sub>) for all further analyses, excluding the longer chained FAs (C<sub>24</sub> to C<sub>28</sub>) which  
301 originate from terrestrial organic matter (Meyers 2003).

302 GC-IRMS data were screened to identify FA groups with <sup>13</sup>C-depleted values typical for  
303 organic compounds produced by MOB. All of the <sup>13</sup>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  $^{13}\text{C}$ -depleted FA groups and  $\text{CH}_4$   
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,  $\text{O}_2 < 1 \text{ mg l}^{-1}$ , 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  $\text{C}_{16}$  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 ( $\text{C}_{24}$  to  
329  $\text{C}_{28}$ ), but the overall composition was similar to samples from Western and Central Europe and  
330 *n*- $\text{C}_{16}$  remained the dominant FA. These interregional differences are confirmed by the PCA  
331 analysis based on relative FA abundances (Fig. 2). Longer chain FAs ( $\text{C}_{24}$ ,  $\text{C}_{26}$ ,  $\text{C}_{28}$ ) were all  
332 characterized by positive axis 1 scores and negative axis 2 scores, in agreement with positive

333 axis 1 scores for most of the Fennoscandian samples. C<sub>20</sub> and C<sub>22</sub> also followed a similar  
334 distribution in our dataset as the longer chain FAs. C<sub>22:1</sub> and C<sub>18</sub> were characterized by high  
335 axis 1 and 2 scores, indicating that these compounds had a different distribution in the lake  
336 sediments than the rest of the FAs. FAs with negative axis 1 scores, e.g. C<sub>15</sub>, C<sub>16</sub>, or C<sub>18:2</sub> form  
337 another, more heterogeneous group. The observation that most of the Swiss lake sediment  
338 samples were also characterized by negative axis 1 scores indicates higher relative abundances  
339 of these compounds in the Swiss sediment samples. The littoral sample of Stora Vänstern (stv)  
340 was different in its FA composition from all other samples (Fig. 2). However, this sample was  
341 already identified as potentially contaminated by older sediments and sediment redeposition in  
342 the field and characterized by sandy material. We therefore excluded this sample from further  
343 analyses. The deep-water sample of Glimmingen (GLI), which is plotted in a similar area of the  
344 PCA biplot as stv, contained also sandy material, although to a lesser extent. Although GLI  
345 showed a different FA composition than the other lakes, the sample was not apparent as an  
346 outlier in the relationships between FAs and CH<sub>4</sub> concentrations and therefore retained in  
347 further analyses. Since the aim of the study was to assess whether with higher CH<sub>4</sub>  
348 concentrations <sup>13</sup>C-depleted FA groups become more abundant relative to other FAs typically  
349 produced by aquatic organisms (see Sections 1 and 2) we eliminated longer chain FAs (C<sub>24</sub>,  
350 C<sub>26</sub>, C<sub>28</sub>) originating from terrestrial environments from further analyses.

351 Most of the individual FAs showed a similar range of  $\delta^{13}\text{C}$  values (Fig. 3). However,  
352 C<sub>16:1 $\omega$ 7c</sub>, C<sub>16:1 $\omega$ 5c/t</sub>, and C<sub>18:1 $\omega$ 7c</sub> were more depleted in <sup>13</sup>C values (Fig. 3), with median  $\delta^{13}\text{C}$   
353 values of -45.5 ‰, -59.6 ‰, and -41.5 ‰, respectively. The rest of the analysed FAs were less  
354 <sup>13</sup>C-depleted with median  $\delta^{13}\text{C}$  values between -36.6 and -30.3 ‰. All of these <sup>13</sup>C-depleted  
355 FAs are known to be produced by MOB, although not exclusively (see Section 5). Comparison  
356 of FA abundances with in-lake CH<sub>4</sub> concentrations therefore focused on these <sup>13</sup>C-depleted  
357 FAs. C<sub>16:1 $\omega$ 8c</sub> and C<sub>18:1 $\omega$ 8c</sub>, 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<sub>4</sub> concentrations 10 cm below the sediment surface and C<sub>16:1ω7c</sub> and the sum of <sup>13</sup>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<sub>16</sub> 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<sub>24</sub>-C<sub>30</sub> from waxy coatings of land plants (Meyers  
398 2003). C<sub>22:1</sub>, which together with C<sub>18</sub> 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<sub>18</sub>  
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<sub>24</sub>, C<sub>26</sub>, C<sub>28</sub>) 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<sub>4</sub> concentrations in  
409 lakes.

410 The relatively lower  $\delta^{13}\text{C}$  values of  $\text{C}_{16:1\omega7\text{c}}$ ,  $\text{C}_{16:1\omega5\text{c/t}}$ , and  $\text{C}_{18:1\omega7\text{c}}$  (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.  $\text{C}_{16:1\omega7\text{c}}$ , 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).  $\text{C}_{16:1\omega5\text{c/t}}$  was also found to be associated with MOB type I  
417 (Bodelier et al. 2012), and  $\text{C}_{18:1\omega7\text{c}}$  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).  $\text{C}_{18:1\omega7\text{c}}$  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  $\text{CH}_4$  concentrations, we focused our numerical  
421 analyses on these compounds, which were more depleted in  $^{13}\text{C}$  and were characterized by a  
422  $\delta^{13}\text{C}$  signature typical for organisms incorporating  $\text{CH}_4$ -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  $\text{C}_{16:1\omega7\text{c}}$ ,  $\text{C}_{16:1\omega5\text{c/t}}$ , and  $\text{C}_{18:1\omega7\text{c}}$  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  $\text{CH}_4$  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  $^{13}\text{C}$ -  
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  $^{13}\text{C}$ -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  $\text{C}_{18:1\omega7c}$ . 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  $\text{C}_{18:1\omega7c}$  is produced by all  
452 three MOB types in our study lakes, since, in principle, the apparent correlation of  $\text{C}_{18:1\omega7c}$  to all  
453 MOB types could also result from production by one MOB type only.  $\text{C}_{16:1\omega7c}$  is only related  
454 significantly to MOB type Ib. This suggests that MOB type Ib may be one of the main producers  
455 of  $\text{C}_{16:1\omega7c}$  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  $\text{C}_{16:1\omega5c/t}$  was not clearly related to MOB abundance, although the  
459 depletion in  $^{13}\text{C}$  supports that the compound contains  $\text{CH}_4$ -derived carbon. In general,  $\text{C}_{16:1\omega5c/t}$   
460 was not very abundant in the analysed sediments.  $\text{C}_{16:1\omega5}$  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  $\delta^{13}C$  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  $^{13}\text{C}$ -depleted FAs were observed in oxic deep-water  
490 and littoral sediments (Fig 4).  $\text{CH}_4$  can be oxidized in both oxic and anoxic conditions, but  
491 aerobic  $\text{CH}_4$  oxidation is considered to be the dominant process, oxidizing up to 99 % of  $\text{CH}_4$   
492 produced in lakes (Bastviken et al. 2008; Brees et al. 2014). The growth of aerobic MOB is not  
493 only limited by  $\text{CH}_4$  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  $\text{CH}_4$  availability,  
496 explaining the highest abundances of  $^{13}\text{C}$ -depleted FA groups observed for sediments deposited  
497 under oxic condition. In the lakes where anoxic conditions develop, the  $\text{CH}_4$  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  $\text{CH}_4$  oxidation (Rudd et  
500 al. 1976; Amaral and Knowles 1995) at the sediment-water interface and in stratified, anoxic  
501 lakes  $\text{CH}_4$  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 surficial 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  $^{13}\text{C}$ -depleted  
510 FAs observed for our dataset in oxic samples overlain by relatively  $\text{CH}_4$ -rich water.  
511 The abundances of  $\text{C}_{16:1\omega7c}$  and of the sum of  $^{13}\text{C}$ -depleted FAs in the surficial sediment layers  
512 were robustly correlated with  $\text{CH}_4$  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  $\text{CH}_4$  richness of sediments,  
515 and supply of  $\text{CH}_4$  to the uppermost sediment layers, also promote higher abundances of  $\text{CH}_4$ -  
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  $^{13}\text{C}$ -depleted FA groups  
521 relative to other FAs produced in freshwater ecosystems increases with increasing  $\text{CH}_4$   
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  $\text{CH}_4$ -derived  
524 carbon in lacustrine sedimentary organic matter, originating from MOB, increases with  
525 increasing in-lake  $\text{CH}_4$  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  $^{13}\text{C}$ -depleted FAs. However, our analyses also indicate that  
528 the proportion of  $^{13}\text{C}$ -depleted FA groups was highest in oxic sediment samples deposited in  
529 environments with relatively high  $\text{CH}_4$  concentrations, suggesting that the proportion of  $\text{CH}_4$ -  
530 derived sedimentary organic carbon may also be elevated in these sediments. In contrast, lower  
531 proportions of these  $^{13}\text{C}$ -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  $^{13}\text{C}$ -depleted FA groups and in-lake  $\text{CH}_4$  concentrations are observed for  
534 sediments deposited in oxic and anoxic sediments. This implies that relationships between the  
535 proportion of  $\text{CH}_4$ -derived carbon in aquatic organic matter and  $\text{CH}_4$  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.

538           The robust correlations observed between CH<sub>4</sub> concentrations of the sediments and the  
539 abundance of <sup>13</sup>C-depleted FAs suggest that these relationships are not just with CH<sub>4</sub>  
540 abundances at or above the sediments. Instead, CH<sub>4</sub>-rich lakes, characterized by high CH<sub>4</sub>  
541 production and concentrations in the sediments, seem to be generally characterized by higher  
542 abundances of <sup>13</sup>C-depleted FA groups in the surface sediment, although we again observed  
543 different relationships between the abundance of these FAs and CH<sub>4</sub> concentrations in the  
544 sediments for oxic and anoxic environments.

545           Our findings have two potential implications for approaches reconstructing past changes  
546 in CH<sub>4</sub> availability in lakes based on geochemical analyses of lake sediments. First, they  
547 confirm that higher abundances of <sup>13</sup>C-depleted FAs, from FA groups that are known to be  
548 produced by MOB, can be found in the sediments of small lakes under higher CH<sub>4</sub>  
549 concentrations. This agrees with earlier interpretations that the observed relationships between  
550  $\delta^{13}\text{C}$  values of unspecific deposit- or filter-feeding invertebrate groups and estimates of in-lake  
551 CH<sub>4</sub> abundance (van Hardenbroek et al. 2013; Schilder et al. 2015) can be explained by higher  
552 MOB abundances under higher in-lake CH<sub>4</sub> concentrations. The results therefore support that  
553 reconstructions of  $\delta^{13}\text{C}$  values of CH<sub>4</sub>-sensitive invertebrate groups, such as Chironomini and  
554 *Daphnia*, may provide information on past changes in in-lake CH<sub>4</sub> concentrations in small  
555 temperate lakes, as has been suggested in earlier studies (e.g. van Hardenbroek et al. 2013;  
556 Schilder et al. 2015), since the  $\delta^{13}\text{C}$  values of these organism groups can be expected to be  
557 strongly driven by the availability of <sup>13</sup>C-depleted lacustrine organic matter, which, as our  
558 results suggest, is related to CH<sub>4</sub> concentrations. Second, our results support that lipids  
559 originating from MOB may be more abundant in environments with high CH<sub>4</sub> concentrations  
560 in the water or the sediments, and that lipid records may provide insights into past changes in  
561 CH<sub>4</sub> concentrations in lakes. Although FAs have been reported to decay rapidly in lake  
562 sediments (e.g. Muri and Wakeham 2006) other lipid groups produced by MOB or  
563 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<sub>4</sub> cycling of lakes. However,  
566 our results also suggest that the relationship between the abundance of lipids originating from  
567 MOB and CH<sub>4</sub> 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 <sup>13</sup>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<sub>4</sub> 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<sub>4</sub> concentrations, it is unclear how the observed relationships were  
576 influenced by the production of C<sub>16:1 $\omega$ 7c</sub>, C<sub>16:1 $\omega$ 5c/t</sub>, and C<sub>18:1 $\omega$ 7c</sub> 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<sub>4</sub> 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

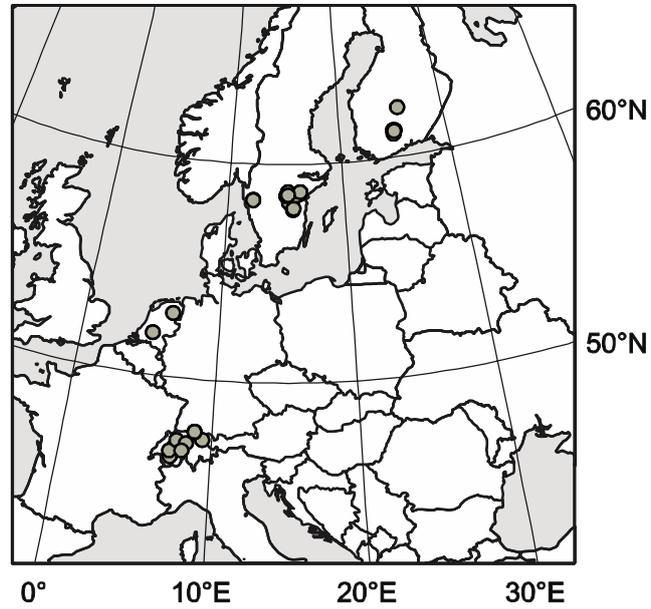


Fig. 1. Study sites located in Finland (6 lakes), the Netherlands (2), Sweden (11) and Switzerland (10).



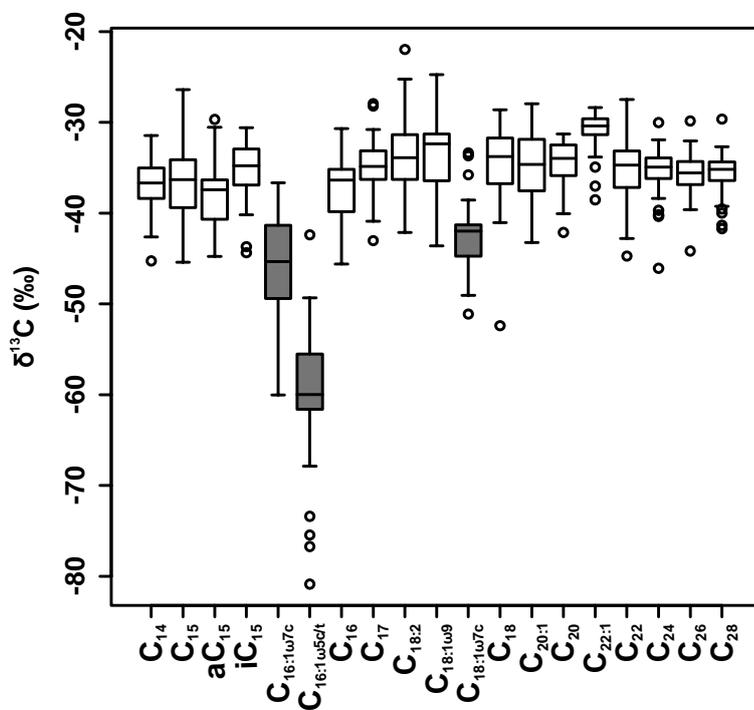
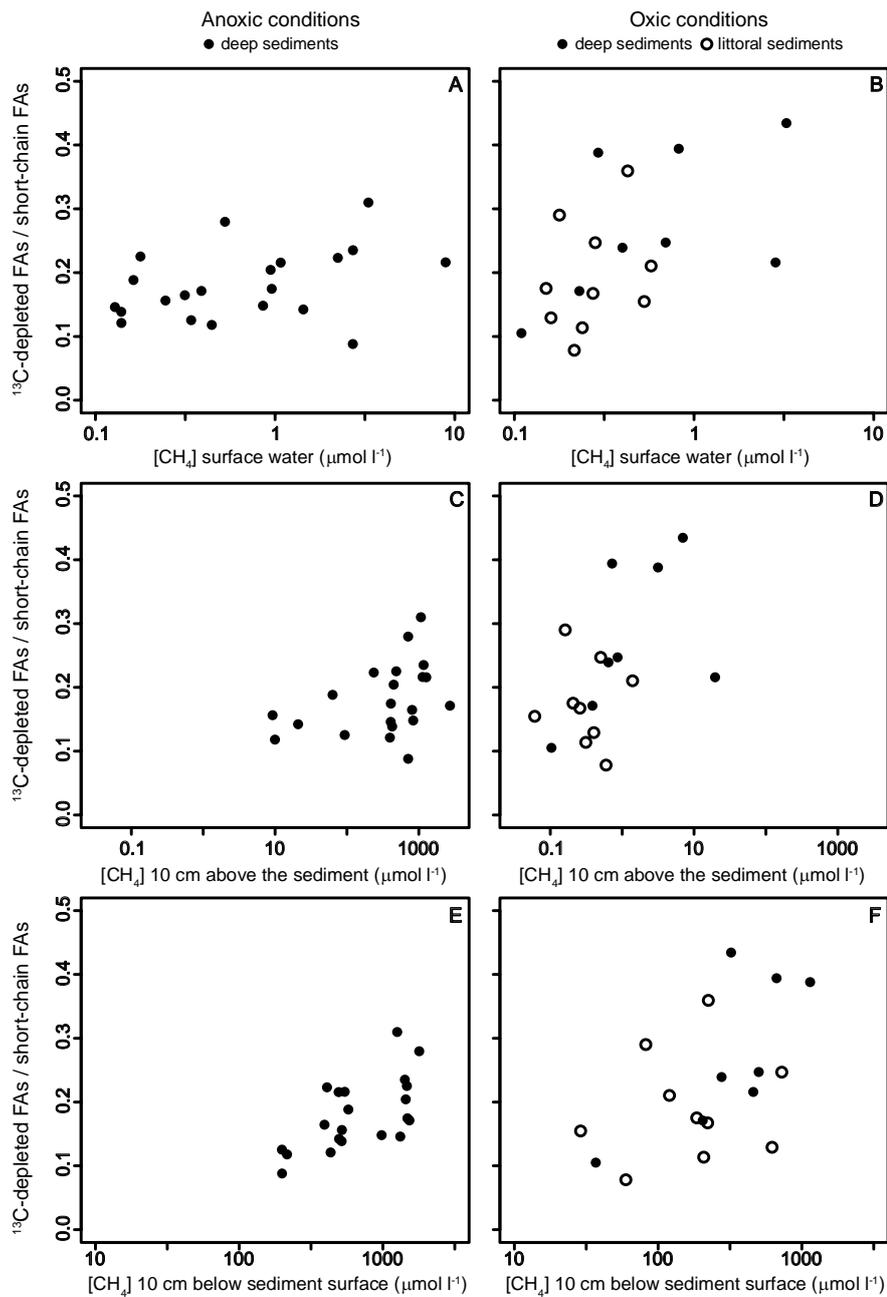


Fig. 3. Boxplots indicating the distribution of FA  $\delta^{13}\text{C}$  values in deep water sediment samples from the 29 examined lakes. Central horizontal lines indicate median values and the bottom and top of boxes the 25th and 75th percentiles, respectively. Circles indicate samples more than 1.5 times the interquartile range above the third and below the first quartile, respectively. Boxplots for the  $^{13}\text{C}$ -depleted FAs that are discussed in detail in the text are marked in grey.



**Fig. 4.** Total abundance of  $^{13}\text{C}$ -depleted FAs ( $\text{C}_{16:1\omega7c}$ ,  $\text{C}_{16:1\omega5c/t}$ , and  $\text{C}_{18:1\omega7c}$ ) in the sediment samples compared with  $\text{CH}_4$  concentrations ( $[\text{CH}_4]$ ) 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<sub>16:1 $\omega$ 7c</sub>, C<sub>16:1 $\omega$ 5c/t</sub>, C<sub>18:1 $\omega$ 7c</sub>, and the sum of these <sup>13</sup>C-depleted FAs.

	C <sub>16:1<math>\omega</math>7c</sub>	C <sub>16:1<math>\omega</math>5c/t</sub>	C <sub>18:1<math>\omega</math>7c</sub>	Sum
<b>MOB Ia</b>	-	-	0.50**	0.42*
<b>MOB Ib</b>	0.42*	-	0.63***	0.53**
<b>MOB II</b>	-	-	0.62**	-
<b>Total MOB</b>	-	-	0.69***	0.45*

**Table 2** Spearman rank correlation ( $r_s$ ) values for relationships between relative abundances of FAs in the sediments and CH<sub>4</sub> 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<sub>4</sub> concentrations are calculated for relative abundances of C<sub>16:1 $\omega$ 7c</sub>, C<sub>16:1 $\omega$ 5c/t</sub>, and C<sub>18:1 $\omega$ 7c</sub>, the sum of these <sup>13</sup>C-depleted FAs.

<b>All lakes</b>	C <sub>16:1<math>\omega</math>7c</sub>	C <sub>16:1<math>\omega</math>5c/t</sub>	C <sub>18:1<math>\omega</math>7c</sub>	<b>Sum</b>
CH <sub>4</sub> surface water	(0.34*)	-	(0.34*)	(0.40*)
CH <sub>4</sub> +10cm	-	-	-	-
CH <sub>4</sub> -10cm	(0.43*)	-	-	(0.36*)
<b>Oxic sediments</b>	C <sub>16:1<math>\omega</math>7c</sub>	C <sub>16:1<math>\omega</math>5c/t</sub>	C <sub>18:1<math>\omega</math>7c</sub>	<b>Sum</b>
CH <sub>4</sub> surface water	0.57*	-	-	0.62*
CH <sub>4</sub> +10cm	-	-	-	0.56*
CH <sub>4</sub> -10cm	0.57 *	(0.51*)	-	0.59*
<b>Anoxic sediments</b>	C <sub>16:1<math>\omega</math>7c</sub>	C <sub>16:1<math>\omega</math>5c/t</sub>	C <sub>18:1<math>\omega</math>7c</sub>	<b>Sum</b>
CH <sub>4</sub> surface water	-	-	0.55*	-
CH <sub>4</sub> +10cm	(0.46*)	-	-	(0.46*)
CH <sub>4</sub> -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	Abbreviation	Country	Lon		Altitude m a.s.l.	Lake area (km <sup>2</sup> )	Max. depth (m)	O <sub>2</sub> bottom (mg l <sup>-1</sup> )	Core depth (m)	Organic matter C/N	CH <sub>4</sub> -10 cm (μmol l <sup>-1</sup> )
			decimal °E	°N							
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	mmn	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	MOB Ib	MOB II	Total MOB	Presence of MOB	Methanogens
		copies per g org C	Nested PCR	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