

Master's thesis

**A stable isotope study of the hydrological and carbon
cycle in meromictic lake, Lovojärvi**

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ABSTRACT

Lake Lovojärvi, at its deepest point of 17.5 m, is permanently stratified forming two water layers. The mixolimnion (the upper layer, from 0 to 10 m depth) is affected by seasonal changes while the monimolimnion (bottom layer, from 10 to 17.5 m depth) does not mix and is not affected by seasonal changes. As a result, the lake stores large quantities of dissolved inorganic carbon and methane. I used a multiple stable isotope approach to gain insight into the carbon cycle and water balance of Lovojärvi. The $\delta^{18}\text{O}$ and $\delta^2\text{H}$ profiles reveal that at the 7 m depth in the mixolimnion, there is a sub-surface stream whose isotopic characteristics are similar to surface streams from a nearby mire, suggesting that this water could be coming from the mire. Measurements of $\delta^{13}\text{C-DIC}$ and of concentrations of DIC and CH_4 were used to trace carbon flow in the lake. Variations in the epilimnion of DIC concentration and of $\delta^{13}\text{C-DIC}$ (ranging from -15.42 to -5.75‰) are explained by photosynthesis, while the strongly enriched $^{13}\text{C-DIC}$ and high concentrations of DIC and CH_4 in the deep water are explained by organic matter oxidation and methanogenesis. Methanotrophy appears to be an important source of carbon to the food web evidenced by the depleted $^{13}\text{C-zooplankton}$ (-38.2‰).

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TIIVISTELMÄ

Lovojärven syväne on kerrostunut pysyvästi kahteen erilliseen vesikerrokseen. Pintakerroksen mioksolimnionissa (0 - 10 m) on veden vuodenaikojen mukaista sekoittumista, pohjan monimolimnionissa (10 - 17.5 m) ei ole vuodenaikaisvaihtelua eikä veden sekoittumista. Tämän seurauksena järveen on varastoitunut runsaasti liuennutta epäorgaanista hiiltä (DIC) ja metaania (CH₄). Käytin hiilen ja veden stabiili-isotooppeja hiilen kierron ja vesitaseen tutkimiseen Lovojärvässä. Veden δ¹⁸O ja δ²H isotoopit osoittavat, että 7 m syvyydessä mixolimnionissa on virtaus, jossa veden isotooppiarvot ovat samankaltaisia kuin läheiseltä suolta tulevan puron veden isotooppiarvot. Tämä viittaa siihen, että vesi voi olla peräisin suolta. Epilimnionin DIC konsentraation vaihtelu ja δ¹³C-DIC:n vaihtelu (-15.42 - -5.7‰) selittyvät pääosin fotosynteesin hiilen otolla, pohjan voimakkaasti rikastunut δ¹³C-DIC ja korkeat DIC ja CH₄ pitoisuudet selittyvät orgaanisen aineen hapettumisella ja metanogeneesillä. Eläinplanktonin matala δ¹³C arvo (-38.2‰) osoittaa metaaninhapetuksen olevan tärkeä ravintoverkon hiilen lähde.

Contents

1. INTRODUCTION	5
2. BACKGROUND	5
2.1. Stable isotopes	5
2.1.1. <i>Fractionation</i>	6
2.1.2. <i>Kinetic and equilibrium isotope fractionation</i>	6
2.2. Carbon dynamics in a lake	7
2.2.1. <i>Dissolved inorganic carbon (DIC)</i>	7
2.2.2. <i>Particulate organic carbon (POC)</i>	8
2.2.3. <i>Dissolved organic carbon (DOC)</i>	8
2.2.4. <i>Methane and carbon dioxide production in lakes</i>	9
3. MATERIAL AND METHODS	12
3.1. Study lake Lovojärvi	12
3.2. Sampling.....	13
3.2.1. <i>Field measurements of physical variables</i>	13
3.2.2. <i>Dissolved gases</i>	13
3.2.3. <i>Ebullition</i>	14
3.3. Stable isotope analysis	14
3.4. Sedimentation.....	14
3.5. Zooplankton	14
4. RESULTS	15
4.1. Lake stratification.....	15
4.1.1. <i>Mixolimnion</i>	15
4.1.2. <i>Monimolimnion</i>	16
4.1.3. <i>Secchi disc depth</i>	16
4.2. Stable isotopes and DIC concentration	16
4.3. Methane in Lovojärvi	18
4.4. Sedimentation and zooplankton in Lovojärvi	19
4.5. $\delta^{13}\text{C}$ -DIC, $\delta^{18}\text{O}$ -H ₂ O and $\delta^2\text{H}$ -H ₂ O of inlets, epilimnion and outlet.....	19
4.6. $\delta^{18}\text{O}$ -H ₂ O and $\delta^2\text{H}$ -H ₂ O of the three inlets compared with water at 7 m depth ..	19
5. DISCUSSION	20
5.1. Lovojärvi meromixis and stratification	20
5.2. Hydrodynamics of Lovojärvi using stable isotopes	20
<i>Mixolimnion</i>	20
<i>Sub-surface flow in the mixolimnion</i>	21
5.3. Carbon dynamics in Lovojärvi	22
<i>Biogeochemical and physico-chemical processes in the mixolimnion</i>	22
<i>DIC and $\delta^{13}\text{C}$-DIC in the monimolimnion</i>	23
<i>Methane oxidation</i>	23
<i>Carbon balance</i>	23
ACKNOWLEDGEMENTS	24
References	25

1. INTRODUCTION

Carbon, the most abundant element in organisms, occurs in different chemical forms in aquatic systems. Hence the carbon cycle is so dynamic and it involves a complex of processes that connect with the atmosphere. Various interactions of dissolved inorganic carbon (DIC) and methane (CH₄) exist with biota in the water column and sediments. Carbon input and output can occur via gas exchange, inflow and outflow streams, runoff and permanent burial in sediments. Transformation amongst DIC, particulate organic carbon (POC) and dissolved organic carbon (DOC) occurs as a result of processes such as photosynthesis, respiration and organic matter oxidation. These processes lead to fractionation of stable isotopes of carbon. The natural variations in the proportions of the two carbon stable isotopes, ¹²C and ¹³C, can thus help to understand the interactions between the various carbon forms.

In the boreal region, where the majority of lakes are small and shallow (Kortelainen *et al.* 2004), the high rate of oxidation of terrestrially fixed carbon leads to high DIC concentration in lake water. Quite often boreal lakes have anaerobic hypolimnetic waters as a result of eutrophication caused by human activities in the lake catchments. The high rate of anaerobic organic matter decomposition in the deep water leads to high emissions of carbon gases CO₂, and CH₄, from these lakes (Huttunen *et al.* 2003, Rantakari and Kortelainen 2005). Striegl and Michmerhuizen (1998) have reported weathering and hydrological input (such as ground water and surface streams) as other sources of these carbon gases that can be locally significant. Lake Lovojärvi in southern Finland has extremely high concentration of these gases in the deepest point of the lake, which evokes research into the possible sources of the gases and how they are circulated in the lake.

Stable isotopes have been extensively applied to describe biogeochemical processes in aquatic systems. McKenzie (1982) reported that the carbon isotope composition of DIC is determined by the photosynthesis and respiration cycle. Preferential uptake of ¹²C during photosynthesis accounted for the enrichment of ¹³C-DIC in surface waters while oxidation of photosynthetically produced organic matter as it sinks releasing ¹²C-enriched CO₂ accounted for depletion of ¹³C-DIC in the underlying waters. Assayag *et al.* (2008) have also applied stable isotopes to study the hydrological budget of Lac Pavin using δ¹⁸O-H₂O to constrain the water balance as well as stratification and mixing dynamics in the lake.

In this study I used water stable isotopes to provide insight into the hydrodynamics of Lovojärvi (mixing dynamics, stratification, sub-surface water inputs and evaporation). δ¹³C-DIC, DIC and CH₄ concentration were used to trace biogeochemical processes (photosynthesis, respiration, methanogenesis, methanotrophy) in the water column of Lovojärvi.

2. BACKGROUND

2.1. Stable isotopes

Isotopes are atoms of the same element that differ in atomic mass due to differences in the number of neutrons contained in the atomic nucleus. For example, carbon has three most abundant isotopes ¹²C, ¹³C and ¹⁴C. Isotopes whose number of neutrons (N) and protons (Z) are the same tend to be stable. Stable isotopes are atoms that are energetically stable and do not decay, and hence are not radioactive (Michener and Lajtha 2007).

Stable isotopes have been widely used for studying the origins and cycling of organic matter in the biosphere. Many processes in the environment can be studied by use of stable

isotopes since they occur naturally (they are integral constituents of water molecules, not something that is dissolved *in* the water like other tracers that are commonly used in hydrology) (Assayag *et al.* 2008). They act as natural dyes or tracers that can give details of the origins and fates of element as they circulate in the biosphere. Stable isotopes of elements such as oxygen, hydrogen, carbon, nitrogen, and sulfur have been of special interest because they cycle tightly with organic matter. Their isotopic compositions are reported as "delta" (δ) values in parts per thousand (denoted as ‰) enrichments or depletions relative to a standard of known composition. δ Values are calculated as:

$$\delta X (\text{‰}) = (R_{\text{sample}}/R_{\text{standard}} - 1) * 1000 \quad (1)$$

Where X can be ^{13}C , ^{15}N , ^2H , ^{18}O etc. and R is the ratio of the heavy to light isotope in the sample or standard e.g. $^{13}\text{C}/^{12}\text{C}$.

$$\delta^{13}\text{C}_{\text{sample}} = \{ (^{13}\text{C}/^{12}\text{C}_{\text{sample}}) / (^{13}\text{C}/^{12}\text{C}_{\text{standard}}) - 1 \} \times 1000 \quad (2)$$

By definition the standard is therefore 0‰ and a positive ‘ δ ’ value means that the sample contains more of the heavy isotope than the standard while a negative ‘ δ ’ value means that the sample contains less of the heavy isotope than the standard. For carbon, the international standard is Pee Dee Belemnite, a carbonate formation, whose generally accepted absolute ratio of $^{13}\text{C}/^{12}\text{C}$ is 0.0112372. Materials with ratios of $^{13}\text{C}/^{12}\text{C} > 0.0112372$ have positive delta values, and those with ratios of $^{13}\text{C}/^{12}\text{C} < 0.0112372$ have negative delta values (Peterson and Fry 1987, Coplen 1996, Michener and Lajtha 2007). For hydrogen and oxygen in water, the standard used is most commonly standard mean ocean water (SMOW), an average value for the isotopic composition of ocean water.

2.1.1. Fractionation

The partial separation of isotopes of the same element during processes such as evaporation and precipitation due to different isotopes having different masses is referred to as isotope fractionation (<http://ethomas.web.wesleyan.edu/ees123/isotope.htm>). Biogeochemical processes by microorganisms may also influence the isotopic composition of an element such as carbon. For example, Barker and Fritz (1981) reported enrichment in ^{13}C by 5.0 to 29.6‰ of CO_2 produced from methanotrophy

When a molecule is vibrating, the energy of its vibration is controlled by its vibration frequency hence light atoms vibrate faster than heavier ones. Since heavy atoms have lower energies, they form more stable and strong bonds. Bond strength differences and velocities among isotopes lead to fractionation or isotopic variations between the source and product in chemical processes (Peterson and Fry 1987, Michener and Lajtha 2007). The reactants/substrates will be enriched in the heavy isotopes while depleted in the lighter isotopes. Isotopic fractionation is given as

$$\delta_{p-s} = R_p/R_s \quad (3)$$

Where, R is the ratio of heavy to light isotopes in the instantaneous product (R_p) and substrate (R_s) (Michener and Lajtha 2007).

2.1.2. Kinetic and equilibrium isotope fractionation

Atoms undergo transformation and displacements creating new linkages with other atoms during chemical reactions while during physical processes it is the states (gas, liquid, solid) that change. There are two different types of processes of isotope fractionation that is Kinetic and Equilibrium isotope fractionation.

In kinetic isotope fractionation there is a difference in behavior resulting from the

differences in speed between molecules containing different isotopes (e.g. in a gas, those molecules containing the lighter isotope move fractionally faster than the molecules containing the heavier isotope). In equilibrium isotope fractionation, there are differences in thermodynamic properties of molecules with different isotopes. The extent of thermodynamic property difference varies with temperature and is significant at low temperatures and insignificant at high temperatures. The largest differences in behavior occur for isotopes that have the largest relative difference in mass. There is much more fractionation between ^1H and ^2H (one of which is twice as heavy than the other), than in oxygen, where the difference in mass is only $(18-16)/16 = 1/8$ (<http://ethomas.web.wesleyan.edu/ees123/isotope.htm>).

In nature, when water evaporates, the water vapor becomes enriched in molecules carrying the lighter isotope ($^1\text{H}_2^{16}\text{O}$) as compared to the water it leaves behind ($^1\text{H}_2^{18}\text{O}$, $^1\text{H}_2^{17}\text{O}$, $^2\text{H}_2^{16}\text{O}$, $^2\text{H}_2^{18}\text{O}$). When water vapor condenses into water, it becomes enriched in molecules carrying the heavier isotope as compared to the vapor it leaves behind. In lakes CO_2 emission to the atmosphere and uptake by primary producers both favor $\delta^{12}\text{C}$ hence leaving lake water dissolved inorganic carbon enriched in ^{13}C (Herczeg 1987). Fractionation factors and the isotopic composition of two substances are related according to:

$$\alpha (\text{A-B}) = (1000 + \delta(\text{A})) / (1000 + \delta(\text{B})) \quad (4)$$

Where A and B represent different substances

2.2. Carbon dynamics in a lake

Autochthonous (photosynthesis in the lake) and allochthonous (terrestrial) inputs are the major sources of carbon in lakes. Carbon exists as both organic and inorganic forms. Organic forms are POC and DOC while the inorganic form is DIC. Carbon in lakes is continuously circulating and recycling. While some of it, as it is respired by anaerobic and aerobic consumers, may be lost to atmosphere as CO_2 and CH_4 (Murase *et al.* 2003), the other part may be lost through groundwater seepage, to other lakes through rivers, or by sedimentation (Dillon and Molot 1997).

The location and hydrology of lakes greatly influences the relative input of the different carbon forms i.e. DIC, DOC and POC. For example in the northern latitudes the input is mostly DIC due to high soil respiration, ground water flow and carbonate weathering while in the humid tropics and boreal forest (without carbonate) DOC is the most important input (Tranvik *et al.* 2009).

2.2.1. Dissolved inorganic carbon (DIC)

Dissolved inorganic carbon exists as carbonate, bicarbonate and CO_2 . Shifts amongst the three forms follow the laws of the CO_2 -bicarbonate-carbonate equilibrium and are indirectly affected by pH and the photosynthetic and respiratory activity of aquatic organisms.

Carbon dioxide fixation by photosynthesizing phytoplankton has a major influence on the DIC pool. Other sources such as microbial utilization of allochthonous carbon (respiration and fermentation) especially in lakes with long hydraulic residence times may also contribute to internal increment of DIC (Cole *et al.* 2002). Weathering and hydrological input can also be locally significant (Striegl and Michmerhuizen 1998, Kortelainen *et al.* 2006, Pasche *et al.* 2011). Photo-oxidative production of DIC from DOC is another source (Graneli *et al.* 1996). Solar radiation of UV-B (short wavelength), UV-A (long wavelength), and PAR (photosynthetically available radiation) plays a very

important role in the photochemical degradation of organic matter to DIC (Graneli *et al.* 1996). Methane oxidation also adds to internal DIC increment as CO₂ is a by-product.

Photosynthesis and chemosynthesis may be the main processes consuming CO₂ but also emission to the atmosphere may be periodically important (Lampert and Sommer 2007). Methanogenesis is another important process that consumes CO₂ forming CH₄.

2.2.2. Particulate organic carbon (POC)

Particulate organic carbon can be either living or dead matter (detritus). Detritus is either formed within the lake as a result of primary production (autochthonous) or imported from outside the lake (allochthonous) consisting of plant or animal material, such as small pieces of insect exoskeletons, debris from leaves residues originating from the forest litter (Lampert and Sommer 2007).

Changes in the POC pool occur because of death, feeding, parasitism and or direct evacuation from the lake by an output such as a river. POC can be transformed into DIC by respiration and into DOC via secretion, excretion, and autolysis. POC is lost from the pelagic region and imported into the benthic zone through sedimentation. Sedimented POC may be re-suspended into the pelagic region by wind-driven water currents and by occasional benthivory by pelagic animals (Lampert and Sommer 2007).

2.2.3. Dissolved organic carbon (DOC)

Dissolved organic carbon is a mixture of various substances such as biologically reactive low-molecular-weight compounds and refractory high-molecular-weight humic substances. Humic substances make up about 4 to 50% of DOC in most surface waters. In the Boreal region, humic substances can contribute 90% of lake DOC. The high water color in most lakes is due to the nature of DOC that mainly originates from terrestrial ecosystem (Houser *et al.* 2003). The simple configuration of DOC enables its transportation via runoff making allochthonous input the most important source of DOC (Lampert and Sommer 2007). Other sources are secretion and excretion by organism from all trophic levels and autolysis of detritus.

Uptake by heterotrophic microorganisms, especially bacteria, is the most important sink for DOC although some protists and pigmented mixotrophic algae can also use it (Lampert and Sommer 2007). Excretion products are rapidly utilized by bacteria and therefore do not accumulate as the more refractory substances do. The microorganisms present in lake waters or in sediments largely participate in the recycling of DOC in a system, either by using it in respiration or growth, or by producing it during photosynthesis.

The carbon unused during these metabolic activities is deposited at the bottom of the lake as POC. For example, data collected from lake-sediment studies in Finland by Kortelainen *et al.* (2004) showed that aquatic ecosystems were second only to peat-lands in storing the largest areal carbon stocks (19 KgC m⁻²). The unused carbon may also be evacuated towards other systems through water currents coming out of the lake (Kortelainen *et al.* 2004, Cole *et al.* 2007).

Houser *et al.* (2003), having found that hypolimnetic accumulation of DIC and CH₄ positively correlated with epilimnetic DOC decline, argued that allochthonous inputs in the lake provide DOC that is a major support to metabolism in the hypolimnion.

2.2.4. Methane and carbon dioxide production in lakes

Carbon dioxide and CH₄ are important greenhouse gases that occur naturally in the atmosphere. They are produced naturally in soils, sediments and waters in various microbiological processes (as seen above in respiration and methanogenesis). Increasing atmospheric loading of these gases is a major global concern since they influence the earth's radiative balance (Rasmussen and Khalil 1984, Leemans *et al.* 1996, Conrad 1996).

In lakes, these gases may come from different sources. The most common is as a result of terrestrially fixed carbon sinking and simultaneously accumulating as sedimentary organic matter (Kortelainen *et al.* 2004, Cole *et al.* 2007), which then undergoes decomposition. This process is a collective effort of fermentative bacteria, sulfate-reducing bacteria and methanogenic archaea (Lauro *et al.* 2010). Lakes can also act as sources for carbon gases to the atmosphere hence playing a major role in regulating regional carbon balances (Bastviken *et al.* 2004).

Methane production is strictly an anaerobic process that can only occur in environments having low concentrations of electron acceptors such as manganese and iron oxides, oxygen, nitrate and sulfate while electron donors, particularly hydrogen (H₂) and acetate should be in high concentration (Capone and Kiene 1988). Lake sediments are the main sites for methane production although methanogenesis can also occur in the anoxic water column (Wand *et al.* 2006). This implies that the methanogens possess specialized physical properties to maintain buoyancy in the water column (Winfrey and Zeikus 1979).

2.2.4.1. Methanogens and methanogenic substrates

Methanogens are prokaryote producers of methane. They are strictly anaerobic respiring archaea, which produce CH₄ as the end product (Liu & Whitman 2008). Phylogenetic results obtained from the analysis of rRNA and other genes have put methanoarchaea in an ancient monophyletic lineage with the Euryarchaeota (Hedderich and Whitman 2006). They are classified into five well-established orders: *Methanobacteriales*, *Methanococcales*, *Methanomicrobiales*, *Methanosarcinales*, and *Methanopyrales*. Methanogens belonging to different orders possess different cell envelope structures, lipid composition, substrate range, and other biological properties.

There is great diversity in methanogens but substrate utilization is restricted to a number of substrates, mainly CO₂, methyl-group containing compounds, and acetate. If organic substances like carbohydrates, long-chain fatty acids and alcohols are to undergo methanogenesis they need to be processed first by eukaryotes (bacteria). In turn methanogenesis provides energy for growth to the eukaryotes (Liu and Whitman 2008). The carbon precursor used by methanogens determines the pathway of methanogenesis and hence its classification (Whiticar 1999).

Methanogens that use H₂ as the primary electron donor to reduce CO₂ to CH₄ are referred to as hydrogenotrophs. Many of these can also use formate as the major electron donor. Hydrogenotrophic methanogenesis occurs in a series of steps where CO₂ is reduced successively to methane through the formyl, methylene, and methyl levels (Liu and Whitman 2008). The overall equation for the hydrogenotrophic methanogenesis:



Acetoclastic methanogens utilize acetate as the major intermediate in the anaerobic food chain. Approximately two-thirds of the methane that is produced from biogenic processes is derived from acetate (Liu and Whitman 2008). *Methanosarcina* and *Methanosaeta* are the only two genera known to use acetate for methanogenesis. They

oxidize the carboxyl-group to CO₂ and reducing the methyl group to CH₄. *Methanosarcina* is a relative generalist that prefers methanol and methylamine to acetate, and many species also utilize H₂ while *Methanosaeta* is a specialist that uses only acetate (Liu and Whitman 2008).



2.2.4.2 Ecology of methanogens

Complex polymers are first broken down to simple sugars such as glucose, lactate, and volatile fatty acids (acetate, propionate and butyrate). Hydrogen is produced in these processes. Methanoarchaea in such ecosystems, are working at the terminal step where they convert the fermentation complex of organic polymers to CH₄ and CO₂ (Hedderich and Whitman 2006).

Accumulation of H₂ inhibits further fermentation of volatile fatty acids (Hedderich and Whitman 2006). Therefore presence of methanogens prevents this H₂ inhibition as they rapidly metabolize the H₂ maintaining its partial pressure well below toxic levels (10⁻³ - 10⁻⁴ atmospheres). However when sulfates are present, sulfate-reducing bacteria are better competitors and H₂ oxidation with sulfate as electron acceptor is more thermodynamically favorable than when CO₂ is (Lovley and Klug 1983, Hedderich and Whitman 2006). Sulfate-reducing bacteria also out-compete methanogens for other important substrates like acetate and formate. The oxidation of H₂ with nitrate, Fe³⁺, and Mn⁴⁺ as electron acceptors is also thermodynamically more favorable than methanogenesis. The denitrifying and iron- and magnesium-reducing bacteria also out-compete the methanogens when these electron acceptors are present (Hedderich and Whitman 2006).

In anaerobic environments, sulfate-reducing bacteria, iron- and magnesium-reducing bacteria, and denitrifying bacteria rapidly consume the entire sulfate, Fe³⁺, Mn⁴⁺ and nitrate hence establishing conditions for methanogenesis. Since CO₂ is a major fermentation end product, it is never limiting thus methanogenesis is dominant in anaerobic environments (freshwater sediments found in lakes, ponds, marshes, and rice paddies) that contain large amounts of easily degradable organic matter (Hedderich and Whitman 2006).

In freshwater ecosystem where there are low sulfate concentrations in both the sediments and the water column, hydrogenotrophic methanogenesis (H₂/CO₂) is the predominant pathway while acetate may be a minor precursor (Wand *et al.* 2006).

2.2.4.3 Methane oxidation

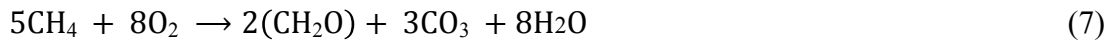
Methane produced in aquatic environments is consumed in both aerobic and anaerobic compartments (Wand *et al.* 2006). About 45 to 100% of CH₄ produced in the water column of lakes and/or derived from benthic flux is oxidized (Bastviken and Likens 2009). Kankaala *et al.* (2006) obtained about the same results for a stratified humic lake, with 80% of the CH₄ that diffused from the sediment consumed by methanotrophs in the water column and only 20% released to the atmosphere.

Methane oxidation in freshwaters is most active in the vicinity of the oxic-anoxic interface, where both CH₄ and O₂ are available (Rudd *et al.* 1974, Lidstrom and Somers 1984). Anaerobic CH₄ oxidation is also a significant process that has been reported mostly in marine sediments. Iversen and Jørgensen (1985) found a sharp maximum oxidation of CH₄ at the sulfate-methane transition coinciding with the sulfate reduction maximum. Oxidation was low in the sulfate zone. An association of microorganisms carries out

anaerobic CH₄ oxidation. Using microscopic techniques, Boetius *et al.* (2000) showed that a consortium of archaea and sulfate-reducing bacteria were responsible for anaerobic CH₄ oxidation.

Methanotrophs that use CH₄ produced by methanogens as a carbon source and electron acceptor are also heterotrophic bacteria that specialize in using one-carbon compounds (methane, methanol, methylamine, formaldehyde, formic acid). This process inputs CH₄ into the lake food web especially in stratified humic lakes. This is shown by the strong correlation of bacterial production in the water column with CH₄ concentration (Kankaala *et al.* 2006). This also correlates to zooplankton biomass implying that methanotrophs offer a significant source of carbon to zooplankton in stratified humic lakes (Bastviken *et al.* 2003, Kankaala *et al.* 2006, Pimenov *et al.* 2010). Jones and Grey (2004) using stable isotope analysis of chironomid larva also found that biogenic CH₄ played an important role as a carbon source to benthic food webs. Microbial consumption is a major sink for CH₄ and oxygen as shown by the equation:

Aerobic CH₄ oxidation



Anaerobic CH₄ oxidation



2.2.4.4 Methane emissions

It is difficult to estimate emission from aquatic ecosystems since there are at least four emission pathways, which may be regulated differently: ebullition flux, diffusive flux, storage flux and flux through aquatic vegetation (Kankaala *et al.* 2003). However variables such as DOM, CH₄ concentration, and volume fraction for storage and water depth for ebullition can be used to predict methane emission (Bastviken *et al.* 2004).

Ebullition is quantitatively the most significant source of atmospheric CH₄ with 40-60% of open water emission from an average lake (Bastviken *et al.* 2004). This is because CH₄ is relatively insoluble with saturation in fresh water of about 1.6 mol m⁻³ at 20 °C. High concentration at depth produces bubbles that are lost by ebullition to the atmosphere. However CO₂ whose saturation is about 39 molm⁻³ at 20 °C can accumulate at depth (Casper *et al.* 2003).

2.2.4.5 Storage and fluxes to atmosphere

Small and shallow lakes in the boreal region have higher CH₄ fluxes per unit area than larger ones. Methane concentrations and fluxes to the atmosphere are also higher in nutrient rich calcareous lakes (Juutinen *et al.* 2009). Most of the carbon gas fluxes occur during turnover periods although the efflux of CH₄ and CO₂ can also be significant in summer if there is an extreme rain episode (Ojala *et al.* 2011).

2.2.4.6 Carbon dioxide in boreal lakes

The high oxidation rate of terrestrially fixed carbon to CO₂ accounts for the high CO₂ concentration in boreal lakes. These lakes play a significant role in transferring terrestrially fixed carbon to the atmosphere (Kortelainen *et al.* 2006, Tranvik *et al.* 2009, Einola *et al.* 2011).

Precipitation is an important driver for carbon balance in most lakes. Increase in precipitation results into high input of allochthonous carbon in lakes and hence high CO₂ emission (Einola *et al.* 2011). Sedimentation, mineralization and subsequent CO₂ emission to the atmosphere are important carbon loss processes. Mineralization of allochthonous

organic carbon regulates its export to other systems (e.g. to the sea) and may also affect net exchange of CO₂ between the atmosphere and the boreal landscape (Algesten *et al.* 2004).

Sediment respiration, which contributes to high CO₂ concentrations of hypolimnetic waters, is one of the major attributes to CO₂ super-saturation in lakes. This saturation increases with precipitation and nutrient availability (especially phosphorus). Nutrients such as nitrogen and phosphorus increase in a lake due to organic matter decomposition mainly from terrestrial ecosystem, which is enhanced by precipitation. This increases oxygen consumption until eventually it can become exhausted. Anaerobic decomposition then starts in the sediments and water column liberating CH₄ and CO₂ as end products whose supersaturation results in emission to the atmosphere (Rantakari and Kortelainen 2005).

Most significant emissions of CH₄ and CO₂ are experienced in regions where lake catchments have been greatly disturbed by human activities such as agriculture. High CO₂ fluxes in the Boreal zone have also been found in catchments rich in peatlands and managed forests (Huttunen *et al.* 2003). Net evasion of CO₂ to the atmosphere implies it is supported by some input. Cole and Caraco (1998) have suggested three input models that explain the sources of CO₂ evaded from a lake if the changes in standing stock of DIC are insignificant: (1) net heterotrophy model: respiration on mainly allochthonous inputs within the lake exceeds the net photosynthetic sink of CO₂; (2) CO₂ injection model: inflowing ground water carries in excess CO₂ that is sufficient to support the efflux; (3) alkalinity consumption model: inflowing ground water and surface water carry in bicarbonate, which meets a source of acidity with the lake, thereby generating the excess CO₂.

3. MATERIAL AND METHODS

3.1. Study lake Lovojärvi

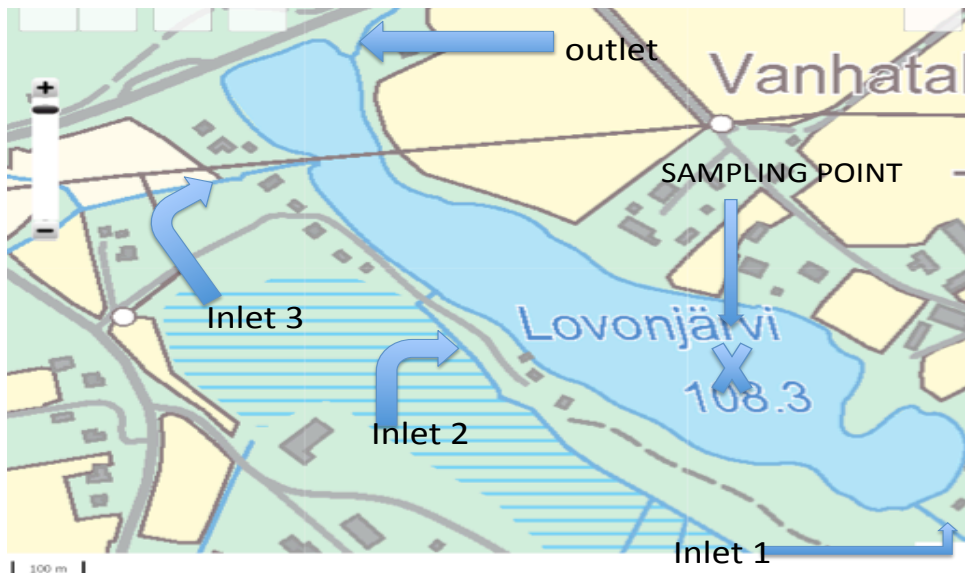


Figure 1. Location of lake Lovojärvi (<https://tiedostopalvelu.maanmittauslaitos.fi/tp/kartta>).

Lovojärvi (Fig. 1) is a small lake (54000 m²) in southern Finland (61° 04'N, 25° 02'E). It is in a glaciofluvial depression that forms an elongated narrow kettle-hole in an esker which rises about 50 m above lake level giving shelter to the lake (Simola 1979, Hakala 2004). The lake is 650 m long in NW-SE direction and 120 m wide. Its maximum

depth (locates near the SE end of the basin) is 17.5 m, and drainage area is 7.2 km². It has a mean depth of 7.7 m and it is 108 m above sea level (Ilmavirta & Kotimaa 1974).

The lake has four inlets and one outlet. The inlet from Lamminjärvi mire is the major one. The input to the lake is mainly water that drains from agricultural fields and it passes through bogs thereby having high concentrations of humic substances and dissolved ions (Hakala 2004). The other three inlets are small and during most of the sampling time they were dry (no flow). The lake experiences strong thermal stratification leading to oxygen deficiency below 5 m. The main cause of this is the lake's sheltered position and morphometry (Saarnisto *et al.* 1977).

3.2. Sampling

Sampling began on the 4 May 2011 and continued every second week of the month for 4 months. The final sampling was in September 2011. Data for pH and conductivity depth profiles were collected by technicians from Lammi station while the rest of the data were collected by me and Dr. Hannu Nykänen.

3.2.1. Field measurements of physical variables

Depth profiles of water temperature, pH, conductivity, dissolved oxygen (O₂) and redox –potential were measured with a YSI 556 mps model (Yellow Springs Instruments) at 0.5 m depth intervals. Transparency/euphotic zone was determined using a secchi disk.

3.2.2. Dissolved gases

Water samples for determination of CH₄ and DIC (free CO₂, bicarbonate (HCO₃⁻) and carbonate (CO₃²⁻)) were collected in duplicate using equipment that was designed so that two 60 ml plastic syringes (Terumo medical corporation, Sommerset, England) equipped with stopcocks (Codan, Berkshire, England) were attached to the bottom of a vertical 1.4 m long heavy iron rod. A rope was attached to the both outer plunger ends. Syringes were filled by hauling the rope that was attached to plunger ends from surface. The other rope fastened to the upper end of the rod was used to suspend the sampler to desired depth. Samples were chilled in a dark cooler containing ice for transport to the laboratory. Gas samples (CH₄ and CO₂) were analyzed within 4 to 6 hours.

In the laboratory, the filled syringes were partially discharged to hold 30 ml of bubble-free water that never had contacted with air. The samples were kept for 15 minutes at 20 °C and then 0.3 ml of 1% HNO₃ was added. 30 ml of nitrogen gas (99.5%) was added to each syringe and equilibrated by shaking the syringes vigorously for 3 minutes. Well-mixed headspace gases from the syringes were injected into pre-evacuated 12-ml Labco exetainer vials. Samples from over-pressurized exetainers were then delivered by 222 XL auto-sampler through a 1 ml Valco 10-port valve into an Agilent II 6890N (Agilent Technologies) gas chromatograph (GC) network system equipped with a flame ionization detector (temperature 210 °C) for CH₄ analysis and thermal conductivity detector for CO₂ analysis (temperature 120 °C, oven 40 °C, plot Q capillary column, flow rate 12 ml min⁻¹, He as a carrier gas). The concentrations of CH₄ and CO₂ in the lake water were calculated using the Bunsen adsorption coefficient for CH₄ and the Henry's law constant for CO₂ (<http://www.mpch-mainz.mpg.de/~sander/res/henry.html>) at appropriate equilibration and lake temperatures. (Standard methane 503 +/-2 mol ppm in N₂ ground gas (AGA Company) and standard CO₂ 4100 mol ppm).

3.2.3. Ebullition

Ebullition was measured continuously with submerged funnel gas collectors. The collector consisted of an inverted funnel (diameter 20 cm) attached to a PVC tube (length 60 cm). A polypropylene syringe at the upper end of the tube equipped with a syringe with a three-way stopcock permitted checking of the volume of trapped bubbles and could be used to obtain gas samples for further CO₂ and CH₄ concentration analyses with the GC. A set of 6 funnels with syringes interconnected was suspended at the deepest point for trapping of ebullition. Funnels were checked at 2 to 4 weeks intervals.

3.3. Stable isotope analysis

A Limnos sampler was used to collect water samples for stable isotope analysis (SIA) of $\delta^{13}\text{C}$ of DIC and $\delta^{18}\text{O}$ and $\delta^2\text{H}$ of water. Duplicate samples from Limnos sampler were transferred to vials containing helium using a syringe connected to a needle. The samples were kept on ice in a cool-box until they were transported to the laboratory for analysis. Gas bench analyser connected to isotope ratio mass spectrometer (IRMS) at University of Jyväskylä was used for $\delta^{13}\text{C}$ - DIC analyses. Samples for SIA $\delta^{18}\text{O}$ and $\delta^2\text{H}$ were collected in vials kept on ice until transferred to the cold storage room in the laboratory. Analyses of $\delta^{18}\text{O}$ and $\delta^2\text{H}$ were done at the Laboratory of Chronology of Finnish Museum of Natural History.

3.4. Sedimentation

Three open tubes for sediment trapping were suspended to collect particulate organic carbon (POC) for 41 days. The 4.5 cm radius tubes were suspended at 12 m depth. The collected samples were stored in the freezer. Stable isotopes of carbon and nitrogen and their percentages in particulate organic matter were analyzed in order to estimate carbon and nitrogen recycling using the EA-IRMS at the University of Jyväskylä.

3.5. Zooplankton

Zooplankton were sampled using a 100 μm mesh net sampler from depths 1 m to 3 m.

4. RESULTS

4.1. Lake stratification

Lake Lovojärvi was permanently stratified throughout the entire ice-free sampling period that began in early May (spring) and ended in September (autumn) 2011. During stratification two water masses were formed in the mixolimnion, the warm epilimnion and the cold hypolimnion. The two layers are separated by a metalimnion where exists a steep temperature gradient. The stratification is shown by depth profiles of temperature, DO, pH and conductivity (Fig. 2).

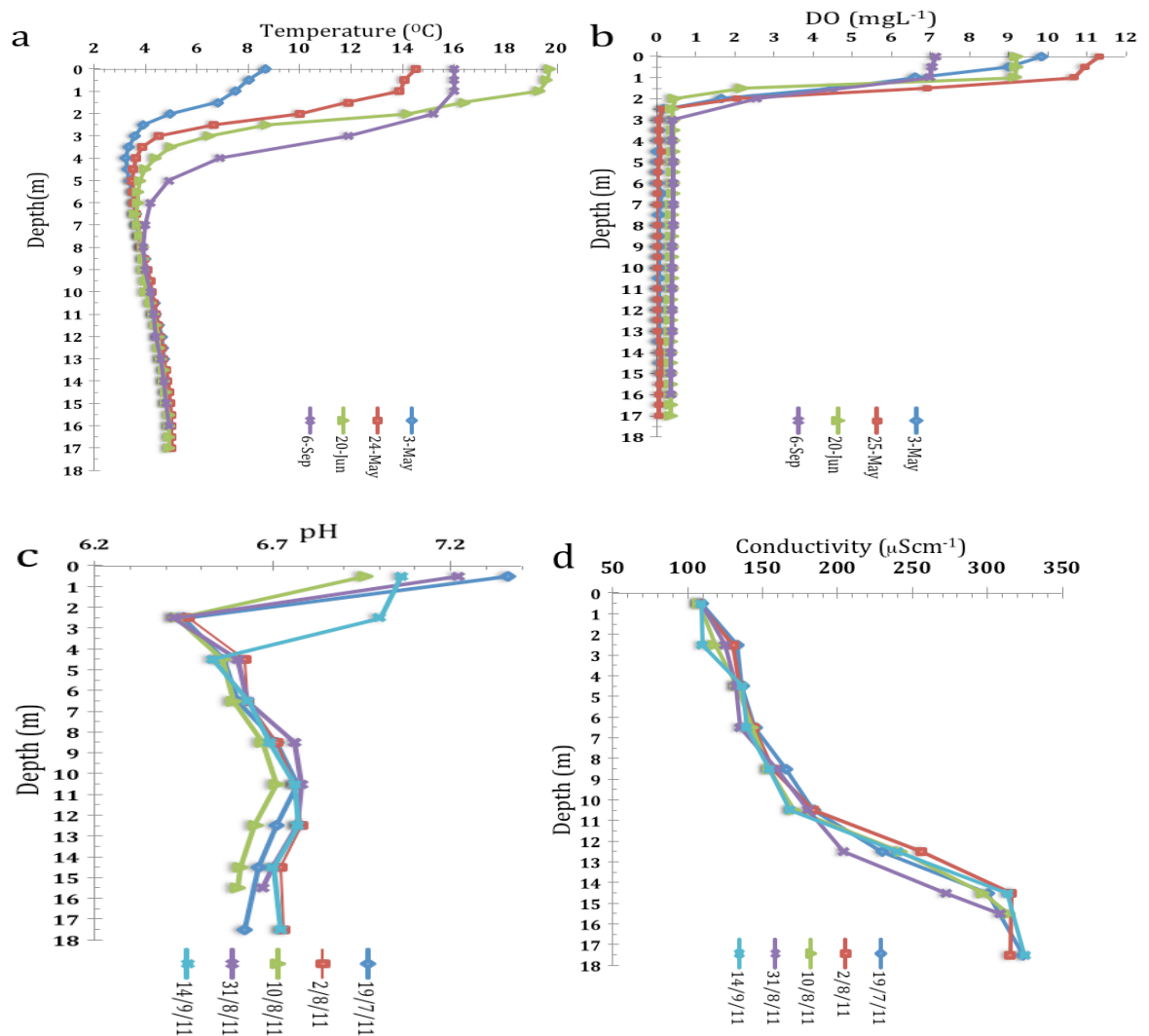


Figure 2. Lake Lovojärvi, depth profiles of: (a) Temperature, (b) Dissolved oxygen, (c) pH and (d) Conductivity.

4.1.1. Mixolimnion

The surface mixed layer of the epilimnion experienced seasonal variation in temperature, DO, pH and conductivity. At the start of spring, the epilimnion was not clearly distinct as seen from May 3 temperature profile. The whole water layer from 0 to 4 m was in transition. The surface water then warmed up very quickly such that by late spring (May 24), through summer (June 20), the epilimnion was distinct at a depth that

extended from 0 to 1 m at temperatures 14 °C and 19.6 °C for May and June respectively. By autumn (Sept. 6), the depth of the epilimnion had increased by 0.5 m to 1.5 m at 16 °C (Fig. 2a). Dissolved oxygen concentration was high while the pH was neutral in the epilimnion (Fig. 2b & c).

The metalimnion was typical with steep temperature gradients that varied in depth thickness with changing seasons. In late May, it extended from 1 to 4 m, in June from 1 to 4.5 m and in Sept. from 1.5 to 8 m (Fig. 2a). DO and pH also steeply decreased in the metalimnion.

In the hypolimnion, the water temperature was stable at about 4 °C. For most of the seasons the hypolimnion started from about 5 m to the bottom except for autumn when it started from 8 m. In September the metalimnion was also exceptionally thicker indicating the autumn mixing of the upper water mass.

4.1.2. Monimolimnion

The pH decreased from around 10-11 m depth while temperature slightly increased but conductivity strongly increased with depth (Fig. 2c & d). At this depth exists the chemocline (strong chemical gradient), which indicates the meromictic nature of Lovojärvi. This separates the mixolimnion (upper water mass which completely mixes) from the monimolimnion (denser bottom water mass which does not mix). In the monimolimnion, temperature, DO, conductivity and other factors do not change with seasons as observed in the mixolimnion.

4.1.3. Secchi disc depth

Measurements of transparency using the secchi disk, measured to a depth of 1.08 ±0.08 m (± Stdev.). This euphotic zone depth is same as the thickness of the epilimnion layer.

4.2. Stable isotopes and DIC concentration

Both oxygen and hydrogen stable isotopes of water show similar trends, which differentiate the mixolimnion from the monimolimnion at 10 m depth (Fig. 3). The epilimnion, metalimnion and hypolimnion are prominent in the mixolimnion even with isotope data. In the epilimnion and metalimnion the isotope profiles vary as seasons change. On the other hand, in the lower part of the mixolimnion and monimolimnion the profiles were almost constant over time.

In the epilimnion from 0 to about 2 m, the $\delta^{18}\text{O}\text{-H}_2\text{O}$ (Fig. 3a) is higher than in other layers; the isotope signatures range from -11.5‰ to -9.4‰. A steep gradient exists in the metalimnion at a depth of 2 to 4 m where isotopes are depleted to about -11.75‰ and then remain relatively constant in the hypolimnion from 4 to 10 m depth. In the monimolimnion, the values of $\delta^{18}\text{O}\text{-H}_2\text{O}$ were slightly decreasing and were superimposed from 10 m to the lake bottom.

The $\delta^2\text{H}\text{-H}_2\text{O}$ showed a similar trend to that of $\delta^{18}\text{O}\text{-H}_2\text{O}$ (Fig. 3b). The enriched epilimnion had values of about -85‰ for May, -79‰ for June and -75‰ for Sept. These signatures decreased along the metalimnion to about -88‰. In the hypolimnion, the signatures increased for all of the three profiles ranging from -88‰ to -81‰. The isotope values gradually decreased to about -88‰ in the monimolimnion.

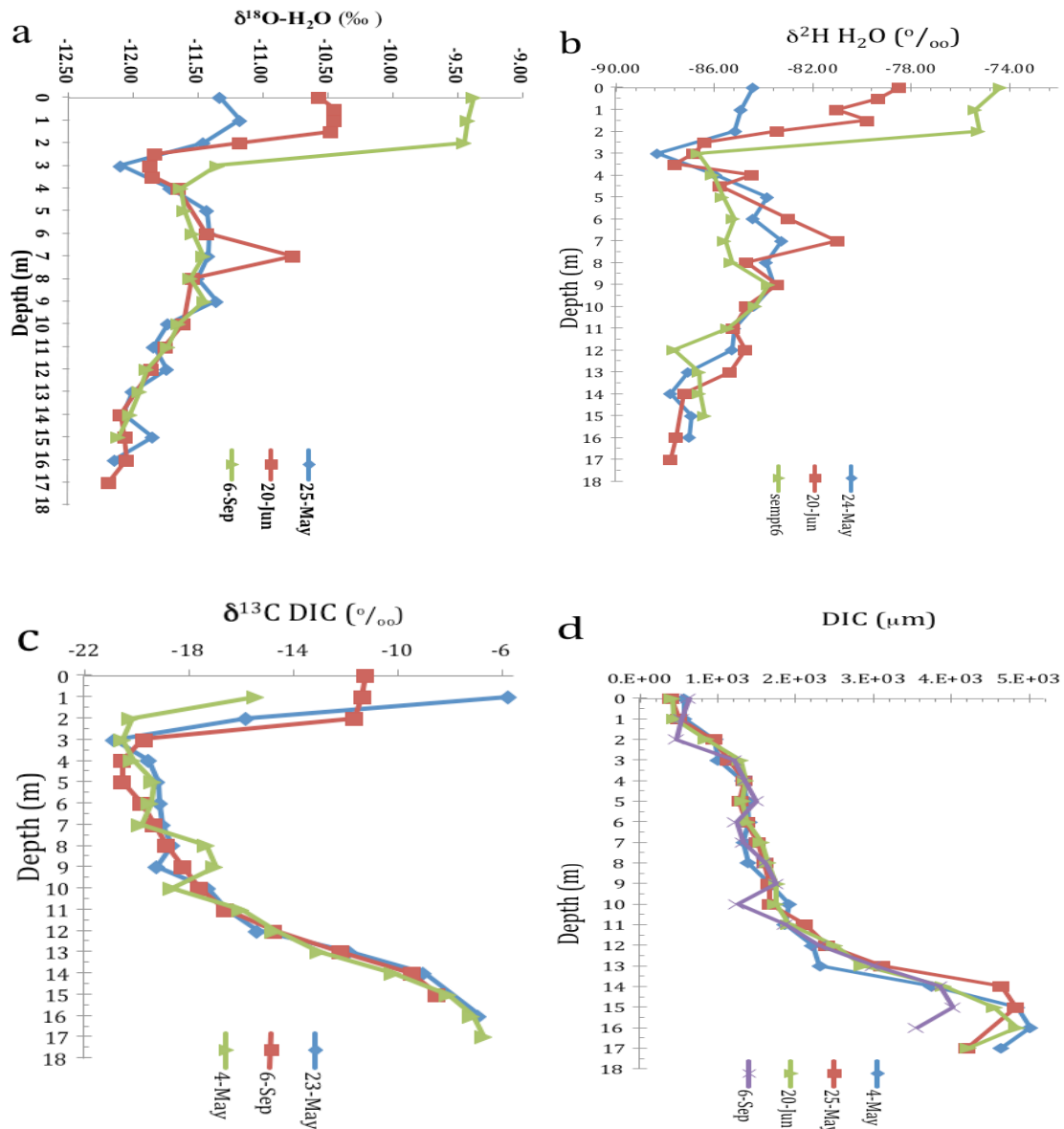


Figure 3. Lake Lovojärvi, depth profiles of: (a) $\delta^{18}\text{O}$ of water, (b) $\delta^2\text{H}$ for water, (c) $\delta^{13}\text{C}$ of DIC and (d) DIC concentration

The $\delta^{13}\text{C}$ -DIC profiles show the chemical stratification of the mixolimnion being divided into 2 layers with a transition separating them (Fig. 3c). The $\delta^{13}\text{C}$ -DIC ranged from -15.42‰ to -5.75‰ in the epilimnion. The $\delta^{13}\text{C}$ -DIC sharply decreases in the metalimnion. The hypolimnion had $\delta^{13}\text{C}$ -DIC that gradually increased. In the monimolimnion, the $\delta^{13}\text{C}$ -DIC profiles are superimposed and sharply increased from about -18‰ to -6‰ towards the bottom.

Dissolved inorganic carbon profiles are nearly superimposed in both water masses (Fig. 3d). DIC concentration in the epilimnion was stable at about $400 \mu\text{mol L}^{-1}$. It increased in the metalimnion reaching to about $1200 \mu\text{mol L}^{-1}$ in the hypolimnion where

was fairly stable (slightly increasing). In the monimolimnion the DIC concentration strongly increased, with values ranging from about 1750 $\mu\text{mol L}^{-1}$ to 5020 $\mu\text{mol L}^{-1}$.

4.3. Methane in Lovojärvi

Even though the amount of methane in both the epilimnion and metalimnion seems almost insignificant (Fig. 4a), it is important to note that its concentration was relatively high in the epilimnion, depth 0 to 1 m (Fig. 4b). It decreased in the metalimnion (1 to 2 m). Methane then gradually increased in the hypolimnion from about 0.14 to 254 $\mu\text{mol L}^{-1}$. There was a strong increase in the monimolimnion ranging from about 200 to 3100 $\mu\text{mol L}^{-1}$ (Fig. 3a).

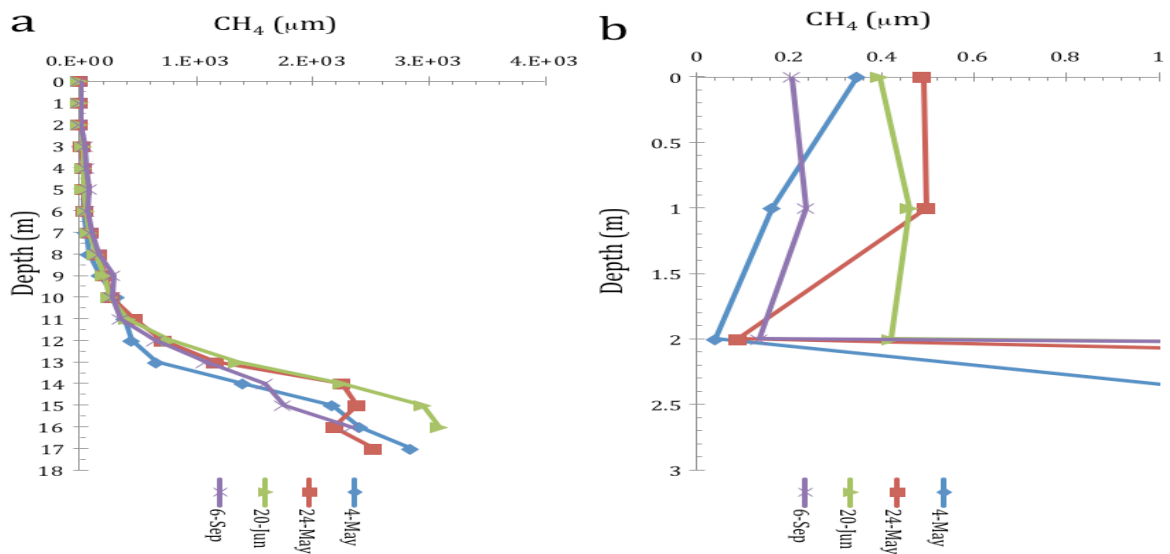


Figure 4. (a) CH₄ depth profiles of lake Lovojärvi, and (b) magnification of CH₄ depth profiles from 0 to 3 m depth (5/2011 to 9/2011).

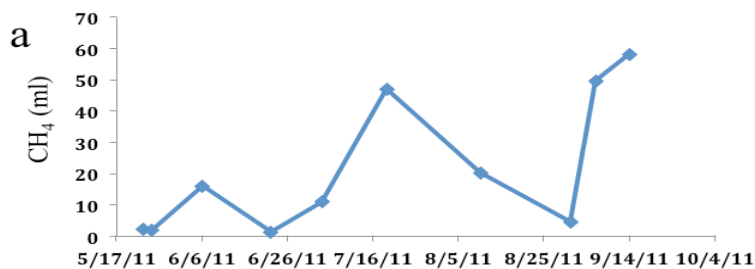


Figure 5. Mean daily methane ebullition during the ice-free period

Ebullition was low during the spring and increased significantly towards summer reaching maximum values in autumn (Fig 5). Highest variations from the daily mean were experienced during days of maximum ebullition. On average, $5.27 \pm 6.32 \text{ mg C m}^{-2} \text{ d}^{-1}$ in the form of CH₄ were released from Lovojärvi.

The estimated total diffusive emission for the open water period (224 days) was 1 g C as CH₄, and 70 g C as CO₂ based on surface gas concentrations, temperature and pH. (Hannu Nykänen, personal communication).

4.4. Sedimentation and zooplankton in Lovojärvi

The 41 days of sediment trapping revealed sedimentation rates of $0.317 \text{ g C m}^{-2} \text{ day}^{-1}$ and $0.029 \text{ g N m}^{-2} \text{ d}^{-1}$ in Lovojärvi (These values are roughly 4 and 8 times higher than in Lake Valkea-Kotinen, in Evo forest area (L. Arvola, pers. com.). Stable isotope analysis of the trapped POM yielded $\delta^{13}\text{C}$ - and $\delta^{15}\text{N}$ -organic matter of -31.36% and 4.11% respectively.

The average stable isotope signatures of zooplanktons collected from littoral and pelagial zones of Lovojärvi were $-38.2 \pm 0.42\%$ for $\delta^{13}\text{C}$ and $10.7 \pm 0.19\%$ for $\delta^{15}\text{N}$. Separate analyses of *Chaoborus sp.* showed $\delta^{13}\text{C}$ values of -37.6% and $\delta^{15}\text{N}$ of 11.2% .

4.5. $\delta^{13}\text{C}$ -DIC, $\delta^{18}\text{O}$ -H₂O and $\delta^2\text{H}$ -H₂O of inlets, epilimnion and outlet

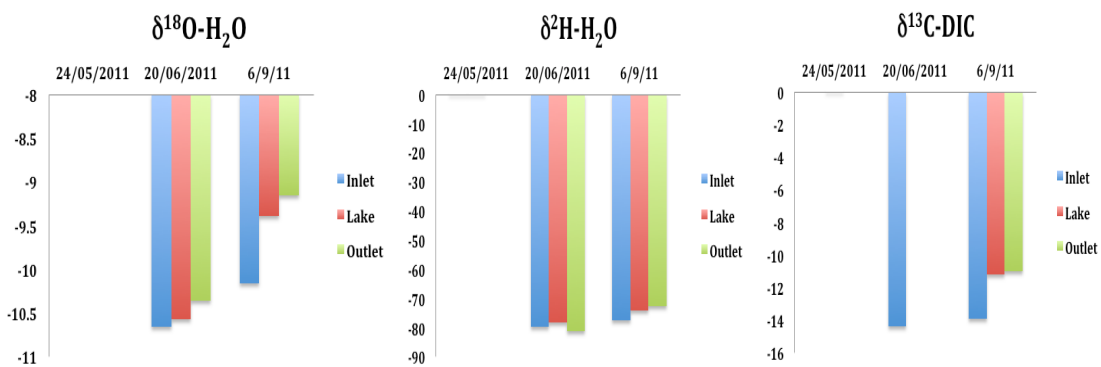


Figure 6. Stable isotopes $\delta^{13}\text{C}$ -DIC, $\delta^{18}\text{O}$ -H₂O and $\delta^2\text{H}$ -H₂O in Lovojärvi surface water, the inlet from Lamminjärvi and the Lovojärvi outlet.

Water in the inlet was slightly more depleted in all the three isotopes than in the surface and outlet water while outlet water was the most enriched (Fig. 6).

4.6. $\delta^{18}\text{O}$ -H₂O and $\delta^2\text{H}$ -H₂O of the three inlets compared with water at 7 m depth

Table 3. Stable isotope signatures of water at 7 m depth compared with water from the three inlets

	24/05/2011		20/06/2011		06/09/2011	
	$\delta^{18}\text{O}$	$\delta^2\text{H}$	$\delta^{18}\text{O}$	$\delta^2\text{H}$	$\delta^{18}\text{O}$	$\delta^2\text{H}$
Inlet1	-11.61	-86.4	-10.66	-79.8	-10.17	-77.7
Inlet2	-11.21	-83.5	–	–	–	–
Inlet3	-11.78	-84.6	-11.39	-83.1	–	–
7 m depth	-11.42	-83.3	-11.65	-81.0	-11.54	-85.6

Table 3 compares the water stable isotopes of the three inlets to Lovojärvi with that of the water at 7 m depth for the months of May, June and September.

5. DISCUSSION

5.1. Lovojärvi meromixis and stratification

The hydrodynamics of Lovojärvi are mainly influenced by its morphometry and location, and by human activities in its catchment. The lake is in a kettle-hole and sheltered by an esker and forests which prevent wind-induced mixing. The lake therefore experiences strong thermal stratification during the ice-free period.

The results obtained here show that Lovojärvi is meromictic, divided into mixolimnion and monimolimnion. The high concentration of ions in the monimolimnion makes this water mass denser than that of the mixolimnion lying above it hence preventing the two water masses from mixing. Evidence of high ion concentration is shown by conductivity depth profile (Fig. 2d). The conductivity increases with depth and this increment is strong especially below 10 m. These results are in agreement with those of Ilmavirta & Kotimaa, (1974) who reported significantly high concentrations of N, P, Ca, and Mg in the deeper waters of Lovojärvi.

The mixolimnion may be completely mixing during autumn turnover while during thermal stratification it separates into epilimnion, metalimnion and hypolimnion. The metalimnion is a transition zone between the epilimnion and the hypolimnion; it experiences a steep gradient in temperature, DO and pH. Each of these layers experienced unique physico-chemical and biological events. The epilimnion is in direct contact with the atmosphere and is affected by seasonal changes. The observed relatively high oxygen concentration, pH and low DIC in the epilimnion compared to other layers/depths are due to primary production by photosynthetic algae since enough light energy penetrates through (secchi depth is 1.08 ± 0.08 m) and other necessary nutrients are available.

The pronounced oxygen deficiency below 2 m creates anaerobic conditions in the hypolimnion and monimolimnion. In this water mass, respiration exceeds photosynthesis hence depleting oxygen (O_2) and producing CO_2 . Temperature slightly increased in the hypolimnion and monimolimnion, this might have been due to high rate of organic matter break down by active microbial organisms generating heat during metabolism (Wand *et al.* 2006). It could also have been due to some heat input at depth from sediments warmed slightly by deep ground heat and never exposed to cooler water during winter mixing. Groundwater inflow may also have contributed to temperature increase. The DIC which is an end product during organic matter break down contributed to the low pH due to formation of carbonic acid.

5.2. Hydrodynamics of Lovojärvi using stable isotopes

The $\delta^{18}O-H_2O$ and δ^2H-H_2O in this study were used to reveal stratification and mixing dynamics in Lovojärvi. The mixolimnion is distinguishable from the monimolimnion at a depth of about 10 m (Fig. 3). They also provided insight in the water balance, i.e. water inputs such as surface stream inflow, subsurface water inflow and precipitation, and water outputs such as evaporation.

Mixolimnion

Seasonal variations of $\delta^{18}O-H_2O$ and δ^2H-H_2O in the epilimnion may be due to water inputs by surface streams, precipitation and by evaporation. At the beginning of spring (May) isotopes values in the epilimnion were relatively lower than other months (Fig 3). This could be as a result of melt water from snow since snow is usually more depleted in heavy isotopes ([http://science.uwaterloo.ca/~jjgibson/mypdfs/Springer book chapter.pdf](http://science.uwaterloo.ca/~jjgibson/mypdfs/Springer%20book%20chapter.pdf)).

From Fig. 6, it is also seen that the major inlet from Lamminjärvi was bringing in water more isotopically depleted water than that in the epilimnion. Therefore the existence of isotopically enriched water in the epilimnion must be accounted for by some other factor, either evaporation or precipitation. Both evaporation and precipitation can account for the enrichment of the epilimnion. During evaporation, $\delta^{16}\text{O}$ and $\delta^1\text{H}$ are preferentially removed with the water vapor enriching the remaining water with the heavier isotopes. During precipitation the heavier isotopes are preferentially converted into water droplets hence rainwater is isotopically heavy. (http://www.science.uwaterloo.ca/~jjgibson/mypdfs/Springer_book_chapter.pdf).

To find out what exactly was happening in Lovojärvi, the relationship between $\delta^{18}\text{O}-\text{H}_2\text{O}$ and $\delta^2\text{H}-\text{H}_2\text{O}$ isotopes in precipitation that has not been evaporated (Craig 1961) was used to establish whether evaporation or precipitation contributed to the enrichment of the epilimnion (Fig. 7)

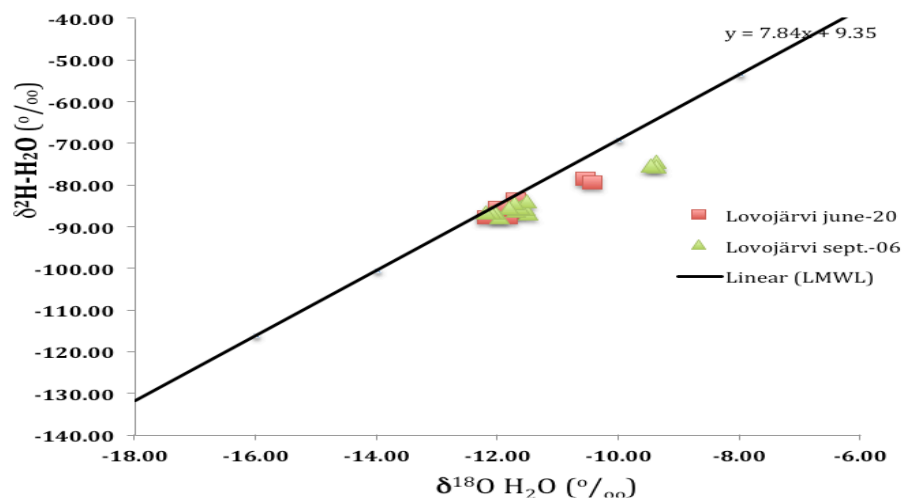


Figure 7. The Local Meteoric Water Line for southern Finland, Espoo (Kortelainen 2007) and water stable isotopes from Lovojärvi.

From the graph, $\delta^{18}\text{O}-\text{H}_2\text{O}$ and $\delta^2\text{H}-\text{H}_2\text{O}$ isotopes for Lovojärvi offset below the meteoric water line implying that the isotopic enrichment was due to evaporation.

Sub-surface flow in the mixolimnion

The $\delta^{18}\text{O}$ and $\delta^2\text{H}$ depth profiles (Fig. 3a & b) reveal intrusion of enriched water into Lovojärvi at a depth of about 6 to 8 m. The mean values for the sampling period at the 7 m depth were $-11.63 \pm 0.13\text{‰}$ and $-83.27 \pm 2.29\text{‰}$ for $\delta^{18}\text{O}-\text{H}_2\text{O}$ and $\delta^2\text{H}-\text{H}_2\text{O}$ respectively. These signatures are closely similar to the values obtained for ground waters of southern Finland (Kortelainen 2007) of $-11.79 \pm 0.09\text{‰}$ for $\delta^{18}\text{O}$ and $-84.10 \pm 1.1\text{‰}$ for $\delta^2\text{H}$. Comparing water at 7 m depth with the three inlets (Table 3), it shows a similarity to water of inlets 2 and 3 that come straight from the esker as surface streams. However, we were unable to sample ground water from the mire, which would have given us greater accuracy to suggest that the subsurface water inflow is from the mire nearby the esker. This seepage water may also be responsible for the apparent enrichment of $^{13}\text{C}-\text{DIC}$ observed below the 7 m depth. It may as well affect the microbial community of this region especially when it is oxygenated. This would create an oxic-anoxic environment that would favour methane oxidation at this point since both CH_4 (produced in sediments and water column) and O_2 are available (Rudd *et al.* 1974, Lidstrom and Somers 1984). This water may also favor the continuous stratification since its density and other physical

properties like temperature and conductivity may be different from that already existing in the lake. Further study should be done to find out more about this seepage and the impact it has on the hydrology and carbon cycle in the lake.

5.3. Carbon dynamics in Lovojärvi

In this study we used $\delta^{13}\text{C}$ -DIC, and DIC and CH_4 concentrations, to follow carbon flow in the lake. Input and output processes that determine DIC and CH_4 concentrations account for the relative enrichment or depletion of $\delta^{13}\text{C}$ -DIC in a lake at a given time. DIC and CH_4 increase with depth and maximum concentrations occur in the monimolimnion. DIC and $\delta^{13}\text{C}$ -DIC depth profiles are mainly controlled by photosynthesis and organic matter oxidation even though processes especially in the epilimnion had strong seasonal patterns.

Biogeochemical and physico-chemical processes in the mixolimnion

The concentration of DIC was low in the epilimnion while there was enrichment of ^{13}C -DIC (Fig. 3c). The major output process of epilimnetic DIC is uptake by phytoplankton although emission to the atmosphere can also sometimes be significant. The preferential ^{12}C uptake during photosynthesis and CO_2 diffusion to the atmosphere leads to ^{13}C -enrichment of epilimnetic DIC (McKenzie 1982). The variations in the available $\delta^{13}\text{C}$ -DIC of the epilimnion show how seasonal changes affect the activities taking place. In spring and summer, increase in light energy leads to increase in photosynthesis hence the enrichment of $\delta^{13}\text{C}$ -DIC.

The slight increase of DIC and the corresponding strong depletion of $\delta^{13}\text{C}$ -DIC, low dissolved oxygen and pH in the metalimnion indicate photosynthesis is reducing while respiration and organic matter oxidation are becoming dominant. Organic matter undergoes partial oxidation to DIC as it sinks. This oxidation has little impact in form of fractionation (Peterson and Fry 1987). The $\delta^{13}\text{C}$ -allochthonous organic matter is $\sim -27\%$ in Finland. $\delta^{13}\text{C}$ -POM in Lovojärvi was -31.36% , similar to that reported previously in some Finnish lakes (Jones *et al.* 1999). DIC from such oxidation has $\delta^{13}\text{C}$ of about -15% . CH_4 oxidation yields light DIC as well. Both of these DICs combined account for the -21% depletion (Fig. 2c).

The subsurface inflow at 7 m depth in the hypolimnion also contributes to increase of DIC in this zone (Fig. 3d). The $\delta^{13}\text{C}$ -DIC of this groundwater seems to be heavier than that of the water in the hypolimnion at 7 m depth. This is counter balanced by the relatively light DIC from methane oxidation explaining the slight enrichment observed in the hypolimnion.

There are a number of possible explanations for the increase of CH_4 observed in the epilimnion (Fig. 4b) where the high O_2 concentration means no CH_4 presence would be expected. In a recent study, Grossart *et al.* (2011) recorded CH_4 oversaturation in a well-oxygenated water column of Lake Stechlin. The CH_4 maxima coincided with oxygen oversaturation. They also conducted field and experimental investigations which both showed that microbial CH_4 production could also occur in well-oxygenated waters. Bastviken *et al.* (2004) argued that methane comes from epilimnetic sediments. Bubbles of ebullition that manage to escape oxidation and reach the epilimnion, may remain attached to the water surface due to surface tension, which might also explain the observed CH_4 increment in the upper epilimnion (H. Nykänen, pers. Com.). There is also a possibility that the surface stream may be contributing some CH_4 . I also suggest the boundary layer theory whereby if particulate matter, even though in oxygenated waters, each has a layer

that surrounds it where the flow of water reduces from turbulence, to lamina and zero at the object surface. In this bubble, nutrients and gases get depleted creating an anaerobic environment which leads to methanogenesis. So even though the water may be oxic, anaerobic microclimates in the epilimnion may still permit some methanogenesis.

DIC and $\delta^{13}\text{C}$ -DIC in the monimolimnion

Concentrations of DIC, $\delta^{13}\text{C}$ -DIC and CH_4 are high in the monimolimnion due to anaerobic oxidation of organic matter. Preferential use of ^{12}C -DIC during methanogenesis accounts for the $\delta^{13}\text{C}$ -DIC enrichment of the monimolimnion.

Methane oxidation

The $\delta^{13}\text{C}$ - CH_4 in Lovojärvi was -50‰, -63‰ and -74‰ at 1 m, 10 m and 15 m depth respectively (L. Arvola, pers. com.). This enrichment from the bottom to epilimnion is indicative of methane oxidation by aerobic and anaerobic methanotrophs. Anaerobic CH_4 oxidation is a significant process that has been reported mostly in marine sediments at sulfate-methane transition (Iversen & Jørgensen 1985) carried out by a consortium of archaea and sulfate-reducing bacteria (Boetius *et al.* 2000). In Lovojärvi, below 2 m oxygen is totally depleted hence any kind of CH_4 oxidation should be anaerobic except for the seepage at 7 m which may bring in oxic water. The aerobic and anaerobic methanotrophs are in turn an energy source in the food web. The -38.2‰ signature of ^{13}C -zooplankton is more depleted than that of POM, -31.36‰. If zooplankton were solely feeding from a source mainly contributed to by POM, then their ^{13}C would be enriched by about 1‰ (DeNiro and Epstein 1978). This means that the zooplankton are obtaining carbon from a more depleted source. Interestingly zooplankton seem to prefer lighter source to the heavier POM source. Since methanogenesis due to fractionation produces methane that is very much depleted (Whiticar 1999), then methanotrophs are always ^{13}C depleted. This therefore suggests that methanotrophs are important energy sources to the zooplankton. These results are in agreement with previous studies such as (Bastviken *et al.* 2003, Jones and Grey 2004).

Carbon balance

Sedimentation and ebullition data are used to estimate carbon balance in Lovojärvi. Particulate organic matter sedimentation rate in Lovojärvi was $0.317 \text{ g C m}^{-2} \text{ d}^{-1}$ of carbon. This is relatively high from the mixolimnion to the monimolimnion. The POM undergoes anaerobic bacterial mineralization and methanogenesis producing CO_2 (DIC) and CH_4 . Cole *et al.* (2002) suggested that this is a major source to internal increment of DIC in lakes with long hydraulic residence times. Our results further imply that carbon is recycling in that if POM is respired as CO_2 and CH_4 in the bottom, then other ions are left back buried all the time thus keeping nutrients and other ions in the lake bottom. This internal carbon cycling creates a density difference between the monimolimnion and mixolimnion hence supporting meromixis. The present trend of conductivity (high nutrient /ion concentration) in Lovojärvi was also accounted for as a result of soaking of hemp and flax in the lake as well as agriculture and human settlement in catchment in early years (Simola 1979).

Estimated diffusive emission for open water period (224 days) was 1 g C as CH_4 , and 70 g C as CO_2 based on surface gas concentrations, temperature and pH (H. Nykänen, pers. com.) while ebullition released 1.2 g C m^{-2} as CH_4 for the 224 days. This rate of ebullition is quite small compared to the range of 0.09 and $92 \text{ g C m}^{-2} \text{ yr}^{-1}$ (Bastviken *et al.* 2004) indicating that most of the CH_4 produced in Lovojärvi undergoes oxidation.

In summary, water stable isotopes analyses in this study revealed stratification and mixing dynamics and water balance in the lake while the stable isotope of carbon for DIC and methane revealed chemical and biological processes. Some of the most interesting findings were that in the hydrological cycle as results from our study suggest there is sub-surface inflow of ground water from the esker. Methanotrophy appears to be an important energy source in the lake food web according to the strongly depleted ^{13}C of zooplankton and enrichment of $^{13}\text{C}\text{-CH}_4$ in the epilimnion. Biochemical processes including photosynthesis in the epilimnion and methanotrophy in the water column, as well as sedimentation followed by microbial metabolism of organic matter, are the major processes maintaining the high gas concentrations in the lake. High gas retention in the water column is supported by physical factors like temperature, pressure and the bathymetry of the lake basin. Early stratification ensures lower temperatures beneath the epilimnion, which increases dissolution of gases while high pressure at the bottom of the lake also ensures high gas retention hence high gas concentration.

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