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Recycling and fluxes of carbon gases in a stratified boreal lake following experimental carbon addition

H. Nykänen[1], **S. Peura**[1,2], **P. Kankaala**[3], **and R. I. Jones**[1]

[1]University of Jyväskylä Department of Biological and Environmental Science, Jyväskylä, Finland
[2]Department of Ecology and Genetics, Uppsala University, Uppsala, Sweden
[3]Department of Biology, University of Eastern Finland, Joensuu Campus, Joensuu, Finland

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Correspondence to: H. Nykänen (hannu.k.nykanen@jyu.fi)

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Abstract

Partly anoxic stratified humic lakes are important sources of methane (CH_4) and carbon dioxide (CO_2) to the atmosphere. We followed the fate of CH_4 and CO_2 in a small boreal stratified lake, Alinen Mustajärvi, during 2007–2009. In 2008 and 2009 the lake received additions of dissolved organic carbon (DOC) with stable carbon isotope ratio ($\delta^{13}C$) around 16 ‰ higher than that of local allochthonous DOC. Carbon transformations in the water column were studied by measurements of $\delta^{13}C$ of CH_4 and of the dissolved inorganic carbon (DIC). Furthermore, CH_4 and CO_2 production, consumption and emissions were estimated. Methane oxidation was estimated by a diffusion gradient method. The amount, location and $\delta^{13}C$ of CH_4-derived biomass and CO_2 in the water column were estimated from the CH_4 oxidation pattern and from measured $\delta^{13}C$ of CH_4. Release of CH_4 and CO_2 to the atmosphere increased during the study. Methane production and almost total consumption of CH_4 mostly in the anoxic water layers, was equivalent to the input from primary production (PP). $\delta^{13}C$ of CH_4 and DIC showed that hydrogenotrophic methanogenesis was the main source of CH_4 to the water column, and methanogenic processes in general were the reasons for the ^{13}C-enriched DIC at the lake bottom. CH_4 and DIC became further ^{13}C-enriched in the anoxic layer of the water column during the years of DOC addition. Even gradient diffusion measurements showed active CH_4 oxidation in the anoxic portion of the water column; there was no clear ^{13}C-enrichment of CH_4 as generally used to estimate CH_4 oxidation strength. Increase in $\delta^{13}C$-CH_4 was clear between the metalimnion and epilimnion where the concentration of dissolved CH_4 and the oxidation of CH_4 were small. Thus, ^{13}C-enrichment of CH_4 does not reveal the main location of methanotrophy in a lake having simultaneous anaerobic and aerobic oxidation of CH_4. Overall the results show that organic carbon is processed efficiently to CH_4 and CO_2 and recycled in the anoxic layer of stratified boreal lakes by CH_4 oxidation. In spite of this, increased DOC input led to increased greenhouse gas release, mainly as CO_2 but also as CH_4. Due to the predominantly anaerobic CH_4 oxidation, a relatively small amount of CH_4-derived

biomass was produced, while a large amount of CH_4-derived CO_2 was produced in the anoxic bottom zone of the lake.

1 Introduction

A large number of small lakes is typical of boreal and arctic regions (Downing et al., 2006). Due to a high load of allochthonous dissolved organic carbon (DOC) from their forested and peatland-dominated catchments, most of these small lakes are highly humic, brown-water lakes (Kortelainen, 1993). Thus, the lakes are integral parts of terrestrial carbon cycling in the landscape and can return a substantial proportion of the carbon originally fixed in their catchment areas back to the atmosphere (Algesten et al., 2003; Huotari et al., 2011). In contrast, the sediments of small lakes function as a permanent sink and store of carbon (Kortelainen et al., 2004). As concentrations of allochthonous DOC in the lakes in many boreal regions are reported to be increasing (Vuorenmaa et al., 2006; Monteith et al., 2007), there is a need for better understanding of carbon cycling in the lakes.

During summer, the water columns of small, sheltered brown-water lakes are typically steeply stratified with respect to light penetration, temperature and chemical properties (Salonen et al., 2004). Under stratified conditions microbial processes also differ considerably according to depth and oxygen availability. Anaerobic microbial decomposition of organic matter in the sediment and deep water layers yields high accumulation of carbon dioxide (CO_2) and methane (CH_4) in the anoxic hypolimnion (Houser et al., 2003). In freshwater lakes methanogenesis is the main process in anaerobic organic matter degradation (Capone and Kiene, 1988), based either on acetoclastic (acetate as terminal substrate) or hydrogenotrophic (H_2 and CO_2 as terminal substrates) pathways. Furthermore, both processes consume and release CO_2 in a chain of processes leading to CH_4.

Methane-oxidizing bacteria (MOB) use both CH_4 and molecular oxygen, so they occur where both CH_4 and oxygen coincide (Hanson and Hanson, 1996). During the stratification period the metalimnetic oxycline is a site of active CH_4 oxidation, which is often seen as minimum CH_4 concentration in this layer (Bastviken et al., 2008). In addition, CH_4 can be oxidized anaerobically (anaerobic oxidation of methane, AOM) by anaerobic methanotrophic archaea (ANME) using electron acceptors other than oxygen (Liikanen et al., 2002; Eller et al., 2005; Caldwell et al., 2008; Schubert et al., 2011, 2012). Furthermore, nitrite reducers can provide molecular oxygen directly for methanotrophs in anoxic systems (Ettwig et al., 2010). Also micro-aerobic CH_4 oxidation is possible in anoxic water columns (Blees et al., 2014)

At an annual scale most boreal lakes are significant sources to the atmosphere of both CO_2 (Kortelainen et al., 2006; Huotari et al., 2011) and CH_4 (Bastviken et al., 2004; Juutinen et al., 2009), although during the summer stratification period they may occasionally be sinks of atmospheric CO_2 due to photosynthetic uptake in the shallow euphotic layer and low gas transfer velocities between epilimnion and hypolimnion (Ojala et al., 2011; Huotari et al., 2011; Kankaala et al., 2013a). When considering the fluxes of radiatively important trace gases, the transformation of CH_4 by MOB to their cell material and to CO_2 is important, because CH_4 is 25 times more active as a greenhouse gas than CO_2 in a time horizon of 100 years (IPCC, 2007). Seasonally, the greatest CH_4 emissions to the atmosphere have usually been measured immediately after ice-melt, and also during the autumnal overturn (Kankaala et al., 2006a; Juutinen et al., 2009; Karlsson et al., 2013). In autumn a high proportion of dissolved CH_4 is oxidized in the mixed water column when plenty of both CH_4 and oxygen are simultaneously available for MOB in the same location (Kankaala et al., 2006a, 2007). In general, a major part of the CH_4 produced (50–100 %) is apparently oxidized in the lake water column (Kankaala et al., 2006a, 2007; Bastviken et al., 2002; Shubert et al., 2011, 2012) before reaching the atmosphere. CH_4 carbon in microbial biomass is a temporal and dynamic form of carbon storage. Since MOB use CH_4 as their sole carbon and energy source, a share of methane-derived carbon (MDC) is incorporated into their biomass which forms an important carbon and energy source for lake food webs in small lakes (Bastviken et al., 2003; Jones and Grey, 2011; Taipale et al., 2008;

Kankaala et al., 2013b). Moreover, in the euphotic zone of the water column, CO_2 derived from CH_4 can be incorporated into the biomass of algae. Thus, a major part of the CH_4 produced seems to be recycled in lakes. Despite this, CH_4 emissions from lakes have been estimated to contribute as much as 8–48 Tg year^{-1} (6–16 %) to the global natural CH_4 emissions (Bastviken et al., 2004), although also smaller estimates of 3.7–10 Tg year^{-1} have also been given (Juutinen et al., 2009).

The knowledge that biogenic CH_4 has a strongly negative stable carbon isotope value ($\delta^{13}C$) compared to other carbon forms in ecosystems has been widely utilized in biogeochemical and ecological studies. CH_4 production and consumption processes give a particular signal to its bulk isotopic composition in the water column, and this signal is then reflected in the isotopic composition of microbes oxidizing CH_4 and also in higher trophic level consumers. It is possible to track the production pathway, especially if the isotopic composition of CO_2 is also known (Whiticar, 1999). In methane oxidation the lighter carbon (^{12}C) isotope is preferentially consumed, increasing the proportion of the heavier ^{13}C in the residual CH_4 (Whiticar, 1999; Bastviken et al., 2002). Fractionation against the heavier isotope has also been demonstrated for anaerobic microbial oxidation of CH_4 (Holler et al., 2009). Thus, the existence of CH_4 oxidation can be verified and an estimation of the fraction of oxidized CH_4 can be calculated from measured $\delta^{13}C$-CH_4 values. The isotopic composition of DIC ($\delta^{13}C$-$\sum CO_2$ or $\delta^{13}C$-DIC) is determined by the original substrate $\delta^{13}C$, respiration, photosynthesis and diffusion; $\delta^{13}C$-DIC is also linked to CH_4 by its use in CH_4 production, and when CH_4 is oxidized aerobically or anaerobically, isotopically depleted CO_2 is produced. Carbon isotope analyses have shown that MDC can be > 50 % of the carbon biomass of crustacean zooplankton and chironomid larvae in some lakes (Jones and Grey, 2011; Taipale et al., 2011) and up to 20 % of carbon biomass of fish (Jones and Grey, 2011). However, according to Jones and Grey (2011) evidence of MDC in consumers higher in the food chains is still scarce, and is also not incorporated into lake food web models. The possible contribution of anaerobic oxidation of CH_4 (AOM) to form MDC and to lake carbon cycles in general is even less studied than that of aerobic methane oxidation

(AMO). Thus the fate of biogenic CH_4 is important in stratified lake carbon cycles and needs further study.

Here we report results from a study of carbon cycling in a small boreal forest lake from 2007 to 2010. During the second and third year the DOC concentration was experimentally increased by addition of cane sugar ($\delta^{13}C$ ca. −12 ‰) to test the effect of increased DOC load on lake ecosystem functions and also to be able to trace the fate of DOC in the lake by its $\delta^{13}C$ signal. Peura et al. (2014) found from this same experiment that after DOC enrichment, diffusive CH_4 and CO_2 fluxes increased, epilimnetic bacterial production increased, DIC became ^{13}C-enriched, and there was also transfer of added carbon to consumers by bacterial usage of DOC. Here we present detailed results for carbon gas (CH_4 and DIC) dynamics in the whole lake water column during the experimental period, based on both the isotopic signature of C ($\delta^{13}C$) and mass balance calculations. We used $\delta^{13}C$-CH_4 to estimate processes involved in CH_4 formation, its vertical and temporal pattern in the water column and the strength of CH_4 oxidation, as well as possible effects of CH_4 oxidation on formation of microbial biomass and on the isotopic composition of DIC. While $\delta^{13}C$ values of microbes utilizing CH_4 and DIC (acetogens, methanogens, methanotrophs and algae) are not easily measurable, $\delta^{13}C$ of POM, DOM and zooplankton are shown to set frames for their possible isotopic composition. These findings are then tied to carbon flow estimates for the lake.

2 Materials and methods

2.1 Site and manipulation

The study was done in a small polyhumic headwater lake with sampling from spring 2007 to autumn 2010, during the ice-free period, and occasionally during the winter ice cover periods. Total amounts of oxygen, DIC, methane and DOC are presented to the first open water measurement in spring 2010, $\delta^{13}C$ of gases to the end of 2009, while production and consumption results are only from the ice-free stratification periods during 2007–2009 when measurements of CH_4 consumption by a diffusion gradient method were possible from 1 May to 31 October in 2007 and 2008, and from 1 May to 30 September in 2009.

Lake Alinen Mustajärvi (Fig. 1) is a small (area 0.7 ha, volume 31 000 m^3) headwater lake located in a boreal coniferous forest area in southern Finland (61°12′ N, 25°06′ E; 129.4 m a.s.l.). The catchment area ($< 0.5\,km^2$) consists of over 90 % mixed spruce, pine and birch forest and less than 10 % peatlands. The lake is covered by ice for 5.5–6 months each year from late November to late April. During the ice-free period the lake is steeply stratified with respect to temperature and oxygen; there is low stable temperature, darkness and anoxic conditions in the hypolimnion, while the eplimnion is aerobic and supports photosynthesis, although the dark water colour restricts the euphotic zone. Alinen Mustajärvi is spring meromictic and thus transition from under ice stratification to summer stratification is rapid, while overturn in autumn mixes aerated surface water to the bottom of the water column and deeper water masses can come into contact with the atmosphere. The littoral zone of the lake is narrow, reaching to a depth of only 1.5–1.6 m, and the vegetation is dominated by sparse stands of *Nuphar lutea* (L.), *Carex* species and submerged *Sphagnum*. Weather data are from the nearest weather station at Lammi Biological Station (61°03′ N, 25°02′ E, 125 m a.s.l.), some 18 km from the lake. Average annual temperature for the period 1981–2010 was 4.2 °C and precipitation 645 mm, of which 326 mm was during 1 May to 30 September (Pirinen et al., 2012). During this study, the precipitation for the same period was 323.7, 306.9 and 324.0 mm for 2007, 2008 and 2009, respectively. A small ditch (10 cm deep and 30 cm wide) drains some water from the lake.

An annual addition of 22 g carbon m^{-2} as cane sugar (Demerara Sugar, Danisco sugar) was made to the lake during the open water periods in 2008 and 2009. Sugar was added to the lake monthly, six times during each open water period. Each monthly addition was 66 kg of sugar containing 28 kg of carbon, equivalent to a concentration of 2 mg C L^{-1} in the epilimnetic water or a mean daily loading of 0.07 mg C L^{-1} of DOC to the epilimnion. Sugar was first dissolved in lake water in a large tub and the sugar solution was then pumped to the lake epilimnion by bilge pump and dispersed manually from a tube at a height of 1 m above the water surface from a rowing boat. Mixing was ensured by vigorous rowing while the sugar was being added. The first addition was on 15 May 2008 and the last on 9 October 2009. All measurements were made before sugar additions, so that after each sugar addition there was always a minimum of 2 weeks when the lake was not disturbed. The added carbon was intended to mimic increased loading of labile allochthonous carbon sources due to changed precipitation, or to altered thawing and melting patterns, changing runoff and carbon flows in the catchment area. However, the cane sugar had δ^{13}C around −12 ‰, while allochthonous (terrestrial) organic carbon entering the lake has δ^{13}C around −27 ‰, so the added sugar also served as an isotopic tracer for carbon transformations in the lake.

2.2 Measurements

All variables were measured from over the deepest point of the lake (6.5 m). All sampling was done between 08:30 and 11:00 (GMT + 2 h).

2.2.1 Physical variables

Temperature and oxygen concentration were measured at 0.5 m intervals with a YSI 55 probe (Yellow Springs Instruments; accuracy ±0.3 °C, $\pm 0.3\,\mathrm{mg\,O_2\,L^{-1}}$ or ±2 % of reading) starting from the lake bottom. Because oxygen measurement with this device does not guarantee when the water is totally anoxic, redox measurements were also made monthly during 2007–2008 with a WTW Multiline P3 and Redox electrode SenTix ORP directly from the water collected in a 2 L Limnos tube sampler from 1 m intervals. Additional measurements made in 2009 (J. Saarenheimo, personal communication, 2010) confirmed anoxic conditions in the lake hypolimnion during the whole study during stratification periods. The temperature profile of the water column at 1 m intervals from surface to the depth of 6 m was logged with a Vemco Minilog-II-T from spring thawing in 2007 to the start of the ice-covered period in 2008; logging during winter was successful only from depths of 1, 2 and 4 m.

2.2.2 Chemical analyses

Samples for water chemistry were collected with the Limnos sampler. Water collected for pH, DOC and nutrient analyses was pooled from the epilimnion (during 2007 and 2008 sample depths were 0, 1, and 2 m, thereafter 0 and 1 m), metalimnion (during 2007 and 2008 sample depths were 3 and 4 m, thereafter 2 and 3 m) and hypolimnion (during 2007 and 2008 sample depths were 5 and 6 m, thereafter 4, 5 and 6 m) (Table 1). Nutrient concentrations ($P-PO_4^-$, $N-NO_3+N-NO_2$, NH_4, P_{tot}, N_{tot}) were measured by standard methods (http://www.sts.fi/). Light penetration was measured as Secchi-disc depth.

2.2.3 Primary production and community respiration

Primary production (PP) was measured with the inorganic ^{14}C-uptake method (Keskitalo and Salonen, 1994), and community respiration as an increase in DIC concentration during 24 h incubation in the dark with DIC analysed according to Salonen (1981). PP and community respiration were measured using water collected from 0, 0.5, 1 and 2 m depths, and incubations were made at the corresponding depths. For cumulative net production and respiration, the daily averages were multiplied by the number of days in the month and these values were summed for each study period.

2.2.4 Methane and DIC concentrations and $\delta^{13}C$

Concentrations of CH_4 and DIC in the water column were measured from samples taken once or twice per month at 1 m intervals into 50 mL gas-tight polypropylene syringes. These were kept under crushed ice prior to analyses (max 4 h) and concentration was analysed with the headspace equilibrium technique and gas chromatography (Agilent 6890N equipped with FID and TCD, details in Ojala et al., 2011). Before adding the N_2 headspace, the water was acidified with HNO_3 to convert all DIC to CO_2 for analyses with TCD. The CH_4 concentration in the water was calculated as described by Huttunen et al. (2001a). Samples for δ^{13}C-DIC were taken from the Limnos sampler directly by 5 mL syringe to vials having a helium atmosphere and 0.15 mL of H_3PO_4. In 2007 samples were taken to a depth of 5 m and were analysed at the University of Helsinki by E. Sonninen. During the rest of the study samples were taken to a depth of 6 m and were analysed at Jyväskylä similarly as in Helsinki with a GasBench II connected to a Thermo Finnigan XP Advantage, using the same in-house carbon standard, $CaCO_3$. Samples for δ^{13}C-CH_4 were collected once a month during the open water period from 2008 to 2009; generally depth intervals were 1 m, and once 0.5 m. For CH_4 isotopic analyses, 30 mL water samples from the Limnos tube sampler were taken into 60 mL syringes. In the laboratory, 30 mL of N_2 headspace gas was added into the syringes via 3-way stopcocks and after shaking the headspace gas was injected into pre-evacuated LABCO exetainers (12 mL). Analyses of δ^{13}C-CH_4 were done similarly and with the same isotopic ratio mass spectrometer and PreCon unit as described in Kankaala et al. (2007). The same gas cylinder of standard for CH_4 was used as an in-house standard during the study to ensure consistency. δ^{13}C of POM, DOM and zooplankton was determined as in Peura et al. (2014). Results are reported relative to the VPDP scale.

$$\delta^{13}\mathrm{C} = \left(\frac{\left(\frac{^{13}\mathrm{C}}{^{12}\mathrm{C}}\right) \mathrm{sample}}{\left(\frac{^{13}\mathrm{C}}{^{12}\mathrm{C}}\right) \mathrm{standard}} - 1 \right) \cdot 1000 \tag{1}$$

CH_4 concentration and δ^{13}C-CH_4 in bubble collectors (design described in Huttunen et al., 2001b) were determined twice during 2008 after 7–20 day deployments and again in spring 2009. The lowest rim (area covering 0.03 m^2) of the collector was at a depth of 0.5 m. Sub-samples were taken from the upper part of the collector and CH_4 concentration and δ^{13}C-CH_4 analyses were made as described for analyses of dissolved CH_4. Efflux of CH_4 and CO_2 during the ice-free period was calculated using the boundary layer diffusion equations presented by Kling et al. (1992) and Phelps et al. (1998), and their calculation is described in Peura et al. (2014).

2.2.5 Biofilm, algae and surface sediment $\delta^{13}C$

Biofilm was scraped by spatula from surfaces of ropes and incubation support tubes in autumn 2009. This represents material accumulated during summer, probably consisting of algae, microbes and some zooplankton, and thus integrates various processes in the lake water column. Algae was sampled on 1 July 2009 straight from a surface scum, and represents photosynthetic material at the lake surface. Floating material from the bottom was taken from the Limnos tube sampler in early spring under ice (6 April 2010). *Chaoborus* were sampled from near the lake bottom by net. For isotopes analyses samples were frozen and then freeze-dried before analysis by EA IRMS.

2.3 Calculations

2.3.1 Amount of oxidized CH_4 during stratification period

An estimate of CH_4 oxidation was derived from estimation of turbulent diffusion of CH_4 across the concentration gradient in the water column and by comparing predicted and observed concentrations in the water column at each meter during the ice-free period (Kankaala et al., 2006a). Estimation of CH_4 oxidation by this method was only possible during the stratification period. Results were compared to data from 2007 when concentration changes during 24 h incubations in glass syringes were measured in the laboratory at temperatures prevailing in the lake (Kankaala et al., 2013b).

2.3.2 Amount of methane production and process pathway

CH_4 production at the lake bottom was based on the amount of CH_4 oxidized in the water column and the estimated surface flux (Bastviken et al., 2002) during the stratification period. An oxidation-based estimate of production was possible because that lost in ebullition was small (results from funnel deployments) and also water flowing out from the lake contained only a small portion of CH_4 according to the low concentration of CH_4 in the epilimnetic water and the small lake outflow. Estimation of the process pathway in CH_4 production was based on Whiticar et al. (1986). Here, the assumption was that CH_4 production in the sediment surface and deep water column affected $\delta^{13}C$-CH_4 and DIC in the bottom water, and thus they were used as values following from methanogenesis. An estimate of hydrogenotrophic production of CH_4 was calculated from $\delta^{13}C$-CH_4 and $\delta^{13}C$-CO_2 (Whiticar et al., 1986; Conrad, 2005)

$$\alpha_{CO_2-CH_4} = \frac{\delta^{13}C_{CO_2} + 1000}{\delta^{13}C_{CH_4} + 1000} \tag{2}$$

where $\alpha_{CO_2-CH_4}$ = apparent carbon fractionation factor by hydrogenotrophs. In freshwater sediments, $\alpha_{CO_2-CH_4} > 1.065$ indicates hydrogenotrophy as the dominant pathway, while $\alpha_{CO_2-CH_4} < 1.055$ indicates dominance of acetatoclastic methanogenesis (Whiticar et al., 1986).

2.3.3 CO_2 flows from organic matter degradation leading to CH_4 formation

Fermentation processes produce H_2 or acetate from organic matter. In the hydrogenotrophic pathway ($CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$) [R1], the required hydrogen (H_2) production also generates CO_2: $2CH_2O + 2H_2O \rightarrow 2CO_2 + 4H_2$ [R2]; thus the complete hydrogenotrophic pathway produces one mole of CO_2. Formation of acetate ($4H_2 + 2CO_2 \rightarrow CH_4COOH$) [R3] for the acetoclastic pathway ($CH_3COOH \rightarrow CH_4 + CO_2$) [R4] consumes two moles of CO_2 but produces one mole of CH_4 and CO_2 which is compensated by CO_2 production in the H_2 formation [R2] needed for acetate. Thus, according to Chanton et al. (2005), both processes producing CH_4 can be written as $2CH_2O \rightarrow CH_4 + CO_2$, [R5] and the CO_2 produced in the whole chain from organic matter leading to CH_4 formation is same as the CH_4 produced, irrespective of the pathway.

Carbon dioxide produced in CH_4 oxidation was estimated for the aerobic and anaerobic parts of the water column based on general equations for CH_4 oxidation: $CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O$ [R6] and $CH_4 + SO_4^{2-} \rightarrow HCO_3^- + HS^- + H_2O$ [R7]. Thus in theory aerobic and anaerobic processes produce one mole of C from one mole of consumed CH_4. However, in practice the portion of CO_2 is smaller, as some CH_4-C is retained in the biomass of methanotrophs.

2.3.4 Bulk amount of methane-derived biomass and CO_2, and $\delta^{13}C$ of MOB and CO_2 in the water column

Growth yield of methanotrophs was estimated from literature values. In aerobic and anaerobic CH_4 oxidation, all CH_4 is converted to either biomass or CO_2. In general, carbon conversion efficiency (CCE) expressed as percentage of carbon incorporated into cell material for microbial growth on CH_4 varies from 19 to 70 % (Leak and Dalton, 1986; Roslev, 1997). Rudd et al. (1974) estimated that one third of CH_4 carbon goes to biomass in lake water column CH_4 oxidation. Kankaala et al., 2013b used a range of 10–40 %. Here a CCE value of 44.9 % was used for aerobic oxidation (Leak and Dalton, 1986). For AOM there is energy limitation, doubling times are high and CCE is small, 99 % of carbon goes to CO_2 and only 1 % to formation of anaerobic methane oxidizer (ANME) biomass (Knittel and Boetius, 1999). Thus 1 % was used here for the value of carbon incorporation to biomass in anaerobic oxidation. In earlier studies AOM did not result in the assimilation of carbon from ^{14}C-CH_4, while 30–60 % was assimilated in aerobic oxidation of CH_4 (Panganiban et al., 1978).

A range for possible $\delta^{13}C$ of methanotrophic biomass was derived from measured water column $\delta^{13}C$-CH_4 and literature values for fractionation between CH_4 and methanotrophic biomass. $\delta^{13}C$ of biomass is 12.6 ‰ lighter for soluble methane mono ogygenase (sMMO) and 23.9 ‰ lighter for particulate methane mono oxygenase (pMMO) than is source CH_4 (Alperin et al., 1988). Anaerobic CH_4 oxidation leads to smaller depletion in $\delta^{13}C$ of methanotrophic biomass, since α_{ox} is 1.009–1.012 ‰

(Alperin et al., 1988); however, Holler et al. (1999) obtained values of 1.012–1.039 for marine anaerobic sediments, and thus values from 1.009 to 1.039 correspond to the range of possible fractionations. Here the expression: $\varepsilon \sim (\alpha - 1) \cdot 1000$ is also used (ε = fractionation).

A substantial portion of CH_4 carbon is assimilated to biomass in aerobic oxidation, while the remaining carbon is lost in respiration as MD-CO_2. The CCE value cited above (Leak and Dalton, 1986) was used to estimate the portion of CO_2 produced in aerobic CH_4 oxidation, this being 100 % – 44.9 % = 55.1 % as CO_2-C. This was also used in mass balance calculation of the δ value of CO_2 produced.

In anaerobic oxidation almost all CH_4 is estimated to produce CO_2 with biomass gain only around 1 %. For CO_2 from anaerobic oxidation, δ^{13}C-CO_2 was calculated by mass balance equation when the amount of oxidized CH_4, its δ^{13}C and amount of MD-biomass carbon is known. Since only 1 % goes to biomass formation, the δ^{13}C value of the CO_2 produced should be almost the same as that of the original CH_4.

Production of biomass and CO_2 was divided through the water column by assuming that CH_4 oxidation was anaerobic below depths at which measured redox turned negative. The depth of the detection limit (0.33 mg L^{-1}) for our O_2 measurementis also shown.

3 Results

3.1 Water column variables

The average water column temperature gradient during stratification was similar during the study years (Fig. 2a). Data logged from spring 2007 to autumn 2008 show the general pattern of temperature profile development in the water column (Fig. 3): water surface temperatures started to decrease in August, while temperatures at greater depths increased until cooling of the air eventually led to cooling of water masses towards autumn. Unlike other depths where temperature decreased toward autumn, temperature at the bottom (6 m) increased until the end of October, but decreased rapidly in November. In winter, the coldest temperature of 2.2 °C was measured on 11 April 2008 at 1 m, while the temperature remained above 3.8 °C at 4 m depth.

Oxygen concentration was below the detection limit at the bottom during the stratification period (Fig. 2b). The total amount of oxygen in the water column (Fig. 4a, Table 1) and the depth of the oxygenated layer (Fig. 5a) increased towards autumn, while at 6 m depth the water was aerobic during overturns in autumn 2007 and 2009, but not in 2008 (Fig. 5a). During the study, the thickness of the aerobic layer decreased (Figs. 2b and 5a). The change in oxygen profile was not due to a change in thermocline depth (see Fig. 2), but due to changes in oxygen consumption, dissolution or its production pattern. Minimum concentrations of oxygen during the open water period were at the end of July, and also after ice-melt in spring 2009 and 2010 (Fig. 4a). The maximum amount of oxygen in the water column was in May in 2007 and during November in 2008 and 2009 (Fig. 4a). Redox potential was negative at 5 and 6 m depths during the stratification period (Fig. 2c); during 2009 redox was already negative below 1 m depth (Fig. 5b). Water level fluctuation was monitored in 2008 and 2009; in 2008 water level increased 10 cm from spring to autumn, while it remained quite stable in 2009 (Fig. 4a). Secchi disc transparency decreased from 2.1 m in 2007 and 2008 to 1.5 m in 2009, so the euphotic zone changed accordingly. However, water colour in the hypolimnion decreased during the study (Table 1). During the stratification period, the water column was clearly stratified with regard to different carbon forms, colour, pH, concentration of dissolved gases and nutrients (2007 data; Table 1). All amounts were highest at the bottom, except oxygen and $NO_2^- + NO_3^-$, which were lowest at the bottom (Table 1). Water was acidic, but less so in the hypolimnion. DOC was the dominant carbon form in the water column (10.1–20.1 mg L^{-1}), with average totals in the water column during the stratification period of 83.4, 87.9 and 88.5 g m^{-2} in 2007, 2008 and 2009 respectively (Table 1). Total amount of DOC decreased clearly from spring to winter during 2007, but in 2008 and 2009 the decrease was minor (Fig. 4b). POC was the smallest fraction of carbon in the water column, being 0.6, 0.7 and 1.5 mg L^{-1}

C in epi-, meta- and hypolimnion in 2007 (Table 1). Average amount of POC in whole water column was totalling 5.7, 7.1 and 6.8 $\mathrm{g\,m^{-2}}$ in 2007, 2008 and 2009 (Table 1). Regarding nutrients, the sum of NO_2^- and NO_3^- increased from 2007 to 2009, but the total N, NH_4^+-N, total P and PO_4^--P showed no systematic trend during the study. pH decreased slightly from the 2007 value, with the greatest decrease in the epilimnion and hypolimnion.

3.2 Primary production and respiration

PP was 20.1, 18.5 and 17.6 $\mathrm{g\,C\,m^{-2}}$ in 2007, 2008 and 2009 respectively (Table 2). Consequently, the amount of sugar carbon added in 2008 and 2009 (22 $\mathrm{g\,C\,m^{-2}}$) was comparable to the amount of carbon derived from PP. In 2007, 2008 and 2009 community respiration was 21.2, 28.3 and 14.3 $\mathrm{g\,C\,m^{-2}}$. Respiration was higher than the PP in 2007 and 2008. Because dark respiration in 2009 showed net uptake of CO_2 below 1 m, integrated PP and respiration are calculated for the upper 1 m of the water column.

3.3 Gaseous carbon flows

Dissolved C gas concentrations were highest in the hypolimnion (Table 1, Fig. 6). Methane concentration was highest at the bottom (Figs. 6a, c, e and Fig. S1b in the Supplement), and there was a steep decrease in concentration from the bottom to a depth of 3 m; above 3 m the concentration was stable to the surface (Fig. 6a, c and e, inserts). In general the amount of dissolved gaseous carbon increased during the study; CH_4 increased from 7.2±1.6 to 9.1±2.5 g CH_4-C $\mathrm{m^{-2}}$ from 2007 to 2009 (Fig. 4b, Table 1). Total average DIC in the water column increased from 31.1±3.6 $\mathrm{g\,m^{-2}}$ in 2007 to 33.2±4.5 $\mathrm{g\,m^{-2}}$ in 2008 and finally to 35.7±4.6 $\mathrm{g\,m^{-2}}$ in 2009 (Table 1, Fig. 4b). Emissions during the stratification period also increased; most important was the diffusional escape of CO_2, which increased from 28.3 $\mathrm{g\,C\,m^{-2}}$ in 2007 to 76 $\mathrm{g\,C\,m^{-2}}$ in 2009, while CH_4 emissions were smaller and increased from 0.9 $\mathrm{g\,C\,m^{-2}}$ in 2007 to 1.6 $\mathrm{g\,C\,m^{-2}}$ in 2009 (Table 2).

Both CH_4 production and oxidation increased from 2007 to 2009; around 97 % of CH_4 produced was oxidized (Table 2). The average in situ production was 161–317 mg CH_4-C m^{-2} d^{-1}. Oxidation patterns were similar during the study years, oxidation being higher in autumn while the minimum oxidation was measured in early summer (Fig. 5c–e). In general, aerobic oxidation was only 6–30 % of all CH_4 oxidation, and was higher in early summer. The estimate for CH_4 oxidation of 28.8 g CH_4-C m^{-2} obtained with the syringe incubation method during the ice-free period in 2007 (back calculated from Kankaala et al., 2013b) was bigger for this longer period, and thus in the same range as those given here by the diffusion gradient method. Based on the bubble collectors there was no clear ebullition at the lake, but there was an increase in CH_4 concentration in funnels, compared to that in the corresponding surface water: 12.9 ± 2.0 µmol CH_4 in the water and 23.5 ± 2.3 µmol CH_4 ($n = 3$) dissolved in the gas collectors on 30 October 2008. The increase during the 2 weeks of deployment by 0.4 mg m^{-2} d^{-1} was considered to be so small that it was not added to CH_4 flux or production estimates.

Since 67–92 % of oxidation during the stratification period was anaerobic, and anaerobic oxidation clearly produces more CO_2 than biomass, total CO_2 production from anaerobic CH_4 oxidation was substantial, while that from aerobic oxidation was small (Table 2, Fig. 7).

Oxidation of CH_4 returned almost all MDC carbon to the water column (Table 3; Fig. 7), mostly as CO_2. This CO_2 (and lack of photosynthesis) is probably seen as increased concentration at depths of 3 m in plots of DIC concentration (Figs. 6a, c, e and S1a). Biomass formed anaerobically was clearly smaller than that formed aerobically (Table 3, Fig. 7a–c). The amount of biomass from aerobic CH_4 oxidation was 75–95 % of all CH_4-derived biomass carbon. Biomass δC from CH_4 oxidation is relatively ^{13}C-depleted, since its location is at depths where isotopic fractionation in CH_4 oxidation is minimal or even reversed. Here the range used for microbial biomass δC estimate is wide (30 ‰), but in any case the most depleted MOB biomass is formed in the deep portion of water column.

Methanogenesis and its preceding steps use CO_2 from sediment or the deep water column, but also release CO_2. The net release of CO_2 related to methanogenesis increased from 2007 to 2009 (Table 2).

3.4 δ^{13}C of CH_4 and DIC

In general, δ^{13}C in CH_4 increased from 2008 to 2009 (Fig. 6d and f) and the difference was statistically significant for depths 4 m (+4.2 ‰), 5 m (+6.9 ‰) and 6 m (+5.5 ‰) (independent sample t test, $p < 0.05$, df = 11). However, CH_4 at 2 m was 15 ‰ lighter in 2009 than in 2008 (t test, $p < 0.05$, df = 11; Fig. 6d and f). Like CH_4, hypolimnetic DIC became ^{13}C-enriched during the study (Fig. 6d and f). In 2008 there was enrichment of 1.9 ‰ at 5 m compared to 2007, and between 2007 and 2009 differences were statistically significant for depths 3 m (+2.9 ‰), 4 m (+4.4 ‰) and 5 m (+3.6 ‰), but not in the surface layers. There was also statistically significant ^{13}C-enrichment in DIC from 2008 to 2009 at 4 m (+2.7 ‰), 5 m (+1.7 ‰) and 6 m (+1.4 ‰).

In early summer δ^{13}C-CH_4 was lowest at the bottom, but later in summer the most ^{13}C-depleted CH_4 values were measured from 5 m depth (Figs. 8b and S1). δ^{13}C-CH_4 at the bottom increased from August 2008, while at 5 m depth δ^{13}C-CH_4 decreased until autumn overturn induced ^{13}C-enrichment. In 2009 the most ^{13}C-depleted values were at 5 m (Fig. 8b). There was no statistically significant difference in δ^{13}C-CH_4 between depths of 6 and 5 m, but there were differences between 6 m and all other depths in 2008. In 2009 δ^{13}C-CH_4 values at 5 and 4 m did not differ from values at 6 m, while there were statistically significant differences with other depths. Average δ^{13}C-CH_4 values at the bottom were −75.0 ± 1.9 ‰ in 2008 and −70.0 ± 1.1 ‰ in 2009. Mechanically-released bubbles gave a corresponding δ^{13}C-CH_4 value of −73.6 ‰ (n = 2) in 2008. The average of the most enriched δ^{13}C-CH_4 in the water column was −33.3 ± 9.2 ‰ in 2008 and −45.4 ± 9.3 ‰ in 2009. The fractionation factor (α_{ox}) for whole water column CH_4 oxidation (calculated from the difference between bottom δ^{13}C-CH_4 and most enriched ^{13}C-CH_4 during the stratification period)was 1.043 in

2008 and 1.026 in 2009. Almost all of the fractionation occurred in the metalimnion just below the aerobic layer (Fig. 9), where the amount of CH_4 was only 1/200–1/300 of that at the bottom, and thus a relatively small oxidation of a small amount of CH_4 led to large ^{13}C-enrichment. It was not possible to calculate α_{ox} for the anaerobic part of water column where δ^{13}C-CH_4 remained similar even though the oxidation of CH_4 ranged from no oxidation ($f_{ox} = 0$) to almost all oxidized ($f_{ox} = 1$) (Fig. 9). The maximum average fractionation was between the bottom and 2 m; ε was 37.9 ‰ in 2008 and 17.0 ‰ in 2009. The average depth of the most enriched CH_4 followed changes in the oxygen-depth profile in the lake, and increased from 2.2 m in 2008 to 1.7 m in 2009. The location of the most enriched CH_4 was narrow (Fig. S1), and the true maximum value could have been missed with our 1 m sampling resolution.

DIC was heavier in 2008 than in 2007 in the whole water column, but there was a statistically significant difference in ^{13}C-DIC only at a depth of 5 m (−20.4 ‰ in 2007 and −18.5 ‰ in 2008; t test, $p = 0.031$, df = 13). In 2009 DIC was also heavier than in 2007 at all depths, but the difference was statistically significant only for depths 3, 4 and 5 m. DIC also became ^{13}C-enriched between 2008 and 2009, but the difference was significant only at 4 m. The average δ^{13}C-DIC at the bottom during the stratification period was −12.4 ± 0.6 ‰ in 2008 and −11.0 ± 0.7 ‰ in 2009. (In 2007 ^{13}C-DIC was not measured from the depth of 6 m). The difference (~ 6 ‰) between 5 and 6 m DIC values was statistically significant (paired samples t test, $p < 0.05$, df = 9 for 2008 or 6 for 2009). Averages of the most depleted DIC values in the water column were −24.9 ± 1.7 ‰, −23.2 ± 2.2 ‰, and −21.2 ± 2.0 ‰ in 2007, 2008 and 2009, respectively. The change in the water column δ^{13}C-DIC was smooth compared with the change in δ^{13}C-CH_4 (Figs. 6b, d and f and S1). The depth of the most ^{13}C-depleted DIC was 1.5–2 m lower than that the depth of the most ^{13}C-enriched values of CH_4. On 27 August 2008 when sampling resolution was 0.5 m, this difference was 1.5 m (min. δ^{13}C-DIC at 4 m and max. δ^{13}C-CH_4 at 2.5 m; Fig. S1). On average, the most depleted δ^{13}C-DIC values were measured from 3.75, 3.75 and 3.67 m depths in 2007, 2008 and 2009 respectively.

The fractionation factor ($f_{CO_2-CH_4}$), between average bottom $\delta^{13}CO_2$ and $\delta^{13}C$-CH_4, decreased from 1.068 ± 0.002 in 2008 to 1.064 ± 0.002 in 2009, indicating that CH_4 was mainly from hydrogenotrophic processes, but there might have been a slight shift towards more acetogenesis in CH_4 production.

3.5 $\delta^{13}C$ of CH_4 derived C

Depending on the processes responsible for CH_4 oxidation, the microbial biomass using CH_4 as a carbon source could have had $\delta^{13}C$ ranging from −114 to −79 ‰ (Table 3, Fig. 7b and c). Similarly, DIC derived from CH_4 oxidation could have had $\delta^{13}C$ from −68 up to −38 ‰ in aerobic CH_4 oxidation, while CO_2 from anaerobic CH_4 oxidation had almost similar $\delta^{13}C$ value as the original CH_4 since only 1 % went to MOB carbon (Table 3). The amount of oxidized CH_4 in different regions of the water column shows that there was a change in the pattern of CH_4 oxidation during the study. Methane-derived biomass at greater depths had the lowest $\delta^{13}C$ (Fig. 7b and c), and most of this biomass was at depths below 3 m. Similarly, depleted CO_2 was formed at the bottom where production from CH_4 oxidation was also highest. A small amount of CH_4-derived CO_2 was produced in shallower water (Fig. 7a–c), where $\delta^{13}C$-CO_2 increased. In the illuminated layer of active photosynthesis, CH_4 oxidation was also minimal due to lack of CH_4. Thus the effect of methanotrophy on DIC production and algal biomass $\delta^{13}C$ was small at the surface as was formation of methanotrophic biomass. In 2009 CH_4 oxidation could have been more active in the euphotic zone, and thus could have affected the algal and zooplankton $\delta^{13}C$.

3.6 $\delta^{13}C$ of other carbon pools

Average POM and DOM $\delta^{13}C$ values, calculated according to the division of the lake water column into epi-, meta and hypolimnion, were quite similar, DOM being moderately lighter than POM. Both were also slightly more ^{13}C-depleted in the metalimnion than in the epi- and hypolimnion.

δ^{13}C-POM measurements on 15 September 2009 from different depths down the water column at 1 m resolution corresponded well to averages for the meta- and hypolimnion, but differed in the epilimnion (Fig. 6f). This is possibly due to different processes in the epilimnion affecting the POM δ^{13}C in autumn when the water layers are mixing, which is not strongly reflected in ice-free average values.

Algae sampled on 1 July 2009 straight from a surface scum had δ^{13}C value of −29.8 ‰ compared with δ^{13}C-DIC of −13.7 to −14 ‰ at the same time at the same depth; thus fractionation between DIC and algae was ∼ 17 ‰. Material floating above the bottom sediments (probably sedimented algal material from the previous summer) sampled in early spring under ice (6 April 2010) was depleted to −32.8 ‰. Biofilm from aluminium tubes used in incubation experiments had δ^{13}C of around −24 ‰ at the surface (with contact to the atmosphere) and −27.2 ‰ at 0.2 and 1 m depths, but was depleted to −36.0 ‰ at 2 m. Larvae of the phantom midge (*Chaoborus* sp.), migrating daily between the bottom and the oxygenated surface, had average δ^{13}C of -31.2 ± 3.8 ($n = 20$) between 14 August 2007 and 3 May 2010, but individual δ^{13}C values ranged from −37.9 to −25.4 ‰.

4 Discussion

4.1 Effect of added carbon

In general, the addition of sugar carbon changed processes in the lake, but CH_4 oxidation and MDC formation were essentially similar to the reference year 2007, and typical of this kind of stratified lake.

CH_4 efflux during the stratification period doubled from 2007 to 2009, while CO_2 efflux almost tripled. Calculations based on global warming potential for a 100 year period (one gram of CH_4 corresponds to 25 g of CO_2; IPCC, 2007), show that CO_2

was the most important greenhouse gas emitted from the lake and even more so after sugar addition. Emission of CO_2 was substantial, but was smaller than that measured by Eddy Covariance at the nearby lake Valkea Kotinen (Huotari et al., 2011). However, methods based on surface concentration generally give lower estimates of fluxes for CO_2 and CH_4 than Eddy Covariance (Schubert et al., 2012). Furthermore, efflux was calculated only for the stratified period, and fluxes during overturn when water masses rich in CO_2 and CH_4 come into contact with the atmosphere are not included, so our values certainly underestimate annual emissions.

Although DOC amount was increased by sugar addition, there was no clear increase in epilimnetic heterotrophy, probably due to shortage of mineral nutrients (Peura et al., 2014). Addition of labile carbon as sugar probably increased nutrient competition between bacteria and algae favouring bacteria, and this decreased the amount of nutrients from 2007 to 2008 (Table 1) as demonstrated by Tammert et al. (2012) in mesocosm experiments with glucose addition.

Our addition of $44\,\mathrm{g\,C\,m^{-2}}$ during 2008 and 2009 led to $\sim 60\,\mathrm{g\,C\,m^{-2}}$ increase in C fluxes to the atmosphere during the stratification period. Peura et al. (2014) explained the increased flux in Alinen Mustajärvi by increased anaerobic respiration and fermentation in the hypolimnion and by increased degradation of DOC in the meta- and hypolimnion. Furthermore, the thinner epilimnion increased DIC-rich water masses in the upper water column, and this physical change also increased efflux of CO_2 and CH_4. Here it was possible to study the fate of CH_4 by methane oxidation, and as production of CH_4 increased, both aerobic and anaerobic CH_4 oxidation increased leading to a substantial increase in CO_2 formation. However, most of this increase in CO_2 production from CH_4 was in the anaerobic bottom layers and upward diffusion from there can partly account for the increased concentration of CO_2 in surface layers and the increased CO_2 efflux.

During this study the hypolimnetic δ^{13}C-DIC and δ^{13}C-CH_4 increased and thus the added ^{13}C-enriched sugar carbon had clear effects on the anaerobic zone of the lake. To our knowledge there are no other data covering two years of δ^{13}C-CH_4 and δ^{13}C-

DIC values from lakes to compare if this kind of fluctuation in δ^{13}C occurs naturally in stratified lakes, but unless autochthonous or allochthonous carbon inputs are changing it is unlikely. Direct aerobic respiration of the cane sugar would produce an enriched ^{13}C signal in DIC, as cane sugar is ~ 16 ‰ heavier than the natural DOM or POM in the lake; in fact enrichment was clear in the hypolimnion but not clear in the epilimnetic DIC. Thus enriched DIC was available to be incorporated into algae, possibly seen in POM δ^{13}C values following those of DIC at 0–2.5 m depth (Fig. 6f), and generally becoming enriched during the study (Peura et al., 2014).

It is unclear whether the ^{13}C-enrichment in CH_4 and DIC in deep layers (4, 5 and 6 m) was due to changes in the microbial and algal biomass and zooplankton food web structure, as documented for the epilimnion (Peura et al., 2014), leading to sedimentation of this enriched carbon source towards the bottom. The change could also have been due to a direct effect of the added carbon source, which partly flocculated and sunk to the bottom where it was used as a substrate in methanogenesis. A third explanation could be a change in the lake anaerobic metabolism due to physical changes, leading gradually to a shift from hydrogenotrophic methanogenesis towards acetoclastic methanogenesis, as fractionation factors were shifting in the direction of acetoclastic methanogenesis. In addition, increased CH_4 production leads to gradual enrichment of δ^{13}C of CH_4 and DIC because the carbon source gets progressively enriched as the light isotopes are used preferentially. Evidence for this increased use of carbon comes from the increased C fluxes and the decrease in total amount of TOC towards 2009. However, with current data the ultimate reason for the hypolimnetic enrichment in δ^{13}C of CH_4 and DIC remains unresolved.

The first δ^{13}C-DIC and δ^{13}C-CH_4 measurements in 2008 were before sugar addition and from a depth of 6 m. There was a small increase in bottom δ^{13}C-DIC after carbon addition in spring 2008 as there had been in the reference year 2007 before any additions were made, whereas δ^{13}C-CH_4 decreased at the depths of 5 and 6 m.

In the euphotic zone, the ^{13}C-enrichment of DIC can be explained by CO_2 uptake in photosynthesis and diffusional losses to the atmosphere, both leaving the remaining

DIC enriched. A Keeling plot estimate for δ^{13}C-DIC produced from dark incubation of epilimnetic water in situ in 2009 (data not shown) gave an estimate for respired δ^{13}C-DIC of −14.1 ‰; together with the preferential diffusional losses of light $^{12}CO_2$ to atmosphere this could well lead to the δ^{13}C-DIC values detected in the epilimnion.

4.2 Recycling of carbon in the water column

As oxidation (and production) of CH_4 was high before sugar carbon addition to whole lake, it is evident that Alinen Mustajärvi was not only a natural "hot spot" for methanogenesis, but also capable of oxidizing considerable amounts of CH_4 and processing it to biomass and CO_2. High CH_4 production is possible as the bottom receives new organic carbon sedimenting from the surface at the same time as older carbon is processed. Furthermore, even though the increase in lake bottom temperature from spring to late autumn is small (from 4.3 to 5.8 °C in 2007 and from 4.3 to 5.2 °C in 2008), with the general Q_{10} value of 4 (Yvon-Durocher et al., 2014) the production rate would increase 13–23 % thus maintaining production of CH_4 from older stores when substrate rain ceases in late autumn. Even though the measurement site was the deepest point of the lake, the difference in depth is not so great that the deep point will receive additional sediment from the sides.

Almost all of the CH_4 produced in the small stratified lake Alinen Mustajärvi was oxidized in the water column, as seen from the low surface concentrations, small CH_4 effluxes and also the clear ^{13}C-enrichment of CH_4 up the water column. The in situ incubation method to estimate the CH_4 oxidation in 2007 and the independent calculation by the diffusion gradient method gave comparable results, so we are confident that our oxidation estimates are reliable. Production and oxidation of CH_4 was an important part of the lake carbon metabolism and quantitatively and qualitatively affected the carbon cycle.

Most CH_4 oxidation was in the anaerobic portion of the water column. The high proportion (~ 97 %) of CH_4 from the total production during the summer stratification period that was oxidized is consistent with other studies; e.g. from a Japanese lake, where 74 % of all CH_4 was oxidized (Utsumi et al., 1998) and from a Finnish lake, where on an annual basis 79 % was consumed in the water column by methanotrophs (Kankaala et al., 2006a). Schubert et al. (2011, 2012) also stressed the importance of CH_4 oxidation, which was consuming 75 % of the CH_4 in Lakes Lugano and Rotsee.

The oxidation measured in Alinen Mustajärvi (annually and per day) was at the upper end of the range reported previously from lakes, whereas the emissions of CH_4 to the atmosphere were at the lower end of the reported range (Bastviken et al., 2004). However, even after the substantial DOC addition as sugar, the CH_4 emissions are more at the level of estimates by Juutinen et al. (2009) than those of Bastviken et al. (2004) for boreal lakes. Bastviken et al. (2011) estimated ebullition to be ∼ 88 % of all emissions of CH_4 to the atmosphere from lakes at the same latitude as Alinen Mustajärvi. Rasilo et al. (2014) used an estimate for ebullition of 9 % of total efflux for Canadian boreal lakes. Ebullition was insignificant in Alinen Mustajärvi, as it was in the nearby lake Valkea-Kotinen (Kankaala et al., 2006a). Carbon input as peat from degrading lake shores led to huge ebullition from Siberian thaw lakes (Zimov et al., 1997), but our sugar addition was dissolved and easily degradable carbon, which was used at least partly in the aerobic zone by microbes (Peura et al., 2014); more recalcitrant peat is a less readily available carbon source and also forms physical barriers on the lake bottom.

Schubert et al. (2012) reported CH_4 oxidation of $5.3\,\mathrm{g\,C\,m^{-2}}$ in the oxic layer and $24.8\,\mathrm{g\,C\,m^{-2}}$ in the whole water column in Lake Lugano, with efflux of $4.1\,\mathrm{g\,C\,m^{-2}}$. In Lake Rotsee oxic oxidation was $7\,\mathrm{g\,C\,m^{-2}}$ and that of the whole water column $33.0\,\mathrm{g\,C\,m^{-2}}$, and efflux $5.4\,\mathrm{g\,C\,m^{-2}}$ (Schubert et al., 2011). These estimates are similar to ours for Alinen Mustajärvi and similarly showed the overwhelming importance of AOM in stratified lakes with anoxic bottoms producing CH_4 and at same time being capable of oxidizing CH_4 anaerobically in the anoxic water column (or sediment) by electron acceptors other than oxygen. Methane oxidation in Alinen Mustajärvi during the stratified period was similar to that found by Liikanen et al. (2002) for the profundal water column of eutrophic Lake Kevätön.

In Alinen Mustajärvi more CH_4 was oxidized in the anaerobic part of the water column which does not support earlier findings that CH_4 oxidation in freshwaters is most active in the vicinity of the oxic–anoxic interface or oxycline, where both CH_4 and O_2 are available (Rudd et al., 1974; Lidstrom and Somers, 1984; Bastviken et al., 2008). Liikanen et al. (2002) also reported highest oxidation rates in a eutrophic lake hypolimnion during stratification when the bottom had the highest CH_4 concentrations. Even though in Alinen Mustajärvi the change inδ^{13}C-CH_4 showing CH_4 oxidation was greatest in the oxycline, the amount of CH_4 there was so much less than at the bottom that the actual quantity of CH_4 oxidized there was small.

The detection limit for our oxygen measurements leaves open the possibility that there was still some residual O_2 available for oxidation. Blees et al. (2014) explained CH_4 oxidation in Lake Lugano by (micro-)aerobic methane oxidation (MOx), in the zone where oxygen concentration was sub-micromolar and not detectable with traditional techniques. This might have been the case in our study, since we were not able to measure sub-micromolar concentrations of O_2. However, there are other indicators that the lake hypolimnion was truly anaerobic: redox was negative, there was sulphide in water column, and pH in the hypolimnion was higher in line with production of basic cations by AOM. Blees et al. (2014) did not report redox values from their study. However, in our study there was CH_4 oxidation in layers where redox was negative. Thus, the explanation for the CH_4 fate may be anaerobic oxidation of CH_4 (Eller et al., 2005) or nitrite reducers providing directly molecular oxygen for methanotrophs in anoxic systems (Ettwig et al., 2010). There was a suite of alternative electron acceptors available, of which nitrate was measured, while the smell of H_2S compounds was evident in samples from depths of 4 to 6 m and was also measured in 2013 (A. Rissanen, personal communication, 2013). Furthermore, humic substances can act as regenerable electron acceptors in recurrently anoxic environments as here (Klüpfel et al., 2014).

Isotope enrichment factors for CH_4 oxidation cannot distinguish distinct aerobic or anaerobic methane-oxidation pathways (Feishauer et al., 2011). There was no, or only a minor, change in δ^{13}C-CH_4, so stable isotopic enrichment did not conclusively show that CH_4 was oxidized microbiologically in Alinen Mustajärvi, even though we could measure clear CH_4 oxidation. Besides possible CH_4 production in the water column, one reason for the lack of clear enrichment of δ^{13}C-CH_4 could be that anaerobic oxidation with low sulphate concentration can lead to ^{13}C-depletion of CH_4, as Yoshinaga et al. (2014) found for sulphate-limited AOM in marine sediments. In Alinen Mustajärvi, sulphate concentration decreased from the aerobic layer to the anaerobic layer and that of sulphide increased (A. Rissanen, personal communication, 2013) thus showing the possibility of AOM by sulphate above a threshold limit of 0.5 mM of sulphate (Yoshinaga et al., 2014).

The isotope data from Alinen Mustajärvi support a view of active recycling of carbon in the lake. CH_4 diffusing from the sediment continuously removes light C isotopes from the bottom. These are mostly retained within the system higher in the water column by oxidation products, as isotopically light DIC, and in biomass of MOB and their consumers. Ultimately the MOB biomass used by grazers is returned to the sediment as faeces and zooplankton carcasses. The fate of isotopically light DIC in the deep water is probably also due to some as yet unidentified sink (Peura et al., 2012), since CO_2 is already quite ^{13}C-enriched below the euphotic zone. In Alinen Mustajärvi less than two grams of CH_4 m^{-2}, with δC ~ 23 ‰ lighter than either allochthonous or autochthonous organic carbon sources, was released to the atmosphere. Furthermore, slow degradation of carbon within the sediment column leads to release of relatively light isotopes compared to bulk sediment carbon. In contrast to these depleted carbon flows, 30–70 g of CO_2-C m^{-2} was released and this was ~ 10 ‰ enriched relative to organic carbon sources. It therefore follows that allochthonous carbon flow into the lake must correspond to that lost in CO_2 emissions, otherwise the lake and its sediments would progressively develop a lighter isotopic composition than the surroundings. In fact, δ^{13}C in lake sediments is generally lighter than allochthonous and autochthonous carbon from the upper layers of lake water column, which Lehman et al. (2002) explained by selective preservation of less reactive compounds depleted in ^{13}C. However, ^{13}C-depleted biomass from aerobic and anaerobic oxidation of CH_4 and from carcasses and faecal pellets at the bottom of lakes could also explain this ^{13}C-depletion of organic carbon in lake sediments.

The location of the most active CH_4 oxidation zone also affects zooplankton consumption of methanotrophs. In Alinen Mustajärvi, CH_4 oxidation takes place also in the suboxic and anaerobic zones, mostly below 3 m, where CH_4 is most ^{13}C-depleted. Thus the oxidation products (DIC and MDC in biomass) also there have the most negative δ^{13}C. Zooplankton in the lake descends to the oxic–anoxic interface to feed and to avoid predation pressure by invertebrate predators (Salonen and Lehtovaara, 1992). But where is the isotopically light biomass C from anaerobic CH_4 oxidation in the anoxic deep water column? In general biomass gain from ANME may be only about 1 % of oxidized CH_4 (Knittel and Boetius, 1999). One likely fate of this biomass derived from anaerobic oxidation can be consumption by zooplankton during autumnal overturn when the water layer is mixed and methanotrophs become more widely accessible to grazers and deplete their δ^{13}C (Kankaala et al., 2007; Taipale et al., 2008).

The measured δ^{13}C values for CH_4 and CO_2 are a result of many processes and it is difficult to establish an isotopic baseline from where change might be measured. The pattern for δ^{13}C-CH_4 is clearer, since the production is mainly in the bottom sediments or in deep water layers and its fate is in the water column. Schubert et al. (2011) also reported light ^{13}C-DIC (−19 ‰) in Lake Cadagno sediments combined with isotopically enriched values for the residual CH_4. Schubert et al. (2011) found methane ^{13}C-enriched from −71.8 to −42.6 ‰, (thus $\varepsilon = 1.031$), and proposed that AOM takes place in the uppermost sediment layers. We found oxidation in the water column, but the locations of the most enriched CH_4 and the most depleted DIC were separated, possibly due to processes consuming DIC.

The most enriched DIC was at the bottom, where ^{13}C-enrichment of DIC is related to anaerobic processes. Ratio of acetoclastic methanogenesis to hydrogenotrophic methanogenesis is typically 2 : 1 for freshwater sediments (Nusslein and Conrad, 2000). Both of these pathways with their preceding pathways use CO_2, even though some CO_2 is also released. Thus, at the lake bottom acetogenesis and hydrogenotrophic methanogenesis are consuming CO_2 and preferentially ^{12}C from DIC and thus enriching the remaining DIC. Enrichment of DIC is also supported from the carboxyl group, released as CO_2 in acetoclastic methanogenesis. The carboxyl group has been shown to enrich in relation to source material $\delta^{13}C$ by 12 ‰ in experiments (Blair et al., 1985). Furthermore, lithotrophic acetogenesis ($CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O$ [R8]; $\Delta G_o = -111\,\text{kJ}\,\text{mol}^{-1}$) may outcompete hydrogenotrophic methanogenesis ($\Delta G_o = -131\,\text{kJ}\,\text{mol}^{-1}$) for hydrogen-supplying substrates and thus CH_4 is produced from this acetate rather than straight from H_2 and CO_2. This sequence of processes has been hypothesised to prevail in low temperature ecosystems like peat and boreal lake sediments (Nozhevnikova et al., 2003). Enriched DIC has also been found from hypereutrophic lake bottoms; Gu et al. (2004) explained ^{13}C-DIC enrichment by methanogenesis, wind mixing, high phytoplankton productivity and by lack of external loading.

Most CH_4 production was in the surface sediment, but there was probably some methanogenesis in the water column, as seen from $\delta^{13}C$-CH_4. Usually the lowest $\delta^{13}C$-CH_4 was measured at the bottom where CH_4 was produced but there were exceptions to this, as in the three lakes studied by Bastviken et al. (2008). As deeper DIC was clearly more ^{13}C-enriched (average difference ∼ 6 ‰ between 5 and 6 m), than in the overlying depths, use of this heavier DIC in methanogenesis would mean that CH_4 produced by any of the processes would lead to formation of heavier $\delta^{13}C$-CH_4 thus leading to enrichment at 6 m compared to 5 m, where $\delta^{13}C$-DIC is lighter due to CH_4 oxidation already producing lighter DIC.

5 Conclusions

The amount of CH_4 produced was comparable to that of PP before carbon addition, after carbon addition CH_4 production almost doubled, while PP decreased. The highest concentration of gases was at the bottom. DIC at the bottom was isotopically enriched and became more depleted below the metalimnion but more enriched again towards the surface. Methane in the bottom was isotopically light and hydrogenotrohic methanogenesis was the main source of CH_4. Methane became enriched in the oxycline, where the amount of CH_4 decreased substantially, and became slightly more depleted again towards the surface. An isotopically enriched carbon source signal was seen in CH_4 and DIC as an increase in $\delta^{13}C$ at the bottom of the water column. Most CH_4 oxidation occurred in the anoxic hypolimnion; however, fractionation of $\delta^{13}C\text{-}CH_4$ in the water column did not reflect this. Oxidation of CH_4 led to substantial formation of depleted CO_2 in the hypolimnion, whereas biomass formation of methanotrophs was mostly in the metalimnion of the water column. This leads to low $\delta^{13}C$ of zooplankton grazing on methanotrophs. In general, CH_4 oxidation, mainly in anoxic or suboxic water column recycles carbon efficiently within the stratified lake even after substantial C addition. Despite this increased carbon input increases effluxes of CH_4 and especially CO_2 to the atmosphere.

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Table 1. Characteristics of the epi-, meta- and hypolimnion of Alinen Mustajärvi during summer stratification 2007. Values represent means ± SD. Water column total amounts of carbon and nutrients and averages for colour and pH are for epi- meta- and hypolimnion during the stratification period for years 2007–2009. Note that epi- meta- and hypolimnion were sampled by different patterns in 2009. Division into epi-, meta- and hypolimnion depths in second row under corresponding year. Number of analyses: $n = 12$–13 in 2007 and 2008, $n = 9$ in 2009.

	2007 (concentrations)			Total amounts			
	Epilimnion (0, 1, 2 m)	Metalimnion (3, 4 m)	Hypolimnion (5, 6 m)	Measure	2007 (0–2/3–4/5–6 m)	2008 (0–2/3–4/5–6 m)	2009* (0–1/2–3/4–6)
DOC (mg L^{-1})	10.3 ± 0.7	11.3 ± 0.7	20.3 ± 1.6	g m^{-2}	83.4 ± 5.7	87.9 ± 3.9	88.5 ± 4.7
POC (mg L^{-1})	0.6 ± 0.2	0.7 ± 0.2	1.5 ± 0.3	g m^{-2}	5.7 ± 1.0	7.1 ± 1.0	6.8 ± 1.1
CH_4 (mg C L^{-1})	0.01 ± 0.00	0.1 ± 0.1	3.5 ± 0.7	g m^{-2}	7.2 ± 1.6	8.5 ± 1.5	9.1 ± 2.5
DIC (mg C L^{-1})	1.2 ± 0.5	4.2 ± 0.9	10.2 ± 1.3	g m^{-2}	31.1 ± 3.6	33.2 ± 4.5	35.7 ± 4.6
O_2 (mg L^{-1})	7.1 ± 0.9	0.8 ± 1.0	0.2 ± 0.0	g m^{-2}	21.8 ± 5.5	17.0 ± 4.0	13.9 ± 2.8
Total N (µg L^{-1})	422.5 ± 86	673.5 ± 81	2572.0 ± 187	mg m^{-2}	7328 ± 354	6811 ± 467	7130 ± 1336
NO_2^-+NO_3^-(µg L^{-1})	16.0 ± 9.8	13.3 ± 6.9	12.1 ± 2.7	mg m^{-2}	82 ± 35	99 ± 43	113 ± 67
NH_4^+ (µg L^{-1})	34.3 ± 44.2	214.7 ± 61	1515.6 ± 179	mg m^{-2}	3525. ± 313	3458 ± 319	3596 ± 1119
Total P (µg L^{-1})	11.5 ± 3.8	19.5 ± 2.8	131.2 ± 16	mg m^{-2}	324 ± 31	289 ± 42	342 ± 111
PO_4^- (µg L^{-1})	1.1 ± 0.3	1.2 ± 0.4	65.8 ± 14	mg m^{-2}	166 ± 29	136 ± 28	167 ± 87
Colour (mg Pt L^{-1})	103.5 ± 13	140.0 ± 10.2	291.3 ± 17	mg Pt L^{-1}	104/140/291*	103/127/277	103/140/217
pH	5.1 ± 0.1	5.0 ± 0.3	5.9 ± 0.2	pH	5.1/5.0/5.9	4.9/5.0/5.8	4.9/4.9/5.7

* Colour and pH are shown separately for epi-, meta- and hypolimnion.

Table 2. Amount of CH_4 ($g\,C\,m^{-2}$) emitted, produced and oxidized, and oxidized CH_4 as % of total. Carbon dioxide diffusive flux, primary production, community respiration, release in CH_4 formation, release in CH_4 oxidation and carbon added to the lake as cane sugar.

$g\,C\text{-}CH_4\,m^{-2}$				$g\,C\,m^{-2}$					
Diff. flux	produced	oxidized ox./anox./ total	% oxidized ox/anox./ total	CO_2 flux	PP	Community respiration	CO_2 released in CH_4 formation	CO_2 released in CH_4 oxidation	Added cane sugar C
0.9	26.6	7.9/17.7/25.7	30/67/97	28.3	20.1	21.2	12.8	25.7	–
1.5	34.4	9.2/23.7/32.9	27/69/96	47.6	18.5	28.3	17.2	32.9	22
1.6	48.7	2.9/44.6/47.5	6/92/97	67.0	17.6*	14.3*	24.3	47.5	22

* PP and respiration measurements in 2009 to depth of 1 m.

Table 3. δ^{13}C (‰) of CH_4 and CO_2 from the lake bottom and of maximum (CH_4) and minimum (CO_2) in the water column. Amount of biomass derived from aerobic and anaerobic CH_4 oxidation and its δ^{13}C value with range of two fractionation factors. Amount of oxidation-derived CO_2 and estimates for its δ^{13}C with two fractionation factors in aerobic and anaerobic CH_4 oxidation.

Year	$\delta^{13}CH_4$ (‰)		$\delta^{13}CO_2$ (‰)		Carbon amount from oxidized CH_4 ($g\,C\,m^{-2}$)		δ^{13}C from oxidized CH_4 (‰)		
	bottom	max.	bottom	min.	[a,b] biomass aerobic/ anaerobic/ total	[c] CO_2-C aerobic/ anaerobic/ total	[d] bm. C aerobic + anaerobic	[e] CO_2 aerobic	[f] CO_2 anaerobic
2007	ND	ND	ND	−24.9 ± 1.7	3.6/0.2/3.7	4.4/17.6/22.0	ND	ND	ND
2008	−75.0 ± 1.9	−34.3 ± 9.2	−12.0 ± 1.0	−23.9 ± 2.2	4.2/0.2/4.4	5.1/23.4/28.5	−114 to −84	−68 to −43	−75
2009	−70.0 ± 1.1	−45.4 ± 9.3	−10.6 ± 1.6	−21.2 ± 1.1	1.3/0.4/1.7	1.6/44.2/45.8	−109 to −79	−63 to −38	−70

[a, b, c] Biomass amount based on CCE = 44.9 % (Leak and Dalton, 1986), and 1 % for anaerobic CH_4 oxidation (Knittel and Boetius, 1999).

[d] Biomass δC estimate with fractionation factors −9 to −39 ‰.

[e] δ^{13}C of biomass from aerobic methane oxidation based on two source mixing model using CCE value of 55.1 % (Leak and Dalton, 1986) and from amounts of biomass and δC-CO_2.

[f] Since 99 % of CH_4 go to CO_2 in anaerobic oxidation, biomass δC is ~ same as source $\delta^{13}CH_4$.

[e, f] Calculated by two source mixing model and δC of biomass.

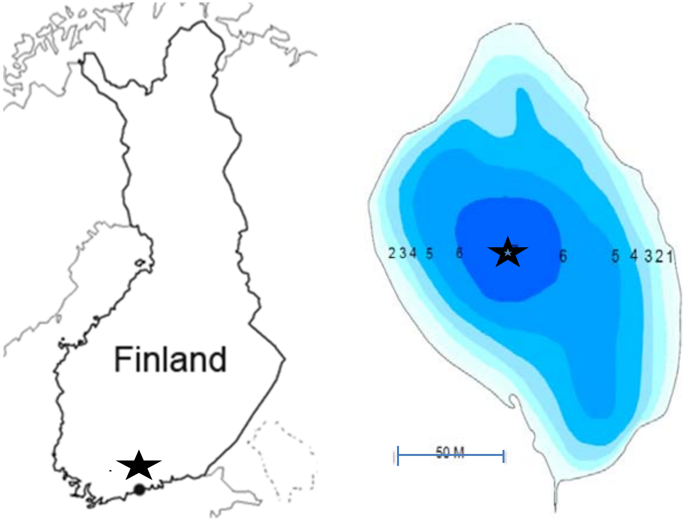

Figure 1. Location of Alinen Mustajärvi (left) and depth profiles (right) with sampling point marked by star.

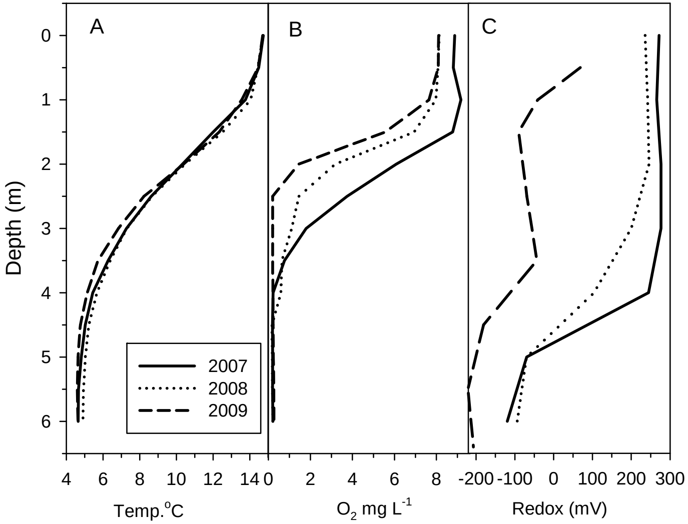

Figure 2. Water column temperature **(a)**, oxygen concentration **(b)** and redox potential **(c)** from 2007 during the stratification period to 2009. In 2009 redox is average from measurements made in 14.5, 13.7 and 11.8.

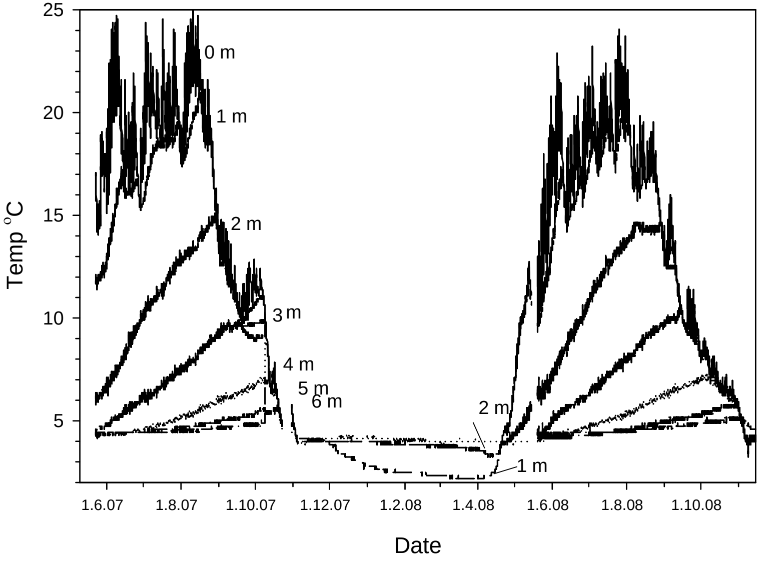

Figure 3. Water temperatures logged from depths 0–6 m from spring 2007 to autumn 2008 in Alinen Mustajärvi. During winter 2007–2008 only depths 1, 2 and 4 m were logged successfully.

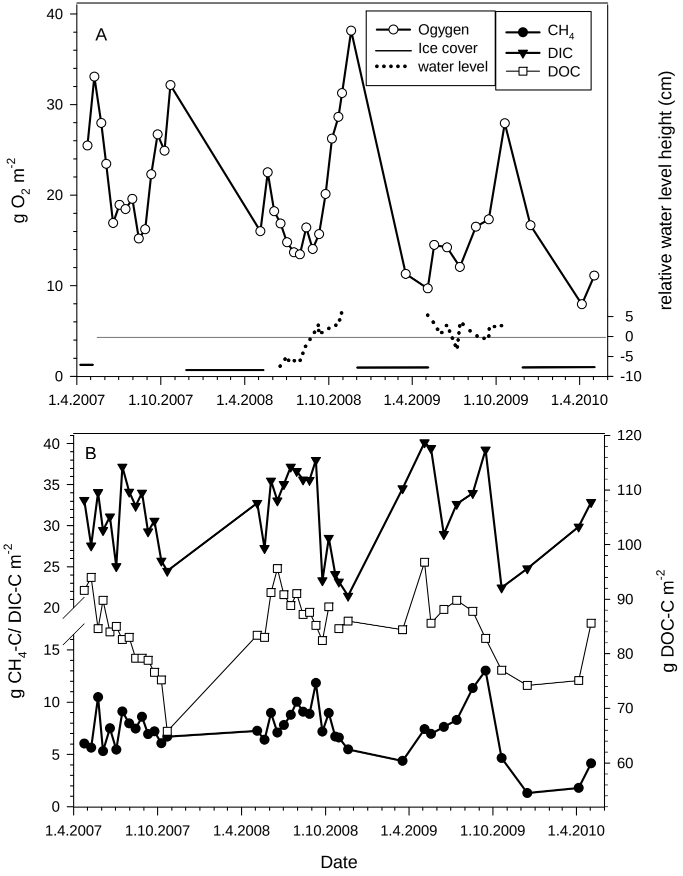

Figure 4. Total amount of oxygen in the water column and relative water level height and ice cover period **(a)**. Total amounts of DIC, DOC and CH_4-C in the water column **(b)**.

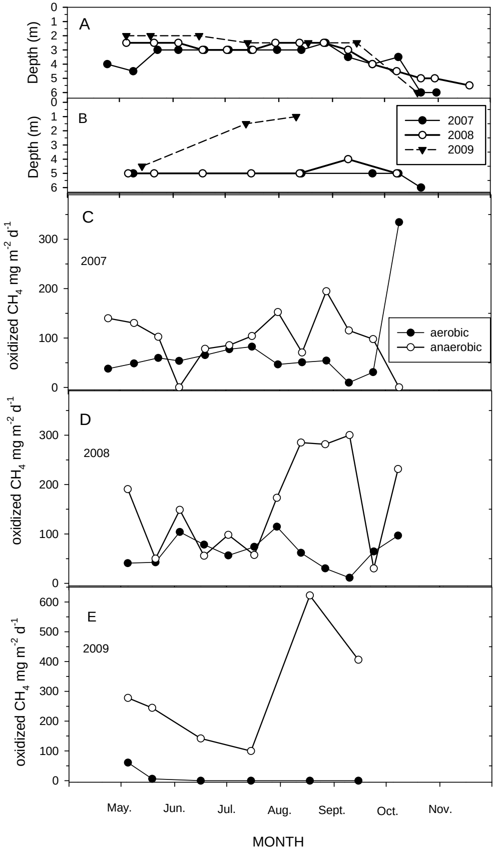

Figure 5. First depth from surface where O_2 concentration was less than 0.33 mg L^{-1} during 2007, 2008 and 2009 **(a)**. Depth where redox (mV) turned negative **(b)**. Amount of CH_4 oxidized aerobically and anaerobically in the water column estimated by diffusion gradient method in 2007 **(c)**, 2008 **(d)** and 2009 **(e)**. Depths within the negative redox zone were considered anaerobic.

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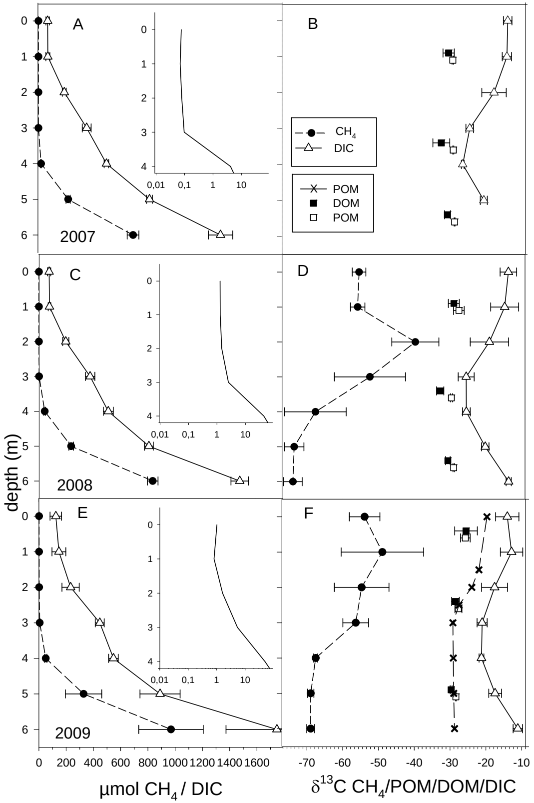

Figure 6. Water column CH_4 and mean concentrations ± S.E. for stratification period for years 2007 **(a)**, 2008 **(c)** and 2009 **(e)**. Superimposed inserts show average CH_4 concentration in upper water column with a logarithmic scale **(b, d, f)**. $\delta^{13}C$ of CH_4 and DIC for the same years. Also shown are $\delta^{13}C$-POM and $\delta^{13}C$ of DOM for composite samples of epi-,meta- and hypolimnion at average depths of sampling. Separately measured $\delta^{13}C$-POM (crosses) from sampling on 15 September 2009 from whole water column is shown in **(f)**.

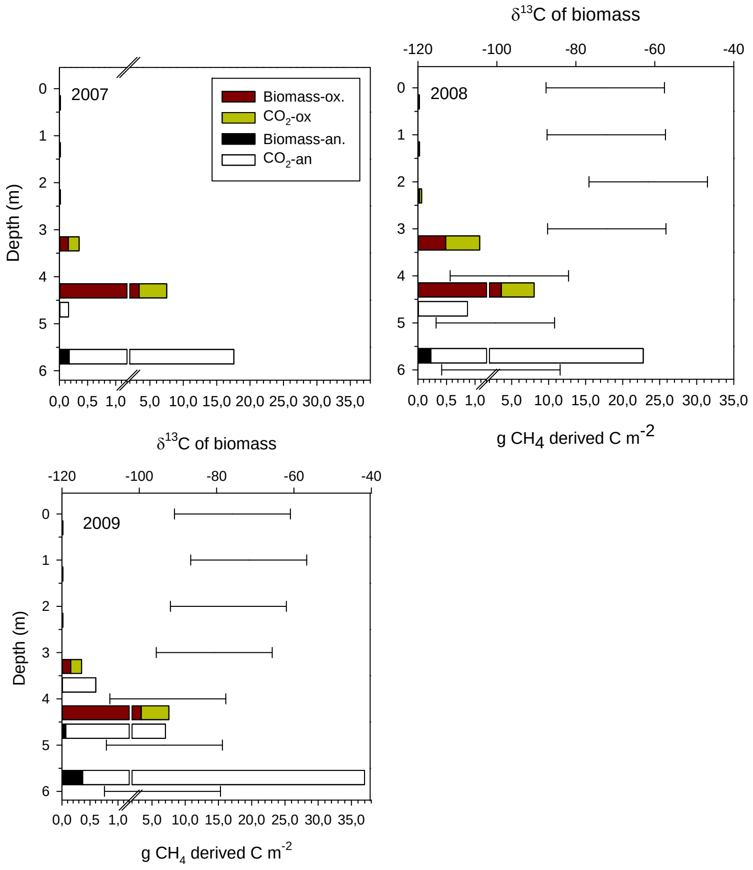

Figure 7. Estimates of the amount of CO_2 and biomass derived from aerobic methanotrophy, and of CO_2 and the amount of biomass derived from anaerobic methanotrophy in 2007–2009. Horizontal lines show estimated range for $\delta^{13}C$ of biomass.

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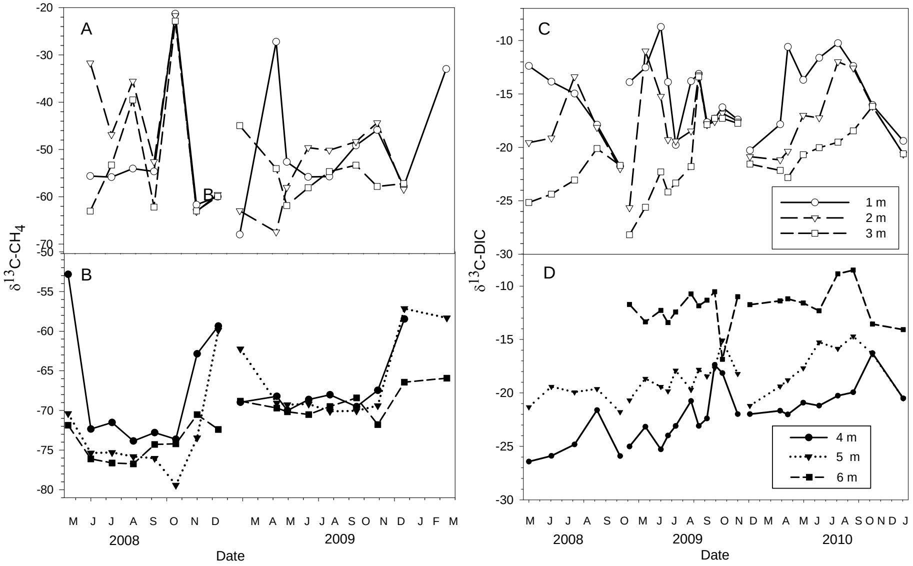

Figure 8. Dynamics of δ^{13}C-CH$_4$ and δ^{13}C-DIC in the water column. δ^{13}C-CH$_4$ at **(a)** depths 1, 2 and 3 m and **(b)** depths 4, 5 and 6 m. δ^{13}C-DIC at **(c)** depths 1, 2 and 3 m and **(d)** depths 4, 5 and 6 m.

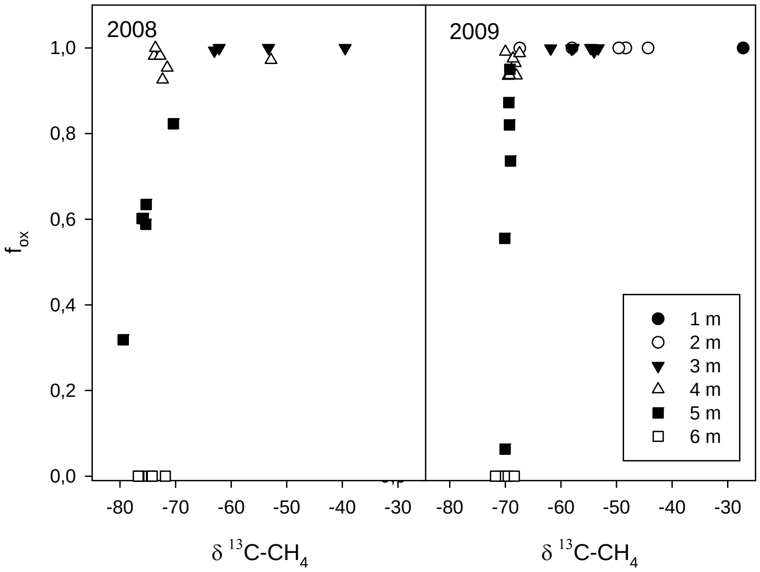

Figure 9. Fraction of oxidized CH$_4$ at different depths and corresponding δ^{13}C-CH$_4$ in 2008 and 2009.