

An inter-regional assessment of concentrations and $\delta^{13}\text{C}$ values of methane and dissolved inorganic carbon in small European lakes

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Abstract Methane (CH_4) and carbon dioxide emissions from lakes are relevant for assessing the greenhouse gas output of wetlands. However, only few standardized datasets describe concentrations of these gases in lakes across different geographical regions. We studied concentrations and stable carbon isotopic composition ($\delta^{13}\text{C}$) of CH_4 and dissolved inorganic carbon (DIC) in 32 small lakes from Finland, Sweden, Germany, the Netherlands, and Switzerland in late summer. Higher concentrations and $\delta^{13}\text{C}$ values of DIC were observed in calcareous lakes than in lakes on non-calcareous areas. In stratified lakes, $\delta^{13}\text{C}$ values of DIC were generally lower in the hypolimnion due to the degradation of organic matter (OM). Unexpectedly, increased $\delta^{13}\text{C}$ values of DIC were registered above the sediment in several lakes. This may reflect carbonate dissolution in calcareous lakes or methanogenesis in

deepwater layers or in the sediments. Surface water CH_4 concentrations were generally higher in western and central European lakes than in Fennoscandian lakes, possibly due to higher CH_4 production in the littoral sediments and lateral transport, whereas CH_4 concentrations in the hypolimnion did not differ significantly between the regions. The $\delta^{13}\text{C}$ values of CH_4 in the sediment suggest that $\delta^{13}\text{C}$ values of biogenic CH_4 are not necessarily linked to $\delta^{13}\text{C}$ values of sedimentary OM but may be strongly influenced by OM quality and methanogenic pathway. Our study suggests that CH_4 and DIC cycling in small lakes differ between geographical regions and that this should be taken into account when regional studies on greenhouse gas emissions are upscaled to inter-regional scales.

Keywords CH_4 · CO_2 · Carbon cycling · Greenhouse gas · Stable carbon isotopes · Lakes

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Introduction

Lakes are a significant source of methane (CH_4) to the atmosphere (Bastviken et al. 2004) and either a source or a sink of carbon dioxide (CO_2) (Raymond et al. 2013). In recent years, there has been an increasing interest in understanding the factors influencing CH_4 and CO_2 concentrations in lakes and in quantifying regional and global greenhouse gas output from lakes to the atmosphere (e.g. Juutinen et al. 2009; Bastviken et al. 2011; Raymond et al. 2013). However, so far only a few standardized multi-lake datasets are available providing a detailed assessment of CH_4 and dissolved inorganic carbon (DIC) concentrations in lakes required for these estimations and for constraining factors influencing greenhouse gas production and

emissions in different lake types and in different geographical and climatic regions.

Regional datasets describing greenhouse gas concentrations in lakes have been developed e.g. for boreal areas in Europe (for CH₄ e.g. Juutinen et al. 2009; Kankaala et al. 2013, for CO₂ e.g. Sobek et al. 2003; Rantakari and Kortelainen 2008). However, it remains unclear if these studies are representative for other regions. The few existing multi-regional datasets suffer from the data having been collected by a range of different methods and from some variables having been measured only in a limited number of the lakes (e.g. Bastviken et al. 2011; Raymond et al. 2013). Furthermore, the large datasets that are available usually examine greenhouse gas concentrations only in the surface water, especially those studies focusing on CO₂ (e.g. Sobek et al. 2003; Rantakari and Kortelainen 2008; Whitfield et al. 2011). Systematic multi-lake studies in different regions would help to identify how CH₄ and DIC concentrations vary in lakes across environmental gradients and how they interact with other variables that are believed to be linked to the carbon cycling in the lakes. For example, recent studies showed that CH₄ and CO₂ fluxes from lakes depend on temperature (Marotta et al. 2014; Yvon-Durocher et al. 2014). Furthermore, in a large study based on 207 lakes in Finland, Juutinen et al. (2009) reported that higher CH₄ concentrations and fluxes to the atmosphere were observed in nutrient-rich calcareous lakes, which are rare in the boreal regions of Fennoscandia. Similarly, concentrations of DIC are expected to vary between different geological and biogeographical regions as well as within the water column of a lake (e.g. Kortelainen et al. 2006; Myrbo and Shapley 2006; López Bellido et al. 2009).

This paper presents a dataset describing both CH₄ and DIC concentrations at multiple depths in the water column in 32 small lakes in late summer. Furthermore, the carbon isotopic composition ($\delta^{13}\text{C}$) of CH₄ and DIC was measured in the water column and for CH₄ in the sediment to provide insights into processes potentially influencing CH₄ and DIC concentrations within and between lakes. The study sites are located across a large latitudinal gradient, and the dataset includes both boreal/hemiboreal soft-water lakes from Fennoscandia, which have received a relatively large amount of attention in previous research, and western and central European hard-water lakes, which have been studied less extensively in multi-lake surveys. We concentrated on small lakes (0.3–340 ha surface area) because of their high number and importance as a landscape feature, especially in formerly glaciated regions (Verpoorter et al. 2014), and because they have been shown to be significant but understudied sources of greenhouse gases (Bastviken et al. 2011). The consistent approach with minimal methodological variability enables (1) an assessment of the

range and variations of late summer concentrations and $\delta^{13}\text{C}$ values of CH₄ and DIC encountered in small lakes in northern, central, and western Europe and (2) a comparison of these values between lakes subject to different climate, geology, and water column oxygenation. Such multi-lake datasets are essential for placing observations from individual well-studied lakes in the context of the wider range of CH₄ and DIC concentrations possible in temperate lake ecosystems.

Methods

Study sites

A total of 32 small lakes in Finland, Sweden, Germany, the Netherlands, and Switzerland were studied in August–September 2010 (Sweden), August 2011 (the Netherlands, Germany, and Finland), and September 2011 (Switzerland) (Fig. 1). All lakes were sampled during the late summer stratification to obtain comparable measurements from the different sites. Furthermore, late summer CH₄ concentrations in stratified lakes are important for assessing the amount of CH₄ released from dimictic and monomictic lakes during the autumn overturn, when CH₄ accumulated in the deepwater layers under anoxic conditions is released to the atmosphere (e.g. Schubert et al. 2012; Encinas Fernández et al. 2014).

The Fennoscandian lakes are located on Precambrian bedrock covered by Quaternary deposits in the boreal/hemiboreal zone and generally characterized by humic

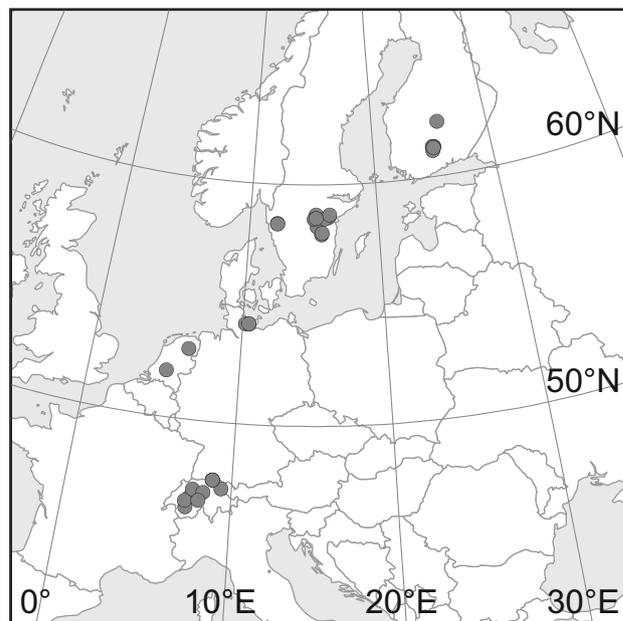


Fig. 1 Geographical location of the 32 study lakes

water and naturally low pH. The Swiss, Dutch, and German lakes are located on sedimentary bedrock or Quaternary deposits in the temperate zone. Lake surface area ranges from 0.3 to 340 ha (median 19 ha) and maximal depth from 2 to 30 m (median 16 m, Online Resource 1). Trophic status of the lakes varies from oligotrophic to hypertrophic (3–150 $\mu\text{g TP l}^{-1}$ in the surface water). Spatial patterns of diffusive CH_4 flux from surface water to the atmosphere during fieldwork are described in a different study (Schilder et al. 2013) with whole-lake flux estimates ranging between 0.03 and 2.8 $\text{mmol m}^{-2} \text{day}^{-1}$.

Lakes were divided into two groups based on the underlying geology. Calcareous lakes include the Swiss and German lakes, one lake in the Netherlands (De Waay), and two lakes in Sweden (Hargsjön and Illersjön) located on calcareous bedrock or carbonate-rich soils (Online Resource 1). Non-calcareous lakes included the Finnish and other Swedish lakes, and one lake in the Netherlands (Hijkermeer) located on carbonate-poor bedrock and deposits. Furthermore, the lakes were divided into oxic and hypoxic lakes depending on whether oxygen concentration was higher or lower than 5 % of saturation in the deepest part of the basin.

Field sampling

The sampling at each lake was conducted during a single day in the deepest part of the basin. First, temperature and oxygen profiles were measured (WTW, Oxi 1970i, Germany). Second, water samples from three depths of the water column were collected using a 5 l water sampler (Uwitec, Austria). In stratified lakes, samples were usually taken in the surface water (ca. 0.5 m below the surface), in the upper hypolimnion, and in the bottom water (ca. 0.5–1 m above the sediment). In unstratified lakes or in lakes with a very shallow hypolimnion, only a sample from the surface and a sample from the bottom water were obtained. Conductivity (WTW LF 330 with TetraCon® 325 conductivity measuring cell, Germany) and pH (pHScan 2, Singapore) were measured immediately in the field.

For the analysis of concentrations and $\delta^{13}\text{C}$ values of CH_4 and DIC, 60 ml of water was immediately collected from the water sampler using a plastic syringe (Becton–Dickinson, USA) and injected into a 118 ml acidified N_2 -filled glass vial for storage following Bastviken et al. (2008). Prior to sampling, 200 μl H_3PO_4 (85 %) was injected into the vials to ensure that all DIC in the sample water would be converted to CO_2 . The vials were capped with a thick massive butyl rubber septum (10 mm thick; Apodan, Denmark) in the laboratory, and the residual air was replaced with N_2 by repeated evacuation using a vacuum pump followed by an addition of N_2 through a

tubing connected to syringe needles (0.6 mm diameter) piercing the septa. After evacuating and filling with N_2 gas for the third time, the overpressure in the vial was allowed to equilibrate with the ambient air pressure by piercing the septum with a needle. The ambient air pressure was recorded.

Samples for the analysis of concentrations and $\delta^{13}\text{C}$ values of CH_4 and DIC in the water layer above the sediment in the deepest part of the basin were collected using a gravity corer (Uwitec, Austria). Water in the corer 10 cm above the intact sediment–water interface was sampled using a tube connected to a syringe with a three-way luer-lock valve. The tube, the valve, and the syringe were rinsed by sucking water into the syringe and ejecting it via the second valve outlet several times, taking care that no gas bubbles remained in the system. Then, 60 ml of water was collected and injected into a 118 ml acidified N_2 -filled glass vial (see above). Samples of CH_4 in the sediment were collected by transferring sediment from the depth of 10–11 cm (ca. 28 cm^3) in the sediment core rapidly into an airtight 130 ml flask. Then, 45 ml of lake water was added into the sediment flask using a syringe attached to a valve in the cap. To remove dissolved CH_4 from this lake water, it was equilibrated with ambient air prior to the injection by filling a 60 ml syringe with 45 ml of lake water and 15 ml of ambient air, shaking this, and refreshing the headspace three times to remove CH_4 from the water. After the addition of the equilibrated lake water into the sediment flask, the flask was rigorously shaken to force CH_4 from the sediment into the headspace. Finally, 45 ml of the headspace was then sampled through the valve using a syringe and injected into a 50 ml glass vial (with 10 mm thick butyl rubber septum; Apodan, Denmark) prefilled completely with saturated NaCl solution for storage (Bastviken et al. 2010), using a second needle to partly drain the NaCl solution.

Concentration and $\delta^{13}\text{C}$ of CH_4 and DIC

CH_4 and CO_2 concentration in the headspace of the storage vials were quantified by gas chromatography using a flame ionization detector with a methanizer (Agilent 6890 N, PlotQ capillary column, with FID for CH_4 and TCD for CO_2 for the samples from the Finnish lakes and GC-FID, Shimadzu GC-8, PoropackN column for the others). The concentration of CH_4 and DIC in the lake water was calculated using the headspace equilibration method (McAuliffe 1971) according to Henry's law describing gas–water partitioning (Stumm and Morgan 1996; see methods in Bastviken et al. 2010). In 15 Swiss, Dutch, and German lakes, duplicate samples from the water sampler were retrieved for assessing the error included in the field sampling and in the measurements. For CH_4 , the concentration

difference between the duplicates was on average 20 % for the surface water samples (characterized by low CH₄ concentrations) and less than 5 % for the bottom water samples. For DIC, the concentration difference between the duplicates varied 6 % on average. The analytical error (σ) based on repeated measurements on standards was less than 1.7 % for CH₄ and less than 2.5 % for CO₂.

For $\delta^{13}\text{C}$ analysis of CH₄ and DIC, a subsample from the headspace was extracted and inserted into a 10 ml exetainer (Labco Limited, UK) filled with saturated NaCl solution using a syringe and a fine needle (0.4 mm diameter). The exetainers were stored upside down in the dark with a small amount of NaCl solution covering the septa. $\delta^{13}\text{C}$ analysis was performed at the Division of Climate and Environmental Physics at the Physics Institute of University of Bern (Switzerland). Between 100 μl and 10 ml of gas from the exetainer, depending on the CH₄ concentration, was injected into a helium carrier gas stream. CO₂ and CH₄ were determined for their isotopic compositions sequentially. For analysis of CO₂, CO₂ was cryogenically separated from the sample gas mixture by means of liquid nitrogen, whereas for analysis of CH₄, CO₂ and CO were removed from the sample gas in Ascarit and Schütze reagent traps, respectively. The sample was then dried in a Mg(ClO₄)₂ trap. Subsequently, the remaining CH₄ (and potentially the higher hydrocarbons) present in the sample was oxidized to CO₂ at 1100 °C in an oven and trapped using liquid nitrogen. Afterwards, the CO₂ was volatilized and via a helium carrier gas stream transferred to the isotope ratio mass-spectrometer (ThermoFinnigan MAT Delta Plus XL, Germany) with several special characteristics for low-volume trace gas isotope analysis described by Leuenberger et al. (2000a, b). The accuracy of the absolute isotope values was relatively poor (1–2 ‰) as the range of the carbon stable isotope composition of the samples exceeded the range of the internal laboratory CH₄ standards used for the calibration. The precision, however, was lower than 0.5 ‰ in most cases. For CO₂, the accuracy was better than 0.3 ‰. Isotopic data are reported in δ units (‰) relative to the VPDB standard (Vienna Pee Dee belemnite). After the analysis, the $\delta^{13}\text{C}$ value of DIC was corrected to account for the fractionation between the aqueous and gaseous CO₂ in the storage vials (Assayag et al. 2006).

Additional chemical analyses

Water samples for nutrient analysis were stored frozen in plastic bottles until analysis, except the Finnish samples which were analysed immediately after the field sampling. Total nitrogen (TN) and total phosphorus (TP) of the lake water were analysed using standard methods: After persulfate oxidation, analysis was conducted colourimetrically using an autoanalyser AA3 (Bran + Luebbe, Germany) for

the Swedish lakes, QuickChem 8000 (Lachat Instruments, USA) for the Finnish lakes, and Skalar (Skalar Analytical, the Netherlands) for the German, Dutch, and Swiss lakes. For the analysis of coloured dissolved organic matter (CDOM), water was filtered through a 0.7 μm glass fibre filter (Whatman GF/F) and stored at 4 °C until analysis within a month. CDOM was indicated by absorbance at 420 nm (ISO 1994). The absorbance was measured in a 1-cm cuvette on an Ultrospec 2100 pro (Biochrom, UK) spectrometer for the Swedish lakes, UV-2100 (Shimadzu, Japan) for the Finnish lakes, and Ultrospec 3100 pro (Biochrom, UK) for the German, Dutch, and Swiss lakes.

C:N ratio and $\delta^{13}\text{C}$ values of sedimentary organic matter (SOM) were analysed from surface sediments (0–2 cm) sampled in the deepest part of the basin. Samples were soaked in a 2.5 % HCl solution for 18 h (Swiss lakes) or 6 h (other lakes) to remove carbonates, rinsed with demineralized water 3–5 times in order to raise the pH to ca. 6, centrifuged to remove excess water, and freeze-dried. Dry sediment (5–10 mg) was transferred into 8 × 5 mm tin capsules (Lüdi Swiss AG, Switzerland) and analysed for $\delta^{13}\text{C}$ values using an Elementar Vario EL Cube or Micro Cube elemental analyser (Elementar Analysensysteme GmbH, Germany) interfaced to a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon Ltd., UK) at UC Davis Stable Isotope Facility (USA). Four laboratory standards (nylon, bovine liver, peach leaves, and USGS-41 Glutamic Acid) of known relation to international standards for VPDB were used as references. Replicate sample measurements on standards ($n = 50$) indicated an analytical error (σ) of 0.05 ‰.

Numerical analyses

Lake surface areas and maximum depths were measured in this study or derived from digitized bathymetric maps obtained from municipal or regional authorities or from literature. Thermocline depth was determined as the depth from the surface where the temperature gradient first reached 1 °C m⁻¹ (Huotari et al. 2009) and oxycline depth as the depth where oxygen concentration first reached less than 5 % of saturation. The strength of the water column stratification was estimated as a Brunt-Väisälä stability frequency (N_s) (s⁻¹) (Spigel and Imberger 1987) calculated from the density gradient across the thermocline using the equation:

$$N_s = \sqrt{\frac{g}{\rho_w} \frac{\partial \rho_w}{\partial z}}$$

where g (m s⁻²) is the acceleration due to gravity, ρ_w (kg m⁻³) the water density, and z (m) the depth. Total lake volume and the volume below the oxycline and the

thermocline were calculated using the 3D Analyst tool in the ArcMap 9.3 software (Esri). Mean annual air temperatures were obtained from national meteorological services. Statistical analyses were made using R (R Core Team 2013). Each variable was tested for normal distribution using the Shapiro–Wilk test, and skewed distributions were \log_{10} transformed. Student's *t* tests were used for testing for statistically significant differences in concentrations and $\delta^{13}\text{C}$ values of CH_4 and DIC and in environmental variables between different lake groups. Pearson's *r* and associated *p* values were used to test for statistically significant relationships between environmental variables.

Mixing model for DIC in deepwater layers

$\delta^{13}\text{C}$ measurements of DIC revealed unexpected vertical profiles in several lakes, with higher $\delta^{13}\text{C}$ values in the deepwater layers than in the overlying water (see “Results” section). To interpret these results and to constrain which in-lake processes could be responsible for these observations, we used a simple two-source mixing model (Fry 2006). Based on this, we estimated how much DIC produced by different processes would be needed to explain the difference in $\delta^{13}\text{C}$ values of DIC between the bottom water (0.5–1 m above the sediment) and 10 cm above the sediment. Potential processes include oxic degradation of organic matter (OM), dissolution of authigenic carbonates, and acetotrophic methanogenesis, as an example of an anaerobic metabolic process releasing CO_2 . CO_2 produced during OM degradation was assumed to have similar $\delta^{13}\text{C}$ values as the SOM of the lake. Dissolved CaCO_3 and CO_2 released during its dissolution were assumed to have $\delta^{13}\text{C}$ values 1 ‰ higher than the HCO_3^- in the surface water (Romanek et al. 1992), which was calculated based on the $\delta^{13}\text{C}$ value and the concentration of DIC and pH in the surface water according to equations in Stumm and Morgan (1996). CO_2 produced as a by-product of acetotrophic methanogenesis was assumed to have a $\delta^{13}\text{C}$ value of 15 ‰ (Wachniew and Róžański 1997).

Results

Lake variables

The studied lakes are situated in different climatic zones and located on variable bedrock and soils. As a consequence, there are major differences in mean annual temperature, CDOM, pH, and conductivity in the surface water between Fennoscandian and non-Fennoscandian lakes and between non-calcareous and calcareous lakes ($p \leq 0.0001$ – 0.002 ; Online Resource 1). Whereas differences in the C:N ratio of

SOM and TN concentrations in the surface water are observed between the lake groups ($p \leq 0.0001$ – 0.02), no significant differences are observed in TP concentrations in the surface water ($p = 0.1$ – 0.3). Furthermore, variables related to lake morphology (lake surface area, max. depth) do not differ significantly between the lake groups ($p = 0.4$ – 1). Most of the lakes (27 out of 32) were defined as thermally stratified, and 24 lakes as having hypoxic conditions above the sediment (Online Resource 1, Fig. 2).

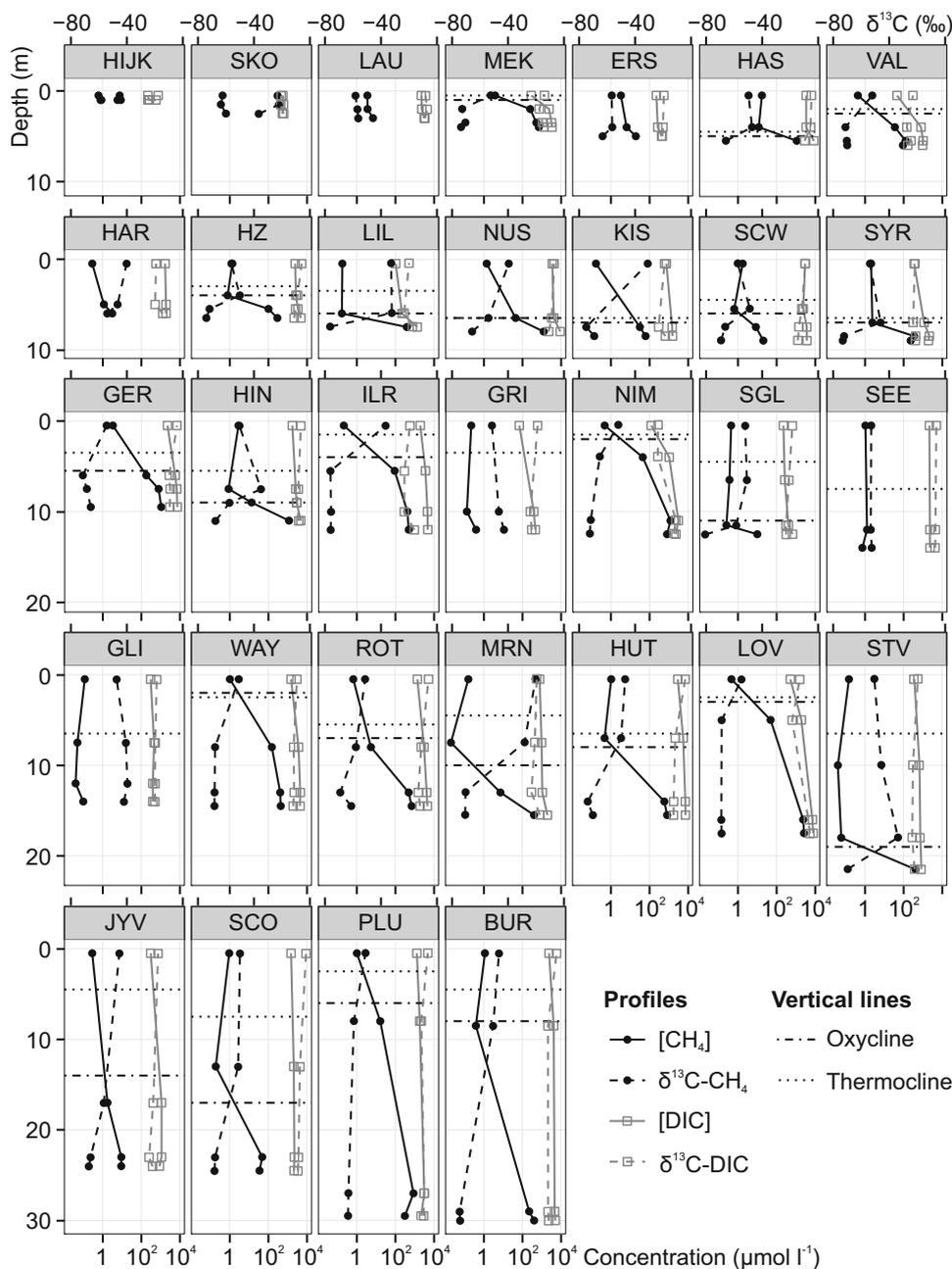
DIC

The surface water concentrations and the $\delta^{13}\text{C}$ values of DIC differed distinctly between the two groups of lakes: Whereas the non-calcareous lakes were characterized by surface water concentrations of 46–793 $\mu\text{mol l}^{-1}$ and by $\delta^{13}\text{C}$ values of -24 to -13 ‰, the calcareous lakes were characterized by surface water concentrations of 1317–3562 $\mu\text{mol l}^{-1}$ and by $\delta^{13}\text{C}$ values of -9 to -3 ‰ (excluding two Swedish lakes, Illersjön and Hargsjön, with $\delta^{13}\text{C}$ values of -20 and -19 ‰, respectively) (Fig. 3). However, no differences between the regions were observed in the concentrations and $\delta^{13}\text{C}$ values of DIC in the bottom water: The concentrations varied between 222 and 7374 $\mu\text{mol l}^{-1}$ and $\delta^{13}\text{C}$ values between -24 and -6 ‰ (Fig. 3). In oxic lakes, the concentrations and $\delta^{13}\text{C}$ values were similar in the surface water and in the bottom water. In hypoxic lakes, DIC concentrations generally increased and $\delta^{13}\text{C}$ values decreased with water depth (Fig. 3). However, in several lakes an increase in $\delta^{13}\text{C}$ values was observed in the deepwater layers (i.e. in the bottom water sample or 10 cm above the sediment) compared to the values in the overlying water (Fig. 2). For example, in 14 out of 32 lakes, the $\delta^{13}\text{C}$ values of DIC directly above the sediment were 1–6 ‰ higher than those in the bottom water.

Methane

CH_4 concentrations generally decreased whereas $\delta^{13}\text{C}$ values of CH_4 increased from the sediment to the surface water (Fig. 4). CH_4 concentrations in the bottom water varied between 0.04 and 2449 $\mu\text{mol l}^{-1}$ and in the surface water between 0.1 and 18 $\mu\text{mol l}^{-1}$. The large variation observed in the samples taken from the bottom water and in those from the upper hypolimnion is clearly related to the varying oxygen conditions at the sampling depth (Fig. 2). In three of the 24 hypoxic lakes, the bottom water samples for gas analyses were retrieved above the oxycline and these samples were characterized by relatively low CH_4 concentrations. The upper hypolimnion was sampled in 21 lakes but only 13 of them were characterized by hypoxic conditions at this depth. In all hypoxic lakes in which the sample from the upper hypolimnion was above the oxycline, CH_4 concentrations were lower at that depth than in

Fig. 2 Water column profiles for CH_4 concentrations (CH_4) and $\delta^{13}\text{C}$ values ($\delta^{13}\text{C}\text{-CH}_4$) as well as DIC concentrations (DIC) and $\delta^{13}\text{C}$ values ($\delta^{13}\text{C}\text{-DIC}$) for each studied lake. Thermocline and oxycline depth are indicated by a horizontal line (see “Methods” section)



the surface water samples. CH_4 concentrations in the surface water were only weakly related to CH_4 concentrations in the bottom water ($r = 0.38$, $p = 0.03$). Surface water CH_4 concentrations were significantly higher in the calcareous lakes than in the non-calcareous lakes ($p = 0.005$, Fig. 4). The $\delta^{13}\text{C}$ value of CH_4 in the sediment in the deepest part of the basin varied between -86 and -61 ‰ and was 31–58 ‰ more negative than the $\delta^{13}\text{C}$ value of the SOM (Fig. 5a). Furthermore, the difference between the $\delta^{13}\text{C}$ values of SOM and CH_4 correlated with the C:N ratio of SOM ($r = -0.39$, $p = 0.03$, Fig. 5b). In the surface water, $\delta^{13}\text{C}$ values of CH_4 varied between -58 and -21 ‰

(Fig. 4). The $\delta^{13}\text{C}$ values of CH_4 in the water column were strongly related to the CH_4 concentrations ($r = -0.83$, $p < 0.0001$, Fig. 6).

Discussion

DIC in the surface water

The calcareous lakes in our dataset have significantly higher DIC concentrations and less negative $\delta^{13}\text{C}$ values of DIC in the surface water than the non-calcareous lakes

Fig. 3 **a** *Boxplots* for DIC concentration (DIC) 10 cm above the sediment, in the bottom water (0.5–1 m above the sediment), in the upper hypolimnion, and in the surface water (0.5 m below the water surface). **b** *Boxplots* for the $\delta^{13}\text{C}$ value of DIC ($\delta^{13}\text{C}$ -DIC) for the same DIC depths. Lakes are grouped into four categories based on underlying geology and oxygen conditions above the sediment. Whiskers of the *boxplots* show data points less than 1.5 times the interquartile range from the box, *circles* indicate more extreme data points. *Numbers* indicate the sample size

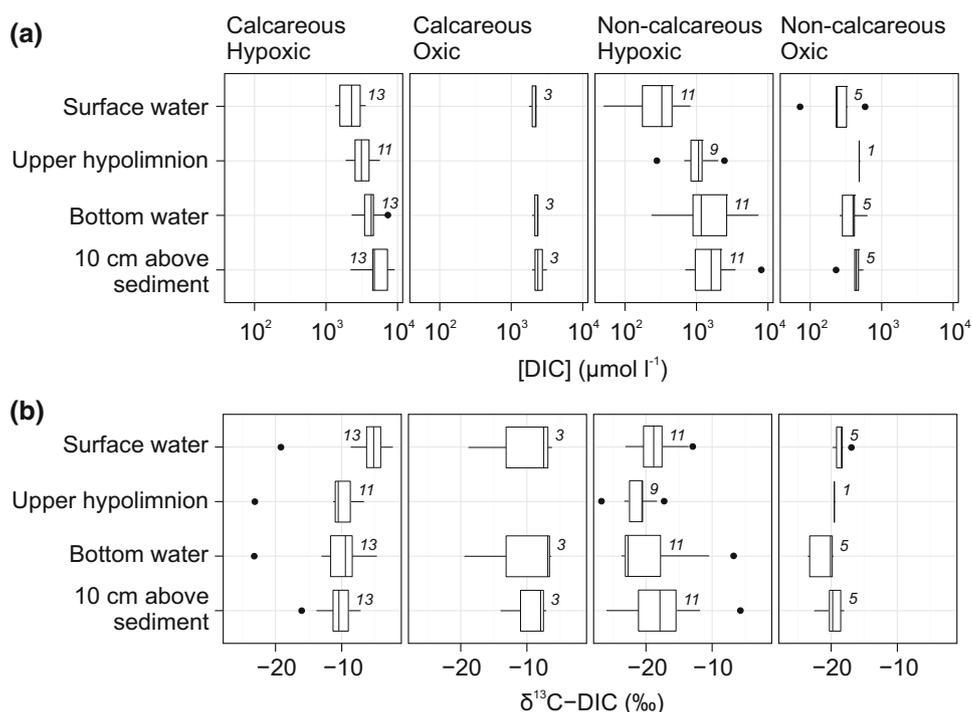
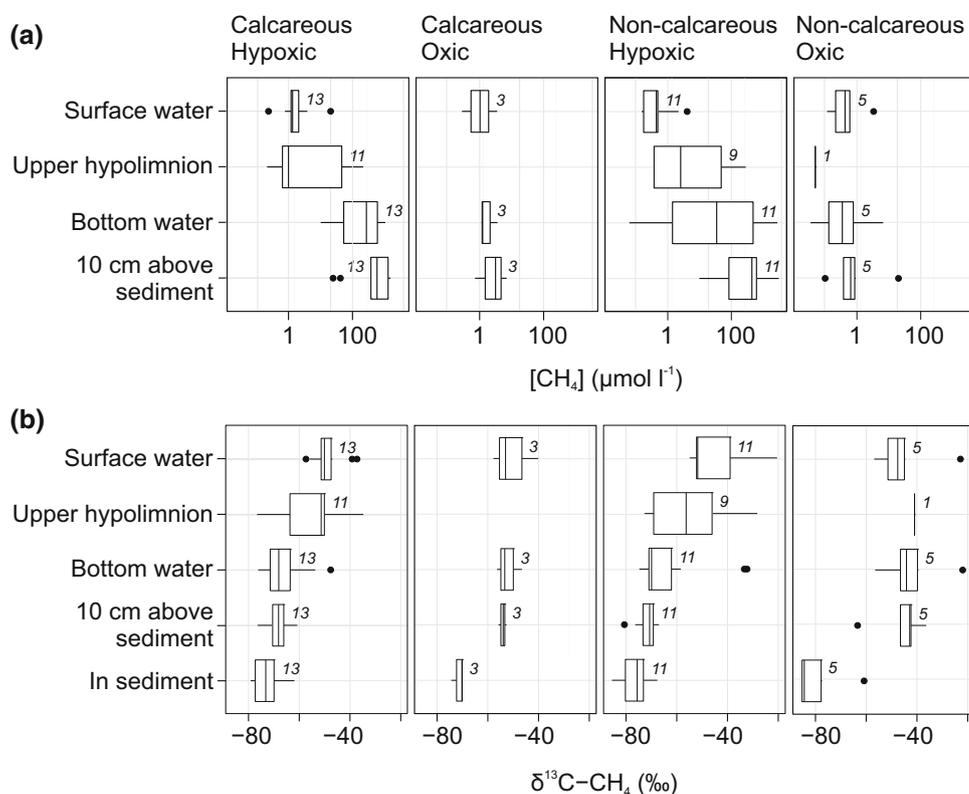


Fig. 4 **a** *Boxplots* for CH_4 concentration (CH_4) 10 cm above the sediment, in the bottom water (0.5–1 m above the sediment), in the upper hypolimnion, and in the surface water (0.5 m below the water surface). **b** *Boxplots* for the $\delta^{13}\text{C}$ value of CH_4 ($\delta^{13}\text{C}$ - CH_4) for the same water depths, together with values measured for CH_4 in the sediment. Lakes are grouped into four categories based on underlying geology and oxygen conditions above the sediment. Whiskers of the *boxplots* show data points less than 1.5 times the interquartile range from the box, *circles* indicate more extreme data points. *Numbers* indicate the sample size



(Fig. 3). The pattern is even clearer when the lakes are grouped based on the geographical location (Table 1). This is expected, since most of the non-Fennoscandian lakes sampled in our campaigns are situated in regions with

calcareous sediments and more intensive land use, where substantial hydrologic DIC inputs into the lake can be important (Stets et al. 2009; Kindler et al. 2011). The $\delta^{13}\text{C}$ value of limestone is typically close to zero, and DIC in

Fig. 5 **a** The $\delta^{13}\text{C}$ value of CH_4 ($\delta^{13}\text{C}-\text{CH}_4$) in the sediment relative to $\delta^{13}\text{C}$ value of sedimentary organic matter ($\delta^{13}\text{C}-\text{SOM}$) and **b** the difference between the $\delta^{13}\text{C}$ values of CH_4 and SOM [$\Delta^{13}\text{C}-(\text{CH}_4-\text{SOM})$] in the sediment relative to the C:N ratio of SOM

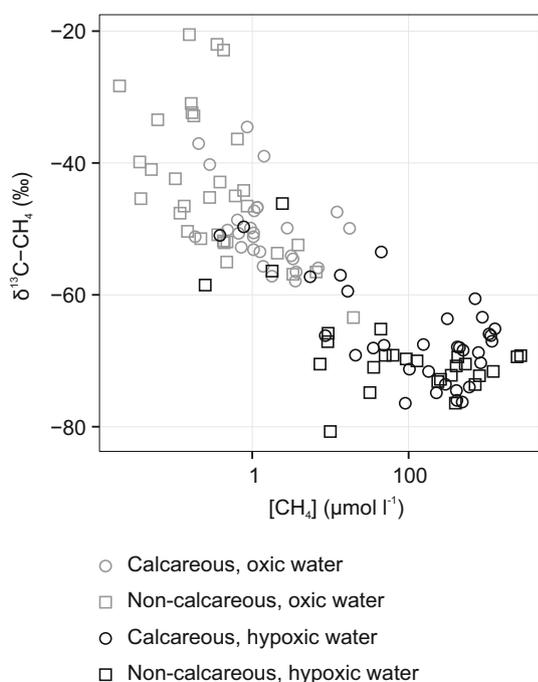
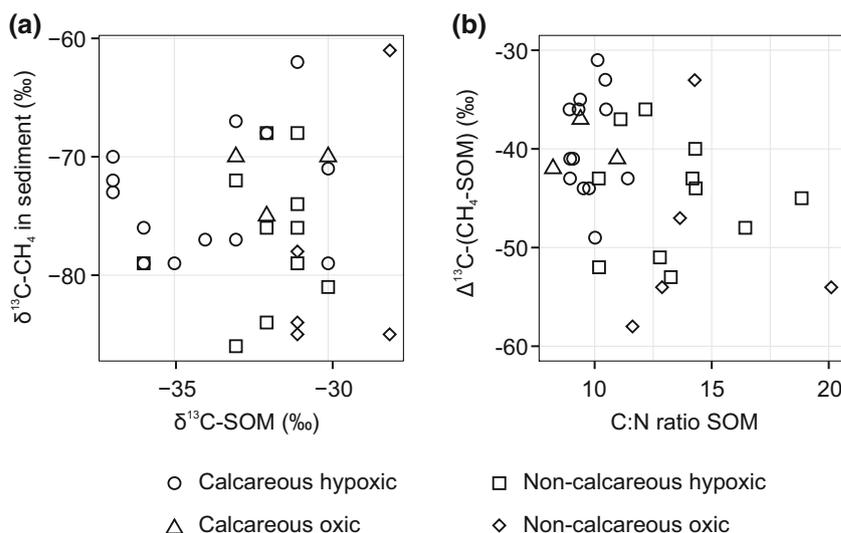


Fig. 6 $\delta^{13}\text{C}$ values of CH_4 ($\delta^{13}\text{C}-\text{CH}_4$) plotted against CH_4 concentrations (CH_4) for all water samples retrieved in this study (including samples from 10 cm above the sediment, the bottom water, the upper hypolimnion, and the surface water). Samples are grouped based on underlying geology and oxygen concentrations at the sampling depth. Grey symbols indicate oxic water and black symbols indicate hypoxic water at the sampling depth

alpine streams in calcareous catchments can have $\delta^{13}\text{C}$ values as high as -2 ‰ (Aucour et al. 1999). It is also possible that the rates of photosynthesis were higher in the non-Fennoscandian lakes due to higher epilimnetic temperatures and higher trophic status, although this was not evident in the TP concentrations in the surface water.

Photosynthetic production preferentially removes ^{12}C from the surface water DIC pool and may therefore lead to ^{13}C enrichment (McKenzie 1982). Furthermore, surface water in most of the non-Fennoscandian lakes was undersaturated in CO_2 during the sampling period (Schilder et al. 2013). Therefore, exchange with atmospheric CO_2 ($\delta^{13}\text{C}$ value of ca. -8 ‰; Valentino et al. 2008) may have led to elevated $\delta^{13}\text{C}$ values of DIC in the surface water of these lakes. On the other hand, the very negative $\delta^{13}\text{C}$ values of DIC in all the Fennoscandian lakes indicate a ^{13}C -depleted inorganic carbon source for these systems. In lakes with high input of dissolved organic carbon (DOC) originating from the decomposition of terrestrial vegetation, the respiration of DOC can contribute CO_2 with relatively negative $\delta^{13}\text{C}$ values (Lennon et al. 2006). Also, DIC produced during CH_4 oxidation has very negative $\delta^{13}\text{C}$ values and can contribute to the surface water DIC pool (Wachniew and Rózański 1997).

DIC in the hypolimnion

As expected, higher concentrations and more negative $\delta^{13}\text{C}$ values of DIC were observed in the hypolimnion of the stratified lakes in our dataset than those measured in the surface waters (Fig. 3). This is a consequence of DIC accumulating in the hypolimnion due to degradation of OM in the water column and in the sediment (Herczeg 1987), since terrestrial and aquatic OM typically have more negative $\delta^{13}\text{C}$ values (ca. -30 to -10 ‰; Meyers and Teranes 2001) than atmospheric CO_2 (-8 ‰; Valentino et al. 2008) and DIC in the surface water due to the discrimination against ^{13}C by terrestrial vegetation and photosynthetic algae. However, in several lakes an increase in the $\delta^{13}\text{C}$ values of DIC was observed in the deepwater layers

Table 1 Concentrations and $\delta^{13}\text{C}$ values of DIC (DIC, $\delta^{13}\text{C}$ -DIC) and CH_4 (CH_4 , $\delta^{13}\text{C}$ - CH_4) in the surface water for the two different biogeographical regions: Fennoscandian lakes in Sweden and Finland and other lakes in lowland Switzerland, the Netherlands, and Germany, and the results of the t test for the differences between the regions (calculated on \log_{10} transformed data)

	Fennoscandian lakes		Other lakes		t test p value
	Mean	σ	Mean	σ	
[DIC] surface water ($\mu\text{mol l}^{-1}$)	513	542	2156	902	<0.0001
$\delta^{13}\text{C}$ -DIC surface water (‰)	-19	2	-7	3	<0.0001
[CH_4] surface water ($\mu\text{mol l}^{-1}$)	0.8	1.2	2.6	4.2	0.0003
$\delta^{13}\text{C}$ - CH_4 surface water (‰)	-44	11	-51	5	0.0477

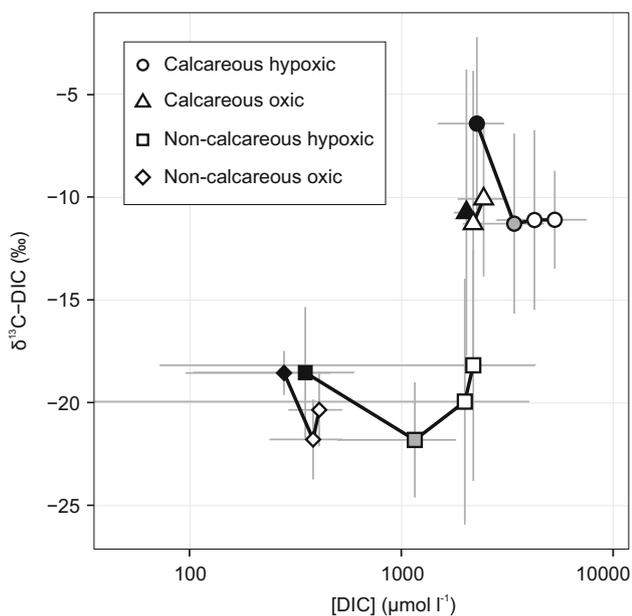


Fig. 7 Average $\delta^{13}\text{C}$ value of DIC ($\delta^{13}\text{C}$ -DIC) plotted against average DIC concentration (DIC) for samples taken at different water depths in the different lake categories. Lakes are grouped based on underlying geology and oxygen conditions above the sediment as in Fig. 3. Averages of each sample depth are connected with a solid black line from the surface water sample (black symbol), to the upper hypolimnion (grey symbol, not shown for oxic lakes), the bottom water sample, and the sample 10 cm above the sediment. Error bars show the standard deviations (for DIC concentrations calculated on untransformed data)

compared to the values in the overlaying water (Figs. 3b, 7; Table 2). Besides CO_2 released during oxic OM degradation, possible other processes that may have affected $\delta^{13}\text{C}$ values of DIC in the deepwater layers of these lakes are dissolution of authigenic carbonates (Oana and Deevey 1960) and CO_2 release or consumption during anaerobic metabolic processes. These metabolic processes include

anoxic respiration (e.g. via reduction of nitrate, iron, manganese, or sulphate), fermentation, and methanogenesis (Enrich-Prast et al. 2009). Both carbonate dissolution and anaerobic processes could produce CO_2 with higher $\delta^{13}\text{C}$ values than oxic OM degradation: Authigenic carbonates reflect the $\delta^{13}\text{C}$ values of the DIC in the surface water (Romanek et al. 1992) whereas enzymatic metabolic processes generally discriminate against ^{13}C (Fry 2006). For example, acetotrophic methanogenesis can generate CO_2 with $\delta^{13}\text{C}$ values of as much as 30 ‰ higher than those of the acetate used as a substrate in the reaction (Conrad 2005; Steinmann et al. 2008). Alternatively, many anaerobic microorganisms, for example hydrogenotrophic methanogenic (Whiticar 1999) or phototrophic sulphate-reducing bacteria (Ormerod 1983; Zyakun et al. 2009), use CO_2 as a carbon source. Their preferential use of ^{12}C can increase the $\delta^{13}\text{C}$ values of the remaining DIC pool.

Using a simple mixing model, we evaluated whether the observed increase in $\delta^{13}\text{C}$ values of DIC in deepwater layers could realistically be explained by OM degradation or carbonate dissolution, or whether anaerobic metabolic processes producing CO_2 with high $\delta^{13}\text{C}$ values need to be invoked to explain our observations. We concentrated on the 14 lakes (11 of them in Fennoscandia) where an increase in the $\delta^{13}\text{C}$ values of DIC above the sediment was observed compared to values in the bottom water (Table 2). First, we estimated the $\delta^{13}\text{C}$ values of DIC produced in these processes and then, the amount of DIC needed to change the $\delta^{13}\text{C}$ value of DIC observed in the bottom water sample to the value measured above the sediment. Based on these calculations, OM degradation cannot explain the increased $\delta^{13}\text{C}$ values of DIC above the sediment, since the estimated values of CO_2 produced during this process are more negative than those of DIC in the bottom water in all the studied lakes. Moreover, CO_2 produced during OM degradation may have even slightly lower $\delta^{13}\text{C}$ values than our estimations based on the $\delta^{13}\text{C}$ values of the SOM, since some ^{13}C fractionation is expected to occur during microbial respiration, especially in lakes with high DOC concentrations (Lennon et al. 2006). In contrast, dissolution of CaCO_3 , estimated to release CO_2 with $\delta^{13}\text{C}$ values of -18 to -2 ‰ in the studied lakes, could explain the increased $\delta^{13}\text{C}$ values of DIC above the sediment in most of the lakes. However, in four Fennoscandian lakes, CO_2 released during the dissolution of CaCO_3 is estimated to have $\delta^{13}\text{C}$ values lower than DIC in the bottom water and in some lakes an extremely large CO_2 release would be needed to explain the observed $\delta^{13}\text{C}$ values of DIC above the sediment (e.g. up to $8000 \mu\text{mol l}^{-1}$ in Lake Kisašjön). Furthermore, it is unlikely that authigenic carbonate precipitation and dissolution play a prominent role in the carbon cycle of the non-calcareous Fennoscandian lakes with low pH. Instead,

Table 2 The $\delta^{13}\text{C}$ values of DIC ($\delta^{13}\text{C}$ -DIC) in the bottom water and 10 cm above the sediment, and the difference between these two samples. In addition, estimates of DIC accumulation by different processes which release CO_2 in the hypolimnion during summer stratification that would be necessary to explain the increase in $\delta^{13}\text{C}$ values of DIC observed 10 cm above the sediment are provided.

Lake code	$\delta^{13}\text{C}$ -DIC bottom water	$\delta^{13}\text{C}$ -DIC 10 cm above sediment	Change $\delta^{13}\text{C}$ -DIC from bottom water to 10 cm above sediment	[DIC] accumulation needed 10 cm above sediment based on the following DIC sources		
	‰	‰		‰	OM degradation $\mu\text{mol l}^{-1}$	CaCO_3 dissolution $\mu\text{mol l}^{-1}$
SKO	-21	-19	2	Not possible	882	38
LIL	-24	-18	6	Not possible	2830	40
GRI	-24	-23	1	Not possible	40	10
MRN	-24	-17	6	Not possible	Not possible	203
KIS	-23	-18	5	Not possible	8252	218
HAR	-20	-14	5	Not possible	Not possible	357
ILR	-24	-17	7	Not possible	Not possible	1034
STV	-23	-22	2	Not possible	106	32
HIJK	-24	-19	5	Not possible	396	34
LOV	-7	-6	1	Not possible	Not possible	357
MEK	-15	-14	1	Not possible	352	118
JYV	-24	-22	3	Not possible	260	78
ROT	-13	-11	1	Not possible	785	210
HIN	-9	-8	1	Not possible	1398	162

Only the lakes where an increase in the $\delta^{13}\text{C}$ values of DIC was observed between the bottom water sample and 10 cm above the sediment are listed. See “Methods” section for further details on the assumptions behind these calculations

anaerobic metabolic processes producing CO_2 with high $\delta^{13}\text{C}$ values could explain the increasing $\delta^{13}\text{C}$ values above the sediment in all of our study lakes. For example, assuming that CO_2 produced as a by-product of acetotrophic methanogenesis is characterized by a $\delta^{13}\text{C}$ value of 15 ‰ (Wachniew and Róžański 1997), only a relatively moderate DIC production would lead to the observed shift in $\delta^{13}\text{C}$ values of DIC above the sediment ($<400 \mu\text{mol l}^{-1}$ in most cases).

Our data suggests that, although the degradation of OM is expected to be the main process responsible for the high DIC values in the hypolimnia of the studied lakes, alternative or more specific mechanisms producing CO_2 with higher $\delta^{13}\text{C}$ values, such as dissolution of authigenic CaCO_3 or acetotrophic methanogenesis, are needed to explain the increasing $\delta^{13}\text{C}$ values of DIC in deepwater layers at some sites. Methanogenesis or other anaerobic metabolic processes seem a probable explanation for the observed patterns in the non-calcareous lakes, where inputs and in-lake production of carbonates are expected to be insignificant. The same anaerobic metabolic processes may have played a role in the calcareous lakes of our dataset. However, due to their naturally high concentration of DIC, a much higher amount of CO_2 with a high $\delta^{13}\text{C}$ value would be needed to cause an increase in the $\delta^{13}\text{C}$ value of DIC. Therefore, the effect of the anaerobic metabolic processes on $\delta^{13}\text{C}$ values of DIC may be less apparent in the calcareous lakes than in the non-calcareous lakes examined in our survey (Fig. 7).

Methane concentrations within the water column

We observed high concentrations and very negative $\delta^{13}\text{C}$ values of CH_4 in the bottom water of our hypoxic lakes whereas low concentrations and less negative $\delta^{13}\text{C}$ values of CH_4 were observed in the oxygen-rich parts of the water column (Figs. 4, 6). This is expected, since CH_4 production and oxidation are strongly controlled by oxygen availability (Rudd et al. 1976; Bastviken et al. 2002; Liikanen et al. 2002). CH_4 production in lakes mainly occurs in the anoxic sediment (Rudd and Hamilton 1978), and a large proportion of CH_4 is oxidized by methane oxidizing bacteria (MOB) when it is transported to the oxic sediment or into the oxic water column (Bastviken et al. 2002). Whereas microbially produced CH_4 has a strongly negative $\delta^{13}\text{C}$ signature, CH_4 in oxic parts of the water column features less negative $\delta^{13}\text{C}$ values since MOB preferentially use CH_4 containing ^{12}C (Rosenfeld and Silverman 1959; Barker and Fritz 1981).

Interestingly, CH_4 concentrations in the surface water of the calcareous lakes were higher than in the non-calcareous lakes (Fig. 4). Again, the pattern is even clearer when the lakes are grouped based on their geographical location (Table 1). Surface water CH_4 concentrations are mainly controlled by diffusive CH_4 flux from the lake surface to the atmosphere and by the CH_4 fluxes from deeper water layers or from the sediment to the surface water (Bastviken et al. 2004). It appears unlikely that the CH_4 concentrations in the surface water of Fennoscandian lakes in our dataset

are lower due to higher diffusive flux to the atmosphere. Although mean wind speeds in the regions where these lakes are located tend to be higher than in lowland Switzerland (Troen and Petersen 1989), many of them are sheltered by forests and probably less exposed to wind. Furthermore, according to Schilder et al. (2013) diffusive CH_4 flux to the atmosphere during our fieldwork was significantly higher from the non-Fennoscandian lakes ($p < 0.001$). The rate of CH_4 production and oxidation in the sediment and in the water column could differ between the regions. Unfortunately, we did not measure CH_4 production or oxidation rates in the studied lakes. However, CH_4 abundance in gas samples obtained in our campaigns for measuring $\delta^{13}\text{C}$ values of CH_4 in the sediment did not differ between the Fennoscandian and the other lakes (with mean values of 7919 ppm and 7031 ppm, and σ of 6664 ppm and 5106 ppm, respectively, $p = 1$). This suggests that sedimentary CH_4 concentrations and CH_4 production rates in the deepest part of the lake basins were similar between the regions.

Alternatively, differences in the diffusive transport of CH_4 to the surface water could explain the observed difference in the surface water CH_4 concentration between regions. In thermally stratified lakes, vertical diffusive gas transport to the surface water is limited (Bastviken et al. 2008). However, in our dataset the stratification strength of the water column (expressed as N_s) did not differ significantly between the Fennoscandian and the other lakes ($p = 0.8$). Furthermore, CH_4 concentration and $\delta^{13}\text{C}$ value profiles suggest that vertical CH_4 transport across the thermocline cannot be the only CH_4 source contributing to concentration and $\delta^{13}\text{C}$ values of CH_4 in the surface water (Fig. 2). In a number of the studied lakes, we observed higher CH_4 concentrations in the surface water compared to those above the oxycline. In previous studies, similar CH_4 concentration profiles with higher values in the surface water than in underlying water layers have been interpreted to be a consequence of lateral transport of CH_4 from littoral sediments or from inflowing drainages (Murase et al. 2003; Bastviken et al. 2008; López Bellido et al. 2012; Huotari et al. 2013). High CH_4 fluxes from the littoral sediments in the non-Fennoscandian lakes can be expected due to higher in situ temperatures and more nutrient-rich sediments, as this promotes methanogenic activity in the littoral zone (Duc et al. 2010). Alternatively, recent publications have suggested that CH_4 production in the oxic parts of the water column may occur in some lakes (e.g. Grossart et al. 2011, Bogard et al. 2014, Tang et al. 2014). High algal productivity may have promoted higher rates of CH_4 production in the oxic surface waters in some of our study lakes (Bogard et al. 2014). However, there is presently only scant evidence that CH_4 production in oxic surface waters is a quantitatively important process in

small lakes, which often have a relatively large littoral area (Bogard et al. 2014; Tang et al. 2014).

Methane in the sediment

No relationship was observed between the $\delta^{13}\text{C}$ values of SOM and of CH_4 in the sediment in the deepest part of the lakes (Fig. 5a), which suggests that the $\delta^{13}\text{C}$ value of SOM is not the main determinant of $\delta^{13}\text{C}$ values of CH_4 in our lakes. The difference between the $\delta^{13}\text{C}$ value of SOM and of CH_4 correlated with the C:N ratio of SOM (Fig. 5b). In sediments containing proportionally more OM from terrestrial sources, characterised by a high C:N ratio, the offset between the $\delta^{13}\text{C}$ value of SOM and CH_4 tends to be larger. SOM in small lakes can be expected to originate from both aquatic and terrestrial sources, and it has been suggested that OM from these two sources differs in its suitability as a substrate for methanogenesis (Duc et al. 2010; West et al. 2012; Guillemette et al. 2013). Aquatic OM can have highly variable $\delta^{13}\text{C}$ values between lakes, and may be either more enriched or more depleted in ^{13}C than terrestrial OM (e.g. Bade et al. 2006). If CH_4 in our lakes is preferentially produced from aquatic OM, and the proportion and the $\delta^{13}\text{C}$ values of aquatic SOM varies between lakes, this could affect the offset between the $\delta^{13}\text{C}$ value of SOM and of CH_4 and explain the absence of a relationship between these two variables in our dataset.

A further factor influencing the $\delta^{13}\text{C}$ values of CH_4 in lakes is the importance of different CH_4 production pathways, which can be expected to vary with SOM quality. Under fully anoxic conditions, the $\delta^{13}\text{C}$ values of pore water CH_4 may be strongly influenced by the dominant methanogenic pathway (Hornibrook et al. 2000). Two main metabolic pathways are recognized for biological CH_4 production: acetotrophic (acetate fermentation) and hydrogenotrophic (CO_2 reduction) methanogenesis. Acetotrophic methanogenesis is expected to lead to CH_4 with less negative $\delta^{13}\text{C}$ values than hydrogenotrophic methanogenesis (Whiticar et al. 1986; Conrad et al. 2007). Whiticar et al. (1986) suggested that differences in the CH_4 production pathway may partly explain the variations they observed in $\delta^{13}\text{C}$ values of CH_4 between freshwater (-65 to -50 ‰) and marine environments (-110 to -60 ‰) and that acetotrophic methanogenesis predominates in freshwaters. However, $\delta^{13}\text{C}$ values remarkably more negative than -65 ‰ have recently been reported for lacustrine CH_4 , leading to the conclusion that hydrogenotrophic methanogenesis might also be a significant pathway in lake ecosystems (e.g. Murase and Sugimoto 2001; Kankaala et al. 2007; Mandic-Mulec et al. 2012). In all our 32 lakes, the $\delta^{13}\text{C}$ value of CH_4 measured in the sediments is more negative than -61 ‰ and in 30 lakes more negative than -65 ‰ (Fig. 5a). Hydrogenotrophic methanogenesis may

be a more relevant pathway for CH₄ production in freshwater sediments if the availability of organic substrates for methanogenesis is low (Hornibrook et al. 1997, 2000; Murase and Sugimoto 2001), which may be the case in our Fennoscandian lakes with a larger proportion of SOM of terrestrial origin. Hydrogenotrophic methanogenesis would be expected to lead to larger offsets between $\delta^{13}\text{C}$ values of SOM and of CH₄ in these sites than in sites with a higher relevance of acetotrophic methanogenesis. Furthermore, seasonal variations in the relative importance of different CH₄ production pathways have been observed. For example, in Lake Bled, a peri-alpine lake in Slovenia, the dominating CH₄ production pathway was reported to change from acetotrophic methanogenesis in spring to over 90 % of hydrogenotrophic methanogenesis in autumn due to the maturation of the SOM (Lojen et al. 1999). Our measurements are based on CH₄ sampled in late summer, which may explain the unexpected negative $\delta^{13}\text{C}$ values observed in our dataset. We do not have information on the stable hydrogen isotope composition of CH₄, which would allow a more rigorous separation of CH₄ originating from acetotrophic or hydrogenotrophic methanogenesis (Conrad 2005). However, our data show that $\delta^{13}\text{C}$ values of CH₄ more negative than reported for freshwater environments by Whiticar et al. (1986) are widespread in small northern, western, and central European lakes.

Conclusions

Our study has documented the wide range of DIC and CH₄ concentration and $\delta^{13}\text{C}$ values encountered in small boreal and temperate lakes in Europe. As expected, variations in DIC concentrations and $\delta^{13}\text{C}$ values within and between lakes can to a large extent be explained by the underlying geology, stratification of the water column, preferential uptake of isotopically light CO₂ in the surface water layers during photosynthesis, and release of isotopically light CO₂ during the degradation of OM. Variations in concentrations and $\delta^{13}\text{C}$ values of CH₄ largely reflect stratification and oxygen availability within the lake basins. However, we also report several unexpected observations. These include higher CH₄ concentrations in the surface water of several sites than observed in deeper water layers. Furthermore, we confirm the results of other studies which found more negative $\delta^{13}\text{C}$ values of CH₄ in lake sediments than reported for acetotrophic methanogenesis, which suggests that hydrogenotrophic methanogenesis may play a significant role in many of our lakes. We also report an unexpected increase in $\delta^{13}\text{C}$ values of DIC above the sediments, especially in many Fennoscandian lakes, and overall higher surface water CH₄ concentrations in our central and western European than in our Fennoscandian lakes. These

latter observations suggest that important aspects of the CH₄ and DIC cycle of small lakes may differ regionally and that this should be taken into account when regional studies on greenhouse gas production, abundance, and fluxes in lakes are extrapolated to different geographical regions (e.g. in upscaling studies).

The dataset on DIC and CH₄ concentrations and $\delta^{13}\text{C}$ values presented in this study is provided in Online Resource 2.

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