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

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

[a] Institute of Plant Sciences and Oeschger Centre for Climate Change Research, University of Bern, Switzerland
[b] Department of Thematic Studies – Water and Environmental Studies, Linköping University, Linköping, Sweden
[c] Department of Microbial Ecology, Netherlands Institute of Ecology (NIOO-KNAW), Wageningen, The Netherlands
[d] School of Geography, Politics and Sociology, Newcastle University, Newcastle, UK
[e] Department of Biological and Environmental Science, University of Jyväskylä, Jyväskylä, Finland
[f] EAWAG, Surface Waters – Research and Management, Kastanienbaum, Switzerland

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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, $C_{16:1\omega7c}$, $C_{16:1\omega5c/t}$, and $C_{18:1\omega7c}$ 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 $C_{16:1\omega7c}$, $C_{16:1\omega5c/t}$, and $C_{18:1\omega7c}$ and the sum of these FAs. For the full dataset these relationships were relatively weak (Spearman's rank correlation (r_s) of 0.34–0.43) 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 0.65) were examined separately. Our results confirm that robust relationships exist between in-lake CH_4 concentrations and ^{13}C-depleted groups of FAs in the examined sediments, agreeing with earlier suggestions that the availability of MOB-derived, ^{13}C-depleted organic matter for aquatic invertebrates increases with increasing methane concentrations. However, we also show that these relationships are complex, with different relationships observed for oxic and anoxic sediments and highest values measured in sediments deposited in oxic environments overlain with relatively methane-rich water. Furthermore, although all three ^{13}C-depleted FA groups identified in our survey are known to be produced by MOB, they also receive contributions by other organism groups, and this will have influenced their distribution in our dataset.

* Corresponding author.
E-mail address: tabea.stoetter@gmail.com (T. Stötter).

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1. Introduction

Methane (CH_4) is a major greenhouse gas and lakes are an important natural source of CH_4 to the atmosphere (Bastviken et al., 2011; Bridgham et al., 2013). CH_4 concentrations in lakes are determined by the rate of biogenic CH_4 production, a process in which methanogens produce CH_4 using the hydrogenotrophic, acetoclastic, or methylotrophic pathway (Borrel et al., 2011), and by the rate of CH_4 diffusion and ebullition into the water column and further into the atmosphere. Another process that can remove CH_4 from lakes is methanotrophy, a microbially mediated process that oxidizes CH_4 with O_2 or other electron acceptors, like nitrate (Hanson and Hanson, 1996; Blees et al., 2014; Oswald et al., 2016). Most of the CH_4 consumption in freshwaters seems to be performed by CH_4-oxidizing bacteria (MOB) under oxic conditions, which have been estimated to oxidize 30–99% of the CH_4 produced in lakes (Bastviken et al., 2008). Biogenic CH_4 has a distinctly ^{13}C-depleted stable carbon isotopic composition, with $\delta^{13}C$ values typically between −80 and −50‰ (vs. VPDB) (Whiticar et al., 1986; Jedrysek, 2005). MOB, which use CH_4 as their energy and carbon source, incorporate CH_4-derived carbon into their biomass. Therefore they are characterized by very negative $\delta^{13}C$ values as well, which can be even lower than those of the original CH_4 due to isotopic fractionation during CH_4 uptake (Summons et al., 1994). Since CH_4 is significantly more depleted in ^{13}C than other carbon sources, $\delta^{13}C$ is a good tracer for CH_4-related processes in lakes. For example, modern and palaeoecological food web studies have used $\delta^{13}C$ analysis to assess the importance of MOB as a food source for aquatic invertebrates in lakes (e.g. Sanseverino et al., 2012; Schilder et al., 2013; Grey, 2016).

Systematic field surveys constraining CH_4 production, abundance and emissions in lakes have only been developed very recently (e.g. Rasilo et al., 2015; Yang et al., 2015; Rinta et al., 2017). Since long instrumental time series are lacking it is challenging to predict how and at which time scales lacustrine CH_4 production and emission will respond to future environmental pressures such as global warming and widespread eutrophication and reoligotrophication of inland waters. Proxy-based reconstructions of past changes in lacustrine carbon cycling have been explored as alternative approaches for constraining how the carbon cycle of lakes, and particularly CH_4 production, oxidation, and uptake in the food web, respond to environmental change. Such studies are particularly relevant for assessing how these processes react on multi-decadal to centennial timescales to global change drivers such as increasing air and water temperatures and anthropogenic nutrient release, since these timescales are not covered by instrumental measurements.

Proxy-based approaches used to constrain past changes in CH_4 availability, production and oxidation in lakes have been based either on geochemical measurements on specific organic microfossil groups (e.g. Wooller et al., 2012; Belle et al., 2014; Rinta et al., 2016; Schilder et al., 2017) or lipid groups (e.g. Bechtel and Schubert, 2009; Hollander and Smith, 2001; Naeher et al., 2014; Davies et al., 2016) that are expected to be related either in their carbon isotopic composition or abundance (or both) with MOB or CH_4-producing microorganisms in lakes. Furthermore, ancient DNA (aDNA) analyses of lake sediments have recently been used to constrain past changes in MOB (Belle et al., 2014). If absolute or relative abundances of these microorganisms are in turn systematically related to CH_4 concentrations in lakes, these and similar approaches may even allow quantitative statements about past changes in CH_4 abundance in lakes based on lacustrine proxy records (e.g. van Hardenbroek et al., 2013; Schilder et al., 2015; Elvert et al., 2016). Recent studies have demonstrated that $\delta^{13}C$ analyses of chitinous remains of aquatic invertebrates may have considerable potential in this respect. Several aquatic invertebrate groups can feed on MOB or other microorganisms such as ciliates feeding on them (e.g. Kankaala et al., 2006; Deines et al., 2007; Deines and Fink, 2011; Jones and Grey, 2011), which leads to strongly ^{13}C-depleted isotope signatures in their chitinous remains (e.g. resting egg sheaths, exoskeleton parts) preserved in lake sediments (Wooller et al., 2012; Belle et al., 2014; Schilder et al., 2015). Reconstructions of changes in $\delta^{13}C$ values of these invertebrate fossils have been used to reconstruct past changes in the relevance of CH_4-derived carbon for different parts of lacustrine food webs. Furthermore, several studies have revealed quantitative relationships between $\delta^{13}C$ values of these remains and measurements of CH_4 abundances, suggesting that estimates of past variations in in-lake CH_4 abundance may be possible based on $\delta^{13}C$ analyses of some of these invertebrate groups. However, the mechanisms that lead from high CH_4 availability in the examined lakes to a higher proportion of CH_4-derived C in invertebrate biomass (and their microfossils) are still poorly constrained. One potential explanation is that CH_4-rich ecosystems are characterized by higher abundances of MOB and therefore also a higher availability of CH_4-derived carbon for filter-feeding and deposit feeding aquatic invertebrates.

Here we present a survey of fatty acid (FA) concentrations and $\delta^{13}C$ values in the surface sediments of 29 lakes from Finland, the Netherlands, Sweden and Switzerland. Concentrations of ^{13}C-depleted FAs are compared with CH_4 concentration estimates in the open water column, above the sediments and in deeper sediment layers (10 cm below the sediment surface). We focus on ^{13}C-depleted FA groups that are produced by MOB but also receive contributions from other organism groups. If there is a higher contribution of MOB to particulated organic matter in the water column and sedimentary organic matter in surface sediments in CH_4-rich systems, as speculated in earlier studies (e.g. van Hardenbroek et al., 2013; Schilder et al., 2013), we expect to see positive relationships between relative abundances of these FAs and CH_4 concentrations in our dataset. Since the ^{13}C-depleted FA groups in our dataset are not strictly limited to MOB we also assess the abundance of MOB in the examined sediments using quantitative polymerase chain reaction (qPCR) to support our interpretations.

2. Study sites and study setup

Surface sediments (0–2 cm) were collected from the deepest part of 29 lakes across Europe, including 2 Dutch (NL), 6 Finnish (FI), 11 Swedish (SE) and 10 Swiss (CH) lakes (Fig. 1). The lakes were sampled in a multi-year campaign and sites were selected to cover a range of small lake types on different bedrocks and with variable water chemistry conditions (e.g. in respect to transparency, pH, conductivity, deepwater oxygen concentrations) in Northern Europe (Finland, Sweden), Western Europe (The Netherlands) and Central Europe (Switzerland). The aim of the survey was to assess whether quantitative relationships existed between invertebrate $\delta^{13}C$ values and variables relevant for determining the overall CH_4 production, abundance and emissions of these lakes (e.g. Schilder et al., 2013; Rinta et al., 2015). Therefore, CH_4 concentrations where measured in the open water column, just above the coring site, and deeper within the sediments (i.e. at 10 cm depth) rather than within the sediment samples that were analysed for invertebrate $\delta^{13}C$ values and that are available for the FA analyses presented in this study. Most of the lakes were thermally stratified and developed anoxic conditions during the summer months. To expand the number of sediment samples accumulated under oxic conditions, near-shore surface sediments from the littoral zone were also analysed for 11 Swedish lakes (lake abbreviations in small

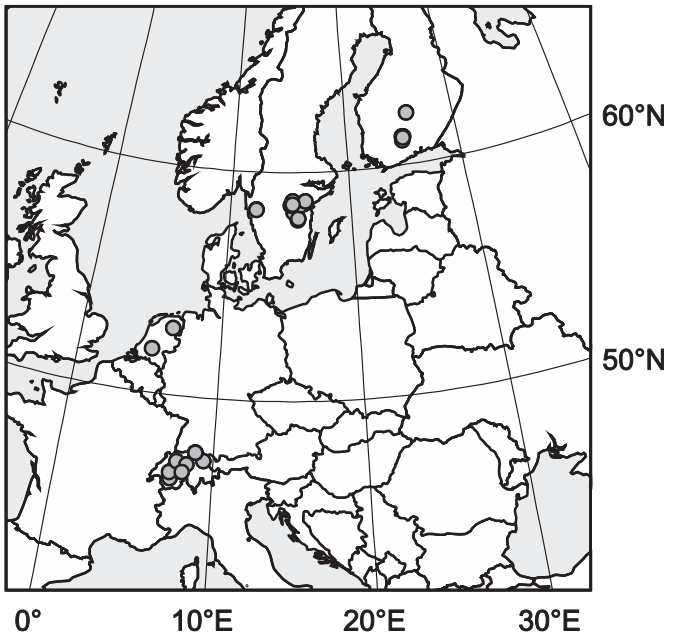

Fig. 1. Study sites located in Finland (6 lakes), the Netherlands (2), Sweden (11) and Switzerland (10).

letters). All of these samples were located above the oxycline. The lakes are all relatively small (average surface area 0.32 km^2), shallower than 32 m, and characterized by variable nutrient concentrations and mixing conditions (Suppl. Table 1). The sampling of the lakes took place during late summer stratification (August–September) in 2010 in Sweden and 2011 in Finland, the Netherlands, and Switzerland. More details on the study lakes and the environmental variables measured during fieldwork are available in Rinta et al. (2015).

3. Methods

3.1. Water chemistry

The lakes were analysed for basic limnological variables including temperature and oxygen profiles, pH and conductivity as well as total nitrogen, total phosphorus and dissolved inorganic carbon (DIC) concentrations in the surface and/or bottom water (presented in detail in Rinta et al., 2015). CH_4 concentrations were measured both in the lake centre and near the shore for surface water (5 cm below water surface), and 10 cm above the sediment-water interface. In addition, CH_4 concentrations were measured for each coring site 10 cm below the sediment-water boundary to provide an estimate about CH_4-richness of the sediments of the different lakes. Samples 10 cm below the sediment surface rather than the 0–2 cm surface sediment sample were collected, since these reflect CH_4 levels in sediments deeper than the surficial zone of oxidation. Furthermore, these sediments were already consolidated and could be transferred to a container for expulsing and subsampling the CH_4 without major loss of CH_4.

Surface water CH_4 concentrations were calculated using the headspace equilibration method (McAuliffe, 1971) by applying Henry's law describing gas-water portioning (Stumm and Morgan, 1996, see methods in Bastviken et al., 2010). The water was sampled with 60 ml syringes 5 cm below the surface in the deep and littoral part of the lakes. 40 ml of water was equilibrated with 20 ml ambient air in the syringes (60 s shaking). A sample of the equilibrated headspace was injected into a glass vial pre-filled with saturated brine solution. Ambient air was collected to correct for background air CH_4 concentrations. Water 10 cm above the sediment surface was sampled from gravity cores with an intact sediment surface both in the lake centre and near-shore environments using a small tube connected to a syringe with a three-way luer-lock valve (Rinta et al., 2015). After rinsing the tube several times and removing any gas bubbles, 60 ml of water were collected above the sediment in the core and injected into 118 ml N_2-filled glass vials holding 0.2 ml phosphoric acid for CH_4 measurements. A standard volume of sediment from 10 cm depth in the cores was collected and rapidly transferred to an airtight 130 ml flask and 45 ml lake water, equilibrated with ambient air in terms of CH_4 concentrations, was added with a syringe attached to a valve in the cap (Rinta et al., 2015). After shaking to force the CH_4 from the sediment into the headspace, 45 ml of the headspace was sampled through the valve and injected into a 50 ml glass vial pre-filled with saturated brine solution, using a second needle to partly drain the brine solution. CH_4 concentrations were measured by gas chromatography with flame ionization detector (GC-FID, Agilent 6890 N, Plot Q capillary column for water samples from Finnish lakes and Shimadzu GC-8, Poropak N column for the others) with a repeatability error below 1.4% for measurements around 100 ppm, 1.7% around 500 ppm and 0.4% around 1000 ppm (Rinta et al., 2015, 2017). Conversions to μmol/l units were made using Henry's law and the common gas law as explained in Bastviken et al. (2008). Concentrations were expressed per unit volume water for samples taken in the water column and per unit volume of wet sediment for sediment samples.

3.2. FA analysis

Sediments for FA analysis were frozen in the field, and stored frozen in the dark until they were freeze-dried in the laboratory. Ca. 1 g freeze-dried sediment was extracted with dichloromethane/methanol (MeOH) in a micro-wave (Anton Paar) and by ultrasonication. Traces of water were removed by running the extract over a Na_2SO_4 column and sulphur was removed using a Cu column. The extract was saponified with 6% KOH in MeOH for 3 h at 80 °C. The acid fraction was extracted from the aqueous phase after the addition of HCl until a pH below 2. After removing water with a Na_2SO_4 column again, the FAs were methylated with 10% BF_3/MeOH to produce methyl esters (FAMEs) (2 h at 100 °C). Lipid concentrations were examined using gas chromatography with flame ionization (GC FID, Schimadzu GC-2010 Plus with Inert Caps 5MS/NP column). Individual compounds were identified with gas chromatography-mass spectrometry (GC-MS, Schimadzu GCMS-QP, 2010 Ultra with phenomenex Zebron phase ZB-5MSi column), based on the retention time and comparison to published mass spectra. Compound specific $\delta^{13}C$ values (‰VPDB) were obtained with GC-IRMS (Agilent Technologies 6890 N with Restek RXi 5 ms column and IsoPrime micromass IRMS) with an analytical error below 1.1‰ (*n*-C25 alkane standard). $\delta^{13}C$ values of FAs were corrected for methylation by examining the methylation-influence on

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*

a standard FA (lauric acid). For identification of multiple bond positions in FAs, an aliquot was derivatised to form 2-alkenyl-4,4-dimethoxyloxazline (DMOX) derivatives. These DMOX derivatives were measured with GC-MS (Schimadzu GCMS-QP, 2010 Ultra with phenomenex Zebron phase ZB-5MSi column) and double bond position identified by comparing the results with published mass spectra and following the rule of Spitzer (1997).

3.3. Molecular analysis

3.3.1. DNA extraction

DNA was extracted using a modification of the method described by Pan et al. (2010) based on the FastDNA spin kit for soil (MP Biomedicals, LLC, Solon, OH, USA). Freeze-dried sediment (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; 2% Polyvinylpyrrolidone K30; 5 mg ml^{-1} lysozyme (added right before use)] was added into a multimix FastPrep tube and incubated at 37 °C for 30 min. MT buffer (122 μl) was added and tubes were shaken in the FastPrep instrument (MP, Biomedicals, LLC, Solon, OH, USA) for 30 s at 5.5 m s^{-1}. Subsequently, samples were centrifuged for 15 min at 10,000 rpm and 700 μl supernatant was collected. The pellet was re-extracted by adding lysis buffer (500 μl) and 50 μl MT buffer to the FastPrep tubes, shaken in the FastPrep instrument for 30 s at 5.5 m s^{-1} again followed by the transfer of the second 700 μl of supernatant into separate Eppendorf tubes. At this step, 2 × 700 μl supernatant was obtained from each sample. 5 μl of 10 mg ml^{-1} freshly made proteinase K was added to each tube. Tubes were incubated at 65 °C for 30 min. Samples were extracted with phenol-chloroform-isoamyl alcohol (25:24:1), followed by a chloroform-isoamyl alcohol (24:1) extraction. 125 μl of 7.5 mol l^{-1} potassium acetate was added, samples were incubated on ice for 5 min and then centrifuged at 10,000 rpm for 10 min. Supernatants (2 × 700 μl per sediment sample) were transferred to new tubes, 700 μl Binding Matrix was added and tubes were mixed for 5 min on a rotator. Binding Matrix, with bound DNA, was pelleted by 1 min centrifugation at 10,000 rpm. The supernatant was discarded and the pellet was resuspended in 500 μl wash buffer. The resulting suspension was added into a Spinfilter, and centrifuged for 1 min at 10,000 rpm. The eluate was discarded and the pellet was washed again in 500 μl wash buffer. After discarding the second eluate, the Spinfilter was centrifuged for another 10 s to dry the pellet. The filter was taken into a new tube and 50 μl of TE pH 8.0 was added. The filter was incubated at room temperature for 1 min and centrifuged for 1 min. The filter was re-eluted in the same way with 50 μl of TE pH 8.0.

3.3.2. Quantitative PCR (qPCR)

Three methanotrophic sub-groups (Ia, Ib, and II) were quantified by pmoA (particulate methanemonooxygenase)-based quantitative PCR based on the assays described by Kolb et al. (2003). The type Ia and II assays were carried out as described by Bodelier et al. (2009b). For the type Ib assay, DNA standards were prepared by dilution of a known amount of PCR product amplified from a reference clone (clone WPBN2, accession KF395333) by using the 189- Mc468 primer set (Kolb et al., 2003). PCRs were carried out in 25 μl reactions containing 12.5 μl 2 × SYBR green mix (AB gene, Epsom, UK), 2.5 μl of diluted DNA template and 0.8 mmol l^{-1} of each primer. The samples were diluted accurately to 1 ng μl^{-1}. The thermal cycle started with an initial denaturation at 95 °C for 15 min, followed by 45 cycles of denaturation at 95 °C for 20 s, annealing at 64 °C for 20 s, and extension at 72 °C for 45 s. Fluorescence was recorded at 84 °C and DNA melting curve analysis was performed at temperatures ranging from 70 °C to 99 °C. All of three assays were performed with a Rotor Gene 6000 thermal cycling system (Corbett Research, Eight Mile Plains, Qld, Australia), where samples were added to aliquots of the master mixture using a CAS-1200 (Corbett Robotics Eight Mile Plains, Qld, Australia) liquid handling system. Every sample was performed in duplicate. Quantification analysis was performed by the RotorGene software. Copy numbers were calculated assuming a PCR product length of 412 bp, 279 bp and 423 bp for type Ia, type Ib and type II methanotrophs, respectively.

3.4. Data treatment and analysis

The examined sediments varied in respect to composition (e.g. carbonate and organic content), texture (fine grained versus coarse, consolidated versus unconsolidated) as well as expected sedimentation rates, with Fennoscandian lakes usually characterized by much lower sedimentation rates than Swiss hardwater lakes (e.g., Lotter et al., 1997; van Hardenbroek et al., 2014; Rinta et al., 2016). Since these factors can strongly affect absolute abundances of sediment components, FAs were expressed as proportion (relative abundances) relative to the sum of other FAs identified by GC-FID/GC-MS.

Variations in the composition of different FA samples were initially examined by principal component analysis (PCA). Variables were centred and analysed using the program Canoco for Windows 4.5 (Ter Braak and Smilauer, 2002). This initial analysis revealed systematic differences in the importance of FAs originating from terrestrial environments between samples from different study regions (see Section 4.1). Since terrestrial input can strongly affect relative abundance data of lacustrine sediment components, and the main interest of this study was on the aquatic components, FA abundances were expressed relative to the sum of short-chain FAs (C_{14} to C_{22}) for all further analyses, excluding the longer chained FAs (C_{24} to C_{28}) which originate from terrestrial organic matter (Meyers, 2003).

GC-IRMS data were screened to identify FA groups with ^{13}C-depleted values typical for organic compounds produced by MOB. All of the ^{13}C-depleted FA groups found in our survey are known to be produced by MOB, but also by other organism groups (see Sections 4 and 5). To confirm that in our dataset they originated to a significant extent from MOB we compared the abundances of these FAs with the number of DNA copies (*pmoA* gene) per g organic matter (org C) measured for different MOB types using Spearman's rank correlation (r_s) and associated p values. DNA copies were expressed relative to total organic carbon content (TOC) to reduce the effects of variable proportions of inorganic sediment components (e.g. clay, silt, sand or autochthonous carbonates) on the results. Organic matter content was measured using loss on ignition at 550 °C (Heiri et al., 2012).

The relationship between relative abundances of ^{13}C-depleted FA groups and CH_4 concentrations was again assessed by calculating r_s and associated p values. r_s values were calculated for the entire dataset but also separately for two categories of sediments: sediments deposited in anoxic deepwater sections of the lake basins (referred to as anoxic sediments samples, $O_2 < 1$ mg l^{-1}, 0.5 m above sediment surface), and oxic deepwater sediments together with near-shore water samples (referred to as oxic sediment samples; see Suppl. Table 1).

Correlations (r_s values) were calculated with the program PAST (Hammer et al., 2001). The results were corrected for multiple testing using the False Discovery Rate method (FDR; Benjamini and Hochberg, 1995) as described in Garcia (2004).

4. Results

4.1. Biomarker composition

The FA composition showed a strong even over odd predominance and maxima at *n*-C_{16} in most of the samples. There were

clear differences in the FA composition between the study areas. The Fennoscandian lakes showed a higher proportion of longer chain FAs (C_{24} to C_{28}), but the overall composition was similar to samples from Western and Central Europe and *n*-C_{16} remained the dominant FA. These interregional differences are confirmed by the PCA analysis based on relative FA abundances (Fig. 2). Longer chain FAs (C_{24}, C_{26}, C_{28}) were all 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

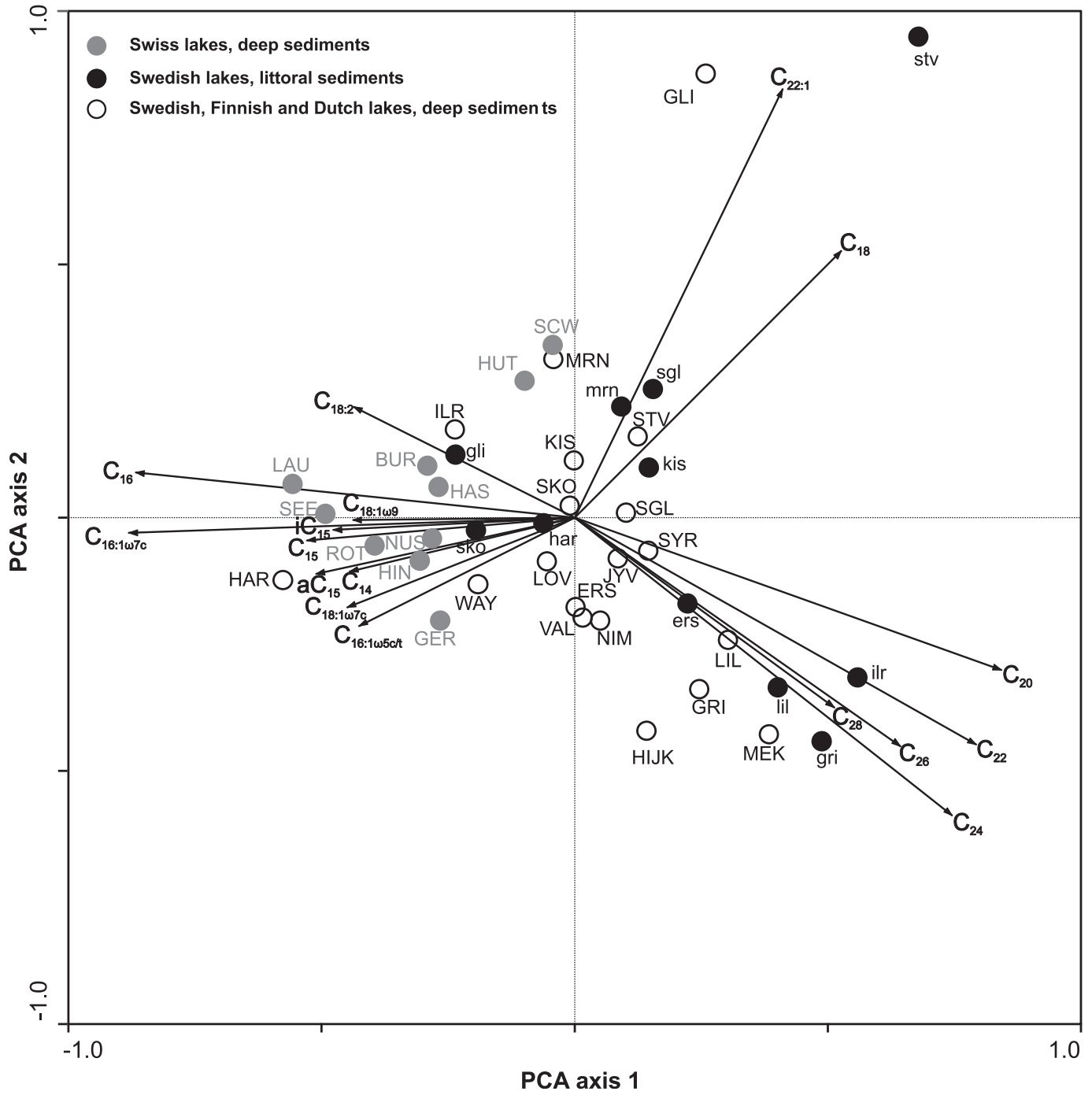

Fig. 2. Principal components analysis (PCA) summarizing variations in relative abundances of FAs in the surface sediment samples. Site names in capital and lowercase letters refer to samples taken in the deepest parts of lakes and littoral samples, respectively.

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 (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., 2009a), were not found in the examined lake sediments or the peak areas of these specific FAs were too low to be quantified and analysed reliably.

The qPCR analyses successfully quantified the number of DNA copies of total MOB in 34 of the examined sediment samples, MOB type Ia in 34, MOB type Ib in 30 and MOB type II in 25 (Supplementary Table S2). However, nested PCR revealed that MOB were present in all but one of the sediment samples. Comparison of the relative abundances of $C_{16:1\omega7c}$, $C_{16:1\omega5c/t}$, and $C_{18:1\omega7c}$ with DNA copies of MOB in the sediments (Table 1) confirmed that $C_{16:1\omega7c}$ was significantly correlated with the abundance of MOB Ib and $C_{18:1\omega7c}$ with MOB Ia, MOB Ib, MOB II and the sum of MOB DNA copies. The sum of the ^{13}C-depleted FAs correlated with MOB type Ia, MOB type Ib and the total number of DNA copies of MOB. $C_{16:1\omega5c/t}$ was not correlated with the overall abundance of any MOB type.

Over the entire dataset, relative abundances of $C_{16:1\omega7c}$, $C_{18:1\omega7c}$, and the sum of ^{13}C-depleted FAs showed weak positive correlations ($r_s = 0.34–0.40$) with surface water CH_4 concentrations (Table 2). $C_{16:1\omega7c}$ and the sum of ^{13}C-depleted FAs were furthermore correlated with CH_4 concentrations in the sediments ($r_s = 0.36–0.43$). However, if the results were corrected for multiple testing none of these relationships remained significant.

If only oxic sediments were examined, r_s values were distinctly higher (0.51–0.62). Both surface water and sedimentary CH_4 concentrations were correlated with $C_{16:1\omega7c}$. Furthermore, the sum of the ^{13}C-depleted FAs was correlated with CH_4 concentrations in the surface water, 10 cm above and 10 cm below the sediment surface. $C_{16:1\omega5c/t}$ was also correlated with CH_4 concentrations in the sediments, but this relationship was no longer significant after correction for multiple testing (Table 2).

For anoxic sediment samples correlations with the abundances of ^{13}C-depleted FAs were also stronger ($r_s = 0.46–0.65$) than observed for the entire dataset. For this group of samples, $C_{18:1\omega7c}$ was correlated with surface water CH_4 concentrations ($r_s = 0.55$) and $C_{16:1\omega7c}$ and the sum of ^{13}C-depleted FAs were correlated with CH_4 concentrations in the lake water 10 cm above the sediments,

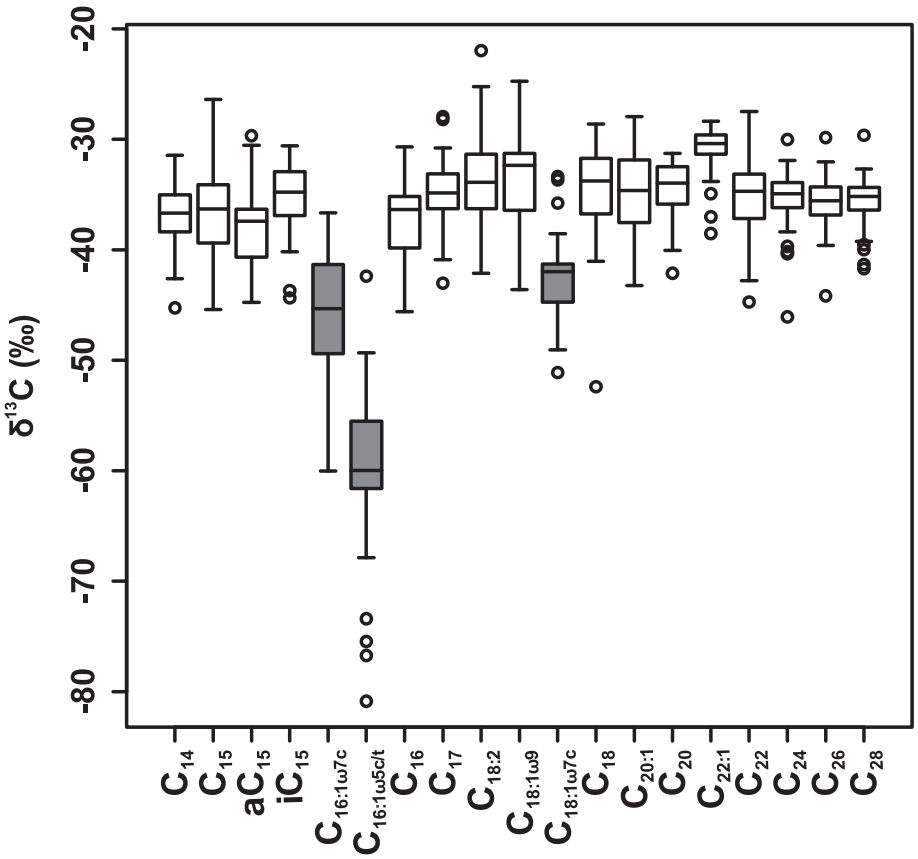

Fig. 3. Boxplots indicating the distribution of FA $\delta^{13}C$ values in deep water sediment samples from the 29 examined lakes. Central horizontal lines indicate median values and the bottom and top of boxes the 25th and 75th percentiles, respectively. Circles indicate samples more than 1.5 times 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.

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.

	$C_{16:1\omega7c}$	$C_{16:1\omega5c/t}$	$C_{18:1\omega7c}$	Sum
All lakes				
CH_4 surface water	(0.34*)	–	(0.34*)	(0.40*)
CH_4 +10 cm	–	–	–	–
CH_4-10 cm	(0.43*)		–	(0.36*)
Oxic sediments				
CH_4 surface water	0.57*	–	–	0.62*
CH_4 +10 cm	–	–	–	0.56*
CH_4 -10 cm	0.57*	(0.51*)	–	0.59*
Anoxic sediments				
CH_4 surface water	–	–	0.55*	–
CH_4 +10 cm	(0.46*)	–	–	(0.46*)
CH_4-10 cm	0.65**	–	–	0.61**

although the latter two relationships were no longer significant after correcting for multiple testing. The strongest relationships for anoxic sediments were apparent between CH_4 concentrations 10 cm below the sediment surface and $C_{16:1\omega7c}$ and the sum of ^{13}C-depleted FAs ($r_s = 0.61–0.65$).

5. Discussion

5.1. FA composition of sediments

The high content of short-chain FAs, with a classical even over odd predominance, and maxima at *n*-C_{16} in the FA composition of the sediment samples, indicates a predominantly autochthonous organic matter production (Stefanova and Disnar, 2000; Woszczyk et al., 2011). The longer-chain FAs, with high axis 1 values in the PCA analysis (Fig. 2), are reported to be derived from terrestrial sources, for example C_{24}-C_{30} from waxy coatings of land plants (Meyers, 2003). $C_{22:1}$, which together with C_{18} shows a different distribution than the rest of the FAs, is known to be produced by zooplankton, copepods and higher plants (Pearson et al., 2007). C_{18} may originate from many sources, mainly freshwater algae (Meyers, 2003). The more heterogeneous group of FAs with negative axis 1 values in the PCA is known to have multiple, mainly autochthonous sources (Pearson et al., 2007; Woszczyk et al., 2011). PCA analysis indicates that FA assemblages from Swiss lakes are generally more strongly influenced by FAs from autochthonous sources, while the Fennoscandian lakes and especially some littoral samples show high relative abundances of terrestrial FAs. As mentioned above (Section 3.4) we therefore eliminated longer-chain FAs (C_{24}, C_{26}, C_{28}) originating from terrestrial environments from further analyses of FA abundances to reduce the effects of varying terrestrial influences on our comparisons between relative abundances of FAs and CH_4 concentrations in lakes.

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 these FAs are, at least partly, produced by organisms incorporating isotopically light carbon, such as MOB. Literature sources confirm that they are produced by MOB, though not exclusively. $C_{16:1\omega7c}$, for example, was found to be associated with MOB type I, and also some MOB type II (Boschker et al., 1998; Bodelier et al., 2009a, 2012). However, this FA has also been reported for phytoplankton, zooplankton, fungi, mycobacteria and higher plants (Volkman et al., 1980; Woszczyk et al., 2011). $C_{16:1\omega5c/t}$ was also found to be associated with MOB type I (Bodelier et al., 2012), and $C_{18:1\omega7c}$ with MOB type II (Bowman et al., 1993; Deines et al., 2007; Bodelier et al., 2009a) and some MOB type Ia (Bodelier et al., 2009a). $C_{18:1\omega7c}$ was also reported to be present in some other bacteria (Zegouagh et al., 2000). As we were mainly interested in relationships between MOB-derived FAs and CH_4 concentrations, we focused our numerical analyses on these compounds, which were more depleted in ^{13}C and were characterized by a $\delta^{13}C$ signature typical for organisms incorporating CH_4-derived carbon. Other chemotrophs, possibly abundant in stratified lakes at the chemocline, have also been reported to produce isotopically light lipids (Enrich-Prast et al., 2009; Zemskaya et al., 2012). However, the highest abundances of $C_{16:1\omega7c}$, $C_{16:1\omega5c/t}$, and $C_{18:1\omega7c}$ were observed in oxic sediment samples and therefore, above the oxycline (and above any existing chemocline) in the lakes (Fig. 4). Also, the correlations of these FAs with MOB concentrations of the sediments (Table 1), and the correlations of the relative abundances of these FAs with in-lake CH_4 concentrations in our dataset (Table 2) support that MOB are a relevant source of these FAs in our study lakes.

5.2. Relationships between FA and MOB abundances

The distribution of MOB types I and II across the entire set of analysed lakes differs from analyses of terrestrial soils where type II usually dominates (Pan et al., 2010; Bodelier et al., 2013). This difference has been observed in freshwater sediments before (Borrel et al., 2011), where MOB type II are less dominant and sometimes even absent (Sundh et al., 2005; Schubert et al., 2010). Some of the genetic clusters belonging to the abundant type Ib MOB have been designated as typical freshwater MOB, only being found in these habitats (Lüke and Frenzel, 2011; Knief, 2015).

Relatively strong correlations ($r_s = 0.42–0.69$) are apparent between relative abundances of ^{13}C-depleted FAs and all three MOB groups (Ia, Ib, II), as well as with the sum of MOB. These relationships are apparent even though different confounding processes potentially affect the relative abundance data of FAs (e.g. in-lake production rates of short chain FAs by organisms other than MOB) and the absolute abundances of MOB DNA expressed relative to the organic content of the sediments (e.g. sediment homogeneity, variable accumulation rates, dilution by terrestrial organic matter). The observed relationships confirm that in our dataset abundances of ^{13}C -depleted FAs are positively related with abundances of MOB as quantified by qPCR.

The strongest and most consistent correlations were observed between MOB abundances and $C_{18:1\omega7c}$. However, the abundances of the different MOB types were strongly inter-correlated ($r_s = 0.67–0.83$). Therefore, this finding does not necessarily indicate that $C_{18:1\omega7c}$ is produced by all three MOB types in our study lakes, since, in principle, the apparent correlation of $C_{18:1\omega7c}$ to all MOB types could also result from production by one MOB type only. $C_{16:1\omega7c}$ is only related significantly to MOB type Ib. This suggests that MOB type Ib may be one of the main producers of $C_{16:1\omega7c}$ in the studied ecosystems. However, significant amounts of this FA have been demonstrated to be produced also by MOB type Ia as well as type II (Bowman et al., 1991; Bodelier et al., 2009a).

The relative abundance of $C_{16:1\omega5c/t}$ was not clearly related to MOB abundance, although the depletion in ^{13}C supports that the compound contains CH_4-derived carbon. In general, $C_{16:1\omega5c/t}$ was not very abundant in the analysed sediments. $C_{16:1\omega5}$ is not abundant in Methylobacter, which is the type Ia genus mostly dominating freshwater sediments (Borrel et al., 2011) and this could explain the lack of relationship of $C_{16:1\omega5c/t}$ with MOB abundance.

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 relationship is possibly driven by $C_{16:1\omega7c}$, since in most sediments this was the most abundant 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 robustly correlated with MOB type Ib than $C_{16:1\omega7c}$, suggesting that the less abundant ^{13}C-depleted FAs, particularly $C_{18:1\omega7c}$, reinforced this relationship.

5.3. Relationships between FA abundances and CH_4 concentrations

As expected we observed positive relationships between the abundance of ^{13}C-depleted FAs and CH_4 concentrations in our dataset. However, only relatively weak relationships were observed when all sediment samples were examined together, with $C_{16:1\omega7c}$ and the sum of ^{13}C-depleted FAs correlating with CH_4 concentrations in the surface water and the sediments and $C_{18:1\omega7c}$ with CH_4 concentrations in the surface water. However, none of these relationships remained significant after correction for multiple testing (Table 2). Relationships between FA abundances and CH_4 concentrations were noticeably stronger and statistically significant when oxic and anoxic sediments were examined separately. The highest correlations between FA concentrations (for $C_{16:1\omega7c}$ and the sum of ^{13}C-depleted FAs) and lake-water CH_4 concentrations were apparent for oxic sediments ($r_s = 0.56–0.62$), whereas observed r_s values were slightly lower (for $C_{18:1\omega7c}$, $C_{16:1\omega7c}$ and the sum of ^{13}C-depleted FAs) for anoxic sediments ($r_s = 0.46–0.55$). These findings agree with the interpretation that the proportion of organic carbon originating from MOB increases in lakes and in lake sediments with higher CH_4 concentrations, as has been suggested to explain observed relationships between estimates of in-lake CH_4 abundance and δ^{13}C values of aquatic invertebrates that can feed on MOB (van Hardenbroek et al., 2013; Schilder et al., 2013). However, our results also suggest that this proportion increases to a different extent in sediments deposited in oxic and anoxic environments. The highest abundances of ^{13}C-depleted FAs were observed in oxic deep-water and littoral sediments (Fig. 4). CH_4 can be oxidized in both oxic and anoxic conditions, but aerobic CH_4 oxidation is considered to be the dominant process, oxidizing up to 99% of CH_4 produced in lakes (Bastviken et al., 2008; Blees et al., 2014). The growth of aerobic MOB is not only limited by CH_4 but also by the availability of electron acceptors, mainly oxygen (Amaral and Knowles, 1995; Hanson and Hanson, 1996). In oxic deep-water and littoral environments oxygen is generally abundant and MOB can profit most from increasing CH_4 availability, explaining the highest abundances of ^{13}C-depleted FA groups observed for sediments deposited under oxic condition. In the lakes where anoxic conditions develop, the CH_4 concentrations in the deep water layers are high during summer stratification, when our samples were taken. However, the absence of oxygen or other electron acceptors can limit CH_4 oxidation (Rudd et al., 1976; Amaral and Knowles, 1995) at the sediment-water interface and in stratified, anoxic lakes CH_4 oxidation is usually most extensive at the oxycline in the open water (Bastviken et al., 2008; Schubert et al., 2010; Milucka et al., 2015). Our results could be explained if MOB occur at higher concentrations where the sediment-water interface coincides with the oxycline than in the open water, since here the sediments would provide a stable substrate for these microorganisms to grow on, and/or if FAs originating from MOB in the open water would be partly decomposed during sedimentation and contribute to a lower extent to FAs measured at the sediment-water interface than MOB growing in the surficial sediment layers. In these situations, a high contribution of MOB-derived FAs would be expected for sediment samples located at or just below the oxycline, as confirmed by the highest abundance of ^{13}C-depleted FAs observed for our dataset in oxic samples overlain by relatively CH_4-rich water.

The abundances of $C_{16:1\omega7c}$ and of the sum of ^{13}C-depleted FAs in the surficial sediment layers were robustly correlated with CH_4 concentrations of the deeper sediment layers 10 cm below the sediment surface, both for the anoxic and oxic sediment samples ($r_s = 0.57–0.59$ and 0.61–0.65, respectively; Table 2; Fig. 4). This suggests that the overall CH_4 richness of sediments, and supply of CH_4 to the uppermost sediment layers, also promote higher abundances of CH_4-derived carbon in sedimentary organic carbon in the surface sediment samples.

6. Conclusions and implications for palaeoenvironmental reconstructions

We show that in 29 small lakes across Europe the abundance of ^{13}C-depleted FA groups relative to other FAs produced in freshwater ecosystems increases with increasing CH_4 concentrations, at least when relationships are examined separately for sediments deposited in oxic and anoxic environments. This is expected if the relative contribution of CH_4-derived carbon in lacustrine sedimentary organic matter, originating from MOB, increases with increasing in-lake CH_4 concentrations. This interpretation is supported by the analysis of the number of DNA copies of MOB in the examined sediments, which in our dataset is clearly correlated with the abundance of ^{13}C-depleted FAs. However, our analyses also indicate that the proportion of ^{13}C-depleted FA groups was highest in oxic sediment samples deposited in environments with relatively high CH_4 concentrations, suggesting that the proportion of CH_4-derived sedimentary organic carbon may also be elevated in these sediments. In contrast, lower proportions of these ^{13}C-depleted FAs were observed in sediments deposited in anoxic sections of the study lakes. Our analyses also show that different relationships between the relative abundance of ^{13}C-depleted FA groups and in-lake CH_4 concentrations are observed for sediments deposited in oxic and anoxic sediments. This implies that relationships between the proportion of CH_4-derived carbon in aquatic organic matter and CH_4 concentrations may also differ between these two depositional environments, at least for sedimentary organic matter 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 δ^{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., 2013) can be explained by higher MOB abundances under higher in-lake CH_4 concentrations. The results therefore support that reconstructions of δ^{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., 2013), since the δ^{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

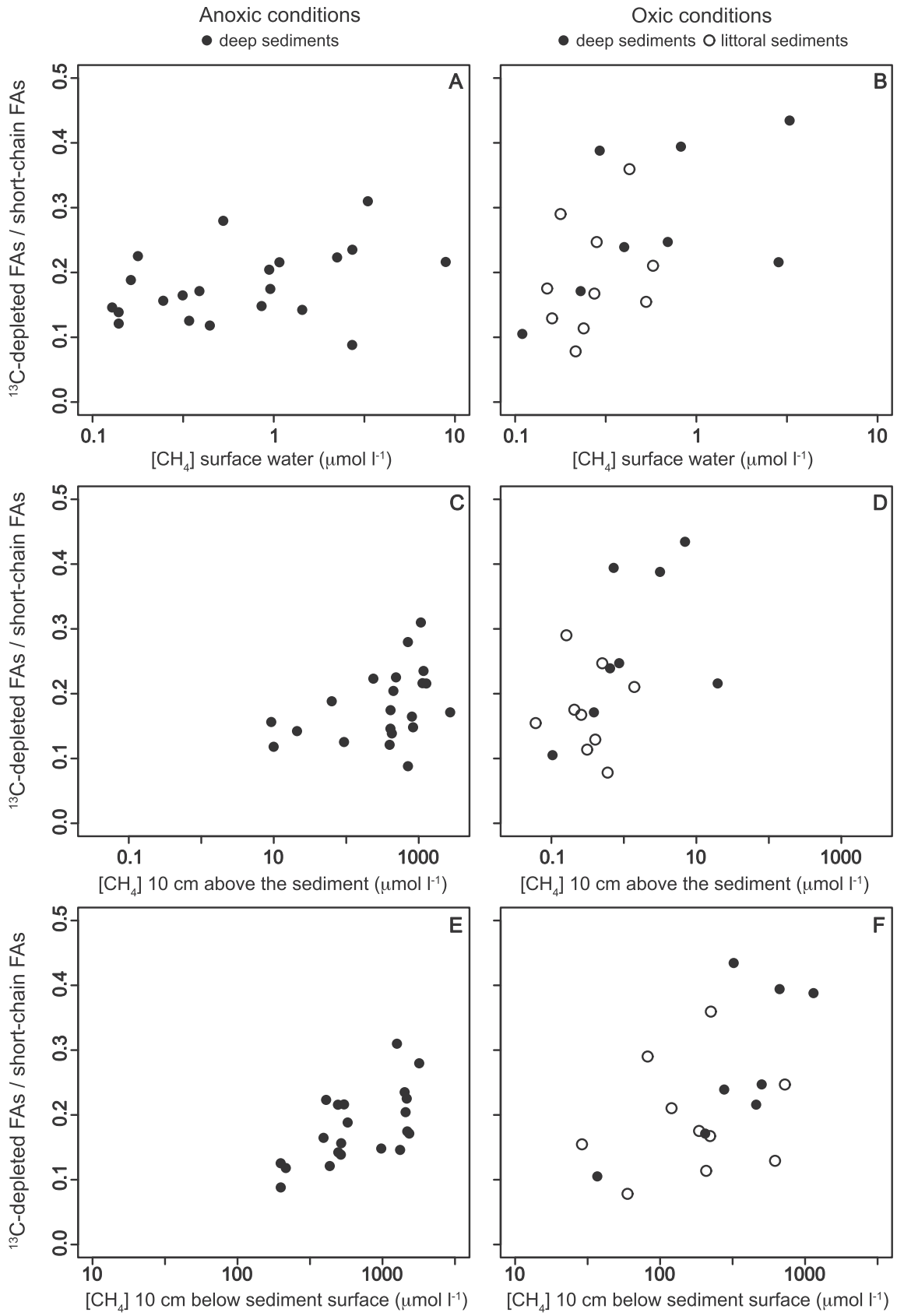

Fig. 4. Total abundance of ^{13}C-depleted FAs ($C_{16:1\omega7c}$, $C_{16:1\omega5c/t}$, and $C_{18:1\omega7c}$) in the sediment samples compared with CH_4 concentrations ([CH_4]) in the surface waters (A–B), 10 cm above the sediment (C–D), and 10 cm below the sediment surface (E–F) (note the logarithmic 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).

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 (GDGTs) (Coolen et al., 2008; Naeher et al., 2012; Sinninghe Damsté et al., 2012) have been analysed in downcore records and related to past variations in CH_4 cycling of lakes. However, our results also suggest that the relationship between the abundance of lipids originating from MOB and CH_4 concentrations may be complex and strongly influenced by oxygen availability at the sediment water-interface and by a different preservation of lipids produced in the open water column compared with those produced in sediments.

A major constraint of our study is that other organisms than MOB can contribute to the three ^{13}C-depleted FA groups detected at our study sites and that FA groups strictly limited to MOB were not detected in our survey. Although the different lines of evidence (FA analyses, qPCR analyses, correlations with CH_4 concentrations) are all consistent with the interpretation that the contribution of MOB-derived organic matter in lake sediments increases with increasing in-lake CH_4 concentrations, it is unclear how the observed relationships were influenced by the production of $C_{16:1\omega7c}$, $C_{16:1\omega5c/t}$, and $C_{18:1\omega7c}$ FAs by other organisms than MOB. Our results should therefore be corroborated by similar surveys focusing on lipid groups specific to MOB such as more specific FAs or specific bacteriohopanepolyols (e.g. methylcarbamate lipids related to C-35 amino-bacteriohopanepolyols, Rush et al., 2016), or alternative approaches which, e.g., assess the abundance of MOB-derived DNA relative to other bacterial and algal DNA in lake sediments. An additional uncertainty associated with our approach is the extent to which catchment carbon cycling reinforced or masked the relationship between FAs and CH_4 concentrations. For example, relationships are noticeably stronger in our dataset when analyses focus on shorter chain FAs only, since longer chain FAs originating from terrestrial plants apparently influence the relative concentrations of FAs in the analysed sediments. Similar effects may affect the relative abundances of short-chain FAs in our data as well. Future studies would therefore ideally aim to more fully quantify varying terrestrial organic matter input between and across lake basins to assess the extent that this organic matter source influences FA abundance analyses in surficial lake sediment samples.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.quascirev.2018.04.029.

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