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Observation of methanogenesis and potential iron-dependent anaerobic oxidation of methane in old lake sediments, a study of two boreal forest lakes

Elias Broman

Degree project in biology, Master of science (2 years), 2013

Examensarbete i biologi 45 hp till masterexamen, 2013

Biology Education Centre and Department of Limnology, Uppsala University

Supervisor: Sebastian Sobek

External opponent: Preetam Choudhary

Abstract

Organic and inorganic carbon can enter inland waters in different ways, and often a considerable amount of this carbon is coming from terrestrial input. Once this terrestrial carbon enters a lake, the carbon may be degraded, mineralized or eventually buried in the sediment. Below the oxic zone of the sediment carbon may be used by archaea to produce methane (CH_4). The CH_4 can then diffuse up in the sediment and escape to the bottom waters, or the CH_4 can be oxidized by bacteria using oxygen as an oxidant. There is also an anoxic process to oxidize CH_4 (anaerobic oxidation of methane: AOM), using sulfate (SO_4) and by recent findings also ferric iron (Fe(III)) as electron acceptors. In this study the main questions of interest were if CH_4 is produced in deep (i.e. old) lake sediments and if CH_4 is oxidized anaerobically using Fe(III) . Two Swedish boreal forest lakes were studied, sediment profiles of CH_4 were conducted in the field (down to 60 cm). Collected sediments were sliced anoxically at different depths and then analyzed for ferrous iron (Fe(II)), Fe(III) and SO_4 . Sediment from different depths was also incubated anoxic in order to test if CH_4 production depends on sediment age. The results show that methanogenic activity occurs by degrading old carbon in deep boreal forest lake sediments, and that a certain part of this might then be oxidized anaerobically. However, all cores exposed a general trend of increasing CH_4 concentrations with sediment depth, indicating that CH_4 production in old sediment layers is greater than AOM. AOM could therefore only act as a partial sink for CH_4 in anoxic deep sediments.

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1. Introduction

1.1 Carbon in lake ecosystems

In all forms of life carbon is the building element making life possible. Considering that carbon dioxide (CO_2) and methane (CH_4) are greenhouse gases the understanding of carbon cycling is widely studied. Carbon can be found in two forms, organic carbon (OC) or inorganic carbon (IC). These two forms can be transformed into each other via e.g. photosynthesis (inorganic to organic) or respiration (organic to inorganic) (Prairie and Cole 2009). In an aquatic system, OC can be classified depending on the size, either particulate; $>0.45 \mu\text{m}$, or dissolved; passes through a $0.45 \mu\text{m}$ filter (Zsolnay 2003). Generally the concentration of dissolved carbon is higher than that of particulate carbon in lakes. The dissolved carbon can be either organic (dissolved organic carbon; DOC) or inorganic (dissolved inorganic carbon; DIC). IC can be produced through respiration by microbes in the water. It may also enter aquatic systems through diffusion of CO_2 from the atmosphere. IC may also enter and leave the system through runoff from either surface or groundwater. OC can be produced internally in aquatic systems through photosynthesizing autotrophs (e.g. algae). OC can also be consumed internally by heterotrophs (e.g. microbes utilizing organic matter as an energy source). A lake dominated by autotrophic production (i.e. GPP (Gross Primary Production) $>$ net heterotrophic respiration) is called a net autotrophic system, while a lake dominated by heterotrophic respiration is called net heterotrophic (i.e. net heterotrophic respiration $>$ GPP). OC can also be transported from the surrounding land or the atmosphere. Terrestrial OC derives mainly from runoff of surface water (Figure 1).

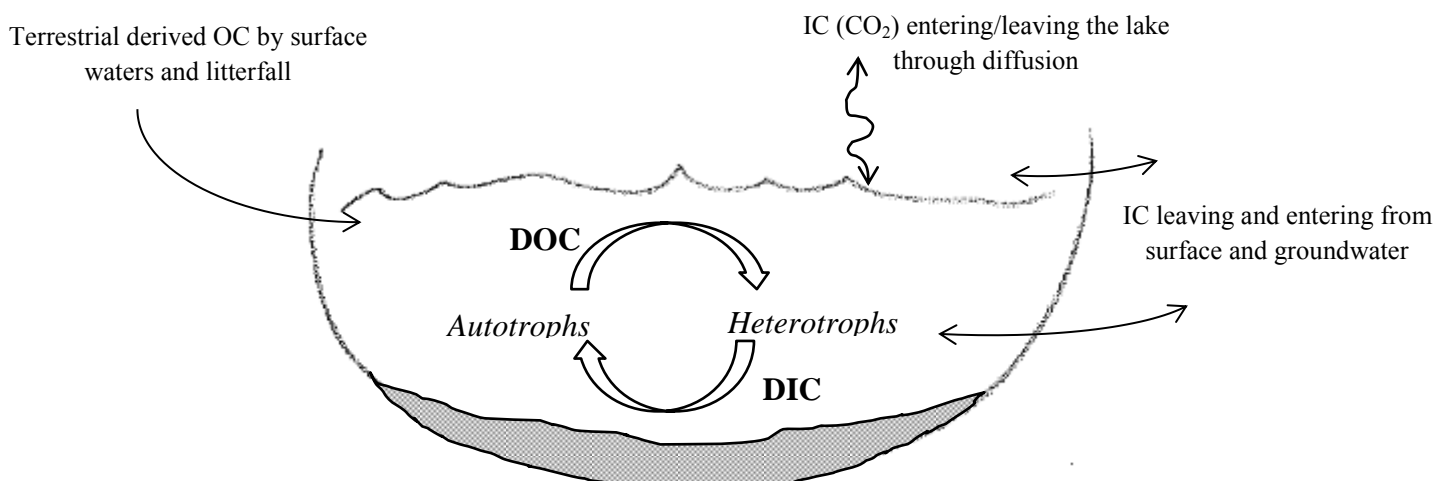


Figure 1 A simplified mixing lake is illustrated. The left side of the lake illustrates OC pathways, while the right side of the lake illustrates IC pathways. Heterotrophs produce DIC, which can be used by autotrophs to produce DOC. DIC that is not consumed by autotrophs can leave the lake through diffusion to the atmosphere. Particulate OC (e.g. from terrestrial sources or deceased organisms) is buried in the lake sediment.

In rivers OC input is mainly from incompletely decomposed carbon from terrestrial sources (e.g. plant litter). The largest pool of carbon is maintained in the sediments of lakes. Here the carbon accumulates over the years and the sediment is increasing in mass over time (Prairie and Cole 2009).

1.2 The role of terrestrial OC

A considerable amount of carbon enters standing (e.g. lakes) and running waters (e.g. streams, which might eventually reach lakes) through terrestrial input, and this carbon is known as allochthonous carbon (Pace et al. 2004; Karlsson et al. 2010). This carbon can be either inorganic or organic. Major pathways for allochthonous inorganic carbon are groundwater while much of the OC is transported via surface water runoff (Sobek 2005). In addition, OC can also enter the system through litterfall. Wallace et al. (1997) demonstrated the importance of litter fall in a study using a canopy cover and a lateral fence to block out all leaf litter from a forest stream. This had observable effects on the abundance and biomass of invertebrates. The allochthonous OC can be either particulate or dissolved and may contain litter from terrestrial plants and soil (Burdige 2007). Known environmental factors affecting the input of allochthonous carbon are soil moisture, topography and temperature. In a study by Jutras et al. (2011) these three parameters were considered to affect the concentration of DOC in running waters. Studies have also shown that the concentration input of allochthonous OC has an effect on food webs in lakes. Autochthonous carbon produced internally in the lake by primary producers has been found to be an insufficient source of OC to support the food webs of lakes (Pace et al. 2004). There have also been observations in lakes that the zooplankton in these systems contained 22-50% carbon derived from terrestrial sources (Pace et al. 2004). However, Brett et al. (2012) estimated through mass flux calculations that approximately 98% of the allochthonous OC is unavailable to the zooplankton, due to mineralization, sediment burial or flush to the sea. Thus the allochthonous OC was not able to support zooplankton production. Brett et al. (2012) also focused on systems with a retention time less than 3 years, which they considered had been overlooked in many studies. Also, DOC derived from terrestrial sources colors the water dark, inhibiting photosynthesis by algae. This has been found to effect even higher trophic levels such as benthic invertebrates (invertebrates residing in the bottom waters near or on the sediment) and fish (Karlsson 2009).

1.3 Expanding the importance of terrestrial OC

The importance of allochthonous OC might be more than just affecting the lake ecosystem itself, mineralization of OC in lake waters also affects the CO₂ budget in the atmosphere. 30-80% of the allochthonous OC that is leached from soils is lost in lakes and running waters, where it later either mineralizes or buries in the sediments (Algesten et al. 2004). Furthermore, Jonsson et al. (2007) assessed the terrestrial derived carbon into a catchment carbon budget and observed that before entering the sea, 45% of the allochthonous OC which leach from soils is mineralized into CO₂ in surface waters.

Because a high amount of the allochthonous OC is mineralized, many standing and running water systems are supersaturated with CO₂ and CH₄ (Sobek et al. 2003; Pavel et al. 2009; Karlsson et al. 2010; Butman and Raymond 2011). In a study by Algesten et al. (2005) focusing on boreal and subarctic lakes, the CO₂ emission was estimated to be ten times higher in the surface water compared to the sediment respiration. This indicates that most of the CO₂ emission occurred via water rather than the sediment. However, Kortelainen et al. (2006) observed the opposite, that CO₂ concentrations were higher near the sediment than that of surface waters. This sediment respiration was concluded to contribute to the supersaturation of CO₂ in lakes. Most of these studied lakes by Algesten et al. (2005) were net heterotrophic, however a couple of the studied lakes were also net autotrophic. The net heterotrophic lakes had higher concentrations of DOC compared to the net autotrophic lakes, this due to these lakes being fueled by allochthonous carbon acting as a source of energy for the microbes. Known environmental factors affecting the CO₂ emission from lake waters are temperature and hydrology. The temperature affects the mineralization of OC to CO₂ (Gudasz et al. 2010), while hydrology, e.g. water residence time determines how much of the OC is mineralized before it is exported to the sea (Sobek et al. 2003; Algesten et al. 2004).

1.4 The role of sediments and the burial of carbon

Although sediments contain a high amount of carbon, sediments also contain many minerals and nutrients, e.g. phosphorus and iron. Approximately 21% of the OC in all sediments (including marine) has been estimated to be bound to iron (Lalonde et al. 2012). When iron is reduced in anoxic sediments, phosphorus has been observed to be released into the water column (Mortimer 1941; Lake et al. 2007). This process is called internal loading and can severely affect the ecosystem and accelerate eutrophication, especially considering that 40% of the phosphorus can be bound to iron in the sediment (Xiang and Zhou 2011). The internal loading process has been observed to be absent if the amount of aluminum is higher than that of iron in the sediment, i.e. most phosphorous being bound to aluminum instead of iron (Lake et al. 2007, Wilson et al. 2008). Sediments may also contain buried environmental pesticides such as PCB, and these pesticides can be resuspended into the water column (Marvin et al. 2004).

Concentrations of sulfate (SO₄) in lakes are generally much lower than in the sea. In sediments the SO₄ is reduced to hydrogen sulfide (H₂S) gas by the sulfate-reducing bacteria. This process is anaerobic and therefore reduction of SO₄ occurs in the anoxic zone of the sediment. Usually this is a few centimeters below the sediment surface. H₂S may then oxidize to SO₄ if it reaches the oxic layer of the sediment (Holmer and Storkholm 2001). Dissolved iron concentrations are moderately high in lake waters, but lake sediments are generally rich in solid iron oxides (Lalonde et al. 2012). Iron has different oxidation states, either as the oxidized form Fe(III), also called ferric iron, and the reduced form Fe(II), called ferrous iron. Oxygen is an effective oxidant for Fe(II), and oxidation occurs rapidly, from minutes to hours depending on the pH (with lower pH resulting in slower oxidation). Other possible oxidants for Fe(II) are nitrite and nitrate, however these are not as important as oxygen (Giblin 2009).

Fe(III) is reduced to Fe(II) below the oxic layer in the sediment, this Fe(II) may then enter the oxic waters through molecular diffusion (Figure 2). Once the Fe(II) reaches oxic waters it is oxidized into Fe(III) and precipitates to the sediment. There Fe(III) binds with phosphorus, to be later once again reduced to Fe(II) in the anoxic layer of the sediment (Giblin 2009).

Another important role of sediments is that they act as a sink for particulate OC and IC, both allochthonous and autochthonous carbon. Particulate carbon settles on the lake sediments, where it either buries or mineralizes. The burial efficiency of OC can be calculated as a ratio of the rate of OC buried in the sediment divided by the rate of OC deposited in the sediment. The OC burial efficiency has been observed to be higher when the sediment is enriched with allochthonous carbon, also the ratio has been found to be higher when oxygen is absent (Burdige 2007; Sobek et al. 2009).

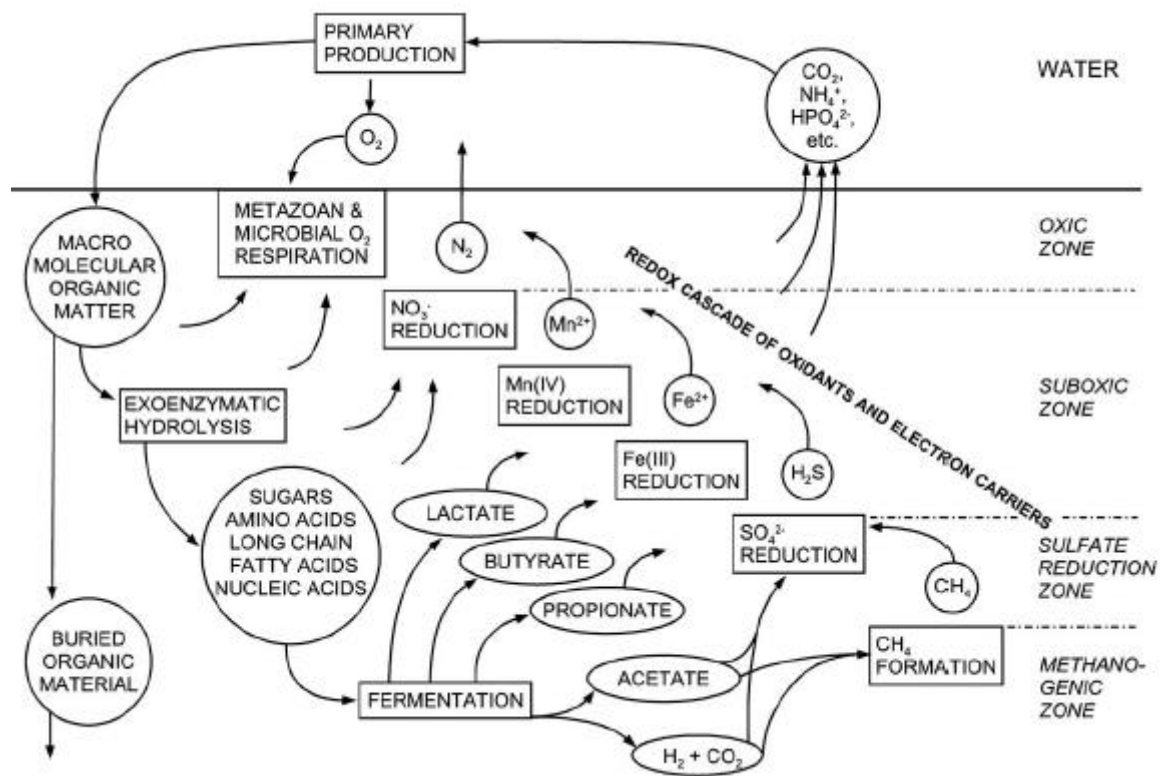


Figure 2 Pathways of OC degradation in sediments, CH_4 is at the end of the redox cascade meaning that processes involving Fe(III) and SO_4 will be favored before CH_4 by microbes. Illustration was reused from Jørgensen (2000), figure 5.11 with kind permission from Springer Science and Business Media.

Particles that settle onto the sediment accumulate over the years and this accumulation can sometimes form visible layers. Dating of sediment age is possible by means of the radionuclides ^{210}Pb , ^{137}Cs or ^{241}Am . Dating of sediment using ^{137}Cs or ^{241}Am is possible due to the test of thermonuclear weapons during the 1954 to 1963 and the Chernobyl fallout 1986. The atmospheric fallout of these isotopes can be measured in the sediments and compared to

dates of weapon testing events. ^{210}Pb occurs naturally and is a decay product of ^{222}Rn in the atmosphere. ^{210}Pb precipitates and falls onto the sediment beds in lakes. Calculating the precipitation rate of ^{210}Pb over the years it is possible to date sediment age by measuring the concentration of ^{210}Pb at different sediment depths. Using dating techniques it makes it possible to observe past states of the lake, looking at concentrations of nutrients and minerals in the sediment from different depths (Appleby 2002).

1.5 Production, consumption and emission of CH_4

1.5.1 Production

CH_4 is a greenhouse gas that has been estimated to have a 25 times greater effect on the atmospheric warming compared to CO_2 (Forster et al. 2007). During the last 300 years CH_4 has been accountable for approximately 25% of the increase in greenhouse gases (Bastviken et al. 2011). CH_4 is produced by anaerobic methanogenic archaea, and higher temperature increases the rate of production (Kelly and Chynoweth 1981). The process of producing CH_4 is called methanogenesis and involves microbial cleaving of the OC compound acetate (CH_3COO) into CH_4 and CO_2 (Bastviken 2009). Another way of generating CH_4 through methanogenesis is when H_2 reacts with CO_2 to form CH_4 and H_2O (Bastviken 2009). Production of CH_4 therefore occurs in the anoxic layer of the sediment and concentration of CH_4 has often been observed to increase over depth. This is because reduction of SO_4 and Fe(III) yields more energy (Figure 2). If the bottom water is anoxic, CH_4 diffusing from the sediment may accumulate there until overturn. Because OC is the substrate for methanogenesis, a high OC burial rate will push fresh (reactive) OC to the deep anoxic sediment. This has been shown to fuel CH_4 emission (Sobek et al. 2012).

1.5.2 Consumption

Oxidization of CH_4 is conducted by both aerobic and anaerobic processes. The aerobic methane-oxidizing bacteria, also called methanotrophs, use CH_4 as an energy source and O_2 as an oxidant. Methods to measure aerobic CH_4 oxidation in lakes have been well studied (Bastviken et al. 2002), and the oxidation of CH_4 occurs in oxygenated waters, especially in the oxic layer of water directly above the sediment. The methanotrophs oxidize CH_4 to methanol, formaldehyde, formate and lastly to CO_2 , and this CO_2 is then released into the water column. Aerobic oxidation of CH_4 increases with temperature, concentrations of O_2 and CH_4 concentration. Compared to methanogenesis, aerobic oxidation of CH_4 is less temperature dependent (Bastviken 2009). Aerobic CH_4 oxidation has been found to support the benthic-pelagic food web in lakes, as CH_4 produced in the benthic zone is consumed by methanotrophs and then transferred with their biomass to the pelagic zone. The carbon from CH_4 then becomes available (in shape of the biomass of the methane-oxidizing bacteria) and

it may in turn be consumed by pelagic zooplankton (Bastviken et al. 2003; Sanseverino et al. 2012).

Anaerobic oxidation of CH_4 (AOM) has been observed in lake sediments (Adler et al 2011; Sivan et al. 2011; Nordi et al. 2013) and in marine sediment incubations (Beal et al. 2009). SO_4 can act as an oxidant for AOM, and this process is conducted by microbial consortia of archaea (commonly called ANME, i.e. anaerobic methanotroph) and sulfate-reducing bacteria (Knittel and Boetius 2009). The optimal temperature for this process has been estimated to be 4-16°C (Nauhaus et al. 2002). However, below the zone of available SO_4 the anaerobic oxidation processes are not fully understood. AOM might potentially also be coupled to denitrification of nitrate to nitrite (e.g. Raghoebarsing et al. 2006). In sediments from Lake Kinneret, Israel it was observed that increasing Fe(II) is correlated with decreasing concentrations of CH_4 in this zone (Adler et al. 2011). Nordi et al. (2013) observed AOM in an iron rich Danish lake, and the results were similar to that of Adler et al. (2011), i.e. that CH_4 was oxidized anaerobically as indicated by decreasing Fe(III) and increasing Fe(II) concentrations. Also, incubations of Lake Kinneret sediments from 25 centimeters depth with an addition of Fe(III) showed that over time Fe(III) was reduced to Fe(II), and when the concentration of Fe(II) started to increase the CH_4 decreased (Sivan et al. 2011). Incubations of marine sediments added with Fe(III) also resulted in AOM (Beal et al. 2009). The conclusions of these findings were that Fe(III) is used as an oxidant in anaerobic CH_4 oxidation. Figure 3 shows graphs of the incubation experiments conducted by Sivan et al. (2011) to demonstrate these processes. This iron-dependent AOM has been suggested by Adler et al. (2011) and Sivan et al. (2011) to be a main factor causing anoxic lake sediments to be a sink for CH_4 .

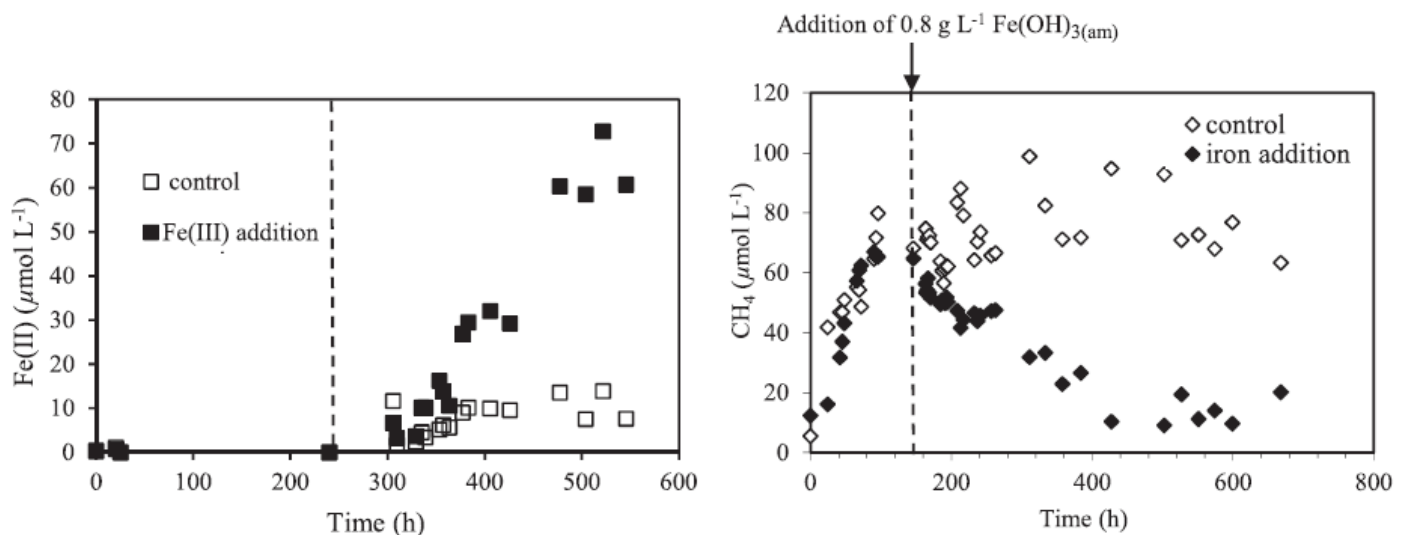


Figure 3 Both the graphs show data from anoxic incubations in 15°C of sediments from Lake Kinneret sliced at approximately 25 centimeters depth. The graph to the left shows that an addition of Fe(III) resulted in the Fe(III) being reduced to Fe(II). The graph to the right shows that an addition of Fe(III) resulted in a decrease of CH_4 concentration over time. Graphs were reused from Sivan et al. (2011), figure 3 and 4 with permission from the publisher. Copyright 2013/2014 by the Association for the Sciences of Limnology and Oceanography, Inc.

1.5.3 Emission

The CH₄ produced by methanogenic archaea in the sediment can escape to the water column and/or the atmosphere by at least four different pathways (Bastviken 2009), three of these pathways are illustrated in figure 4.

1. CH₄ has a low solubility and easily forms bubbles, which initially are trapped in the sediment but eventually overcome the hydrostatic pressure and bubbles up through the water column. This emission pathway largely bypasses methane oxidizers.
2. Mixing of an anoxic hypolimnion with stored CH₄, e.g. when the lake is mixing.
3. CH₄ in the sediment can enter the water column through diffusion, dissolved CH₄ then continues to travel through the water column until it finally leaves the water surface. However, much of the CH₄ that travels through the water column is oxidized.
4. CH₄ may also be transported to the atmosphere by roots of emergent plants.

Especially the ebullition pathway of CH₄ can have a substantial effect on the amount of CH₄ being emitted. Delsontro et al. (2010) studied a Swiss reservoir using gas traps and observed a high rate of CH₄ being emitted through ebullition. In another study by Delsontro et al. (2011) it was observed that CH₄ ebullition increased in the littoral zone nearby river inflows which carried fresh allochthonous OM into the reservoir. This is in accordance with Sobek et al. (2012) who observed that a deposition rate of sediment, and therefore a rapid input of fresh OM to anoxic sediment layers increased methanogenesis.

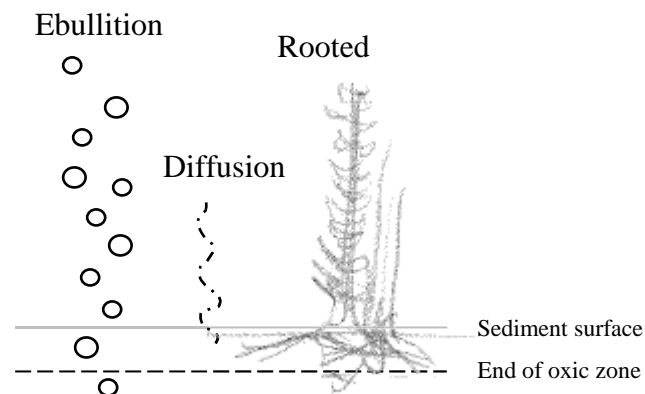


Figure 4 Three of at least four pathways for CH₄ to reach the water surface and/or atmosphere are illustrated. The four pathways are, mixing of an anoxic hypolimnion stored with CH₄, ebullition, diffusion and transport through the roots of plants attached to the sediment floor.

An overview of the processes outlined in this introduction can be seen in figure 5, notice that these processes have been simplified in this introduction (to give a broad overview) and do actually include more intermediate steps.

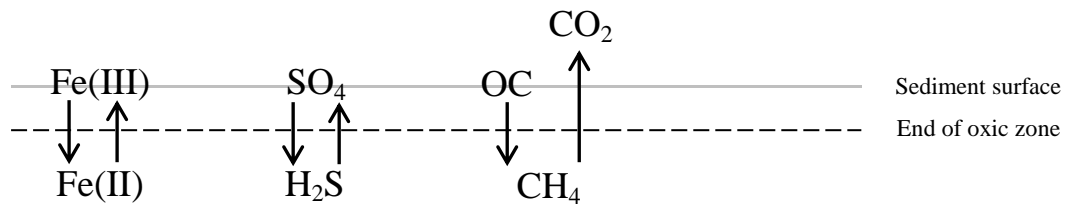


Figure 5 The figure gives an overview of processes described in the introduction. The two lines denote (from top to bottom), ‘sediment’ and ‘end of the oxic zone in the sediment’. Fe(III) is reduced to Fe(II) below the oxic zone, but oxidized to Fe(III) if it reaches oxic sediment or water again. SO₄ is reduced to H₂S below the oxic zone and may then be oxidized to SO₄ if it reaches oxic sediment or water again. OC derived from terrestrial or internal sources in the lake falls on the sediment and may then be used by methanogenic archaea to produce CH₄. If this CH₄ reaches oxic sediment or water it may then be oxidized into CO₂. The OC that is not mineralized will be buried over geological timescales.

2. Aim of study and hypotheses

To date, there are few detailed studies of CH₄ production and consumption over depth in lake sediments. Hence, it is not known until what age (i.e. sediment depth) CH₄ production can be observed, and if the degradation of old sediment OM may contribute to contemporary CH₄ emission. Potentially, CH₄ production in old sediment layers is counterbalanced by AOM via Fe(III), as iron is a very abundant element in lake sediments, particularly in humic-rich forest lakes. The objective was to study CH₄ production and oxidation in dependence of sediment age, by means of sediment pore-water profiles (CH₄, Fe(II), Fe(III) and SO₄) and incubation experiments.

Hypotheses for this study were:

1. There is a net production of CH₄ in deep lake sediments, such that degradation of old OM can fuel CH₄ emission of lakes.
2. Iron mediated CH₄ oxidation (Fe(II) is generated when CH₄ is oxidized with Fe(III) as an oxidant) is an important process in the sediments of boreal forest lakes.

3. Methods

3.1 Study site

In this study two boreal forest lakes were sampled in the area of Skogaryd, near the municipality of Uddevalla, Sweden (Figure 6). This area is part of project Landscape greenhouse gas exchange (LAGGE), which aims to integrate greenhouse gas emissions from aquatic and terrestrial systems into the landscape-scale budget. To do this, a catchment area (in and nearby Skogaryd) in Sweden has been chosen for long term observation.

The lakes Erssjön and Skottenesjö were both sampled for sediment on the 26th September 2012 and during the 4th and 5th February in February 2013. Lake Erssjön was measured for pH (Thermo Scientific ORION) in September 2012 and both lakes were measured for pH during sampling in February 2013. Profiles of oxygen, conductivity and temperature were also conducted using sensors (Hach LDO) attached to a probe (HQ 40d, Hach) during both sampling occasions. At the sampling location Lake Erssjön was found to have a depth around 4 meters, while Lake Skottenesjö had a depth around 2 meters. The sampling point in Lake Skottenesjö was close to the outlet/delta of the Lake Erssjön catchment area (Figure 6). To the east of Gundlebosjön there is an area of agricultural landscape with adjacent streams flowing into the lake and eventually Lake Skottenesjö.

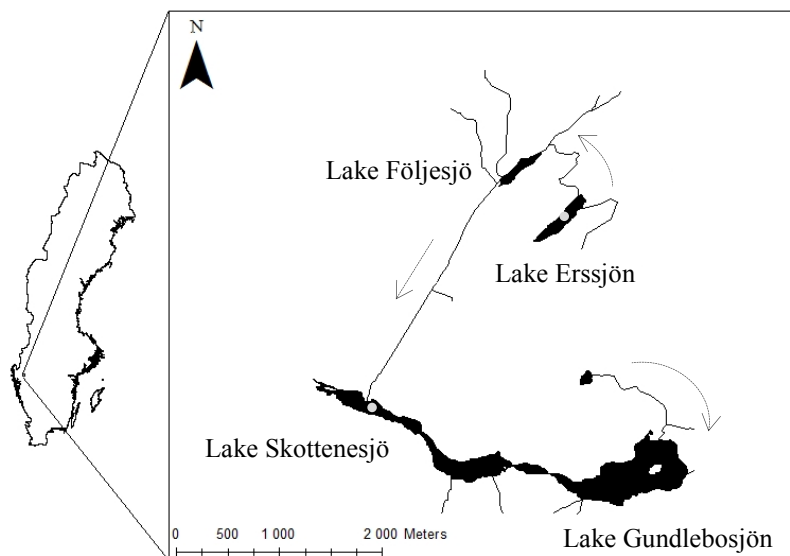


Figure 6 The water system in and nearby Skogaryd. In his study the lakes Erssjön (6.2 ha) and Skottenesjö (35.8 ha) were sampled (light grey circles denotes the small area where sampling occurred). The water from Lake Erssjön flows through the lake/wetland Följesjö and eventually reaches Lake Skottenesjö. WGS 84 coordinates (lat., long.) for Lake Erssjön was 58.37148, 12.16240. The coordinates for Lake Skottenesjö (west area) was 58.35427, 12.13168. The arrows denote the direction of the streams. Between Lake Erssjön and Lake Följesjö the stream passes through a small wetland (not shown on map). The map was adapted from © Lantmäteriet Gävle (2010): Permisson I 2010/0058.

3.2 Sampling

Sampling of sediment was conducted on the 26th of September from a boat using a gravity corer (UWITEC). The plastic tubes used with the gravity corer had a length of 60 cm and an inner diameter of 61 mm. Sediment was also sampled to conduct CH₄ profiling of pore-water (described further down). Two CH₄ profiling cores were taken in each lake, while three sediment cores were taken in Lake Erssjön and two in Lake Skottenesjö. Sediment sampled and collected were closed by rubber stoppers and kept cold (approximately 9°C) for one night before being transported back to the laboratory. In the laboratory the sediment cores were kept closed and stored in 4°C until being further analyzed. Sampling that occurred during the 4th-5th February 2013 was conducted in a similar way, but holes were drilled in the ice to make sampling possible. The sediment cores were 1 m in length and had an inner diameter of 59 mm. During this occasion, samples were taken to measure CH₄ in the water column (described further down). Outdoor temperature was around 0°C, therefore collected sediment cores were kept in this temperature over the night before being transported back to the laboratory.

3.3 Experimental setup

A schematic overview of the experimental setup can be seen in figure 7. The methods are described more thoroughly in this chapter.

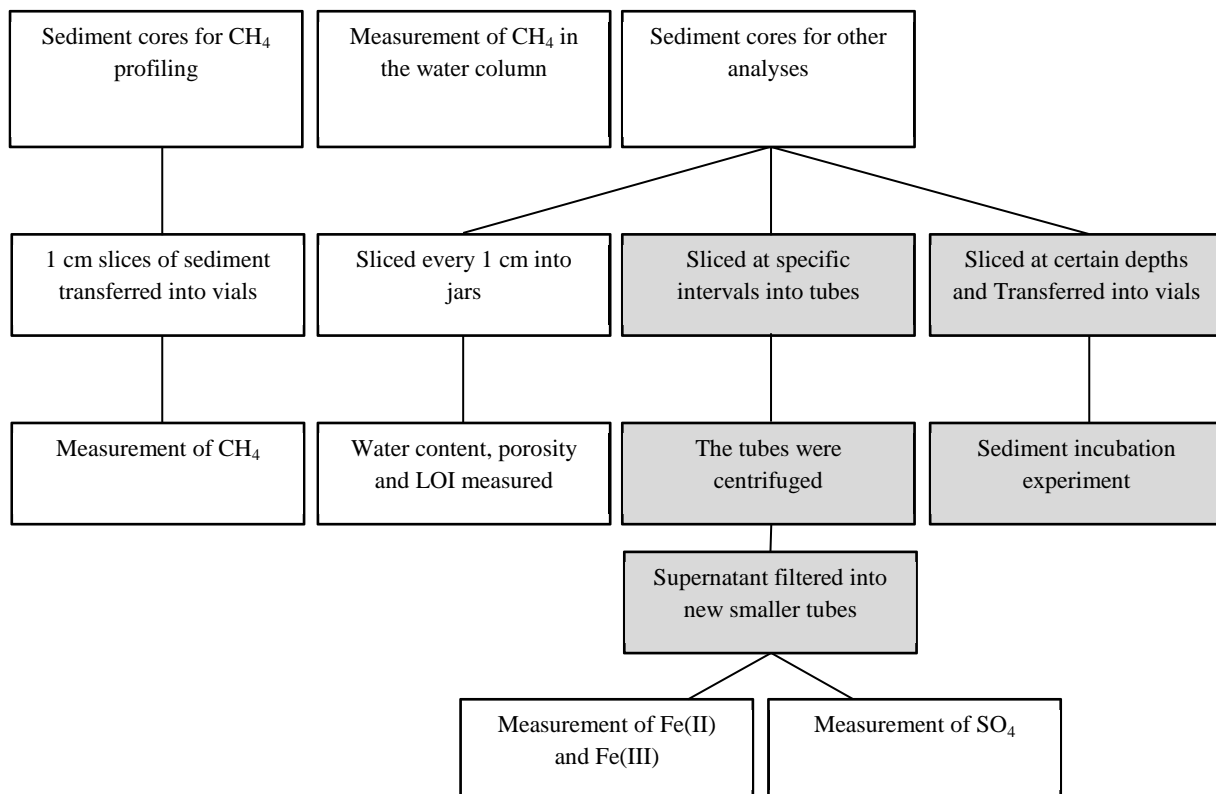


Figure 7 Different cores were used for different analyses, tubes prepared for CH₄ profiling (tubes with 1 cm holes in them) were used to transfer sediment to infusion vials in the field. Sediment collected in regular tubes were transported back to the laboratory and stored until further analysis. 13
Grey boxes denote experiments done in an anoxic condition.

3.3.1 Sediment dating and determination of sedimentation rate

Sediment from Lake Erssjön and Lake Skottenesjö was sampled during the summer of 2010 with a gravity corer (UWITEC). The sediment core was then sliced and transferred to plastic jars every 0.5 cm until 5 cm, then every 1 cm until 20 cm. The samples were then transported back to the laboratory in Uppsala University. In the laboratory each sample was grinded with a mortar and pestle, the grinded sediment was then transferred into 10 ml tubes. The samples were then analyzed for concentrations of ^{210}Pb using gamma spectrometry.

Sediment age over depth, and sedimentation rate was then calculated using the constant rate of ^{210}Pb model (CRS). The CRS model assumes that the atmospheric input of ^{210}Pb is constant and uninterrupted, external ^{210}Pb (not originated inside the lake) fallen on the sediment is from the atmosphere, this ^{210}Pb is undisturbed in the sediment, and decays exponentially over time in the sediment (Appleby 2002). Equations used to calculate the CRS model were in accordance with Appleby (2002):

$$t = \frac{1}{\lambda} \ln \left(\frac{A(O)}{A} \right)$$

where t is the age of the sediment (at a certain depth), λ is the ^{210}Pb radioactive decay constant per year, this value (0.03114) was taken from Appleby (2002). $A(O)$ and A are calculated integrations using the concentrations of ^{210}Pb and the sediment depth, using the following equations:

$$A(O) = \int_0^{\infty} C(m) dm$$

$$A = \int_m^{\infty} C(m) dm$$

where C is the concentration of ^{210}Pb , m is the sediment depth. $A(O)$ will be the total area of graph (total ^{210}Pb per area unit in the sediment) while A will be the accumulated ^{210}Pb at each sediment depth. Sedimentation rate (r) was then calculated using the following equation (Appleby 2002):

$$r = \frac{\lambda A}{C}$$

3.3.2 Water content, porosity and LOI

Water content, porosity and Loss on Ignition (LOI) were determined on sediment cores sampled on the 26th of September 2012. After the sediment had been stored for 15 days one sediment core sampled in each lake was sliced every 1 cm. Each slice was transferred into a plastic jar. The wet sediment was then weighed and later stored in -20°C. After approximately 25-30 days the frozen sediment was freeze dried (Edwards, Mini Fast 680). The dry sediment was then weighed and the water content was then calculated by dividing the water mass by the total mass.

To calculate the porosity, sediment dry bulk density was first calculated. The density was calculated according to Muller et al. (2005):

$$\rho_{sed} = -0.0523 * C_{org} + 2.65$$

Where ρ_{sed} is the sediment dry bulk density, C_{org} is the OC in percentage (calculated after the LOI, 26% for Lake Erssjön and 13% for Lake Skottenesjö). The calculation assumes that the minerals in the sediment consist of a mix between silicate and carbonate minerals having a density of 2.65 g cm⁻³. The organic carbon is assumed to have a density of 1 g cm⁻³ (Sobek et al. 2009).

Porosity (ϕ) was then calculated according to Muller et al. (2005), wc is the water content (%/100):

$$\phi = \frac{wc}{wc + (1 - wc)\rho_{sed}}$$

LOI was conducted on a few of the dry sediment slices. The samples were pre-weighted before being put into a muffle furnace (Nabertherm) at 550°C for 2 hours. During the ignition the organic matter (OM) either oxidizes as CO₂ or becomes ash (Heiri et al. 2001). The organic matter (%) was then calculated using the weight difference before and after being ignited in the muffle furnace. The percentage of OC was then determined by using a factor of 1.72 (Broadbent 1965):

$$OC (\%) = \frac{Organic\ Matter (\%)}{1.72}$$

3.3.3 Concentration of CH₄ in the sediment pore-water and the water column

CH₄ profiling of sediment pore-water was partly conducted in the field (during both sampling occasions). Sediment cores with 1 cm holes covered by water resistant tape were used to collect sediment. A plastic 2 ml syringe (BD Plastipak) was used to withdraw sediment from each hole. The top of the syringe was cut off to make the syringe fit the 1 cm hole. 2 ml of sediment from each 1 cm depth of the sediment core was transferred into 20 ml infusion vials. The vials were closed by 20 mm rubber stopper (Apodan Nordic Pharmapacking) and aluminum caps. Beforehand these vials were pre-filled with 4 ml 0.625 M (2.5 % w/v) sodium hydroxide (MERCK, Sodium hydroxide pellets NaOH) to stop microbial activity in the transferred sediment. Considering that air was let into the vials when the sediment was transferred to the vials, blanks containing air was also taken to correct for the final results. The vials were then transported to the laboratory and stored in room temperature until further analysis.

The vials were then shaken for approximately 1 minute to equilibrate the CH₄ concentration in the gas and aqueous phase. The headspace in the vials was then analyzed in a gas chromatograph (Agilent Technologies, 7890A GC System) equipped with a FID detector. Using the gas law and Bunsen solubility coefficient for CH₄ (Yamamoto et al. 1976) the CH₄ concentration was calculated. Equations to calculate CH₄ concentrations in sediment pore-water can be seen below:

$$CH_4(gas) \text{ mol } L^{-1} = \frac{P * Gas V}{R * T}$$

$$CH_4(aqueous) \text{ mol } L^{-1} = P * \beta * Water V$$

$$CH_4 \text{ pore water } \text{ mol } L^{-1} = \frac{CH_4(gas) + CH_4(aqueous)}{Water V}$$

P is the measured CH₄ in ppm, R is the universal gas constant, T is the temperature of the water in Kelvin. $Gas V$ is the volume of gas in the headspace, while $Water V$ is the volume of liquid in the vial (pore-water and NaCl). β is the Bunsen solubility coefficient for CH₄, the value used for β was calculated using equation 1 from Yamamoto et al. (1976):

$$\beta = A1 + A2 \left(\frac{100}{T} \right) + A3 * \ln \left(\frac{T}{100} \right)$$

$A1$, $A2$ and $A3$ are constants taken from Yamamoto et al. (1976).

For the samples collected on the 4th and 5th of February 2013 the CH₄ in the water column was also measured. 30 ml Infusion vials (Apodan Nordic Pharmapacking) were prepared in the laboratory with a saturated NaCl solution. Each vial was filled completely and closed with a 20 mm rubber stopper and an aluminum cap. In the field lake water was sampled using a Ruttner water sampler at different depths. 40 ml of lake water was directly transferred from the Ruttner water sampler using tubing into a 60 ml syringe (BD Plastipak). The syringe was then filled with 20 ml air, and then shaken for 1 minute (to equilibrate the gas in the water and the air in the syringe). The prepared infusion vials were then hold upside down and the gas phase in the syringe were injected into the vials, while the saturated NaCl solution was released from the vial through a needle in the rubber stopper. Later the vials were analyzed using a gas chromatograph (Agilent Technologies, 7890A GC System) and calculated in the same manner as the CH₄ in the sediment pore-water.

3.3.4 Sediment incubations

The Sediment cores sampled during February 2013 were stored for 40 days at 4°C (the temperature of the sediment at the time of sampling) before the incubation experiment started. Each sediment core was sliced in 20°C at different depths (each slice approximately 1 cm thick) in a nitrogen environment using a glove box (BELLE). Each slice was transferred into a jar, homogenized using a small spoon and then transferred into an empty pre-weighted 30 ml infusion vial (Apodan Nordic Pharmapacking). The vial was closed with a rubber stopper (Agilent Technologies, 7890A GC System) and an aluminum cap. Still inside the glove box, 25 ml of nitrogen were added to the vial (taken directly from the nitrogen inlet in the glove box) using a syringe and a needle, in order to create overpressure and to avoid under pressure during the incubation period. The vials were then weighted after slicing to find out the weight of the transferred sediment. Initial concentrations of CH₄ and CO₂ were then measured using a gas chromatograph (Agilent Technologies, 7890A GC System). The GC also measures O₂ and confirmed the vials to be anoxic. Before each measurement each sample was shaken for approximately 1 minute. This was conducted to equilibrate the CH₄ and CO₂ concentration in the gas and the aqueous phase. CH₄ was calculated in the same manner as for CH₄ pore-water profiles (but was not divided with the water volume). CO₂ was calculated using the same equations as above for CH₄, but instead of the Bunsen solubility coefficient for CH₄, Henry's Law constant for solubility of CO₂ in water was used:

$$kH(T) \text{ mol Kg}^{-1} * \text{bar} = k^{\circ}H * \exp\left(\left(\frac{d(\ln kH)}{d\left(\frac{1}{T}\right)}\right)\left(\frac{1}{T} - \frac{1}{K}\right)\right)$$

where $k^{\circ}H$ is Henry's Law constant for solubility of CO_2 in water at 25°C , $d(\ln kH)/d\left(\frac{1}{T}\right)$ is a temperature dependence constant, T is the temperature of the sample in Kelvin and K is Kelvin at 0°C . The result $kH(T)$ is Henry's Law constant for solubility of CO_2 in water at the temperature of the sample. In this study the temperature was 10°C . Values for $k^{\circ}H$ and $d(\ln kH)/d\left(\frac{1}{T}\right)$ was taken from Lide and Frederikse (1995).

The vials were then left to stand in darkness at 4°C for 14 days and later measured again for CH_4 and CO_2 in the same manner as before using the gas chromatograph. Final units were then calculated as a rate (using the slope coefficient) and expressed in $\text{nmol h}^{-1} \text{g}^{-1}$ dry weight. Water content was measured for the vials used in Lake Skottenesjö again (and not based on the values from September 2012). This was done to get a more precise value of the dry weight for Lake Skottenesjö considering that the sediment was found to be more heterogeneous than Lake Erssjön.

3.3.5 Fe(II) and Fe(III)

Iron analysis was conducted using the ferrozine method revisited by Viollier et al. (2000). Ferrozine is a light yellow powder. Between pH 4 and 9 ferrozine forms a magenta complex with ferrous iron (Fe(II)). Using a spectrophotometer at 562 nm the maximum absorbance can be recorded (Stookey 1970). After analyzing Fe(II) a reducing agent is used to reduce ferric iron (Fe(III)) to Fe(II). A buffer is then used to increase the pH back to a range between 4 and 9. The sample is then analyzed again at 562 nm (Viollier et al. 2000). Because Fe(II) oxidizes to Fe(III) when in contact with oxygen (Giblin 2009), extra care was taken to ensure Fe(II) was not oxidized when the sediment was sliced.

After being stored in 4°C for 40 to 50 days sediment collected in September 2012 was sliced using a glove bag that was filled and continuously purged with nitrogen. Sediment cores collected during February 2013 were stored in 4°C for 6 to 10 days and then sliced in a nitrogen environment using a glove box (BELLE). For both lakes the sediment cores were sliced in 20°C at certain depths, each slice 1 cm thick for sediments sampled in September 2012. Sediment samples sampled in February 2013 were sliced in 1 cm thickness until 10 cm depth, then into 2 cm thick slices (to get enough pore-water for the IC analysis). Each slice was transferred into a 50 ml centrifugation tube (VWR), closed under N_2 atmosphere, and centrifuged anoxic at 4°C and 2000 rpm for 30 minutes. The supernatant was then filtered inside the glove box, through a $0.45 \mu\text{m}$ filter (VWR) and transferred into 15 ml polypropylene tubes (BD falcon) containing 300 μl of the ferrozine reagent. These 15 ml tubes were in advance acid washed with 10 % HCl (SIGMA-ALDRICH, 32 % Hydrochloric acid) for approximately 40 hours. The samples were then analyzed for iron the same day.

To conduct the iron analysis, solutions were prepared according to Viollier et al. (2000). The ferrozine reagent was prepared by adding 0.01 M ferrozine (SIGMA-ALDRICH, Ferrozine 3-(2-Pyridyl)-5,6-diphenyl-1,2,4-triazine-p,p-disulfonic acid monosodium salt hydrate) into 0.1 M ammonium acetate (SIGMA-ALDRICH, approx. 98%). The reducing reagent was prepared

by adding 1.4 M hydroxylamine HCl (SIGMA-ALDRICH, Hydroxylamine Hydrochloride, $\text{NH}_2\text{OH}\cdot\text{HCl}$ ACS reagent) into 2 M HCl (SIGMA-ALDRICH, 32 % Hydrochloric acid). The buffer solution was made by adding a few ml of ammonium hydroxide (SIGMA-ALDRICH, NH_4OH solution 28-30%) into a 10 M ammonium acetate solution, ammonium hydroxide was added until the solution had a pH of 9.5.

Before conducting analyses on the sediment pore-water standards with known Fe(III) concentration was prepared. The standard solution was prepared by adding anhydrous FeCl_3 (MERCK-SCHUCHARDT, Iron(III) chloride anhydrous FeCl_3 for synthesis) into 0,01 M HCl. Measuring the absorbance the standard solutions confirmed that all solutions had been prepared correctly.

To measure the Fe(II) and Fe(III) concentration of the pore-water samples, first 3 ml of the sample with ferrozine reagent was added to a 1 cm cuvette. A few samples had to be diluted slightly to make certain there was enough volume in the cuvette. The sample was then measured in a spectrophotometer (Perkin Elmer Lambda 40, UV Visible Spectrophotometer) at 562 nm. This absorbance yields the value of A_1 , which is comparable to that of the Fe(II) concentration. 450 μl of the reducing agent was then added to the sample, the sample was left to stand for 10 minutes to make sure all Fe(III) is reduced to Fe(II). 150 μl of the buffer solution is then added. The sample was measured again at 562 nm. This will yield the value of A_2 , which is comparable to the total concentration of Fe.

The values of A_1 and A_2 were used to calculate the concentrations of Fe(II) and Fe(III) using equations from Viollier et al. (2000):

$$C_{\text{Fe(II)}} = \frac{A_1 \epsilon_{\text{Fe(II)}} l \alpha - A_2 \epsilon_{\text{Fe(III)}} l}{\epsilon_{\text{Fe(II)}} l \alpha (\epsilon_{\text{Fe(II)}} l - \epsilon_{\text{Fe(III)}} l)}$$

$$C_{\text{Fe(III)}} = \frac{A_2 - A_1}{\alpha (\epsilon_{\text{Fe(II)}} l - \epsilon_{\text{Fe(III)}} l)}$$

where $C_{\text{Fe(II)}}$ and $C_{\text{Fe(III)}}$ are Fe(II) and Fe(III) concentrations in molarity. $\epsilon_{\text{Fe(II)}}$ and $\epsilon_{\text{Fe(III)}}$ are molar absorbance coefficients that can be derived from a calibration curve using standards. In this study, values for $\epsilon_{\text{Fe(II)}}$ and $\epsilon_{\text{Fe(III)}}$ were taken from Viollier et al. (2000). l is the length of the cuvette (optic path length). Finally α is the dilution factor, which can be estimated by adding the reducing agent and buffer solution again after A_2 has been measured. In this study, the value of α used was taken from Viollier et al. (2000).

3.3.6 SO₄

A Sediment core sampled in February 2013 from each lake was sliced in a glove box the same manner as for the iron analysis. The sediment was centrifuged and filtered into falcon tubes (same method as iron analysis). When H₂S in the pore-water is in contact with oxygen it is oxidized to SO₄ (Holmer and Storkholm 2001), therefore extra care was taken to ensure the pore-water stayed under an anoxic condition until analysis. The vials were therefore kept in an anoxic environment (in the glove box's lock unit) until being analyzed the same day. The samples were analyzed for SO₄ using an ion chromatograph (Methrom 883 Basic IC Plus). A few samples had to be diluted slightly to make certain there was enough volume in the vials being used by the IC.

3.4 Data analyses

Production and consumption of CH₄ at different sediment depths (expressed as zones) were estimated using the software PROFILE constructed by Berg et al. (1998). Before running the software CH₄ concentrations of the cores for each lake were calculated into a moving average (3 average each step) to smooth the data. This software uses several parameters to calculate production and consumption zones. Parameters used in the software PROFILE were boundary condition 1, i.e. the top of the boundary and the bottom of the boundary is based on the concentration of CH₄ in the top and bottom of the sediment core. The boundary condition is used in the interpretation of the production and consumption zones. The expression of sediment diffusivity was set to 2, i.e. sediment diffusivity is a function of the porosity squared times the diffusivity in the free water. The diffusivity for CH₄ in water was set to 13.6E-06 and 11.0E-06 cm² s⁻¹ based on 4°C and 11°C (temperature of the sediment during sampling). These diffusivity values for CH₄ were calculated from Broecker and Peng (1974). The significance level was set to 0.05, this determines the detection of the amount of zones.

CH₄ saturation was determined at different sediment depths and calculated using the following calculation:

$$\text{Hydrostatic pressure } Pa^{-1} = h\rho g$$

$$\text{Sediment pressure } Pa^{-1} = h(\rho_{sed} * (1 - wc))g$$

$$p = \text{Hydrostatic pressure} + \text{Sediment pressure} + \text{Atmospheric pressure}$$

$$CH_4 \text{ saturation } mol L^{-1} = p\beta$$

$$CH_4 \text{ saturation } \% = \frac{CH_4 \text{ saturation } mol L^{-1}}{CH_4 \text{ mol } L^{-1}}$$

h is the depth in m, ρ is the density of water in $\text{kg}^{-1} \text{m}^{-3}$ and g is the gravitational constant in m/s . ρ_{sed} is the dry bulk density of the sediment in $\text{kg}^{-1} \text{m}^{-3}$ (as calculated in the water content and porosity section) and wc is the water content (%/100). β is the Bunsen solubility coefficient for CH_4 (as calculated in the concentration of CH_4 in the sediment pore-water section). Following this calculation the CH_4 saturation mol L^{-1} will then be the concentration at which the CH_4 will be saturated. Dividing this saturation concentration with the measured CH_4 concentration will then yield the saturation in %.

The CH_4 production rates determined in the incubation experiment were used in conjunction with the values of CH_4 saturation mol L^{-1} (the concentration at which the CH_4 will be saturated) to model the amount of time required to reach saturation. In this model molecular diffusion of CH_4 was also included. The model was based on the following calculations, first the production in each sediment layer was calculated:

$$\text{CH}_4 \text{ mol L}^{-1}p = p_{water}\text{CH}_4 \text{ mol L}^{-1} + (Tsens * \text{CH}_4\text{rate mol L}^{-1})$$

$\text{CH}_4\text{rate mol L}^{-1}$ is the production rate of CH_4 estimated from the incubation experiment at certain sediment depths. $Tsens$ is a value of temperature sensitivity that was set to 1 for 4°C , because the incubations were incubated at this temperature. This value was then adjusted to 14°C and 24°C using the Q_{10} value, i.e. a factor of 4.1 per 10°C temperature increase (Bastviken 2009). $p_{water}\text{CH}_4 \text{ mol L}^{-1}$ is the pore-water CH_4 concentration from the same sediment depth the incubation was conducted from. Molecular diffusion was subtracted from the value $\text{CH}_4 \text{ mol L}^{-1}p$, based on the following calculations:

CH_4 molecular diffusion

$$= \phi * D_s * \left(\frac{\partial p_{water}\text{CH}_4 \text{ mol L}^{-1} + (Tsens * \text{CH}_4\text{rate mol L}^{-1})}{\partial h} \right)$$

$$D_s = \frac{D_0}{(1.02 * \phi^{-1.81}) * \phi}$$

$$\text{CH}_4 \text{ mol L}^{-1}pd = \text{CH}_4 \text{ mol L}^{-1}p - \text{CH}_4 \text{ molecular diffusion}$$

ϕ is the porosity (%/100), and D_s is the diffusivity of CH_4 in sediment based on equations for clay-silt sediments from Maerki et al. (2004). D_0 is the diffusivity of CH_4 in water and was calculated from Broecker and Peng (1974). The difference of CH_4 in the pore water between

each depth was then then added with the CH₄ production from the depth being calculated. This value was then divided by the sediment height between each layer. Over time, CH₄ was considered to diffuse upwards in the sediment and to be lost either to the bottom water or to aerobic oxidation in surficial sediment layers. The CH₄ concentration in the bottom water used for calculation of diffusivity flux across the water interface was set to 0 for Lake Erssjön and Lake Skottenesjö. Anaerobic oxidation of CH₄ is accounted for since the CH₄ production rates derived from the incubation experiments are net productions, i.e. are the result of CH₄ production and anaerobic oxidation. Downwards diffusion was not included in the model. Accordingly, calculations were:

$$CH_4 \text{ mol } L^{-1}_{t_2} = CH_4 \text{ mol } L^{-1}pd_{t_1} + CH_4 \text{ molecular diffusion below}_{t_1}$$

In this calculation, CH_4 molecular diffusion below _{t_1} represents one time step back, the molecular diffusion of CH₄ from the sediment layer directly below the one being calculated. From the same time step, this value is then added with $CH_4 \text{ mol } L^{-1}pd_{t_1}$. The final value, $CH_4 \text{ mol } L^{-1}_{t_2}$ will represent CH₄ diffused from the underlying sediment layer added with the CH₄ concentration already present. To determine the amount of time to reach CH₄ saturation the following calculation was used:

$$\text{time until saturation} = \frac{CH_4 \text{ saturation mol } L^{-1}}{CH_4 \text{ mol } L^{-1}/t}$$

Where $CH_4 \text{ saturation mol } L^{-1}$ is the concentration at which the CH₄ will be saturated, and t is the amount of time steps used in the model. In this model five time steps were used and expressed as days.

4. Results

4.1 Lake characteristics

During the time of sampling Lake Erssjön had a pH of 6.3 in September 2012 and a pH of 5.4 in February 2013. Lake Skottenesjö had a pH of 6.26 in February 2013. During the sampling in September 2012 both lakes were mixed with an isotherm temperature of 11°C. During the sampling in February 2013 both lakes were stratified with an average temperature of 3°C in Lake Erssjön and 1.2°C in Lake Skottenesjö. Oxygen, conductivity and temperature profiles can be seen in figure 9.

The two lakes had different sediment characteristics (Table 1). At 10.5 cm below the sediment surface the age of the sediment for Lake Erssjön was 116 years, while Lake Skottenesjö showed an age of 89 years at 16.5 cm (Figure 8). The measured and calculated density for each lake also showed differences (Table 1), Lake Erssjön had a sediment dry bulk density of 1.29 g⁻¹ cm⁻³ while Lake Skottenesjö had 1.98 g⁻¹ cm⁻³. The average sedimentation rate (kg m² yr⁻¹) in Lake Skottenesjö was five times higher than that of Lake Erssjön. For both lakes the sedimentation rate declined with depth.

Table 1 Sediment water content, porosity, dry bulk density, OM and calculated OC in the two lakes. The values are shown as an average of data from the September 2012 samples.

	Erssjön	Skottenesjö
wc (%)	92	76
ϕ (%)	93	86
ρ _{sed} (g ⁻¹ cm ⁻³)	1.3	2.0
OM (%)	44	22
OC (%)	26	13

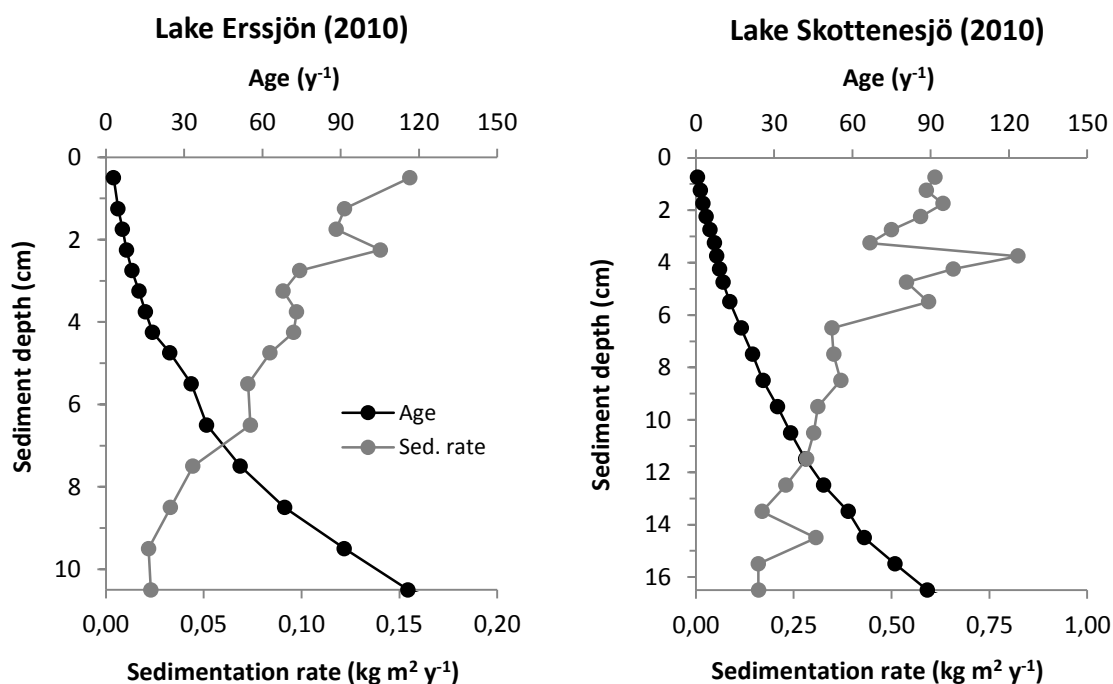
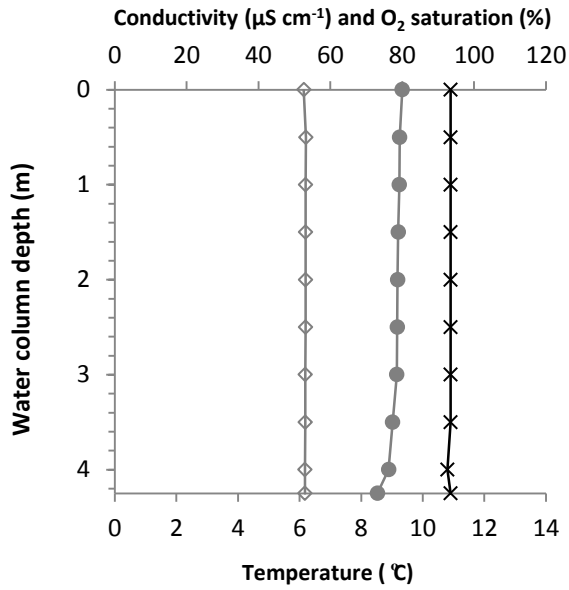
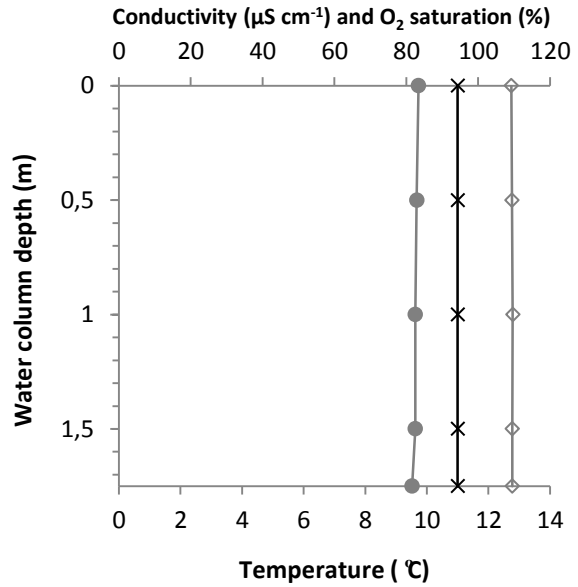


Figure 8 ²¹⁰Pb sediment dating of the lakes conducted during

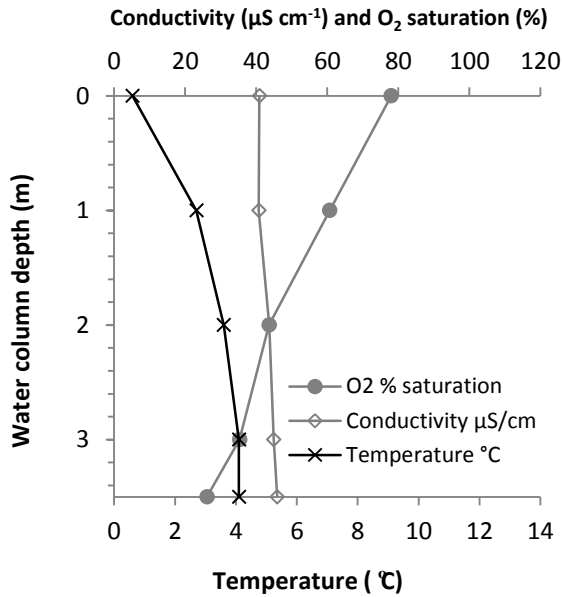
Lake Erssjön, Sep. 2012



Lake Skottenesjö, Sep. 2012



Lake Erssjön, Feb. 2013



Lake Skottenesjö, Feb. 2013

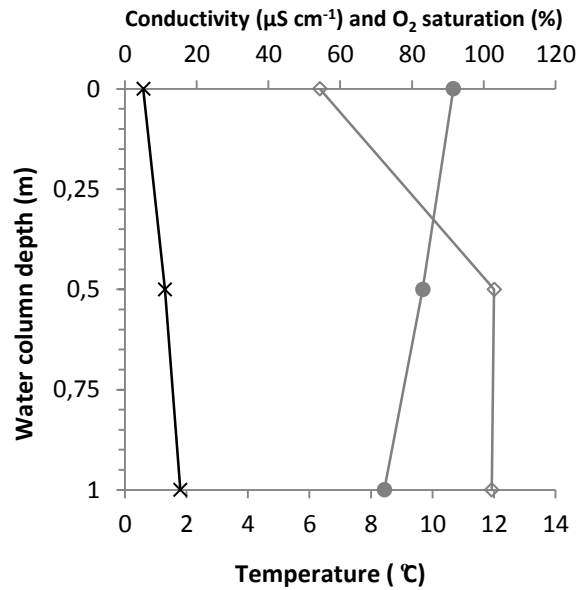


Figure 9 Conductivity, O₂ and temperature profiles were conducted in the water column of both lakes at both sampling moments. Notice the different in depth between the dates (due to a few meters apart for the previous sampling point). Black lines with a black cross denote temperature in °C, grey line with a grey rotated square denotes the conductivity µS cm⁻¹ and grey lines with a grey circle denotes O₂ saturation in percent.

The LOI in Lake Erssjön was on average 44.3% (OC average = 26%) which was around twice as much compared to 21.9% OM (OC average = 13%) in Lake Skottenesjö (Figure 10). The two lakes differed in water content and porosity (Figure 11), with Lake Erssjön being higher (average 92% water content, 93% porosity) compared to Lake Skottenesjö (average 76% water content, 86% porosity). Concentrations of CH₄ in the lake water were highest directly under the ice in Lake Erssjön (51 μmol L⁻¹), but then declined below 1 μmol L⁻¹. Lake Skottenesjö had overall low CH₄ concentrations (<0.42 μmol L⁻¹). For both of the lakes there was a small increase of CH₄ in the bottom waters near the sediment surface (Figure 12). CH₄ blanks (only air) that were measured at both lakes gave a result of 0.33 to 0.43 μmol L⁻¹.

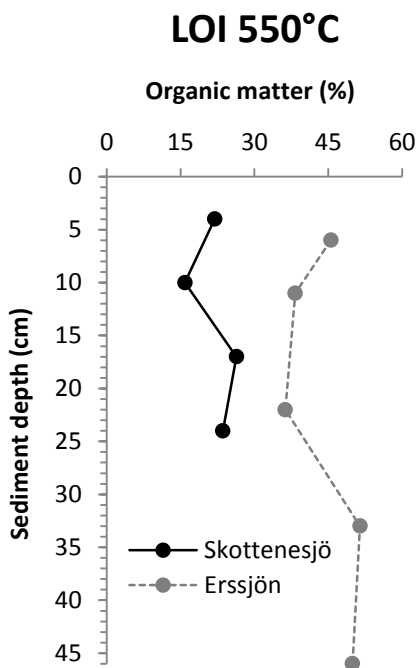


Figure 10 LOI conducted on the cores from each lake sampled in September 2012.

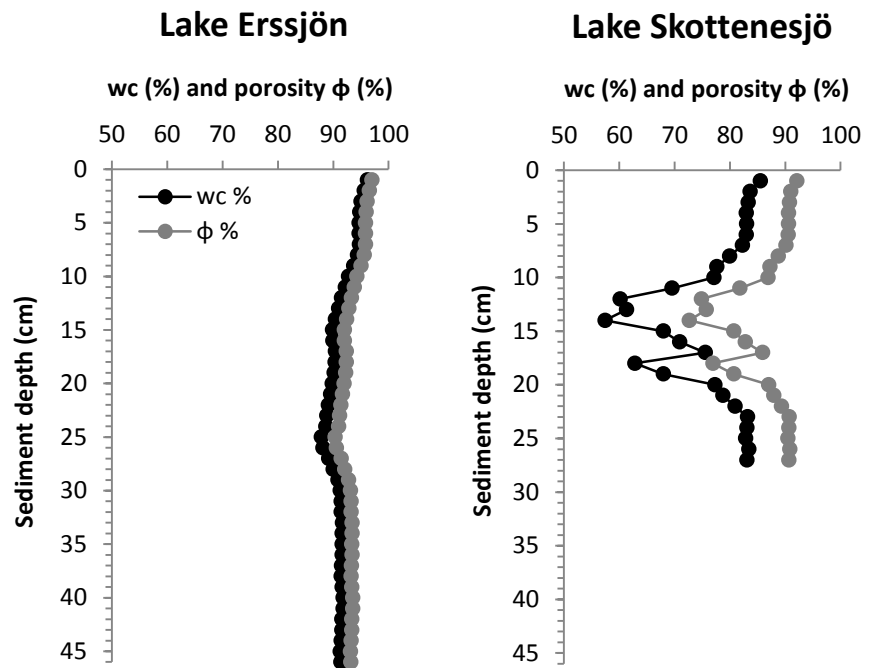


Figure 11 Water content and porosity profiles of sediment sampled in September 2012.

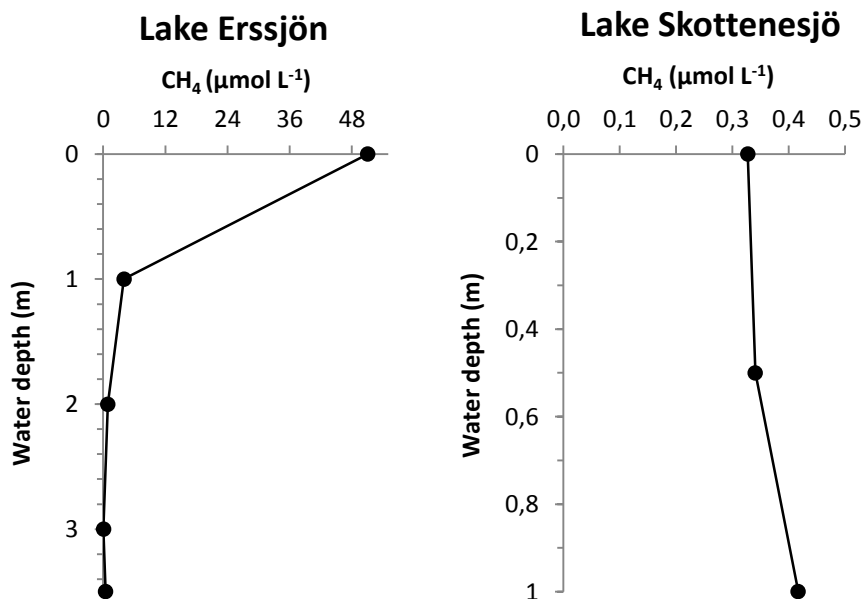


Figure 12 CH₄ in the water column measured in February 2013.

4.2 Sediment pore-water

Saturation profiles of CH₄ for both lakes were also different (Fig 13). The saturation is different for each lake at certain depths, e.g. Lake Skottenesjö showed a higher tendency towards CH₄ saturation around a sediment depth of 18 cm (86% saturation) in a warmer temperature during September 2012, compared to February 2013 (18 cm: 9% saturation). Lake Erssjön had an increasingly higher concentration of CH₄ below 45 cm, but this never reached saturation above 30%. Except one sample in Lake Skottenesjö, none of the lakes reached CH₄ saturation near or above 100% during both sampling occasions.

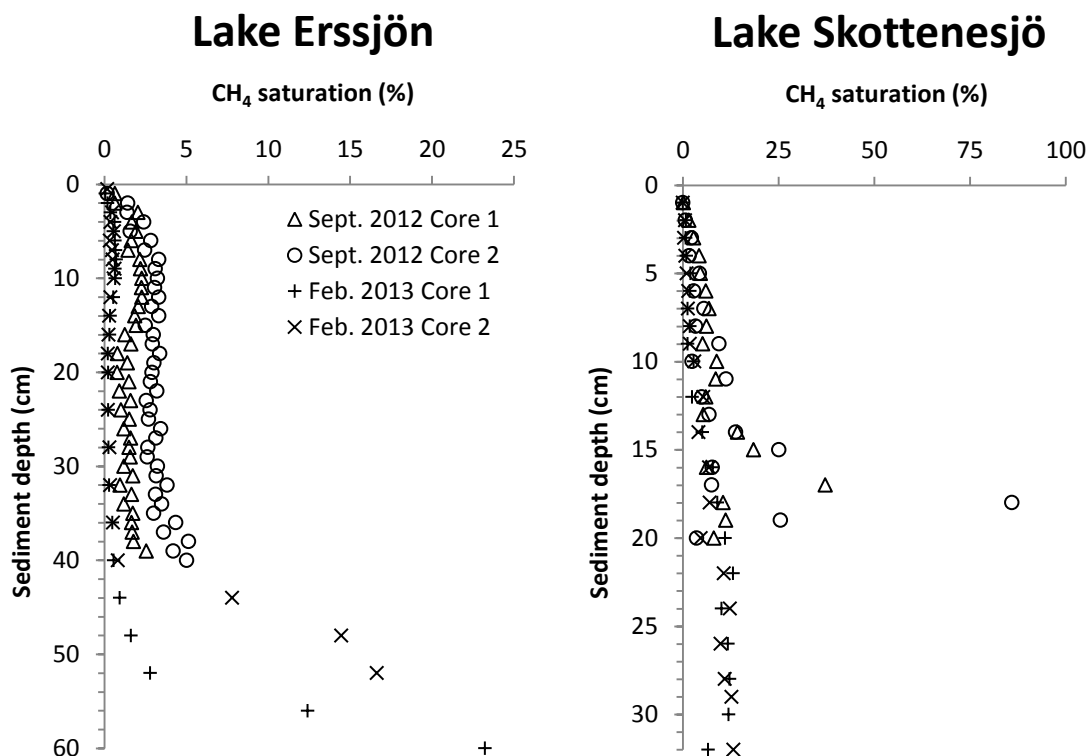


Figure 13 CH₄ saturation from both lakes at both sampling occasions. Triangles and circles denote samples taken in September 2012, while crosses denote samples taken in February 2013. Notice the different scales between the lakes.

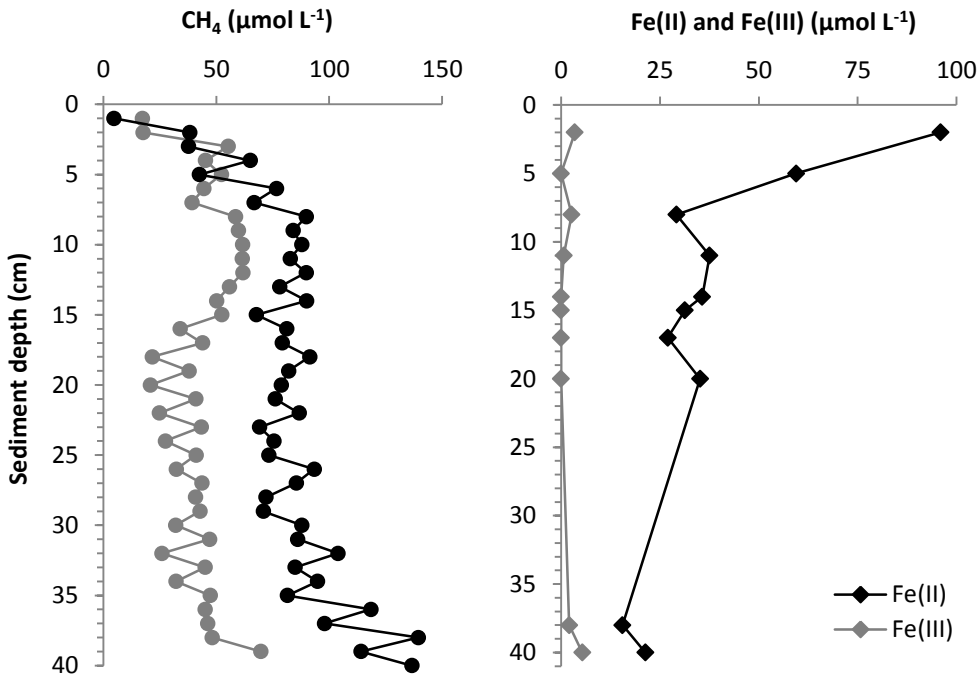
Results of SO₄ in the sediment cores yielded values near the detection limit, except one sample (17.55 $\mu\text{mol L}^{-1}$) in Lake Skottenesjö at 1 cm sediment depth. SO₄ decreased then to be near the detection limit. In figure 14 and 15 profiles of CH₄, Fe(II) and Fe(III) are shown. For iron during the February 2013 sampling the sediment cores had been sliced at an interval of two cm, these slices are shown visually as the average between these two centimeters, i.e. a slice consisting of the centimeters 8 and 9 in the sediment is shown as 8.5 cm in the figures for illustrative purposes. The top portion of the figure shows the profiles for September 2012, while the bottom portion shows profiles from February 2013. CH₄ concentrations were overall slightly higher in Lake Skottenesjö during both sampling occasions (50–400 $\mu\text{mol L}^{-1}$), especially around a sediment depth of 18 cm during September 2012 (500 – 1000 $\mu\text{mol L}^{-1}$,

2000 for one extreme case). For Lake Erssjön CH₄ concentrations were between 50 -150 μmol L⁻¹ for the September 2012 sampling, even lower during the February 2013 sampling (10-50 μmol L⁻¹). During the February 2013 sampling Lake Erssjön was sampled below 40 cm and results yielded a continuous increase of CH₄ concentrations up to 734 μmol L⁻¹ at 60 cm.

The iron profiles showed higher concentrations of both Fe(II) and Fe(III) in Lake Skottenesjö than in Lake Erssjön during both sampling occasions. Lake Erssjön Fe(II) concentrations were below 100 (μmol L⁻¹), while for Lake Skottenesjö concentrations were most of the time nearby or above 100 μmol L⁻¹. Fe(III) concentrations were higher in Lake Skottenesjö (average 28 μmol L⁻¹) compared to Lake Erssjön (average 6 μmol L⁻¹).

Comparing the profiles, Fe(II) decreased with an increase of CH₄ for the Lake Erssjön 2012 sample. For the Lake Erssjön February 2013 sample, in the middle of the core Fe(II) increased, with a decrease of Fe(III). Samples from Lake Skottenesjö showed mirrored patterns for Fe(II) and Fe(III). For the Lake Skottenesjö 2012 sample CH₄ was increasing continuously with depth, while Fe(II) and Fe(III) showed no obvious patterns related to CH₄. For the 2013 sample in Lake Skottenesjö below a sediment depth of 20 cm Fe(II) increased while Fe(III) and CH₄ decreased.

Lake Erssjön 26th September 2012



Lake Erssjön 5th February 2013

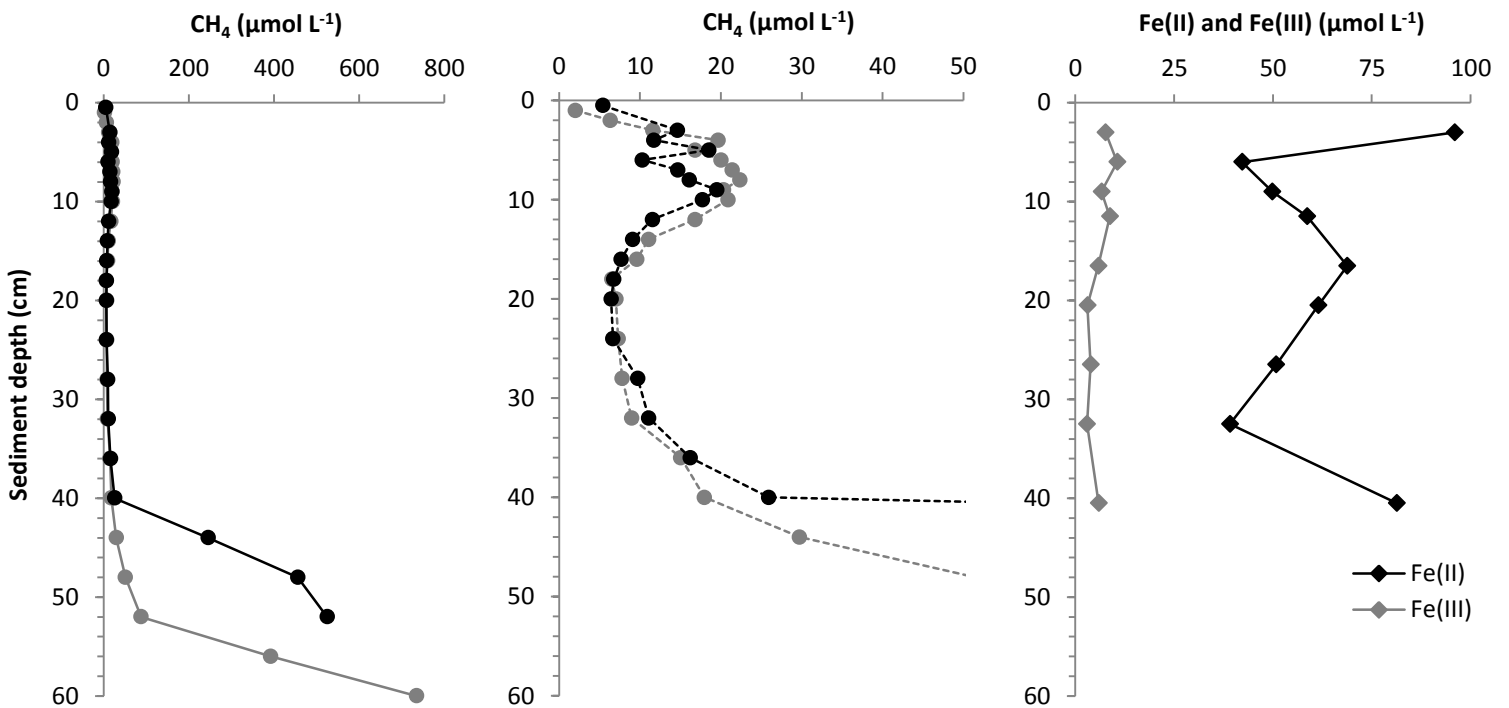
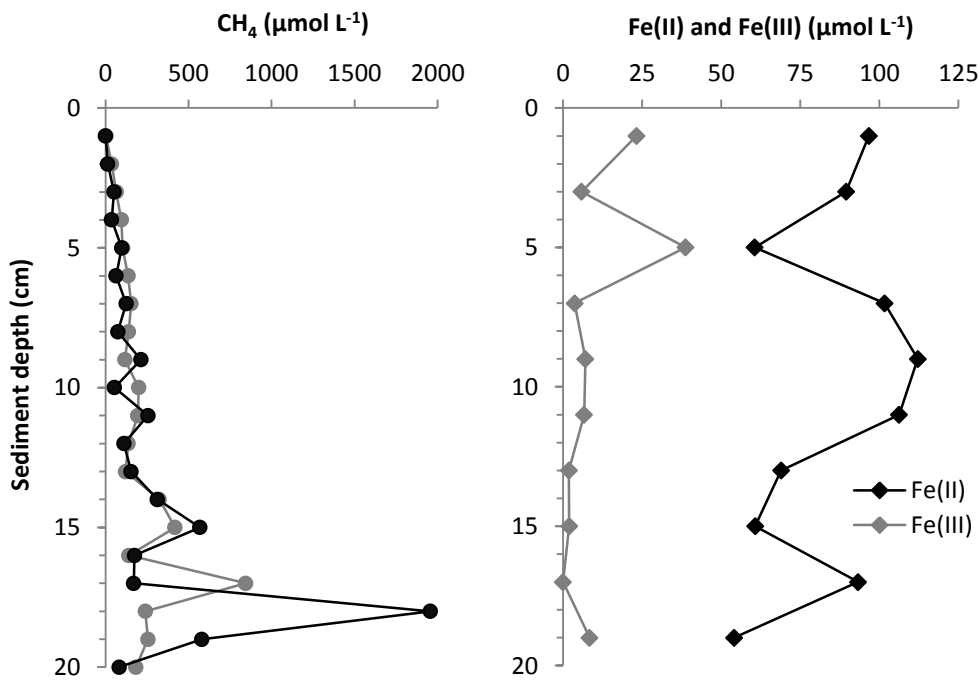


Figure 14 Profiles of CH₄, Fe(II) and Fe(III) conducted on sediment cores from Lake Erssjön. In the graph showing CH₄ profiles the different colors denote two different samples. The second CH₄ graph for Lake Erssjön sampled during February 2013 shows the profiles above 50 cm sediment depth in more detail. Notice the different scales between the sampling dates.

Lake Skottenesjö 26th September 2012



Lake Skottenesjö 4th February 2013

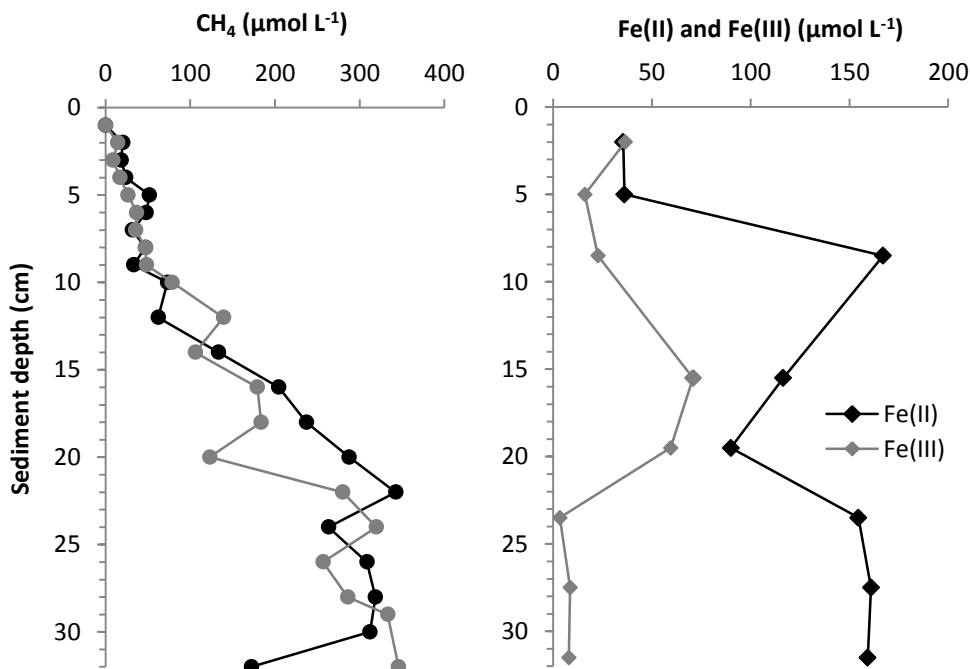


Figure 15 Profiles of CH₄, Fe(II) and Fe(III) conducted on sediment from Lake Skottenesjö. In the graph showing the CH₄ profiles the different colors denote two different samples. Notice the different scales between the sampling dates.

4.3 Production and consumption of CH₄ in the sediment

Production and consumption of CH₄ zones calculated with the software PROFILE yielded several zones (using a moving average of 3 steps) for the samples collected in September 2012. Only one sample in Lake Erssjön 2013 generated statistical significant zones, in the other cores no zones could be detected by the software. The zones indicated consumption (oxidation) below the oxic zone in the sediment. Production and consumption rates (CH₄ nmol h⁻¹ g⁻¹ dw) for samples collected in September 2012 were generally higher in Lake Skottenesjö (production: 15-586 consumption: 115-226) compared to that of Lake Erssjön (production: 1.5-5.6, consumption: 3.5-5.15). The rates were also different in the Lake Erssjön 2013 sample collected in February (production: 2.1, consumption: 0.56-32.55). A detailed table of the zones can be seen in table 2 and figure 16.

The CH₄ production rates from the sediment incubation experiment can be seen in figure 17 and the values of the rates can be found in Table 3. CH₄ production was observed in all vials from all depths during incubation in 4°C for 14 days). Production rates in Lake Erssjön were roughly 5 to 10 times lower compared to that of Lake Skottenesjö. In Lake Skottenesjö the rate of CH₄ production was highest at a sediment depth of 15 cm (up to 12.394 nmol h⁻¹ g⁻¹ dw).

Table 2 The calculated production and consumption rates of CH₄ using the software PROFILE. Samples not listed in the table did not contain any statistical significant zones. Consumption rates are shown with a minus sign. The 'Zone' column denotes the depth in the sediment (cm). For each sample core 1 and core 2 can be compared to figure 14 and 15, where the grey lines are core 1 and the black lines are core 2.

Lake	Zone	CH ₄ (nmol g ⁻¹ h ⁻¹ dw)
Erssjön 2012 1	1 - 8	1.47
	9 - 15	5.62
	16 - 23	-5.15
	24 - 30	1.90
	31 - 39	-3.54
Erssjön 2013 1	1 - 12	2.09
	13 - 39	-0.56
	40 - 50	-1.18
	51 - 60	-32.55
Skottenesjö 2012 1	1 - 12	19.25
	13 - 16	-225.94
	17 - 20	586.97
Skottenesjö 2012 2	1 - 10	15.31
	11 - 20	-115.12

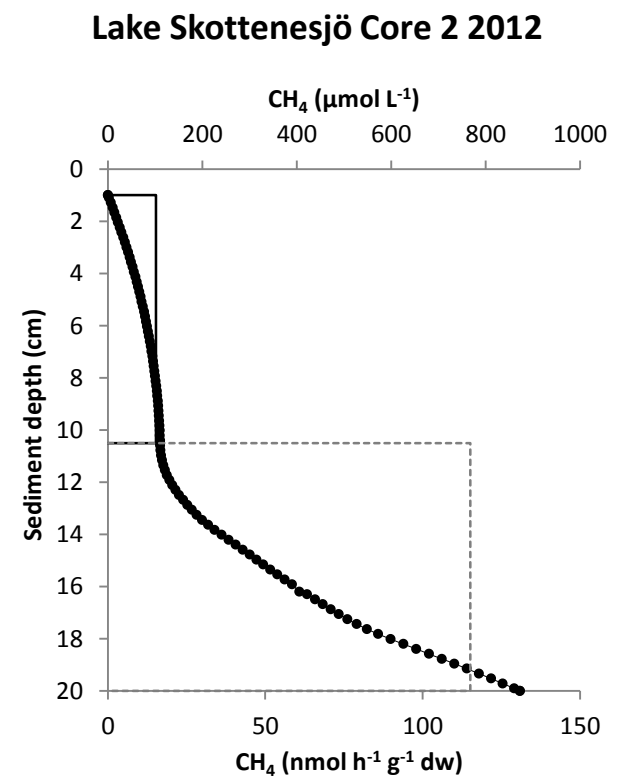
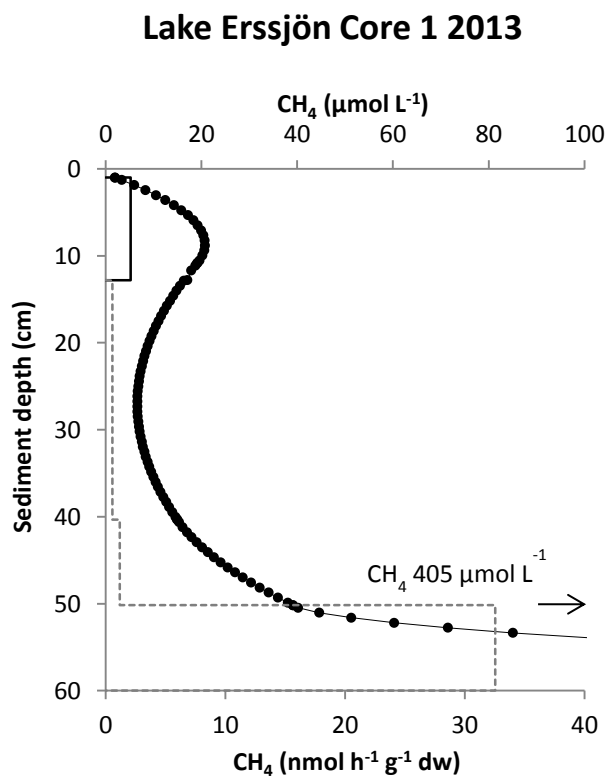
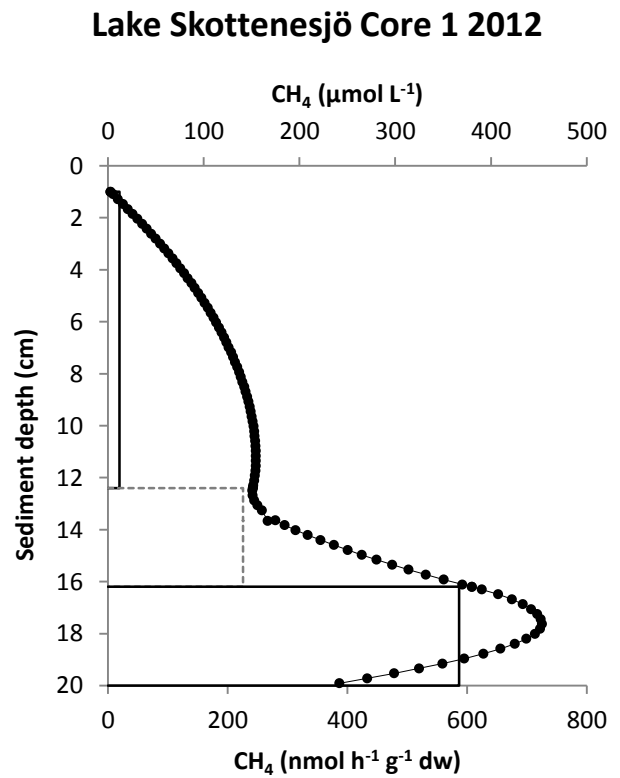
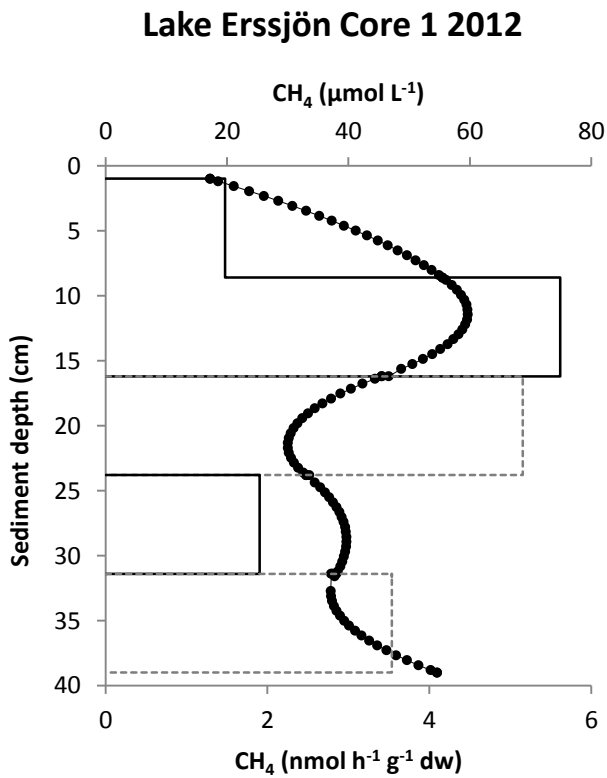


Figure 16 Profiles from production and consumption zones calculated using the software PROFILE. The black circles with a black line denote CH_4 concentrations based on a moving average. Zones with a black line denote a zone of production, while zones with a grey dotted line denotes a zone of consumption. For Core 1 in Lake Erssjön 2013 the CH_4 concentrations increases with depth below 30 cm and eventually reach a concentration of $405 \mu\text{mol L}^{-1}$ at a sediment depth of 60 cm.

Production rates of CH₄ then decreased with depth, but production did not stop below 30 cm (2.9-5.8 nmol h⁻¹ g⁻¹ dw). In Lake Erssjön CH₄ production rates were about 1 nmol h⁻¹ g⁻¹ dw and also decreased with depth but increased slightly below 40 cm (1.14-1.6 nmol h⁻¹ g⁻¹ dw). CO₂ rates from the incubations decreased with depth for Lake Erssjön, but increased with depth for Lake Skottenesjö. Below a depth of 30 cm for Lake Skottenesjö, the variation in CO₂ rates were high. Assuming that the sediment accumulates linearly (based on the data from the sediment dating). A sediment depth of 40 cm for Lake Erssjön will have an age of 413 years, while a depth of 30 cm for Lake Skottenesjö will have an of age of 158 years.

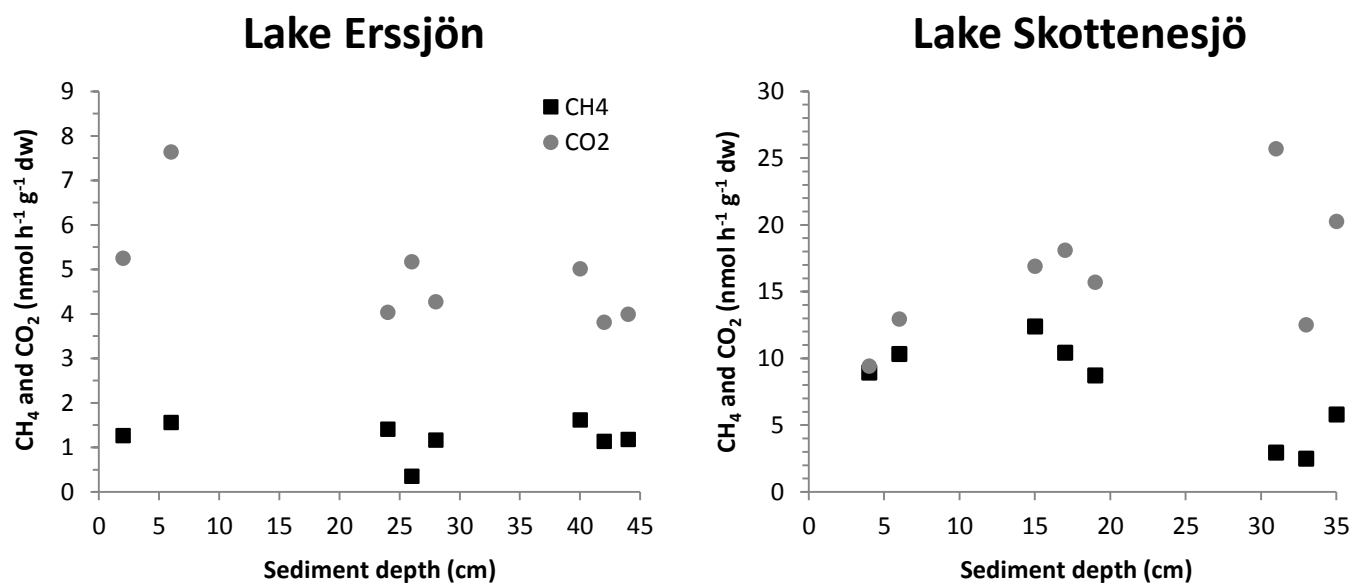


Figure 17 The results from the sediment incubation experiment. Sediment samples collected in February 2013 were incubated for 14 days in 4°C. Black squares denote CH₄ rates, while grey circles denote CO₂ rates. Notice the different scales between the lakes.

Table 3 Results of production rates from the sediment incubations. The units of the rates are expressed as nmol h⁻¹ g⁻¹ dw.

Lake Erssjön			Lake Skottenesjö		
Sed. depth (cm)	CH ₄	CO ₂	Sed. depth (cm)	CH ₄	CO ₂
2	1.262	5.247	4	8.928	9.416
6	1.556	7.632	6	10.314	12.944
24	1.408	4.035	15	12.394	16.882
26	0.348	5.169	17	10.409	18.077
28	1.163	4.269	19	8.712	15.685
40	1.611	5.007	31	2.937	25.692
42	1.136	3.808	33	2.487	12.502
44	1.177	3.987	35	5.793	20.239

The results from modeling the amount of time to reach CH₄ saturation using the production rates and calculated diffusion from the incubation experiment can be seen in table 4. The sediment of Lake Erssjön requires one to a few several years to reach saturation at 4°C, while the sediment in Lake Skottenesjö is able to reach saturation in less than one year. Compared to Lake Erssjön, Lake Skottenesjö has an average of 9.5 and 4.1 times faster to reach saturation at 4°C and 24°C respectively. The amount of days to reach saturation dropped rapidly with increasing temperature. In Lake Skottenesjö, at 24°C saturation was reached at an average of 5 times faster than that compared to 4°C, with an average of 9.5 times faster in the top layers of the sediment. At 24°C Lake Erssjön reached saturation with an average of 9 times faster than that compared to 4°C, with an average of 9.9 above a sediment depth of 40 cm. At 24°C Lake Skottenesjö would be able to reach saturation in less than 1 month, while for Lake Erssjön it would take between 1-2 months.

Table 4 Results from the modeling of the amount of time to reach CH₄ saturation using the production rates from the incubation experiment and calculated diffusion.

Lake	Sed. depth (cm)	Days until saturation		
		4°C	14°C	24°C
Erssjön	2	500	172	54
Erssjön	6	348	127	41
Erssjön	24	603	198	56
Erssjön	26	900	299	76
Erssjön	28	623	218	59
Erssjön	40	304	122	35
Erssjön	42	325	169	54
Erssjön	44	308	200	76
Skottenesjö	4	110	37	10
Skottenesjö	6	97	38	12
Skottenesjö	15	46	25	9
Skottenesjö	17	45	29	11
Skottenesjö	19	44	32	15
Skottenesjö	31	40	35	20
Skottenesjö	33	41	37	22
Skottenesjö	35	60	41	25

5. Discussion

5.1 Differences in lake characteristics

The two studied lakes in this study were different, results from dating of the sediment shows that Lake Skottenesjö has a five times higher sedimentation rate and that the age of the top 50 cm of sediment is less than that of Lake Erssjön (Figure 8). Considering that Lake Skottenesjö is receiving the sediment load from the Lake Erssjön catchment, as well as from a larger agricultural catchment east of the lake (Figure 6), it is reasonable that more allochthonous materials (e.g. OC and minerals) are transported to the lake, therefore increasing sedimentation rate. This also explains why conductivity was different among the lakes at both sampling occasions, with higher conductivity being observed in Lake Skottenesjö. This could also explain differences found in LOI, water content and porosity between the lakes (Table 1).

Concentration and patterns of CH₄ was different between the lakes, with concentrations increasing with depth for Lake Skottenesjö, while Lake Erssjön tended to show an increase in the upper 10 centimeter and then a decrease of CH₄ with depth, but eventually an increase of CH₄ below 50 cm (Figure 14 and 15). A possible reason could be that the sedimentation rate is higher in Lake Skottenesjö, thus increasing the availability of fresh OC to be used in methanogenesis (Bastviken 2009, Sobek et al. 2012). The OM and OC data from September 2012 shows that the sediment in Lake Skottenesjö had a lower percent of OC compared to that of Lake Erssjön (Table 1), still Lake Skottenesjö had higher concentrations of CH₄. This shows that a higher supply of sediment containing comparatively small amounts of OC is more stimulating for methanogenesis than a smaller supply of OC-rich sediment. High sediment deposition pushes relatively young (i.e. degradable) OC to deeper layers in the sediment, and thereby provides methanogens with substrate. Such results are in accordance with Sobek et al. (2012) and literature findings by Bastviken (2009).

Because Lake Skottenesjö is shallower than Lake Erssjön the sediment is likely warmer during warm seasons when the lakes are stratified. CH₄ concentrations were lower in both lakes during the February 2013 sampling, and this can be explained by the cold temperature, as methanogenesis has been reported to be strongly temperature-dependent (Kelly and Chynoweth 1981; Bastviken 2009). Considering that CH₄ in the air for both lakes was measured to be 0.33 to 0.43 $\mu\text{mol L}^{-1}$ the CH₄ profiles in the water column indicate that CH₄ was trapped below the ice in Lake Erssjön, which had a CH₄ concentration of 51 $\mu\text{mol L}^{-1}$ below the ice surface.

5.2 Possibilities of iron-dependent AOM in the studied samples

There are some visible patterns that can be seen in the CH₄ and profiles, and some of these patterns are in accordance with other studies, such as Adler et al. (2011) and Nordi et al. (2013). For example looking at the February 2013 Lake Erssjön profiles in figure 14 between

6.5 cm to 32.5 cm Fe(II) increased while CH₄ and Fe(III) decreased. This pattern of CH₄ decreasing in the anoxic zone is a typical pattern of AOM (Adler 2011). This decrease of CH₄ could also be seen in the top layers of the sediment, reasons for this are available O₂ in the top of the sediment for aerobic oxidation (Bastviken 2009), and that CH₄ is at the end of the redox cascade (Jørgensen 2000). There was an increase of CH₄ below 40 cm in the sediments collected in Lake Erssjön during February 2013. This could potentially be due to AOM in the middle layers of the sediment, such as indicated by increasing rates of Fe(II). This anaerobic oxidation could also be due to the available concentrations of SO₄ considering that SO₄ is also an oxidant for AOM (Adler et al. 2011). However, for all samples the concentration of SO₄ was near the detection limit, except in the top of the sediment for Lake Skottenesjö. A few cm down in the sediment, SO₄ is reduced to H₂S (Jørgensen 2000) and was therefore not detected in the rest of the sediment core.

Looking at the profiles during February 2013 for Lake Skottenesjö (Figure 15) no obvious AOM could be seen above 23.5 cm, however Fe(II) and Fe(III) showed patterns that mirrored each other. Below 23.5 cm the CH₄ concentrations did decrease slightly, with an increase of Fe(II) and decrease of Fe(III). This could potentially be AOM. Samples from 2012 were quite different from the samples collected in 2013, i.e. for Lake Erssjön Fe(II) decreased constantly with depth, while for Lake Skottenesjö Fe(II) increased at certain depths (with no visible effect on CH₄). An explanation for this could be that the warmer temperature of the sediment in September as compared to February had a higher impact on the methanogenic activity, than on that of AOM. During the 2012 sampling both lakes were also mixed (Figure 9), which increases the O₂ in the surface layers of the sediment. Also, the colder temperature lowers the methanogenic activity, which might favor AOM considering that the optimal temperature for AOM in consortia with sulfate-reducing bacteria was observed to be 4-16°C (Nauhaus et al. 2002). Also, considering that Lake Skottenesjö gets a more constant supply of allochthonous materials than Lake Erssjön, and that this pushes fresh OC to the deep sediment layers, methanogenic activity might be more stimulated than AOM in Lake Skottenesjö. A higher concentration of CH₄ did not seem to favor AOM in Lake Skottenesjö considering that a pattern of anaerobic consumption was not obvious from the CH₄ profiles.

For most of the CH₄ pore-water profiles sampled in February 2013, the PROFILE software was not able to identify zones of CH₄ production and consumption, with a significant level set to 0.05 (Table 2). Zones with statistical significance were calculated with the September profiles and one profile from Lake Erssjön 2013 (e.g. Figure 14 and 15). For the Lake Erssjön 2013 and for the second Lake Skottenesjö 2012 sample consumption rates were higher than the production rates, this is likely due to the consumption zone based on the bottom for the profile, i.e. the profile is increasing but because the measurement was interrupted at e.g. 20 cm the zone of production could not be detected below 20 cm. This artifact becomes apparent in figure 16 because the CH₄ curve at the bottom of the profile is not convex, thus it is interpreted as a zone of consumption. The fluttering pattern of the CH₄ profiles also affected the amount of detectable significant zones. A three step moving average decreased this fluttering but also the amount of zones detectable. Testing a five step moving average yielded no significant zones. However, in spite of these subjective interpretations of the profiles, zones of consumption in the Lake Erssjön samples could be found in the middle of the

profiles below the oxic zone using either raw data or 3 step moving average. This strengthens what is visually observable in the CH₄ profiles (Figure 15) that CH₄ seems to be consumed in the middle zone of the profile. These zones indicate the presence of AOM in the sediment.

5.3 Production of CH₄ by degradation of old carbon

Sediment incubations conducted in 4°C for 14 days on the sediment samples collected in February 2012 all yielded production of CH₄ (Figure 17 and Table 3), even in sediment layers below 30 cm (for Lake Skottenesjö) and 40 cm (for Lake Erssjön) which correspond to an age of about 150 and 400 years, respectively. Data from the software PROFILE and the sediment incubations yielded production rates that were considerable higher in Lake Skottenesjö compared to Lake Erssjön (Figure 16 and Table 2). Considering that the sediment in Lake Skottenesjö is younger and that the sedimentation rate is higher, there is a higher supply of fresher OC available for methanogenesis (Bastviken 2009, Sobek et al. 2012). Still the sediment below 30 cm and 40 cm in each respective lake is several hundreds of years old (Figure 8). It is highly likely that the production of CH₄ below these depths is higher in the summer season due to strong temperature dependence of CH₄ production (Bastviken 2009). Looking at the CH₄ profiles (Figure 14 and 15) the concentrations in the pore-water were higher during the summer, which could also indicate that production of CH₄ in both shallow and deep sediments is higher during the summer.

The sediment incubations yielded high production rates of CH₄ in Lake Skottenesjö between a sediment of 15-19 cm. Likewise, in accordance with the observed CH₄ concentrations at these depths almost reaching saturation (Figure 13), and the model to calculate the amount of time to reach CH₄ saturation also shows that the time to reach saturation is less in Lake Skottenesjö than Lake Erssjön (Table 4). Also, at a sediment depth of 15-19 cm, Lake Skottenesjö had a decrease in water content and porosity (Figure 11). This could explain why CH₄ saturation seems to be more prominent in this zone. The lower porosity causes less molecular diffusion, therefore CH₄ is more easily trapped. Compared to Lake Erssjön, Lake Skottenesjö sediment reaches CH₄ saturation on average 9.5 and 4.1 times faster at 4°C and 24°C, respectively. The model also shows that temperature has a high impact on the amount of days to reach CH₄ saturation, with a speed almost 10 times faster to reach saturation at the top layers, above 10 cm, of the sediment in both lakes. Similarly, the data from PROFILE for the Lake Erssjön 2013 sample indicate a lower production and consumption of CH₄ in a cold temperature (4°C in this case). Because Lake Skottenesjö has shallower water depth compared to Lake Erssjön, Lake Skottenesjö is likely to have warmer sediments during warm seasons and thus a higher methanogenic activity.

For figure 17 the production of CH₄ was higher in the top layers of the sediment for Lake Skottenesjö (4-19 cm). A reason for this is that the OM is more fresh compared to the bottom layers (Sobek et al. 2012). Below this depth the production decreased but did not reach zero. However, for Lake Erssjön the production of CH₄ slightly increased below 40 cm, this could be due to decreased AOM (compare to the high Fe(II) concentrations in Figure 14 between ~

10-30 cm, and CH₄ production rates in Table 3). The sediment incubations indicate that there is methanogenic activity in old sediment layers (below 30 and 40 cm, corresponding to an age of 158 and 413 years, respectively) in the two studied lakes, and that in all sediment depths of both lakes methanogenesis is greater than AOM. The incubations (Figure 16) did show some differences in CH₄ profiles compared to the sampled sediment cores (Figure 14 and 15), this is probably because aerobic oxidation of CH₄ was not possible in the enclosed anoxic vials, and that diffused CH₄ was trapped inside the vials.

5.4 Methanogenesis counterbalanced by AOM

Results from this study indicate that methanogenesis is occurring in deep and old sediments in the two studied boreal forest lakes. Results from February 2013 also indicate that AOM may be occurring in the sediment of Lake Erssjön. This AOM seems to be coupled to Fe(III) rather than SO₄ (Figure 14), considering SO₄ concentrations were near the detection limit.

Considering that the rate of CH₄ produced at 26 cm in Lake Erssjön was lower than that of all the other measured depths. The CH₄ pore-water profiles also showed a decreasing curve with one of the lowest concentrations of CH₄ at 26 cm, based on these results it can be suggested that CH₄ was anaerobically oxidized in this zone of the sediment but this AOM is lower than methanogenesis due to there being a net emission of CH₄ (i.e. CH₄ accumulated in all incubation vials over time). Results from the software PROFILE also indicate that there are zones of anaerobic oxidation in the sediment in Lake Erssjön (Table 2 and Figure 16). The results from the Lake Erssjön samples collected in February 2013 are in accordance to the suggestion by Adler et al. (2011) and Sivan et al. (2011) that deep lake sediments might act as a sink for CH₄. In this study methanogenesis was higher than AOM, therefore anoxic lake sediments can only act as a partial sink for CH₄. However, considering the lack of replicates in this study, further studies in deeper sediment layers are required of boreal forest lakes to estimate the potential of the anaerobic CH₄ sink.

5.5 Suggestions for development of future research

There are a few studies that have been conducted in this field (e.g. Adler et al. 2011; Sivan et al. 2011; Nordi et al. 2013), these studies include measurements of the stable carbon isotope ($\delta^{13}\text{C}$) for either DIC or CH₄, which is a good method to observe if the CH₄ has undergone anaerobic oxidation (due to differences in isotopic fraction). Continuing studies in this field could include measurements of stable carbon isotopes. Also, Sivan et al. (2011) and Nordi et al. (2013) measured highly reactive iron bound to particles (by either adding ascorbic acid or HCl to the sediment), this can be used in future studies as this gives an indication of the highly labile iron, which may be available as electron acceptor for CH₄ oxidation. Studies conducted in this field have not looked at the CH₄ production below a 30 cm sediment depth. Sivan et al. (2011) did manipulative incubations in 15°C from a sediment depth of 25 cm which yielded iron-dependent AOM and a production of CH₄. It is suggested that for future

studies concerning iron-dependent AOM and methanogenesis more incubations can be conducted from deeper depths, this to be able to observe possible methanogenesis in deep sediments, i.e. degradation of old carbon. This can then be coupled to AOM in the sediment, and a budget of CH₄ fluxes in the studied deep sediments can be modeled.

6. Conclusions

Methanogenesis was not strongly dependent on sediment age in the two lakes, and occurred in sediment incubations of a sediment depth of 30 and 40 cm, corresponding to an age of about 150 and 400 years, respectively. Also indications of potential AOM were observed in some of the collected sediment cores. AOM was more prominent in sediment cores collected during February 2013 compared to September 2012, potentially related to methanogenesis being more temperature-dependent than CH₄ oxidation. In the analyzed samples this AOM seemed to be coupled to Fe(III). The results show that methanogenic activity occurs by degrading old carbon in deep boreal forest lake sediments, and a certain part of this might then be oxidized anaerobically. However, all cores exposed a general trend of increasing CH₄ concentrations with sediment depth, indicating that CH₄ production in old sediment layers is greater than AOM. AOM could therefore only act as a partial sink for CH₄ in anoxic deep sediments. To elucidate this in more detail, further studies are required.

Acknowledgements

I would like to express deep appreciation to my supervisor Sebastian Sobek for his feedback on the report, advices for practical work and the opportunity he gave me to independently plan, set up and work in the laboratory and field. I thank Hannah Chmiel who assisted me in the field and introduced me to many of the routines in the field and laboratory. I also thank Jan Johansson for assistance in the field, and together with Christoffer Bergvall I thank them for guidance on laboratory instruments and the opportunity they gave me to learn and how to use analytical instruments independently. Also, I thank Stefan Bertilsson who gave me the opportunity to assembly a glove box. Finally I would want to thank everyone else in the Department of Limnology at the Evolutionary Biology Centre, Uppsala University who helped or gave me advice.

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