Methane from wetlands

A study on gross and net methane emissions from organic wetland soils
Preface

This bachelor’s thesis was conducted at the Department of Earth Sciences at University of Gothenburg, during spring 2017, as a final examination at the Bachelors program of Environmental Sciences.
I want to thank my supervisor Tobias Rütting for all the help and the patience. I learnt a lot during these months. Thanks also to the technician David Allbrand for the help during sampling collection I would like to thank Paolo Perrone for teaching me useful data analysis skills; Petar Todorov for all the coffee and the company during some long days in the lab; Daniele Fagiani for coming to Sweden to run Göteborgsvartvet with me a few days before completing the work.
Finally, I want to thank my girlfriend and my family for the support and trust given me during these three years.
Abstract

Atmospheric concentrations of carbon dioxide, methane and nitrous oxide reached levels that are unprecedented in at least the last 800,000 years. Land-use based emissions can be triggered and altered by changes in temperature and climate system. Globally, the single most dominant source of the global methane flux is emissions from wetlands (177 to 284 Tg(CH₄) yr⁻¹). Soil organic matter is a source of carbon dioxide and methane emissions into the atmosphere. To better estimate wetlands future emissions and theirs effect on the climate, it is necessary to gather more knowledge about the processes involved in methane production and consumption. The aim of this project was using stable isotope pool dilution technique to perform measurement of methane flux from different peatlands environment to understand and quantify how soil composition and water content affect methane production and consumption. Measurements showed that methane emissions result from antagonistic but correlated microbial activities. It was noticed that methane production can take place also in drained organic soils. Production takes place in the anoxic zones of water flooded soils by methanogens and methane can be oxidized into CO₂ by methanotrophs in the aerobic zones of wetland soils and in drained soils. Methanogens and methanotrophs seem to be ubiquitous in soils where they remain viable under unfavourable environmental conditions. Methane consumption occurs in flooded and drained soils and can assume a wide range of values. Because of the presence of anaerobic organisms in oxic soils, microbial activity within anoxic microsites is assumable.
Sammanfattning

Under de senaste 800 000 åren har atmosfäriska koncentrationer av koldioxid, metan och lustgas nått ovanliga och extremt höga nivåer. Landbaserade växthusgasutsläpp kan orsakas och påverkas av klimat- och temperaturförändringar. Våtmarker, i synnerhet jordens organiska material, är den dominerande metankällan i atmosfären (177 to 284 Tg(CH4) yr⁻¹). För att kunna förbättra våtmarkers utsläppsuppskattningar och deras klimatpåverkan, är det nödvändigt att öka förståelsen kring processerna relaterade till produktion och konsumtion av metan.

Syftet med projektet var att utföra metanflödemätningar från två olika torvmarker för att kunna förstå och kvantifiera hur markens komposition och vatteninnehåll påverkar produktion och konsumtion av metan. Detta gjordes med hjälp av Stable Isotope Pool Dilution metoden. Mätningarna visade att metanutsläpp resulterar från antagonistiska mikrobiella aktiviteter som kan äga rum både i dränerade och vattenmättade jordar.

Metan produceras från metanogener, anaerobiska bakterier som finns i anoxiska delar av vattenmättade jordar och oxideras av metanotrofer vid aerobiska förhållande. Båda typer av bakterier tycks vistas i båda förhållanden, men blir endast aktiva vid lämpliga konditioner. Metankonsumtion sker både i dränerade och vattenmättade jordar och kan variera mycket. Anaerobiska organismer närvarande vid oxiska jordar kan tyda på mikrobiell aktivitet vid anoxiska miktrositer (microsites).
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Introduction

Anthropogenic greenhouse gas emissions have increased since the pre-industrial era (see Figure 1). From 2000 to 2010 registered emissions were the highest in history. The atmospheric concentrations of carbon dioxide, methane and nitrous oxide reached levels that are unprecedented in at least the last 800,000 years. One of the consequences of this accumulation is leading to an uptake of energy by the climate system (IPCC 2014). Land-use based emissions can be triggered and altered by changes in temperature and climate system.

Soil organic matter is a source of carbon dioxide and methane emissions into the atmosphere (IPCC 2006; DeLaune, K.R. Reddy, C.J. Richardson & J.P. Megonigal 2013; K. Ramesh Reddy & Ronald D. DeLaune 2008). Wetlands represent 6-7% of the total land surface and play a significant role in climate change and carbon budgets (K. Ramesh Reddy et al. 2008). Between 15-22% of terrestrial carbon is stored in wetlands which are the major contributors to the global methane flux. According to IPCC 5AR in the years 2000s, methane released from natural sources represents between the 35 and the 50% of the global CH$_4$ emission of the decade. Globally, the single most dominant source of the global methane flux is emissions from wetlands, which is estimated to be 177 to 284 Tg(CH4) yr$^{-1}$ (IPCC, 2014).

Earlier studies estimated the natural contribution of wetlands to CH$_4$ emission being 55-150 Tg CH$_4$ yr$^{-1}$ (Watson et al., 2000). Methane emissions from drained wetlands are drastically reduced, while the decomposition of dry organic matter increases the emissions of CO$_2$ into the atmosphere (M. Maljanen 2010). With drained conditions the ratio of net primary productivity to soil respiration (production/consumption)

Methane emissions are usually measured in situ by using closed chamber placed over a unit area of wetland, including optionally also vegetation (Ramesh Reddy et al. 2008; R.D. DeLaune et al. 2013). In order to estimate methane fluxes, a net change in methane concentration in the chamber can be measured over a certain period of time. The net methane flux measured accounts for the production but also for the consumption of methane. Methane consumption happens either in the root zone or above the soil-floodwater layer and water column (Ramesh Reddy et al. 2008).

Globally the major sources of atmospheric emission of methane are agricultural lands including rice paddies and natural wetlands such as forested wetlands, riparian wetlands, freshwater marshes, bogs, and fens (IPCC 2013; Milich 1999).

There are three types of methane emissions depending on if their origins are biogenic, thermogenic or pyrogenic. Emission can result from human activities and/or natural processes (IPCC 2013). Biogenic emissions are caused by oxidation of organic material in anaerobic conditions (natural wetlands, ruminants, waste, rice paddies, landfills, rice cultivations and termites). Thermogenic emissions are originated by slow reaction that in geological time scales transform organic matter into fossil fuels. Pyrogenic emission happens during incomplete combustions of organic matter, e.g. biofuel (IPCC 2013). Since each type of emission is characterized by a typical range of isotopic composition ($^{13}$C-$\text{CH}_4$) it is possible to estimate the contribution of each source (IPCC 2013).

Net methane fluxes vary depending on location, hydroperiod, temperature, and other regulators (IPCC 2014, 2013). Hydroperiod (i.e. the flow, the depth, the frequency and duration of standing water condition, the seasonality and the frequency of flooding) can significantly influence methane emission from wetlands (Ramesh Reddy et al. 2008). The lowering of the water tables can somewhat decrease methane emissions (IPCC 2014, 2013). The methane produced can be readily oxidized in surface soil layers. The rate of methane production increases in soils with greater percent of soil pores filled with water, i.e. water saturation (K. Ramesh Reddy et al. 2008).

Methane emission from soils to the atmosphere is a balance between methane oxidation, production and transport within the soil system. Methane is released from anaerobic wetland soils to the atmosphere in three ways: ebullition of gas bubbles
(accounting for 30-85% of the total release), diffusion of dissolved methane, and transport through aerenchyma tissue of some wetlands plants, which is a rather slow process (K. Ramesh Reddy et al. 2008). A wide portion of methane can be oxidized to carbon dioxide when the floodwater is drained or when the soil dries. Entrapped methane can escape to the atmosphere immediately after the floodwater is removed or decreased.

The low solubility of methane in water limits its diffusive transport in the flooded soil, and most methane is oxidized to carbon dioxide.

Globally extensive areas of peatlands and wetlands have been drained and converted into agricultural lands (K. Ramesh Reddy et al. 2008). Drainage of organic rich soils accelerated the decomposition process and emission of carbon dioxide. Many peatlands that have accumulated organic matter for centuries have been oxidized, resulting in soil loss and CO$_2$ emission (K. Ramesh Reddy et al. 2008).

Drainage of peat soils are done for forestry, agriculture or peat extraction and greatly changes the soil's GHG dynamics. After drainage, the decomposition increases and the soil may turn from a carbon sink to net sources of CO$_2$. When the availability of oxygen in peat increases after drainage, there is a decrease in CH$_4$ emissions resulting from a decrease in CH$_4$ production and an increase in oxidation of CH$_4$ by methane oxidizing microbes. (K. Ramesh Reddy et al. 2008). Drained peat soils are often only minor sources or sometimes small sinks of CH$_4$. In order to obtain a more complete picture of the climatic consequences of methane emission from wetlands and to improve the emission estimates of this land-use category, more knowledge about methane production and consumption in wetland organic soil is needed.
Aim and hypothesis

The aim of the project is to perform measurement of methane flux from different peatlands environment in order to understand and quantify how soil composition and water content affect methane production and consumption. 18 soil samples from a bog will be compared to 18 soil samples from a drained fen in order to understand how net emissions reflect gross emission of CH₄ and what role has soil water content and soil organic matter in this relationship. It is expected that in very wet condition there is no consumption and thus net emissions are almost equal to gross emissions (P>C in Figure 2). In dry condition both production and consumption determine the net flux so the intent is to reveal in what extent the relation changes and if there is any correlation with soils specific characteristics. In this study roots and vegetation contribution to methane production and emission was overlooked.

Figure 2. Graph showing the 4 possible dynamics of the amount of methane over time (von Fischer & Hedin 2002)
Types of wetlands and peatlands

In American English, the term swamps is used to identify wetlands with primarily wood vegetation, whereas the term marshes is used to identify wetlands with predominant herbaceous vegetation. These terms are sometimes interchanged in other parts of the world.

Wetlands differ by soil types, climate, hydrologic regime, vegetation, physicochemical properties of water or water category, and anthropogenic disturbance (K. Ramesh)

Figure 3. Soil based subcategories of land use sector. IPCC 2014, 2013
Reddy et al. 2008). There are two main categories and several subcategories of wetlands. Here are most common:

Coastal wetlands
- Tidal salt marshes
- Tidal freshwater marshes
- Mangrove wetlands

Inland wetlands
- Freshwater marshes
- Peatlands
- Freshwater swamps and riparian wetlands

Peatlands are those wetlands with organic soils. Other kinds of wetlands have, as shown in Figure 3, mineral soil. Peatlands have a type of organic soil characterized by the presence of fibrous plant material.

This work is uniquely focused on emissions from peatlands. Bogs are a kind of peatland characterized by spongy peat deposits, acid waters, sphagnum moss (peat moss), and low nutrient status. The main source of water for this wetland is precipitations and they origin because of sphagnum ability to hold water and grow over lakes and ponds or on wet uplands areas. These conditions can generate the deposition of several centimeters of acidic peat. (K. Ramesh Reddy et al. 2008).

Fens are another type of peat-forming wetlands but that are more nutrient rich and eutrophic. They receive nutrients from surrounding watershed through drainage and surface runoff. Compared to bogs, fens are less acidic and can host more diverse
Hydrology of wetlands

Three aspects define wetlands hydrology: hydroperiod, hydrodynamics and source of water. Hydroperiod depends on depth, flow, frequency, duration (amount of time wetlands are in standing water), seasonality, and frequency of flooding (how often water saturation occurs). Hydroperiod has to do with all aspects of water budget, as rainfall, evapotranspiration, and flow, regardless to the water origin. Hydrodynamics take into account direction and velocity of water movements whilst water source can divide water into groundwater or surface water according to its origin (Fig. 4). Wetlands hydrology controls biogeochemical characteristics such as physical, chemical, and biological properties of soil, productivities of biotic organisms, and water quality (K. Ramesh Reddy et al. 2008).

Wetland soils

Soil is the unconsolidated mineral or organic material on the immediate surface on the earth that serves as a natural medium for the growth of plants. Wetland soils are periodically or continuously saturated with water and can be called flooded soils, wetland soils, waterlogged soils, paddy soils, and marsh soils. Lately the term hydric soils is also being used. Wetlands represent approximately the 6% of Earth's land surface and can be found globally in all climates. The physical, chemical and
biological characteristics of wetland soils are important in studying the properties of wetlands (K. Ramesh Reddy et al. 2008).

### Physical characteristics

![Composition of soil volume in mineral and organic soils under drained and flooded condition](image)

Fig. 5. Composition of soil volume in mineral and organic soils under drained and flooded condition. K. Ramesh Reddy et al. 2008

Soil is composed mainly of solid matter, water and air (Fig 5). Solid matter has a component of organic and mineral material in different ratio. Soil aeration is an important process of oxygen diffusion from atmosphere into the soil and it is influenced by the hydroperiod. The air-filled pores of soils are otherwise occupied by water in flooded soils. As shown in the picture above, one-fifth of the volume of organic soils consists of mineral and organic matter, whilst the rest is water and air. In wetland soils oxygen is limited since soils pores are filled with water, which can occupy up to 90% of the soil volume whereas the rest is solid matter. In such oxygen-free environment anaerobes organisms predominate. Relative proportion of air and water per unit volume of soil depends on soil type and hydrologic conditions (precipitation, irrigation or water table). Hydrologic conditions characterize also whether wetland soils are 1) flooded with water depth above soil surface, 2) water saturated with no excess floodwater, or 3) if the water table is below the soil surface.
Condition 1 and 2 define a soil as *hydric* whereas condition 3, depending on soil type and hydrologic condition, is classified as *Aquic* soil (K. Ramesh Reddy et al. 2008).

**Chemical characteristics**

Wetland soils are characterized by conditions, inter alia, such as excessive rainfall, poor drainage and high oxygen demand in the soil. Under these conditions, anaerobic organisms reduce oxidized compounds in order to collect nourishment and energy. If wetlands are drained these reduced compounds are oxidized by chemical or biochemical reactions. The abundance of reduced forms in a soil is an indicator of soil wetness or anaerobic conditions and therefore of hydric soil (Fig. 6).

![Fig. 6 Dominating chemical compound and their redox forms in drained and flooded soils](image)

Ramesh Reddy et al. 2008

Generally, saturated soils have a higher pH, electrical conductivity, and ionic strength but a lower redox potential. Redox potential is the most common parameter used to study the degree of soil wetness or intensity of soil anaerobic conditions since it
reflects the intensity of reduction. Eh (redox potential) usually decreases with time and reaches a steady value.

Microbial metabolism is a series of processes and reactions by which a microbe obtains the energy and nutrients (carbon) necessary for its existence and reproduction. The range of Eh values observed in wetland soils is from +700 to -300 mV. Negative values represent high electron activity and intense anaerobic conditions, typical of permanently waterlogged soils. Positive values represent low electron activity and aerobic, or moderately aerobic, condition typical of wetlands in a transit zone. There are several types of metabolic strategies that microbes can use.

To collect energy, organisms transfer electrons from an electron donor to an electrons acceptor. An electron acceptor is a chemical compound and an oxidizing agent that, by accepting electrons, gets reduced in the process. Aerobic organisms use oxygen as a terminal electron acceptor, while anaerobic organisms use inorganic compounds with a lower reduction potential than oxygen. For this reason, anaerobic respiration is less energy efficient. Facultative anaerobes can use, depending on the environmental conditions, either oxygen or alternative terminal electron acceptors (see Figure 6). Most respiring anaerobes are heterotrophs, although some do live autotrophically. Because of its higher electron affinity oxygen, if available, will be use first. The order of reduction of these compounds depends mainly on their electron affinity (Ramesh Reddy et al. 2008).

**Biological characteristic**

Water saturated soils offer an environment suitable to those microbial populations that have accustomed to anaerobic conditions. Aerobic microorganisms are confined where oxygen is available whilst they die or become inactive in anaerobic conditions. Under these conditions facultative and obligate anaerobic bacteria dominate.
The metabolic activities of anaerobic organisms depend on alternate electron acceptors, such as oxidized forms of nitrogen, iron, manganese, and sulfur. Microbially mediated reaction typical of uplands soil conditions may cease happening and be replaced by other reaction as the reduction of oxidized compounds during respiration processes. This produces reduced compounds.

A type of wetland soil: organic soils

Wetland soils can be divided into the following groups:

- Waterlogged mineral soils
- Organic soils
- Marsh soils
- Paddy soils
- Subaqueous soils

All the 36 soil samples collected and analyzed for this study belong to the second category. Organic soils, known also as peatlands or Histosols, are characterized by high organic matter (more than 12% total carbon) in the upper first meter (K. Ramesh Reddy et al. 2008). These soils originate from accumulation of partially decomposed organic matter. Here decomposition processes are slower than primary productivity. More precisely the top layer (surface horizon) is generally well decomposed; while below fibrous undecomposed material (peat) accumulates. Organic soils have high water-holding capacity and poor drainage (K. Ramesh Reddy et al. 2008).
Anaerobic mineralization processes and methanogenesis

Methane production is the terminal step in the anaerobic decomposition of organic matter in wetland soils. It is believed that the production is uniquely performed by methanogenic anaerobic microorganisms that can be active only in anoxic conditions. These bacteria necessitate on other microbes to provide them with substrate as hydrogen, CO$_2$, formate, acetate, methanol, methylamines and methysulfides (K. Ramesh Reddy et al. 2008). In wetlands soils, methane is mainly produced by transmethylation of acetic acid and partially by the reduction of CO$_2$. As mentioned these anaerobes microbes need other fermentative bacteria to metabolize celluloses, sugars and proteins. Methane production is a four-step process in which several group of microorganisms are involved. The first step is hydrolysis of macromolecules and polymers to which acidogenesis, acetogenesis and methanogenesis follow in order (Serrano-Silva et al., 2014).

The main mode of production comes from the splitting of acetate:

\[
\text{CH}_3\text{COOH} \rightarrow \text{CO}_2 + \text{CH}_4
\]

Acetate comes from fermentation of organic matter.

Alternatively, methane can be produced by reduction of CO$_2$.

\[
\text{CO}_2 + 4\text{H}_2 \rightarrow \text{CH}_4 + 2\text{H}_2\text{O}
\]

Wetland plants play an important role in the emission of methane to the atmosphere. Vegetation is behind the production of the carbon used to produce methane. Plants also facilitate the release of methane by providing a system of interconnected internal gas cavities. Aquatic macrophytes, plants that grow in or near water, are usually fitting in oxygen-limited conditions and thus they dominate in flooded or waterlogged soils. Oxygen is transported through empty cavities of the stems, to the underground parts of the plants. A portion of the oxygen transported downwards is released in the root zone, where it sets off beneficial oxidation processes. Methane produced in the soil moves in the other directions. First through the pores from which oxygen escapes from the plant in the root zone then it goes through the plant aerenchyma system and finally is emitted into the atmosphere. Another portion of the oxygen released into the root zone can be used to oxidize methane before it enters the atmosphere. However, the oxygen can furthermore be used to reactivate alternative electron acceptors. The continuous supply of alternative electron acceptors will decrease the importance of
methanogenesis in the anaerobic mineralization reactions in the root zone and thus reduce methane production and emission (K. Ramesh Reddy et al. 2008). The role of roots and vegetation in the emission of methane was not considered in this paper and work.

Use of stable isotopes

Stable isotopes are extremely useful for studying a wide variety of processes associated with methane production, consumption and transport mechanisms from a small to a global scale (Chanton 2005 and references therein). Isotopes are atoms of the same element that have the same number of electrons and protons but differ in the numbers of neutrons. An element and its isotopes have similar charges but different masses. $^{13}$CH$_4$ designate a molecule of methane in which the atoms of carbon in an isotope with 6 protons and 7 neutrons.

Unstable isotopes (radioactive) are nuclei that spontaneously disintegrate over time to form other isotopes. Radioactive isotopes emit alpha or beta particles and some also gamma radiations during disintegration. Stable isotopes are nuclei that seem not to decay to other isotopes on geologic timescales, but may themselves be produced from radioactive isotopes (Kendall et al. 1998).

There are several definition and notation to express and define the isotopic composition of an element. Exact equations for mixing are written in one notation (the atom percent or AP notation, or also the fractional F notation when atom percent values are divided by 100 (Fry 2006). For this work, it was used the notation $^{13}$F.

The mass differences between isotopes of an element cause small differences in the chemical and physical properties (Kendall et al. 1998). In elements of low atomic numbers, these mass differences are large enough for many physical, chemical, and biological processes or reactions to “fractionate” or influence the relative proportions of various isotopes. Biological processes are good examples of kinetic isotope reactions kinetics. Microorganisms prefer use the lighter isotopic species because of energy economy reasons. This results in significant fractionations between the substrate (heavier) and the biologically mediated product (lighter) (Kendall et al. 1998).
Method

The main experiment on which this work is based is the stable ($^{13}$C) Isotope pool dilution technique. Soil organic matter, soil water content and C-N ratio values have been measured and calculated for each sample. The method section about the isotope pool dilution technique is entirely based on the description of the method found in von Fischer & Hedin 2002. Figure 8 illustrates pool dilution approach for measuring gross methane production and consumption by adding isotope labeled methane ($^{13}$CH$_4$) in the chamber. $P$ stands for methane production rate (constant); $K*m$ describes methane consumption rate where $k$ is the first-order rate constant and $m$ the amount of methane. Panel A in figure 8 shows a situation where methane production and consumption happen simultaneously affecting $m$, the total amount of methane in the system. In panel B, only methane consumption affects the amount of labeled methane $n$. Production of unlabeled methane will also take place, but will not affect the amount of labeled methane. Isotopic fractionation usually slows consumption of labeled CH$_4$ by constant amount (alpha). The advantage of this technique is based on the fact that, unlike for example other chemical inhibition techniques, it does not involve the risk to inhibit simultaneously both production and consumption of methane. This technique has been shown to be most reliable for measuring the amount of the atmospheric methane and the ratio of labeled /unlabeled methane during soil incubation (von Fischer & Hedin 2002). From these data collected during the experiment it is possible to calculate gross methane consumption ($C$) by looking at the loss of labeled methane over time. After an initial diffusive redistribution of the added label gas, in fact only consumption affects the amount of labeled methane present in the chamber. Since production generates only unlabeled methane it is possible to further calculate gross methane production from the dilution of labeled methane over time. In the next
sections the dynamics for each unlabeled and labeled methane and isotopic composition will be explained and described with mathematical models. The functions will be used together with the collected data to obtain gross rate of methane production and consumption for each soil sample.

**Dynamics of unlabeled methane**

The balance between production and consumption of methane is what regulate the changes of methane concentration in the incubation chamber over a certain period of time. It is assumable that the rate of consumption changes together with changes in the methane concentration.

\[ F = P - C \]  (1)

This equation describes the changes in the amount of unlabeled methane over time.

- **F** = net flux rate between soil and atmosphere.
- **P** = gross rate of methane production
- **C** = gross rate of methane consumption

Atmospheric methane concentrations are usually below the \( K_m \) (Michaelis constant used in the Michaelis–Menten kinetics model of enzymatic reactions) so first-order relationship can be used to model consumption:

\[ C_t = k m_t \]  (2)

- **C**\(_t\): instantaneous rate of methane consumption at time \( t \)
- **k**: first order rate constant for consumption
- **m**\(_t\): amount of methane at time \( t \)

In a closed system at a constant volume the change in moles and concentration is equivalent. So the equation above can be modeled as a first-order reaction with respect to the amount of methane. \( K \), the consumption constant, can be used to calculate the consumption rate at a known methane concentration (\( b \), e.g. microL/L).
If $K$ is corrected to the volume of the incubation container ($v$) and by the concentration of interest, then the equation can be expressed in this way:

$$C = bkv \ (3)$$

It is assumable, usually, that methane concentration over the soil surface is equal to the atmospheric concentration of $1.8 \ \mu\text{LL}^{-1}$. The gross consumption rate is thus calculated for $b = 1.8 \ \mu\text{LL}^{-1}$. (von Fischer & Hedin 2002).

The atmospheric methane pool is used as point of reference for measuring gross rates of methane production and consumption.

The instantaneous consumption rate @ in the last two equations is expressed as a function of $m$ and $k$. $K$ includes environmental restrictions (such as oxygen limitation) and is empirically determined. It combines effects of both methane concentration and oxygen concentration.

Incorporating the second equation (2) into the first (1) results in a differential equation that allows representing instantaneous rates as derivatives with respect to time.

$$F = \frac{dm}{dt} = P - km.$$ \ (4)

The solution of the equation is:

$$m_t = \frac{P}{k} - \left(\frac{P}{k} - m_0\right) \exp(-kt) \ (5)$$

$m_0$: initial mass of methane.

$M_t$: amount of methane over time.

The changes in mass can assume one of three qualitatively different dynamics, namely increasing ($P>C$), constant ($P=C$), or decreasing ($P<C$, $P=0$).

The amount of CH$_4$ in the system reaches steady state (if $P$ and $K$ are both not zero) when
\[ m = P/k \] (6)

This equilibrium point for biogenic trace gases is the compensation concentration where production and consumption are balanced in the soil.

**Dynamics of labeled methane**

Since the mass balance equation (4) depends on two unknown variables \( P \) and \( K \) it is not possible to calculate the rates of production and consumption by only following changes in the amount of methane. It is possible to determine \( P \) and \( k \) by combining the mass balance equation with an expression for the changes over time of the ratio of labeled/unlabeled methane (the isotopic composition). In this experiment \(^{13}\)C methane was used as labeled methane. It was assumed that no production of \(^{13}\)C methane took place during the 8h of incubation. The amount of added \(^{13}\)C methane at time \( t \) can be related to the percent abundance of \(^{13}\)C in total methane as:

\[ n_t = m_t (A_{Pt} - A_{Pp}) \] (7)

\( n_t \) = amount of labeled methane
\( m_t \) = total amount of methane
\( A_{Pt} \) = measured atom percent \(^{13}\)C methane in the system
\( A_{Pp} \) = atom percent \(^{13}\)C of methane generated by methane production (natural abundance)

Equation (4), used to model the total amount of methane, can be simplified and applied to show the amount of labeled methane, \( n \), after two modifications. Since there is no production of labeled methane during incubation, it is possible to remove the production term \( P \) from eq. (4). Then the rate of consumption of \(^{13}\)C methane is slowed down by isotopic fractionation by a very small but constant value \( (\alpha) \), as
compared to unlabeled methane ($^{12}$CH$_4$). The fraction coefficient (alpha) derives from
\[ \alpha = \frac{K_{13}}{K_{12}} \]
$K_{12}$ is the first-order rate constant for consumption of $^{12}$CH$_4$ and $K_{13}$ is the first-order rate constant for consumption of $^{13}$CH$_4$.

Here is the equation that describes the instantaneous change in the amount of label methane with respect to time

\[ \frac{dn}{dt} = -k \alpha n \]  
(8)

The solution to this equation (8) shows how the mass of labeled methane changes over time:

\[ n_t = n_0 \cdot \exp(-k \alpha t) \]  
(9)

$n_0$ = initial mass of labeled methane.

In this experiment, though, it was assumed that the enrichment of labeled methane in the system made the fractionation coefficient effect negligible. $N_t$ was therefore calculated considering $\alpha$ as 1, resulting in:

\[ n_t = n_0 \cdot \exp(-k t) \]  
(9a)

Once discovered the equations to calculate the amount of labeled and unlabeled methane it becomes easier to show how the isotopic composition of the system changes with time. The amount of methane in the headspace of the chamber and its isotopic composition are the two values measured during incubation so in order to calculate methane production it is necessary to use the equation for isotopic composition.
Dynamics of isotopic composition

Once the concentration of methane is known (expressed in ppmv) it is possible to obtain the volume of the methane in the head space by multiplying concentration per head space volume. Using the volume of methane in the general gas law will allow calculating the amount of methane (mol) which value, multiplied per fractionation ($^{13}$F) will result in the amount of labeled methane (mol).

The atom percent $^{13}$C methane in the system depends on the ratio between the amount of labeled methane and the natural abundance of atom percent $^{13}$C methane in the system and it is described by the following equation:

$$AP_t = \frac{n_t}{m_t} + AP_p.$$  \hspace{1cm} (10)

From equation (5) and equation (9) the definition of $m_t$ and $n_t$ can be taken and substitute into equation (10) to get

$$AP_t = \frac{n_0 \exp(-kat)}{P - (\frac{P}{k} - m_0 \exp(-kt))} + AP_p.$$  \hspace{1cm} (11)

This equation has two qualitatively different dynamics depending on the size of $P$ (production) respect to $C$ (consumption). If $P$ is greater than $C$, the equation will reveal a decline in the atom percent $^{13}$C over time since production dilutes the labeled methane. This graph (n) shows that when time approaches infinity, the isotopic composition of methane, $AP_t$, approaches the isotopic composition of produced methane, $AP_p$, for all cases except where $P=0$. In general, the rate at which $AP_t$ approaches $AP_p$ is greater when the combined rate of $P$ and $C$
is greater (the turnover rate of the methane pool. The changes in amount can also be expressed with respect to changes in the isotope ratio. The product of methane amount and isotope ratio can be used to determine $k$, while $P$ can be determined by the two measures separately.

**Determination of production and consumption from data**

The gross rates of methane production and consumption can be determined by fitting equations for the amount of labeled methane (9), the total amount of methane (5) to the measured data. Figure 10 illustrates these equations and gives graphic examples of their dynamics.

With a two-step process it is possible to fit the equations to data. First $k$, consumption rate constant, needs to be determined by linear regression from equation (9). The value of $k$ is then combined with measures of the amount of methane and its isotopic composition to determine $P$ using a curve fitting routine. With the help of QtiPlot, a software that analyzes and plots scientific data, a custom fitting with equation (9) was done using the data about the amount of labeled methane. This operation was done for all the 36 soil samples data series. After this it was possible to collect each $k$ and each $R^2$ (correlation coefficient). Each value of $k$ was then inserted as a constant in equation (5) before fitting

![Figure 10. Fundamental equations used in biogeochemistry to calculate gross methane production and consumption and curves examples.](image)

![Figure 11. Graph showing the possible dynamics of isotopic composition 13C methane related to methane amount (Fischer & Hedin 2002).](image)
the curve to each 36 total amounts of methane data series. The result of the fitting was 36 different P (gross rate of production) and their respective $R^2$. The combination of the effects of methane production and consumption drives the initial amount of methane and its isotopic composition through one of four qualitatively different trajectories (Figure 11).
Field Sites and Techniques

Figure 12. Satellite picture over Skogaryd Research Catchment. University of Gothenburg 2017

Skogaryd Research Catchment is an infrastructure for terrestrial and limnological field research, consisting of nine research stations covering the different landscapes and climatic regions in Sweden (Figure 12).

36 soil samples were taken from two different locations (location A and E in Fig. 12). Each sample was taken close to a preexisting chamber frame minding that the height, the vegetation type, and the water content were approximately the same as in the corresponding chamber. This has been done in order to get a more reliable correspondence between the stored field methane emissions from previous monitoring of the University of Gothenburg and the data from the result of this experiment. Unfortunately, not all the previous data was still available so the comparison was not possible.
During soil sampling the vegetation was lifted and removed from the soils. Visible roots were removed once the samples were removed from the ground. The soils were carefully dug out with and collected in a metallic corer (cylinder) and placed in hermetically closed plastic bag. Each corer was previously weighed and numbered. 18 samples were collected in a bog called Mycklemossen (site A) and were named and labelled from 1a to 18a. They correspond to flux data from chamber number 1 to 18. Myckelmossen is a wetland (peatlands) generated by accumulation of peat moss (*Sphagnum, vitmossor*) (Fig. 13).

The second category of samples comes from a drained organic wetland converted into a forest. The samples of this category were taken from two areas of the forest that underwent different experiments and treatments and therefore have different numerations. To facilitate the identification of the samples and to simplify the comparison to previously collected data, samples numbered from 1b-6b belong to one site called drained organic soils B. Samples numbered from 1c-12c were collected close to the control chambers in the ash-treated site named drained organic soil C. Both B and C belong to the area E in Skogaryd Research Catchment. Table 11 in the appendix shows the correspondence between the numeration of the chambers of the stored flux data from Skogaryd interactive map and the numeration given to the respective samples of this experiment.

The samples were stored in GVC (Geovetarcentrum) cooling room for two nights. After that, each sample was weighted. The bottom end and the lateral perforated surfaces of the cores were sealed with parafilm to eliminate gas exchange from soil surfaces that would not have been exposed to the atmosphere. The soil samples were inserted in plastic cylindrical jars and placed vertically in an incubator (INCU-Line IL 250R premium) at a temperature of 14°C to acclimatize for a week. Parafilm was used to cover the opening of the cylinders. The film had a few small holes to allow air exchange but keep the water content. After incubation, in

![Figure 13. Picture taken at Myckelmossen during samples collection.](image-url)
order to create a closed system for the $^{13}\text{CH}_4$ additions, the small chambers were sealed with a lid. A hole with a port for gas sampling placed in the middle of the lid allowed the use of syringes to extract gas.

The atmosphere in the incubation jar was enriched by adding labeled methane at the beginning of the incubation. The target was an isotopic enrichment of 2–10 atom percent $^{13}\text{C}$-methane.

To know the volume of the isotopically labeled methane required for the enrichment it was necessary to calculate the volume of the headspace ($V_{hs}$) inside the jar.

Volume of a cylinder = $\pi * r^2 * h$ \hspace{1cm} (12)

Volume plastic cylinder

$h = 12.6 \text{ cm}$
$r = 4.15 \text{ cm}$

$V = \pi * 4.15^2 * 12.6 = 681.73 \text{ cm}^3$

Volume metal corer

$h = 10 \text{ cm}$
$r = 3.65$

$V = \pi * 3.65^2 * 10 = 418.54 \text{ cm}^3$

Volume headspace = $V_{plastic\ cylinder} - V_{metal\ corer}$

$V_{hs} = 681.73 - 418.54 = 263.196 \text{ cm}^3 (\text{mL}) = 0.2631 \text{ L}$

Once calculated $V_{hs}$, to calculate the amount of methane needed for the enrichment and its volume it was followed the calculation described in R.D. DeLaune et al. 2013. For the first step the Ideal gas law comes to help:

$pV = nRT$ \hspace{1cm} (13)

To use the ideal gas law correctly it is necessary to obtain the partial pressure of the gas using the equation $P_g = C_g * P_a$ \hspace{1cm} (14)

$P_g = \text{partial pressure of a gas}$

$C_g = \text{atmospheric concentration of the gas, CH}_4 (\text{v/v}): 1.8 \text{ mL/L}$

$P_a = \text{atmospheric pressure: 101.325 kPa}$
At this point it is possible to calculate the amount of methane to add by including equation (14) in equation (13)

\[ n = C \cdot P_a \cdot V / (R \cdot T) \] (15)

\[ V = \text{headscape volume: } 0.263 \text{ L} \]
\[ R = \text{gas constant: } 8.31446 \text{ L kPa mol}^{-1} \text{ K}^{-1} \]
\[ T = 287.15 \text{ K} \]

\[ n = 2.01 \cdot 10^{-8} \text{ mol} = 20.1 \text{ nmol} \]

With the amount of moles needed is now possible to calculate the actual volume of methane that is going to be used for the enrichment. This is done once again by using equation (15)

\[ V = nRT/P \] (16)
\[ V = 47.1 \text{ nL} \]

Since it is almost impossible to extract and handle 47.1 nL from a gas bottle, it is necessary to create a gas solution with methane and Helium.

Two empty 250 mL gas bottles were filled with water three times till the top. Each time the water volume was measured. The average value of the volume was taken and appeared to be of about 315.33 mL. To obtain a syringe with 1 mL of a solution with Helium and CH\textsubscript{4} containing 47.1 nL (0.00005 mL) of CH\textsubscript{4} it is necessary to go through two dilutions. The Table 1 shows the ratio of the dilution.

Table 1. Dilution volumetric proportions.

<table>
<thead>
<tr>
<th></th>
<th>CH\textsubscript{4}</th>
<th>Tot Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mL</td>
<td>mL</td>
</tr>
<tr>
<td>Syringe 2</td>
<td>0.00005</td>
<td>1</td>
</tr>
<tr>
<td>2 solution</td>
<td>0.0157665</td>
<td>315.33</td>
</tr>
<tr>
<td>Syringe 1</td>
<td>0.0157665</td>
<td>1</td>
</tr>
<tr>
<td>1 solution</td>
<td>4.971650445</td>
<td>315.33</td>
</tr>
</tbody>
</table>
5 mL was therefore the volume of $^{13}\text{CH}_4$ extracted from the gas bottle. The gas mixture was injected to a gas bag with the help of a 500 mL gas syringe. This has been done in order to keep the internal pressure of the methane always constant so that at each extraction the amount of methane would be the same. 1 mL of solution 2 was injected to each of the 36 samples, previously hermetically closed. To ensure initial distribution of the labeled methane, the atmosphere in the jar was mixed four times with the syringe. Directly after that, a first air sample of 12.5 mL was taken at $t=0$ from each cylinder. After that, to adequately follow changes in methane mass and isotopic composition over the course of the incubation, air samples were taken in four different time moments, following the timetable below. During the waiting time the soils were kept in the incubator. Each time a 12.5 mL air sample was taken, the syringe was removed from the needle, while this was kept inserted through the soil container for a few more seconds. This solution was taken in order to keep constant the pressure inside the cylinder. No adjustment was made to correct the inflow of air which diluted the $^{13}\text{C}$ signal and changed concentration.

Each air samples were transferred into a glass serum vials and sent to UC Davis Stable Isotope Facility, Dept of Plant Sciences to undergo isotope ratio mass spectrometry.

**Soil water content**

Once the air samples were all collected, the soils were placed in an oven for at least 70 hours at a temperature of 60°C in order to calculate soils moisture content. A lower temperature and a longer drying time were preferred to higher temperature and shorter time. Since the soils in question are organic soils this technique minimizes the loss of organic carbon (DeLaune et al., 2013). After drying, all the samples were weighed and soils water content was calculated dividing the difference between the wet and the dry masses by the mass of the dry sample. The result is the ratio of the mass of water to the mass of dry soil.
**Soil organic matter**

To calculate soil organic matter (SOM) for each sample, between 10 and 20 g of soil were taken from each soil and weighed. After this they have been burned for 8 hours at a constant temperature of 550°C. The weight of what was left after combustion was used to calculate the organic content with this equation

\[
\text{SOM} = 1 - \left( \frac{W_f}{W_i} \right) \quad (17)
\]

Where \( W_i/W_f \) is the ratio between the initial and the final weight of each sample (DeLaune et al. 2013).

**Carbon-nitrogen analysis**

To perform CN elemental analysis three replicate for each dried soil sample were taken and finely grinded to obtain a uniform composition. Of each replicate about 10 mg was weighed in tin capsules using an analytical microbalance. The weighed cups were then shaped to little round balls with the help of forceps and placed in 96-wells plates. Tin is important for the increasing the combustion temperature in the elemental analyzer (around 1800 °C). The gas products of combustion, \( \text{N}_2, \text{NO}_x, \text{H}_2\text{O}, \text{O}_2, \text{and CO}_2 \), are carried away by helium and filtered and absorbed through three to obtain only \( \text{N}_2 \) and \( \text{CO}_2 \). These two gases pass along a thermal conductivity detector that produces an electric signal proportional to their concentrations (Radboud University 2017).

All basic data analysis and statistic were performed in LibreOffice Calc. Plotting, curve fitting and part of the statistic tests were performed using open source software QtiPlot.
Result

Model fit

The model fit to measured data was very strong for the vast majority of the samples analyzed. The median coefficient of determination $r^2$ for equation for labeled methane (9) was 0.998 while the one for equation (5) for the amount of methane during time was 0.993. Sample 7a had only two values out of four so it was taken into consideration. For sample 10a it was not possible to obtain IRMS results since methane concentration in the glass vials were higher than 60 ppmv and saturated the instrumentation. For this reason, all the further results that depend on these measurements were not possible to calculate.

The decreasing of $n$, the amount of labeled methane, is shown in figure 14. The graph has been made from values coming from soils sample 6a (bog). The dynamic of the total amount of methane over time, $m$, is shown in figure 15. In the graphs, it is also possible to read the value of the rate of consumption $K$ and the rate...
of production $P$ specific for sample 6a. Sample 6a has a positive and high net flux (so in this example, gross production $>$ gross consumption).

Figure 16 and 17 show data from soils sample 5c (drained fen) and their model fit. In particular, Figure 16 shows the dynamic of labeled methane over time, while Figure 17 shows the dynamic of the total amount of methane over time. In the graphs, it is also possible to read the value of the rate of consumption $K$ and the rate of production $P$ specific for sample 5c. Sample 5c has a negative net flux (gross consumption $>$ gross production).

Figure 16. Dynamic of labeled methane and model fitting of sample 5c.

Figure 17. Dynamic of total amount of methane and model fitting of sample 5c.
Gross production and consumption

Gross rates of production and consumption are presented for the 36 soil samples in Table 4. Rates have been also calculated per soil mass. Histograms (Fig. 18, 19, 20, 21) show graphically the variation of production and consumption rates calculated.

Figure 18. Gross production rates calculated for each bog soil samples.

Figure 19. Gross production rates calculated for each drained fen soil samples.
Figure 20. Gross consumption rates calculated for each bog soil samples.

Figure 21. Gross consumption rates calculated for each drained fen soil samples.
Net flux emissions

Subtracting the consumption rates to the respective production rates it was possible to obtain net flux rates. Table 4 gives a list of the net fluxes for each soil samples. The 36 incubated soils showed a wide range of net methane flux. The positive rates go from a minimum of 5.09 nmol CH4-C h\(^{-1}\) to a maximum of 1324.4 nmol CH4-C h\(^{-1}\). Negative rates start from -15.77 nmol CH4-C h\(^{-1}\) and reach a maximum of -103.4 nmol CH4-C h\(^{-1}\). These values illustrate that methane production and consumption can take place in the same soil in concomitance. Traditional measures of methane flux (e.g. flux chambers) would have only revealed a net consumption or a net production.

To better understand these values, it is possible to relate them to their respective dry soil weights. The principle is that if a soil sample has more mass it will also be proportionally more microbiologically active. In Table 4 it is shown gross production and consumption rates divided per their dry weight.

SOM and water content

Percentages of water content and soil organic matter measured for each soil before incubation are shown in Table 4. A graphic representation of how SOM and soil water content values are spread between soil samples is shown in Figure 22.

Figure 22. Graph showing soil organic matter results and water content for the 36 samples
Correlations

Correlation coefficients were calculated for production and consumption rates with water content, SOM and carbon-nitrogen ratio data (Tab. 2). In the table, P and C stand for production and consumption rate, while a, b, c are used to represent the different soils categories.

Table 2. Correlation coefficients obtained comparing gross rate of production and consumption with soil water content, soils organic matter content and CN ratio values for each sample. Coefficients were calculated for the rate per dry weight values.

<table>
<thead>
<tr>
<th></th>
<th>P (per dry mass)</th>
<th>C (per dry mass)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Correlation</strong></td>
<td><strong>Correlation</strong></td>
<td><strong>Correlation</strong></td>
</tr>
<tr>
<td>P / water content</td>
<td>-0.084138</td>
<td>0.794939</td>
</tr>
<tr>
<td>a</td>
<td>-0.751496</td>
<td>b</td>
</tr>
<tr>
<td>b</td>
<td>0.315573</td>
<td>-0.656401</td>
</tr>
<tr>
<td>c</td>
<td>0.353463</td>
<td>-0.03885</td>
</tr>
<tr>
<td>tot</td>
<td>0.419823</td>
<td>0.090776</td>
</tr>
<tr>
<td><strong>Correlation</strong></td>
<td><strong>Correlation</strong></td>
<td><strong>Correlation</strong></td>
</tr>
<tr>
<td>P / SOM</td>
<td>-0.233164</td>
<td>0.743681</td>
</tr>
<tr>
<td>a</td>
<td>0.160986</td>
<td>b</td>
</tr>
<tr>
<td>b</td>
<td>0.23603</td>
<td>-0.45911</td>
</tr>
<tr>
<td>c</td>
<td>0.151748</td>
<td>-0.07105</td>
</tr>
<tr>
<td>tot</td>
<td>0.26385</td>
<td>0.271635</td>
</tr>
<tr>
<td><strong>Correlation</strong></td>
<td><strong>Correlation</strong></td>
<td><strong>Correlation</strong></td>
</tr>
<tr>
<td>P / CN</td>
<td>0.374064</td>
<td>-0.37699</td>
</tr>
<tr>
<td>a</td>
<td>0.142124</td>
<td>b</td>
</tr>
<tr>
<td>b</td>
<td>0.482933</td>
<td>-0.16181</td>
</tr>
<tr>
<td>c</td>
<td>0.514522</td>
<td>-0.098795</td>
</tr>
<tr>
<td>tot</td>
<td>0.098055</td>
<td>-0.490624</td>
</tr>
</tbody>
</table>

Correlation coefficients were also calculated between gross production and consumption rate. (Tab. 3)

Table 3. Correlation coefficients obtained between gross rate of production and consumption. Coefficients were calculated for the rate per dry weight values.

<table>
<thead>
<tr>
<th>Correlation P and C</th>
<th>R²</th>
<th>(per dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.9931050577</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>-0.565651005</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>-0.4861079128</td>
<td></td>
</tr>
<tr>
<td>Tot</td>
<td>0.9223011649</td>
<td></td>
</tr>
</tbody>
</table>
Methane amount

Average amounts of methane measured during the 8 hours’ incubation are shown in Table 4 and represented in with graphs in Figure 23 and 24.

Figure 23. Average amount of methane measured in bog soils.

Figure 24. Average amount of methane measured in drained fen soils.
### Carbon-nitrogen analysis

Mass percentage ratio between carbon and nitrogen for the 36 samples are shown in Table 4. The results are averages values of the three replicates for each sample.

<table>
<thead>
<tr>
<th></th>
<th>Average CH₄ (nmol)</th>
<th>P (nmol CH₄ day⁻¹)</th>
<th>C (nmol CH₄ day⁻¹)</th>
<th>Net flux (nmol CH₄ day⁻¹)</th>
<th>% water content</th>
<th>% SOM</th>
<th>% C/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>16.46</td>
<td>10.95</td>
<td>0.59</td>
<td>35.13</td>
<td>-24.18</td>
<td>-1.29</td>
<td>298.46</td>
</tr>
<tr>
<td>2A</td>
<td>12.55</td>
<td>13.52</td>
<td>0.29</td>
<td>59.54</td>
<td>-46.01</td>
<td>-0.98</td>
<td>240.07</td>
</tr>
<tr>
<td>3A</td>
<td>17.58</td>
<td>13.13</td>
<td>0.35</td>
<td>55.18</td>
<td>-42.05</td>
<td>-1.12</td>
<td>459.98</td>
</tr>
<tr>
<td>4A</td>
<td>13.11</td>
<td>39.62</td>
<td>0.86</td>
<td>59.10</td>
<td>-19.49</td>
<td>-0.42</td>
<td>271.19</td>
</tr>
<tr>
<td>5A</td>
<td>193.33</td>
<td>730.61</td>
<td>1.37</td>
<td>724.71</td>
<td>5.90</td>
<td>0.01</td>
<td>1042.96</td>
</tr>
<tr>
<td>6A</td>
<td>155.67</td>
<td>1110.52</td>
<td>4.26</td>
<td>416.60</td>
<td>693.93</td>
<td>2.66</td>
<td>1105.72</td>
</tr>
<tr>
<td>8A</td>
<td>24.27</td>
<td>54.98</td>
<td>1.89</td>
<td>71.69</td>
<td>-16.71</td>
<td>-0.58</td>
<td>835.55</td>
</tr>
<tr>
<td>9A</td>
<td>17.58</td>
<td>12.80</td>
<td>0.33</td>
<td>46.63</td>
<td>-33.83</td>
<td>-0.88</td>
<td>347.03</td>
</tr>
<tr>
<td>11A</td>
<td>18.97</td>
<td>17.49</td>
<td>0.87</td>
<td>57.42</td>
<td>-39.93</td>
<td>-1.99</td>
<td>369.72</td>
</tr>
<tr>
<td>12A</td>
<td>15.06</td>
<td>22.96</td>
<td>0.07</td>
<td>79.10</td>
<td>-56.14</td>
<td>-0.18</td>
<td>162.67</td>
</tr>
<tr>
<td>13A</td>
<td>24.55</td>
<td>51.41</td>
<td>0.94</td>
<td>44.61</td>
<td>6.80</td>
<td>0.12</td>
<td>623.01</td>
</tr>
<tr>
<td>14A</td>
<td>48.82</td>
<td>363.10</td>
<td>2.33</td>
<td>358.01</td>
<td>5.09</td>
<td>0.03</td>
<td>291.85</td>
</tr>
<tr>
<td>15A</td>
<td>14.79</td>
<td>19.50</td>
<td>0.05</td>
<td>69.64</td>
<td>-50.14</td>
<td>-0.13</td>
<td>164.63</td>
</tr>
<tr>
<td>16A</td>
<td>182.45</td>
<td>1108.61</td>
<td>3.50</td>
<td>338.53</td>
<td>770.08</td>
<td>2.43</td>
<td>152.69</td>
</tr>
<tr>
<td>17A</td>
<td>318.31</td>
<td>2041.32</td>
<td>74.71</td>
<td>716.92</td>
<td>1324.40</td>
<td>48.47</td>
<td>341.63</td>
</tr>
<tr>
<td>18A</td>
<td>266.42</td>
<td>1670.51</td>
<td>17.48</td>
<td>583.32</td>
<td>1087.19</td>
<td>11.37</td>
<td>260.95</td>
</tr>
<tr>
<td>1B</td>
<td>17.85</td>
<td>11.83</td>
<td>0.43</td>
<td>45.18</td>
<td>-33.35</td>
<td>-1.22</td>
<td>141.25</td>
</tr>
<tr>
<td>2B</td>
<td>11.16</td>
<td>45.69</td>
<td>0.48</td>
<td>106.75</td>
<td>-61.07</td>
<td>-0.64</td>
<td>55.55</td>
</tr>
<tr>
<td>3B</td>
<td>8.09</td>
<td>91.10</td>
<td>0.66</td>
<td>145.08</td>
<td>-53.99</td>
<td>-0.39</td>
<td>119.21</td>
</tr>
<tr>
<td>4B</td>
<td>11.72</td>
<td>30.31</td>
<td>0.41</td>
<td>85.24</td>
<td>-54.92</td>
<td>-0.75</td>
<td>88.11</td>
</tr>
<tr>
<td>5B</td>
<td>15.62</td>
<td>1.36</td>
<td>0.90</td>
<td>17.14</td>
<td>-15.78</td>
<td>-10.40</td>
<td>238.49</td>
</tr>
<tr>
<td>6B</td>
<td>11.72</td>
<td>13.40</td>
<td>0.25</td>
<td>66.30</td>
<td>-52.90</td>
<td>-0.97</td>
<td>84.67</td>
</tr>
<tr>
<td>1C</td>
<td>8.37</td>
<td>44.54</td>
<td>0.44</td>
<td>108.66</td>
<td>-64.12</td>
<td>-0.64</td>
<td>48.48</td>
</tr>
<tr>
<td>2C</td>
<td>5.86</td>
<td>79.43</td>
<td>0.54</td>
<td>151.73</td>
<td>-72.29</td>
<td>-0.50</td>
<td>45.95</td>
</tr>
<tr>
<td>3C</td>
<td>12.83</td>
<td>20.89</td>
<td>0.38</td>
<td>67.51</td>
<td>-46.82</td>
<td>-0.85</td>
<td>32.46</td>
</tr>
<tr>
<td>4C</td>
<td>8.65</td>
<td>49.52</td>
<td>0.47</td>
<td>112.92</td>
<td>-63.40</td>
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<td>0.46</td>
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<tr>
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<td>0.43</td>
<td>171.67</td>
<td>-99.80</td>
<td>-0.60</td>
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Discussion

This work was an application of the stable isotope pool dilution technique described in von Fischer & Hedin 2002. The aim of the experiment was to study the methane production and consumption through the addition of labeled $^{13}$C methane. The equations of the model and the measured data had, for the most, very high $r^2$ values. The fits were very strong. This means that the model satisfactorily explains the essential dynamics of methane production and consumption.

It has already been shown by von Fischer & Hedin 2002 that the technique is not subjected to non-biological factors. An undertaken adjustment of the guide method was the decision to improve the sensitivity by performing longer incubations (8 hours).

The results could show different combinations of methane production and consumption (see Figure 18, 19, 20 and 21). Gross methane production had wider variation, spread in 3 orders of magnitude, while methane consumption varied across a smaller range. Soils with net positive flux showed a wider range of gross production rates (6a, 16a, 17a 18a) but also relatively high consumption rate.

An interesting result is the presence of methane production even in drained soils with negative net flux. The rates of gross production for these samples reached, as in soil sample 11c, 7.19 nmol CH4-C h$^{-1}$. As previously explained two of the regulating factors of methane production and consumption are soil moisture and soil water content. In particular, it emerged that soils that were more active in production processes were generally also more active in consumption. This is not only shown in the production and consumption rates peaks of samples 6a, 14a, 15a and 16a, but also from a high correlation coefficient between general production and consumption rates ($R^2=0.92$).

As described also in Le Mer & Roger 2001, the highest consumption rates have been registered in soils where methane concentration has been much higher than the atmospheric. Higher values of consumption rates dominate, as expected, in soils belonging to the drained fens (b and c) where net flux is always negative but close to zero. Here production rates, although low, coexist with relatively higher consumption rates. Methane production takes place hence also in drained organic soils. A high correlation coefficient ($R^2=0.79$) seems to support the idea that, at least for the samples b, water content could play a positive role in the production rate of drained organic soils. Six of the samples coming from the bog (a) showed a net positive flux (for two of
which methane concentration was so high that it was not possible to perform accurate measurements). A wider variation and higher values of production rates, as it happened for several bog samples (a), were combined with a wide variation and higher values of consumption rates. Contrarily to the results in von Fischer & Hedin 2002, this work results seem to support the idea that high rates of production and high rates of consumption are not necessarily mutually exclusive. A high correlation coefficient ($R^2=0.99$) seems to confirm that there is a positive correlation between production and consumption rates in the samples belonging to the bog (a).

As described by Matthews 2000 and confirmed by von Fischer & Hedin 2002, soil moisture may act both as a methane production and consumption regulator. In fact, also in this work, the obtained soil water content values seem to support the idea that soils with higher water content have lower consumption rates but not necessarily show higher production rate. It has been shown (Andersen et al. 1998) that anoxic microenvironments (microsites) are present within oxic soils and it has been suggested that they could become biogeochemically active rapidly once under anoxic conditions (Yavitt et al. 1995). As supposed also by von Fischer & Hedin 2002 it is not yet excludible to assume that in oxic soils, methane production is uniquely performed by methanogenic anaerobic organisms. It is known that aerobic soils oxidize atmospheric methane, but most of microorganisms involved and their activities are still largely unknown (Le Mer & Roger. 2001). Plausible is hence the potential for non-methanogenic microbes to produce trace quantities of methane (von Fischer & Hedin 2002).

Statistical tests performed, Kruskal-wallis H test, did not show significant results probably because of the peak values and the small sample size. A bigger sample size could have given better correlation coefficients and higher statistical significance. A more accurate description and categorization of vegetation during sampling could have improved results interpretation.
Conclusion

- Methane production can take place also in drained organic soils.
- Methane emissions from are results of antagonistic but correlated microbial activities.
- Methane is produced in the anoxic zones of water flooded soils by methanogens and can be oxidized into CO$_2$ by methanotrophs in the aerobic zones of wetland soils and in drained soils.
- Methanogens and methanotrophs seem to be ubiquitous in soils where they remain viable under unfavorable environmental conditions.
- Methane consumption occurs in flooded and drained soils and can assume a wide range of values.
- Aerobic drained soils oxidize atmospheric methane but their activities appear to be lower.
- Because of the presence of anaerobic organisms in oxic soils, microbial activity within anoxic microsites is assumable
References

Articles


Reports


Books


Websites

Radboud University (2017) *CN elemental analyzer*.

University of Gothenburg (2017) *Skogaryd Research Catchment*
## Appendix

Table 11. Correspondence between this work sampling and Skogaryd interactive maps chambers numeration.

<table>
<thead>
<tr>
<th>A (bog)</th>
<th>B (drained fen)</th>
<th>C (drained fen)</th>
</tr>
</thead>
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<td>35/1b</td>
<td></td>
</tr>
<tr>
<td>2/2a</td>
<td>32/2b</td>
<td></td>
</tr>
<tr>
<td>3/3a</td>
<td>5/3b</td>
<td></td>
</tr>
<tr>
<td>4/4a</td>
<td>3/4b</td>
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<td>8/5b</td>
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<td>6/6a</td>
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<td>C (drained fen)</td>
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