universität wien

MASTERARBEIT / MASTER'S THESIS

Titel der Masterarbeit / Title of the Master's Thesis

„Determination of Methanogenic Pathways in Pürgschachen Moor"

verfasst von / submitted by

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angestrebter akademischer Grad / in partial fulfilment of the requirements for the degree of

Master of Science, (MSc)

Wien, 2021 / Vienna 2021

Studienkennzahl lt. Studienblatt / degree programme code as it appears on the student record sheet:	A 066 299
Studienrichtung lt. Studienblatt / degree programme as it appears on the student record sheet:	Interdisziplinäres Masterstudium Environmental Science
Betreut von / Supervisor:	Univ.-Prof. Dipl.-Geogr. Dr. Stephan Glatzel

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ACKNOWLEDGMENTS

All the practical work was conducted in the Geoecology laboratory in University of Vienna during author's studies for master's degree in Environmental Sciences.

Firstly, I am immensely grateful to prof. Glatzel, for supporting me in a long process of figuring out the path to take with this thesis and for introducing me to the wonderful world of peatlands in general. I want to thank him for providing me guidance and support to meet the challenges that arised during this experiment, even at the times of the global pandemic. It has been a pleasure and an honour to work with him.

Secondly, I want to express my gratitude to Andreas Maier, who, as a field and lab technician, supported this idea and helped with the technical part as well as collecting the samples, when it was not possible for me.

Furthermore, I express gratitude to Robert Petiszka, Claudia Blauensteiner and Raphael Müller for their guidance and assistance with the numerous laboratory analyses.

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ABSTRACT

Intact peatlands sequester approx. 0.37 Gt of carbon dioxide (CO_2) per year and are therefore vital ecosystems in the climate change context. However, drained peatlands turn from being carbon sinks to carbon sources, releasing around 6% of global anthropogenic CO_2 emissions. Six peat mesocosms were observed in laboratory conditions to determine the different methanogenic pathways in Pürgschachen via identification of methane (CH_4) and CO_2 fluxes and ^{13}C isotopes of CH_4 and CO_2 with the removal of vegetation from the experiment group with the aim to assess the significance of vegetation contribution to methane emissions. CO_2 fluxes ranged in the experiment group from 2.4 to 12.2 g m^2 h^{-1} and in the control group from 4.13 to 14.6 g m^2 h^{-1}. CH_4 fluxes ranged from 0.058 to 0.16 g m^2 h^{-1} in the experiment and from 0.046 to 0.751 g m^2 h^{-1} in the control group. For both CO_2 (ß= -1.94, $t_{137.9}$= -3.97, $p < 0.001$ and CH_4 (ß= -0.106, $t_{137.9}$= -4.09, $p<0.001$) treatment had significant effect on the fluxes.

All in all, taking into consideration the aforementioned results and other parameters measured throughout this study, it can be acclaimed that the presence of vegetation (*Callluna Vulgaris)* changes the ability of the peat profile to produce and emit both CO_2 and CH_4. Hydrogenotrophic pathway is dominant methanogenic pathway in Pürgschachen Moor is, although may coexist with the acetoclastic pathway.

ZUSAMMENFASSUNG

Intakte Moore binden ca. 0.37 Gt CO_2 pro Jahr und sind daher im Zusammenhang mit dem Klimawandel wichtige Ökosysteme. Trockengelegte Torfgebiete warden jedoch von Kohlenstoffsenken zu Kohlenstoffquellen und setzen dadurch etwa 6% der weltweiten anthropogenen CO_2-Emissionen frei. Sechs Torf-Mesokosmen wurden unter Laborbedingungen beobachtet, um die unterschiedlichen Pfade des klimawirksamen Treibhausgases Methan (CH_4) im Pürgschachener Moor zu untersuchen. Dafür werden die CH_4 und CO_2-Flüsse und deren ^{13}C-Isotope gemessen. Die Vegetation aus der Versuchsgruppe wurde entfernt, um der Vegetation die Methanemissionen zu bewerten. Die CO_2-Flüsse reichten in der Versuchgruppe von 2,4 bis 12,2 g m^2 h^{-1} und in die Kontrollgruppe von 4.13 bis 14.6 g m^2 h^{-1}. Die CH_4-Flüsse reichten von 0.058 bis 0.16 g m^2 h^{-1} in der Versuchgruppe und von 0.046 bis 0.751 g m^2 h^{-1} in der Kontrollgruppe. Sowohl für CO_2 (ß= -1.94, $t_{137.9}$= -3.97, $p < 0.001$ als auch für CH_4 (ß= -0.106, $t_{137.9}$= -4.09, $p<0.001$hatte die Behandlung signifikante Auwirkungen auf die Flüsse.

Insgesamt kann unter Berücksichtingung der oben genannten Ergebnisse und anderer in dieser Studie gemessener Parameter festgestellt warden, dass das Vorhandsensein von Vegetation (*Calluna Vulgaris)* die Fähigkeit der Torfprofils, sowohl CO_2 als auch CH_4 zu produzieren und zu emittieren, verändert. Die hydrogenotrophe Methanogenese ist der dominante Pfad im Pürgschachener Moor, obwohl er mit dem acetoklastischen Pfad koexistieren kann.

INTRODUCTION

Peatland research is an emerging field of research, especially in the world where combating climate change is more actual than ever. Many researchers have emphasized the importance of peatland ecosystems in the context of climate change. Although many experiments conducted globally address the methanogenic pathways and the isotopic signatures of peatlands, to my knowledge, there has not been many similar studies carried out by viewing specifically the determination of differentiation in methane fluxes between peat and the vegetation.

Peatlands are important ecosystems when it comes to climate change. Healthy (wet) peatlands sequester approx. 0.37 Gt of CO_2 per year. However, when damaged, peatlands turn from being carbon sinks to carbon sources, releasing around 6% of global anthropogenic CO_2 emissions. It is not only about carbon, but peatlands offer many other ecosystem services, vital to a healthy planet. These include, but are not limited to regulating water flows, reducing flood risks and drought, prevent seawater intrusion. Peatlands are also of global significance for biodiversity with the majority of peatland species and habitats rare, threatened or declining. Furthermore, peatlands preserve the past, like an archive of previous records, holding a good record of past biodiversity, condensed in the layers of peat, revealing the history as the research advances through pollen records and human artefacts. (IUCN, 2017)

Although covering approximately 3% of Earth's surface, they are significant carbon reservoirs, storing somewhat 30% of the whole terrestrial carbon pool and store more carbon than all other vegetation combined. In peatlands net primary production (NPP) is greater than organic matter (OM) decomposition. Peatlands have a high rate of carbon accumulation, which supports the growth of peat forming vegetation. These ecosystems play an important role in biogeochemical cycles, identified as important greenhouse gas sources, namely of carbon dioxide and methane. It is mainly the human activities, especially drainage, that affect the peatlands and therefore the whole terrestrial carbon cycle. (Gorham, 1991; O R Kotsyurbenko et al., 2019; Wieder & Vitt, 2006) This significance highlights the importance of peatlands and their research. Therefore, there lies an urgent need to understand the complexity of carbon and methane cycling and the role they play in peatlands and

ecological systems in general. These understandings can help us to in development of a holistic approach to preserving, protecting and restoring these vulnerable yet valuable ecosystems, including decision making regarding restoration, agriculture, natural resource management and more.

The first part of the thesis gives a literature overview of the peatlands and their association with carbon cycle, including the short description of peatlands, carbon and methane cycling, methanogenic pathways and isotopic fractionation.

The second part of the thesis comes to grips with practical work, including the detailed descriptions of methodologies and statistical analysis. The main aim of this study was to determine the different methanogenic pathways in Pürgschachen Moor – an ombrotrophic bog situated in the Austrian Alps. Main objective of this study was to understand the methane fluxes related to the peat and the vegetation. This was achieved by measuring gas fluxes and conducting the isotope analysis. For this, identification of CH_4 and CO_2 fluxes and ^{13}C isotopes of CH_4 and CO_2 in incubated peat mesocosms were carried out as specified in the upcoming chapter. These measurements are the key to determining methanogenic pathways from the incubated cores, which in turn allows to assess the significance of vegetation contribution to methane emissions.

Third part handles the analysis and discussion of the results obtained and concludes the findings of the author's work and leads to evaluate whether *Calluna vulgaris*-covered peat mesocosms have a higher CH_4 flux than the non-vegetated peat and which of the methanogenic pathways is dominant in Pürgschachen Moor ombrotrophic bog.

1. STATE OF KNOWLEDGE

1.1. Peatlands in brief

Peatlands can be found in variety of climates, including arctic, boreal, temperate and tropical. (Leifeld & Menichetti, 2018) revealed in their up-to-date study, that around 83,3% of the peatlands are boreal, 12,7% tropical and 4% temperate. These combined make up a total peatland area of 463,2 million hectares. Although majority of peatlands are found in northern climates and until recently, most of the tropical peatlands were found in South-East Asia, (Dargie et al., 2017) identified an area with peat coverage of approx. $140*10^3$ m^2 in the Congo basin swamp forest. This peatland, known as Cuvette Centrale, is today the most extensive peatland complex in the tropics.

There are numerous classifications for peatland, most relevant in this case would be to divide peatlands to ombrotrophic bogs and minerotrophic fens. Ombrotrophic bogs receive their input of nutrients from the atmosphere and the main vegetation is *Sphagnum* moss. Minerotrophic fens on the other hand, have the input of nutrients from surrounding soils and groundwater. (Gorham, 1991)

Due to waterlogged conditions, all the oxygen is depleted and peatlands become anoxic environments with decreased rate of organic decomposition. Biodegradation in peatlands is usually higher than productivity, which favours lowered rate of remineralization and accumulation of OM, eventually forming peat. (Glaser, 1987; Gorham, 1991)

Once oxygen is depleted, following reactions proceed according to the redox potential (Figure 1). Many of those transformations are mediated by microbes, with populations adapted to anaerobic environments. Methane production is the most important terminal electron sink of anaerobic respiration and methanogenesis is the last reaction to occur, after all other electron acceptors are used up and redox potential drops below 200 mV. Redox potentials are influenced by pH and temperature. (Mitsch, William J, Gosselink, 2015)

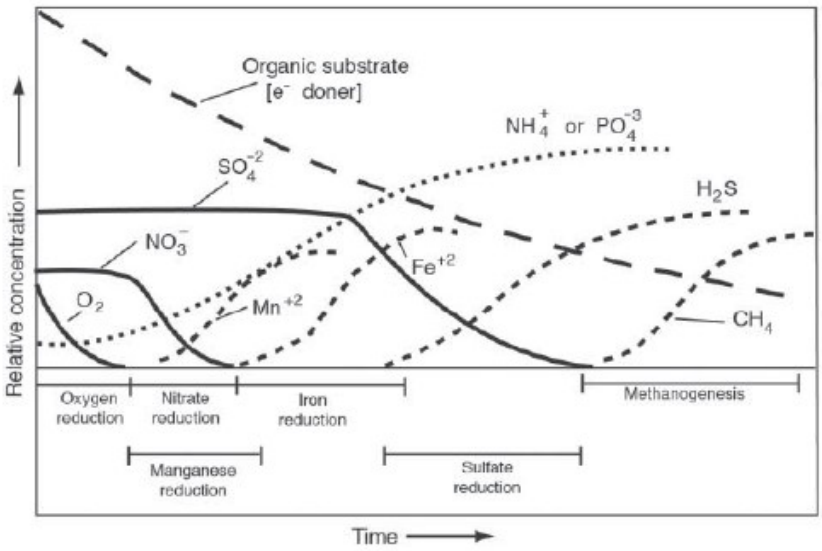

Figure 1. The redox cascade. (Mitsch & Gosselink, 2015)

1.2. Carbon cycling

Peatlands have a very important role in terrestrial carbon cycling, although processes involved can be hard to predict. There is yet a lack of knowledge about the mineralization of OM, consequences of short- and long-term disturbances - all in all a comprehensive overview of the carbon budget.

It is estimated that peatlands hold around 550 Gt carbon. (F. Parish et al., 2008) Groundwater, precipitation and net community productivity are sources of carbon into wetlands. There are several pathways of carbon leaving the system, including emission as greenhouse gas (CO_2, CH_4) and discharge as dissolved organic carbon (DOC). (Limpens et al., 2008)

Complex OM decomposes to aforementioned final products and is dependent on microbiological activity, transport processes of electron acceptors and the quality of OM. Environmental changes can affect microbial activity via nutrient availability and electron acceptors directly or due to changes in vegetation or soil structure indirectly. (Limpens et al., 2008)

C mineralization in peatlands is slow compared to other ecosystems. Litter bag experiments show an average mass loss of about 20%, although the mass decreases exponentially and not linearly. (Blodau, 2002).

Figure 2 illustrates the carbon cycle in peatland environment, both aerobic and anaerobic processes, out of which the latter are timely in the present work. Encircled ones represent relevant gases, and dashed arrows show microbial processes. Dissolved organic carbon (DOC) is leached via groundwater, both from oxic and anoxic layer. Fermentation and methanogenesis are dominant processes in peatlands. Fermentation is carried out by microbes, during which high-molecular-weight carbohydrates are being broken down and made available to other microbes, such as methanogens, to

carry out methanogenesis, which will be explained in more detail in the next chapter. (Blodau, 2002; Limpens et al., 2008; Rydin & Jeglum, 2013)

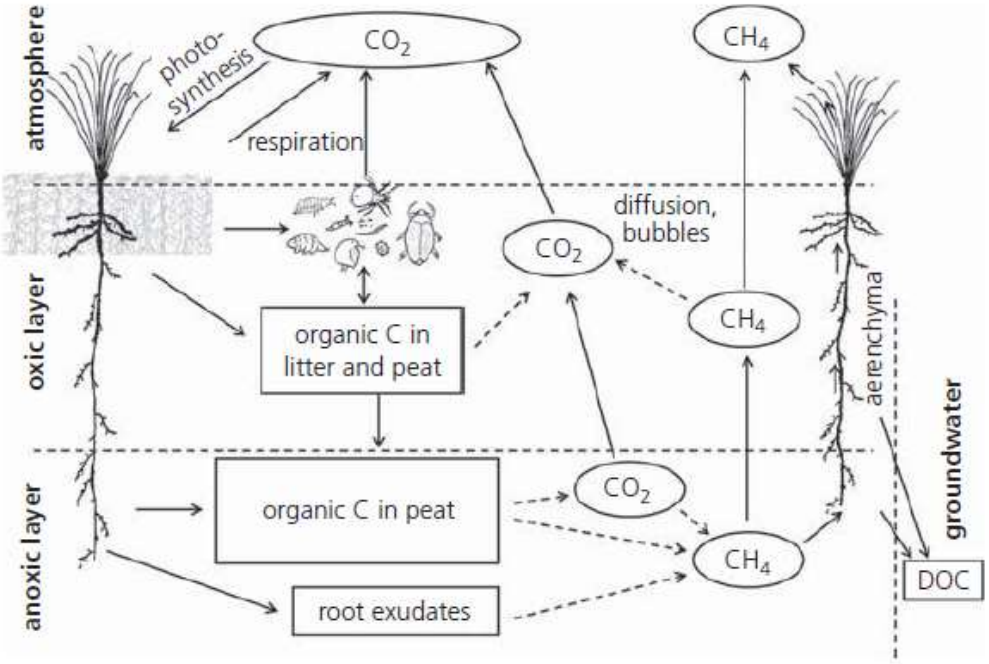

Figure 2. The Carbon cycle in peatlands (Rydin & Jeglum, 2013, p. 264)

1.2.1. Methane in peatlands

Methane is the most abundant organic gas in the atmosphere and an end product in the degradation of OM under anaerobic conditions (Battley, 1995). 20% of the radiative forcing related to climate change can be attributed to increasing levels of methane. Pre-industrial methane levels of 700ppb have raised as high as 1850 ppb according to the report by Intergovernmental Panel on Climate Change (IPCC), with a high annual and seasonal variability, as has been shown to be the case also in peatlands. (Drollinger et al., 2019; IPCC, 2019)

Total methane production is given by (Conrad, 2005):

CH_{4_total}= CH_4 [acetate] + CH_4 [CO_2] + CH_4 [other compounds (i.e. methanol)]

Total methane production can be quantified with measuring stable carbon isotope signatures, which can be further used to determine specific pathways (See Chapter 1.2.2).

Methane fluxes are dependent on the production rate through anaerobic degradation of OM as well as the pathways of transport and consumption in the system. (F. Parish et al., 2008)

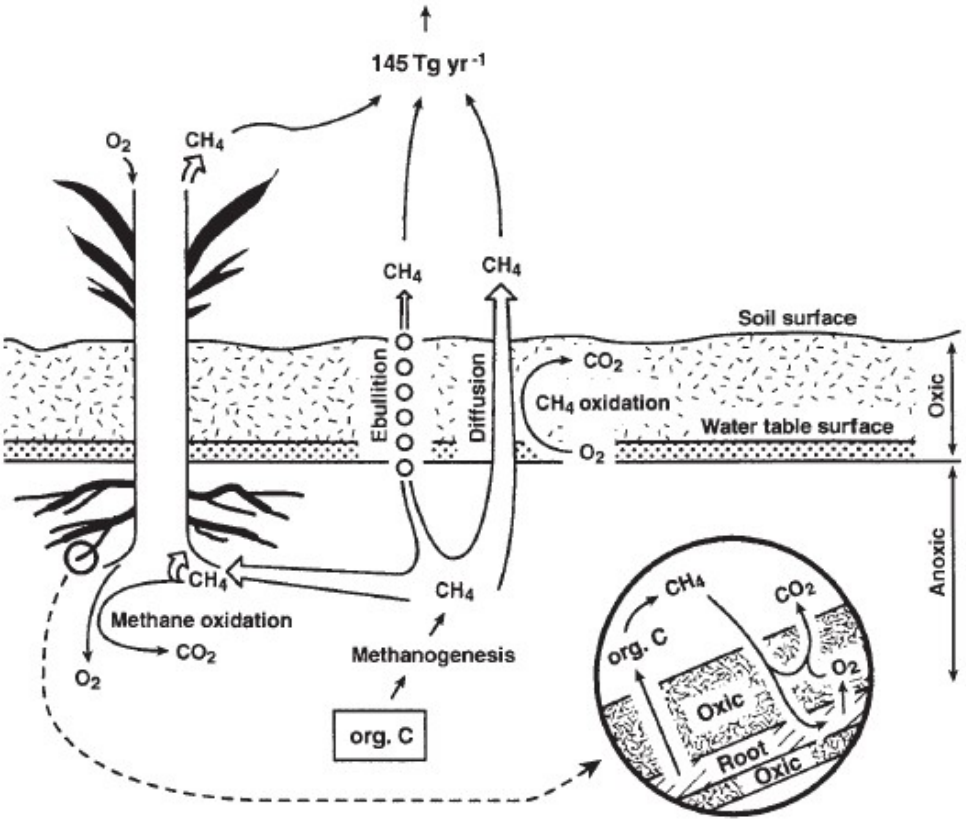

Figure 3. Methane in peatlands. (Whalen, 2005)

Figure 3 depicts different methane emission pathways to the atmosphere (LAI, 2009; Whalen, 2005):

a) emerging from the surface through the water column by diffusion
b) ebullition, which essentially means that methane is released to the surface via bubbles
c) significant amount of methane is released through the plant vascular system, 50-90 % of all methane released from vegetated wetland

Vascular transport is fast and reduces methane residence time in aerobic zone and reduces the amount of methane that is oxidized. Methane transported by ebullition and diffusion more slower and tend to oxidize methane, therefore emission rates are low. (Blodau, 2002)

(Leroy et al., 2017) demonstrated with their study that biotic and abiotic factors control CO_2, CH_4 and DOC emissions in peat mesocosms and that all of those factors interact with one another and the contribution of vegetation and its change should not be neglected in the carbon dynamics in peatlands.

Fluctuations in methane fluxes, but also those of other greenhouse gas (GHG), are due to different influencing factors, such as soil temperature, water table depth, moisture, nutrient input and organic matter accumulation, vegetation characteristics, transportation via vegetation and presence of

sulfate and iron reducers and homoacetogens, that may outcompete methanogens (Fisher et al., 2017; O R Kotsyurbenko et al., 2019; Matthews, 1987; Sebacher et al., 1986) Although, sulfate is believed to be problem mostly in saltwater wetlands. (Mitsch & Gosselink, 2015)

Several studies show that methane production in acidic peat is stimulated by changes in temperature, pH and plant community composition, indicating important role of microbial adaptation. Production of methane is also based on the OM reaching the anoxic zone. Therefore, the primary productivity of biomass and water table depth are important factors here. Organisms producing methane grow in anaerobic conditions, which highly depend on the water level. (Dedysh & Panikov, 1997; R. Parish et al., 2008; Whalen, 2005; Yavitt & Seidman-Zager, 2006)

Products of the complete oxidation of methane are CO_2 and H_2O, following the equation (Cicerone & Oremland, 1988):

$$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O$$

Total methane production can be quantified with measuring stable carbon isotope signatures of carbon dioxide, with known fractionation factors for the conversion of CO_2 and acetate methyl to CH_4, although they vary depending on environmental conditions. Isotopic fractionation of acetate is a challenge, because acetate methyl consumption and production may happen via different pathways (Conrad, 2005).

Study by (Galand et al., 2005) showed that the nutrient-rich mesotrophic site had, by far, the highest rates of CH_4 production. It promotes vegetation growth, where decomposition of organic matter and exudates from plant roots increase the quantity of carbon substrates available for methanogenesis.

(Sebacher et al., 1986) estimated annual methane emissions from northern wetlands to be between 45 and 106 Tg, emphasizing that at higher latitudes the methane fluxes are higher, but for shorter period and at low latitudes the methane fluxes are lower but for longer period. (Matthews, 1987) estimated global methane emissions from wetlands to be 110 Tg of which around 60% are expected to origin from northern peatlands. The most recent estimate by (Kirschke et al., 2013) about the global methane budget in the period 1980-2009, claims natural wetland to be the largest methane source, with emissions of 177-284 Tg per year, which represents approx.. 1/3 of total natural and anthropogenic sources.

(Drollinger et al., 2019) reported annual methane emissions values between 4.40 ± 2.4 and 5.24 ± 2.68 g C m-2 yr-1, showing also pronounced significant (10-fold) seasonal variability, highest values found in summer where the soil temperature is higher. They also identified diurnal variations in the release of CH4 from the Pürgschachen Moor.

1.2.2. Methanogenic pathways

Microbially mediated methanogenesis is a considerable source of methane and has a major role in the global carbon cycle. Methanogens are unique group of microbes responsible for methanogenesis. They can be found in many anaerobic environments – ruminants' intestines, bioreactors, deep ocean and wetlands. Acetate, CO_2 and methylated compounds are precursors for methanogenesis and different substrates are used by different microorganisms (Battley, 1995; Zhang et al., 2019). Therefore, methane is produced mainly by 3 pathways (Ye et al., 2012; Zhang et al., 2019,):

a) acetate disproportionation (acetoclastic/acetotrophic methanogenesis)
b) the reduction of carbon dioxide by dihydrogen (hydrogenotrophic methanogenesis)
c) using methylated compounds (methylotrophic methanogenesis)

Different pathways are subject to different isotopic fractionation characteristics, which can be used to determine the specific pathway. (Conrad, 2005; M. J. Whiticar et al., 1986; Zhang et al., 2019) The ratio of the pathways depend on the relative net production rates of acetate and H_2, because other main precursor, carbon dioxide, is generally not a limiting substrate (Conrad, 2020).

(Zhang et al., 2019) emphasized on the dominance of acetoclastic methanogenesis in peatlands, although variability present in isotope characteristics, increased methanogens and ratio of CO_2 to CH_4 mean that acetoclastic and hydrogenotrophic methanogenesis coexist. Generally, it is found that approximately 2/3 of CH_4 is derived from acetoclastic and 1/3 from hydrogenotrophic methanogenesis when OM is completely degraded. Exceptions and seasonal variabilities may occur (Avery Jr et al., 2003; Conrad, 2005; Oleg R. Kotsyurbenko et al., 2004)

Fresh organic matter from high plant production is thought to fuel acetoclastic rather than the hydrogenotrophic pathway (O R Kotsyurbenko, 2010). In contrast, hydrogenotrophic pathway dominates in less productive peatlands and in those dominated by *Sphagnum* mosses and heath shrubs, where only the new and fresh organic matter is completely degraded and deeper layers the hydrogenotrophic methanogenesis is dominant, since the organic matter is more recalcitrant for degradation and the production of methane happens a lower rates. (Galand et al., 2005; Keller & Bridgham, 2007)

Acetate fermentation is more common in freshwaters due to higher availability of precursor acetate (M. J. Whiticar et al., 1986). Minerotrophic fens have higher plant productivity and fresh OM, indicating dominance of acetate fermentation. In ombrotrophic peatlands with less productive plant communities, e.g. *Sphagnum* and older OM, hydrogenotrophic pathway might dominate (Galand et

al., 2005; Keller & Bridgham, 2007; Ye et al., 2012). (Yavitt & Seidman-Zager, 2006) suspected that pH is the main factor that determines which of the methanogenic pathway dominates.

Research by (Duddleston et al., 2002) showed that methane in Turnagain bog was derived via hydrogenotrophic pathway and not of acetoclastic. Eventually, anaerobically produced acetate was aerobically degraded to CO_2. They indicated that that fermentation may be the primary process of OM degradation and methanogenesis might play only a minor role (Duddleston et al., 2002).

(Chidthaisong et al., 2002) illustrated in their study with rice paddies, that generally soil samples had higher methane production than the roots. When saturating with 5000ppm of H_2, theoretically 1250ppm of CH_4 should have been produced ($CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$). Maximum production was reached at 1200 ppm (96%) and continued to decline onwards. Possible causes have been suggested to be high density of cells (die out), but not the pH (6.5). However, highest methane production after 19 days occurred in methanogenic archaea from roots, showing that there is high potential which is limited by H_2. Furthermore, they reported that in the beginning of methanogenesis, fractionation was small and increased when production was active and remained stable until the end. The latter is proposed to be illustrative of natural environment.

(Lehmann-Richter et al., 1999) reported that excised rice roots produced methane almost exclusively via hydrogenotrophic pathway, as did (Conrad & Klose, 1999). (Gilbert & Frenzel, 1998) stated that only 10% of the methane is produced by the roots.

1.2.3. Acetate

In anaerobic systems, acetate is the most important carbon intermediate. It has a rapid turnover rate due to the uptake by bacteria, however it might also accumulate in some systems, e.g. freshwater or marine sediments due to production and consuming processes caused by temporal separation. (Avery et al., 1999; Lovley & Klug, 1983) Acetate can be produced from organic carbon or CO_2 and consumed by conversion to CH_4, CO_2 or biomass. (Conrad, 2005).

(Duddleston et al., 2002) conducted field measurements to determine acetate formation and methanogenesis in a bog in Alaska and found acetate to be a final step in anaerobic decomposition. Carbon from acetate accumulated up to 120 times faster than from methane, indicating once again the importance of acetate as terminal product of anaerobic decomposition. This research demonstrated low production rates of CH_4, but when accumulated, acetate was quantitatively converted to CH_4, the rates of methanogenesis showed similar results as reported in other wetlands where CH_4 is mainly derived from acetate. (Avery et al., 1999; Duddleston et al., 2002)

However, (Hines et al., 2001) reported that methane production in northern wetlands are not derived from acetate or other compounds, but the latter rather accumulate in high concentrations as it is the foremost terminal organic end product of anaerobic degradation, often even higher that the formation of CH_4. They reported that in northern wetlands, acetate is ultimately aerobically degraded to CO_2 and not to CH_4. They also found acetate accumulation in anaerobic peat to be up to 100-fold more rapid than CH_4 formation, reaching maximum concentrations in the upper layers of peat (6-8 cm). It is later aerobically respired to CO_2 after diffusing to surficial peat and to vascular plant roots. Moreover, the processes related to acetate are controlled by the water table, when the latter is lowering, the extent of aerobic zone is larger, inhibiting anaerobic formation and accumulation of acetate, leading to its aerobic degradation.

In study carried out by (Duddleston et al., 2002), the highest acetate concentrations were observed with the water saturation of peat, and decreased with the dropping water table, presumptively as a result of aerobic oxidation of acetate. Concentrations reached as high as 1.0 mM at16cm depth and below that no acetate accumulation was recognized. On the other hand, anaerobic incubations for 4.5 months at 24ºC did not result in production of methane from acetate (Figure 4). Acetate was the main final product of decomposition and acetate was finally oxidized to CO_2 though aerobic respiration and to some degree, anaerobically via Fe reduction.

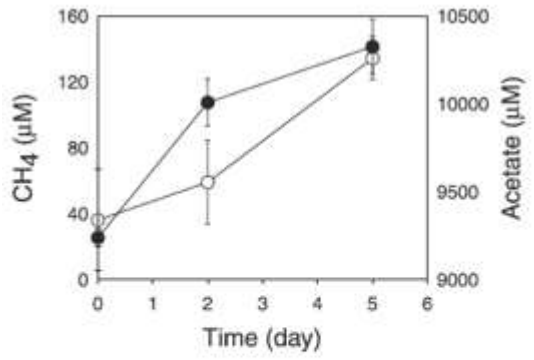

Figure 5. Methane (solid circles) and acetate (open circles) production in slurried peat following long-term incubation. Whole peat from Turnagain Bog was incubated anaerobically at 24°C for 4.5 months prior to creating slurries. Error bars represent ±1 standard deviation of the mean of three replicate vials. Absence of error bars indicates that the error is smaller than the symbol.

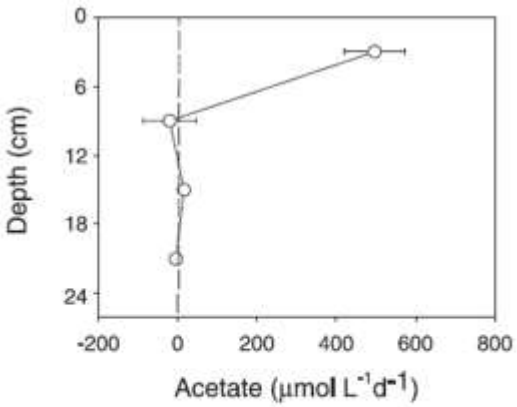

Figure 6. Rate of acetate accumulation in slurried peat with depth. Whole peat samples were separated to represent depths of 0–6, 6–12, 12–18, and 18–24 cm. Error bars represent ±1 standard deviation of the mean of three replicate vials. Absence of error bars indicates that the error is smaller than the symbol.

Figure 4. Acetate levels in an Alaskan bog (obtained from (Duddleston et al., 2002))

(White et al., 2008) suggested that the high methane fluxes can be due to the inhibiting porewater compounds, high levels of DOC and high plant productivity. They further endorse that acetate fermentation is the primary pathway of methanogenesis in bogs. Additionally, they reported

positive relationship between methane emissions and pore water DOC and found no significant correlation with methane fluxes and porewater acetate concentrations in bog mesocosm. (White et al., 2008)

Although acetate is a primary intermediate for acetoclastic methanogenesis, there is evidence that it can also inhibit methanogenesis as a result of converting it to acetic acid at low pH (Bräuer et al., 2004). (White et al., 2008) witnessed this at high acetate porewater concentrations in fen mesocosms, however not on lower levels in the bog mesocosms, where the levels were consistent between the values 20 and 40 mM.

1.2.4. DOC

DOC is made up from different compounds, e.g., cell residues, products of fermentation, humic and fulvic substances, which all arrive from different sources and have different bioavailability as well as ecological functions. The concentration of these different compounds can be seasonally variable and are dependent on the activity of bacteria, including methanogens. (Avery et al., 1999; Blodau, 2002)

DOC is the most labile form of soil OM. In peatlands, DOC plays an important role, as any change in DOC flux ends in the redistribution of terrestrial carbon. Mineralisation of carbon and the production of CH_4 depends on DOC production. (Blodau, 2002)

Fastly rising DOC concentration in the catchment areas of drained peatlands have been observed. (Freeman et al., 2004) did drought-experiment and proposed that labile DOC is released via root exudates, which stimulates microbial activity and boosts OM degradation, principally DOC. (Limpens et al., 2008) found that dominating vegetation affects also the DOC rates, in addition to photosynthetic and respiration rates.

1.3. Isotopes

Isotopes are forms of the same element, that have the same number of electrons and protons, but a different number of neutrons in the nucleus, which make a chemical difference, although they behave the same (Fry, 2006).

Thermodynamic properties of substances depend on their mass and lighter elements have slight mass differences due to isotopic substitution, causing isotopic fractionation in chemical and physical processes, which can be measured. This fractionation of isotopes is a result of the contrast in vapour pressure or the deviation from equilibrium constant between two molecules that contain

isotopes of constituent atoms. The constants of those equilibrium exchanges can be measured or calculated and therefore the variations in the abundance of the element isotopes can be used as a tool to evaluate the biogeochemical processes that the element has gone through in the past. In terrestrial systems, abundance of ^{12}C is measured to be ca. 90 times higher than ^{13}C. (Craig, 1953)

Isotopic gas flux measurements are used to identify a source of the gas. Studying the fluxes of ^{13}C and ^{12}C carbon isotopes allows to get a comprehensive picture of a carbon cycle, in this case to classify methane sources and understand the different pathways of methane production. (Conrad, 2005)

Variations in stable isotope ratios can be caused in several ways from methanogenesis via different production pathways but also the substrate isotopic value and methanogenic community. Via both methanogenic pathways, $\delta^{13}C$ of OM is fractionated and forms enriched $\delta^{13}C$ of CO_2 and depleted δ^{13} of CH_4 (Chidthaisong et al., 2002). Figure 5 depicts a scheme of carbon flow.

Nevertheless, understanding methane isotope biogeochemistry is vital. In rice paddies, roots and rhizosphere are participating in the cycling of methane, as this is where most of the oxidation occurs and methane enters the plants before released to the atmosphere. Additionally, rice roots have active site for methanogenesis and potentially a higher production rate of methane than soils. In rice paddies carbon isotope fractionation factors of 1.045 and 1.060 have been found. (Chidthaisong et al., 2002)

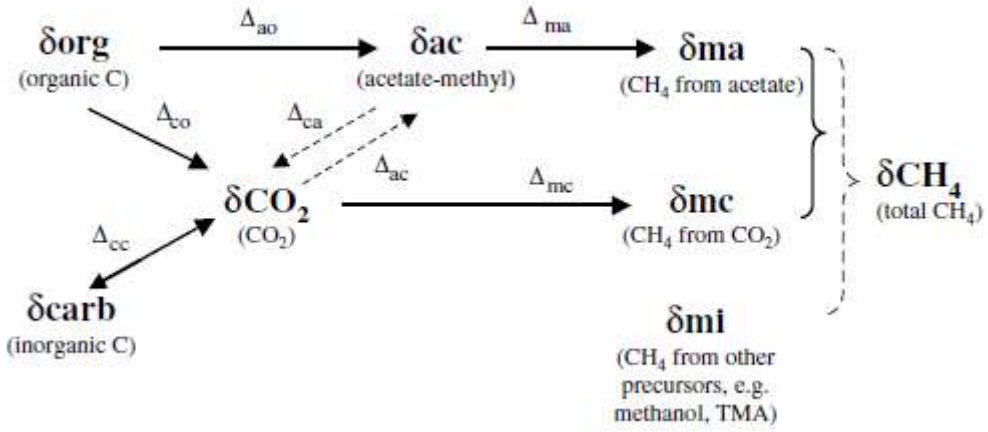

Figure 5. Scheme of carbon flow and the changes of ^{13}C isotope in a methanogenic environment (Conrad, 2005)

Methane sources have different carbon isotopic ratios and those signatures can be used to divide emissions from different sources, although these can vary temporally and spatially. (Fisher et al., 2017)

(Tyler et al., 1997) managed to determine methanogenic pathway via $\delta^{13}C$ isotope in rice paddies and defined isotope mass balance as following (Tyler et al., 1997):

$\delta^{13}CH_4 = \delta^{13}CH_4\{acetate\} * F_{acetate} + \delta^{13}CH_4\{CO_2+H_2\} * (1-F_{acetate})$

in which $\delta^{13}CH_4$ is production by relevant pathway and the fraction from acetoclastic is defined as (Tyler et al., 1997):

$F_{acetate} = CH_4\{acetate\} / [CH_4\{CO_2+H_2\} + CH_4\{acetate\}]$

This calculation is possible due to several studies that have identified values for $\delta^{13}C$. (M. J. Whiticar et al., 1986) considered marine environment to represent hydrogenotrophic and freshwater environment the acetoclastic pathway, and found $\delta^{13}C$ values of -60‰ to -110‰ and -50‰ to 70‰, respectively.

(Fisher et al., 2017) measured methane emissions from different peatlands in Scandinavia, one site had a range of $\delta^{13}C$ of -53 to -76‰ (mean -72‰) whereas the other side ranged from -48 to 112‰ (mean 69.2‰) and proposed it to be due to different emission processes. They found also, that *Eriphorum angustifolum* - vegetated sites had higher methane fluxes than the areas with *Sphagnum*-cover. The first also had isotopic composition more enriched in ^{13}C. However, these results might be biased due to relatively small sample size and many more chambers would be required to provide a more wholesome understanding,

1.4. Hypotheses

Based on the literature research carried out and concluding the state of the art in peatland research discussed in this chapter, the author has posted the following hypotheses and pursues to either accept or reject those.

The main aim of this study is to determine the different methanogenic pathways in Pürgschachen Moor and to understand the methane fluxes related to the peat and the vegetation using isotope analysis. The specific objectives of this study are firstly, to measure isotopic signatures of methane and determine methanogenic pathways through it. For this both methane and carbon dioxide fluxes are measured from all the six cores. Three of the cores, serving as the experiment group, have plants clipped, to kill the plant and decay the roots. The other 3 remaining cores, with *Calluna Vulgaris* cover, are hereon considered as the control group (explained in detail in 2.3). This setup enables to assess the significance of vegetation contribution to methane emissions.

<u>Hypothesis 1:</u> Methane release from the cores with only *Sphagnum* covered peat is significantly different of the cores that have the plant cover (*Calluna Vulgaris).*

This is based on the assumption, that pathways of transport of gases depend upon the plants and also the production rate of OM degradation in anaerobic conditions. Several studies have showed,

that vascular transport can reduce the amount of oxidized methane. (Blodau, 2002; F. Parish et al., 2008) CH_4 production also depends on the amount of OM reaching the anoxic zone to which the plant cover contributes to. (Galand et al., 2005)

<u>Hypothesis 2:</u> On the study site hydrogenotrophic pathway is dominant.

Considering all the above-mentioned findings in literature, it is expected that in Pürgschachen Moor hydrogenotrophic pathway is dominant. (Galand et al., 2010; Keller & Bridgham, 2007) have reported that hydrogenotrophic pathway dominates in ombrotrophic peatlands. Furthermore, peatlands that have less productive plant communities, e.g. *Sphagnum* mosses and heath shrubs, as the dominant vegetation species, hydrogenotrophic pathway might be prevalent (Galand et al., 2005; Keller & Bridgham, 2007; Ye et al., 2012). For hydrogenotrophic pathway, the $\delta^{13}C$ values of are expected in the range from -60‰ to -110‰. If values are significantly higher, then acetoclastic pathway is dominant. This would highlight the importance of roots in the peat bog methane emissions. Isotopic fractionation would be dependent on the emitted CH_4 as plants can significantly alter the fractionation factors.

2. MATERIALS & METHODS

2.1. Study area

The Pürgschachen Moor is an ombrotrophic low-shrub pine bog located in Austrian Alps, close to Ardning village. Geographical coordinates: Latitude 47° 34' 50" N Longitude: 14° 20' 40". It is situated 632 meters above sea level and has an area of 62 hectares, making it the biggest alpine peat bog in Austria. It is a designated Ramsar site since 1991 and is a protected site under Natura 200 as a nature reserve and important bird area. The area is a subject to mean average temperature of 6.6°C and has annual precipitation of1400 mm. Pürgschachen Moor has been established as an emerging research site for University of Vienna to investigate carbon fluxes, full carbon balance and peatland degradation of alpine peatlands since 2015. The site is equipped with eddy covariance tower and gas chambers. Main vegetation consists of *Pino mugo, Sphagnetum magellanici* and *Caricetum limosae.* (Pürgschachen Moor - Austria | DEIMS-SDR, Annotated List of Wetlands of International Importance)

2.2. Sampling & Experiment Setup

In total 6 samples were collected on 8th of May 2020 from Pürgschachen moor, all with the *Calluna vulgaris* plant on them. After the collection, the samples were transported to Vienna and were left to recover in order to allow for the conditions in the samples to adjust and for the vegetation to revive (The recovery period was 2 weeks. Throughout this time period, the cores were watered with additionally collected porewater, in order to maintain anoxic environment needed for methane production. The author is aware, that this might not be representative of field conditions, as the *Calluna vulgaris* rather prefers drier habitat. Albeit, to successfully conduct this experiment, the constant waterlogged conditions were required, to ensure anoxia and therefore methanogenic activity. Throughout this thesis, they are referred to as either peat cores or mesocosms and they can be seen on Photo 1.

Photo 1. Experiment setup in the beginning.

Porewater samples were collected biweekly for further analyses. Into each core, holes were drilled at relevant depths – 10cm and 30 cm. Pipes with holes (Photo 2) were inserted (diameter 0.4 cm) and the end of the pipe was closed with glue, to keep the water inside. Protruding part of the pipe was equipped with a tap, to easily collect the water at scheduled times.

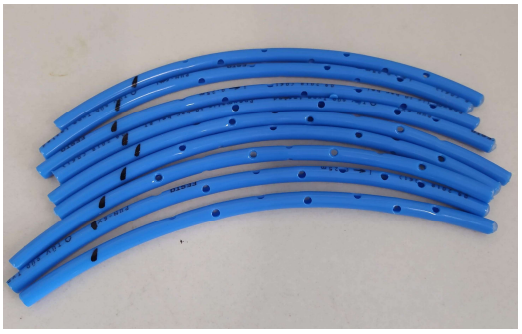

Photo 2. Pipes for pore water sampling

To ensure maximum comparability, all cores were of the same volume of 0.00786 ± 0.0032(SD) m^3. Nevertheless, differences might occur in GHG emissions due to differences of peat composition in different mesocosms. All of the cores had a radius of 7.5 cm. Photo 1 shows all the mesocosms with the pipes installed already at the very start of the experiment.

A headspace with a total volume of 0.006 m^3 was designed. As the Picarro analyzer works on a principle that when a gas sample is taken from the headspace (See 2.3.1), the headspace air is returned to the chamber after the measurement. For this reason, 2 pipes were installed on the top of

the headspace. Longer inlet and shorter outlet pipes had a length of 20.5 cm and 6.3 cm, respectively. Rubber bands were added on each side of the headspace lid to secure it to the headspace assuring no leaking. Photo 3 depicts the top secured to the headspace and the in- and outlet pipes.

Photo 3. Selfmade headspace.

Environmental conditions were kept the same throughout the measurements for all the mesocosms and all of them were kept constantly waterlogged to ensure anaerobic conditions. Furthermore, additional core (Nr 7) was added only with the peat pore water, in order to have same sampling conditions for all the cores and the pore water. From core 7 also flux was measured and water samples taken to analyse with the other samples.

2.3. Gas flux measurements

Each core was measured twice a day, morning and afternoon, with the Picarro isotopic gas analyser (See 2.3.1) to identify the isotopic signatures as well as gas fluxes of CO_2 and CH_4. Prior to each measurement, the chambers were removed from the cores at least 5 minutes to allow free exchange between peat surface and atmosphere in the experiment room. Measurements were repeated for 4 weeks in total, amounting up to 574 hours.

Workflow was the following:

1. Put the headspace on the core
2. Secure the top onto the headspace with rubber bands
3. Wait for the linear increase in concentration

4. Log the start and the end time of the linear increase (4 minutes)
    a. In case of sudden increase, back to step 1
    b. In case not linear, back to step 1
5. Remove the headspace
6. Flush at least 5 minutes with ambient air
7. Continue onto the next core and repeat steps 1-6 until all 6 cores are measured

First week of the experiment served as a baseline for further calculations. This was taken into account during data analysis. After the first week (hour 140) all emergent *Calluna vulgaris* plants were clipped from three cores, leaving the methane efflux to be dependent on only diffusion and ebullition. New emergent vegetation was further clipped every Friday afternoon to ensure enough time for equilibration over the weekend. This will be further mentioned as the "experiment group", consisting of cores 1 to 3. The other cores, namely 4 to 6, where the plant remained untouched, is counted as the "control group". The experiment setup can be seen from Photo 4 with the experiment group on the top and the control group on the bottom (with and without *Calluna Vulgaris*, respectively) and the additional core with only pore water in the bottom of the photo.

Photo 4. Experiment setup after the first week.

Flux rates were calculated by determining the slope of least squares regression line of the gas concentration in the chamber over time. Non-linear slopes ($r^2 < 0.9$) were rejected. All the flux calculations were done using MS Excel software, assuming linear increase in concentration and following the equation:

$$Fc = \frac{\partial [GHG]c}{\partial t_{sec}} \frac{M_m * p}{R * T} \frac{V_c}{A_c} * 3600 \; [gm^{-2}h^{-1}] \quad (1)$$

M_m [g/mol] – molar mass

A_c [m^2] - chamber area

V_m [m^3/mol] - molar volume

V_c [m^3] - chamber volume

R [J/mol*K] – gas constant

P [Pa] – pressure

$\frac{\partial [GHG]c}{\partial t_{sec}}$ – linear increase of gas concentration over time (slope)

The difference in efflux between the control and the experiment group is considered to be representative of the direct CH_4 emissions from the emergent plants.

In addition to CO_2 and CH_4 fluxes, δ^{13} of CO_2 and CH_4 were also recorded. Both values are represented in this thesis as the median with a standard deviation (SD). Isotopic values are reported against Pee Dee belemnite (V-PDB) carbonate, as is common.

2.3.1. Cavity Ring Down Spectometer (CRDS) – Picarro

The Picarro G2132-i isotope analyser was used, which measures $\delta^{13}C$ in both CO_2 and CH_4, and the relevant gas fluxes. It has a method of Cavity Ring Down Spectometry (Figure 6). As every molecule has a unique optical absorption, the gas will be let into the cavity (35 cc), where it is reflected by 3 mirrors. Spectrum is then measured by changing the color of light passing through a sample, that measures the amount of light that is absorbed. Thereafter, the concentration of the gas is determined from the size of each line. It has precise temperature and pressure control and is therefore highly accurate. (Inc., 2018)

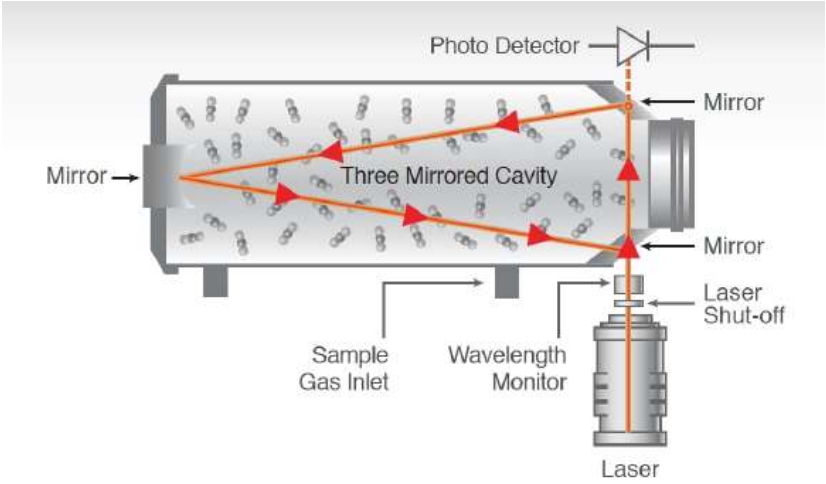

Figure 6. Illustration of the mechanisms of CRDS machine. (Inc., 2018)

2.4. Laboratory analyses

Biweekly porewater samples were collected from all the cores from 2 different depth (10 cm and 30 cm) and frozen for further analysis.Variety of parameters were measured including pH, organic carbon, organic nitrogen and acetate. The pH of each unfiltered porewater sample was determined using a WTW pH 7110 pH meter.

After the flux measurements were completed, the cores were drained of porewater for 24h (Photo 5). Different color of the pore water was observed for different cores.

Photo 5. Draining of the cores.

After this, the peat was removed from the cores and carefully cut into 10-cm sections representing depths of 0–10, 10–20 and 20-30 cm (Photo 6) using electrical knife. The sections were placed on a Petri dish and taken to the Soil Laboratory for further treatment and analyses.

Photo 6. Peat sections after cutting.

2.4.1. Physical properties

Dry and wet mass of the peat were determined by weighing and substantially drying the samples in the oven on two different temperatures – 45°C for air-drying (*lutro)* and 105°C for oven-drying (*atro).* The water content (WC) was calculated using the known equations from (Jury et al., 1991) :

$$WC(\%) = \frac{W_w - D_w}{W_w} * 100 \quad (2)$$

WC – water content (%) D_w – dry weight of peat (atro, at 105'C)

$$WC = \frac{W_m}{D_w} \quad (3)$$

WC – gravimetric water content Wm – mass of the water

W_w – wet weight of peat

The 10cm-depth section contained green *Sphagnum*. Roots of *Calluna vulgaris* were present in each section, decreasing with depth. For every section of each peat core, roots were physically separated from the peat and weighed (Photo 7). From this a percentage was calculated – how much share roots each core has in relation to depth.

Photo 7. Extracted roots.

2.4.2. Porewater DOC

Porewater samples (n=12) were collected at 2 different depths throughout the whole experiment twice a week (Tuesday and Friday). After collection, samples were filtered into 13ml plastic vials with CHROMAFIL® 0.45 µm Xtra PET-45/25 disposable syringe filters to ensure there is only dissolved organic carbon in the samples. Thereafter samples were stored frozen at -18ºC. When sufficient number of samples was collected, they were analysed for dissolved organic carbon (DOC) with Shimadzu TOC/TON Analyzer. Ordinarily, 9ml of sample was required. Although when the required amount was not managed to be extracted due to the filtering capacity, dilution factor 1:3 was used.

The analyzer machine works on catalytic oxidation principle, where at high temperatures (~680ºC) catalyst completes oxidation of all forms of C (to CO_2) and N (to NO/NO_2). The products react thereafter with ozone (O_3) with a product of excited state of NO_2 (NO_2*). When the latter returns to original state, the emitted light energy is detected and measured by chemiluminescence. In this experiment, DOC is measured as non-purgeable carbon (NPOC) where acid is added and samples

purged with synthetic air in order to release inorganic carbon, which is then measured by NDIR detection, consisting of light source, cell and detection portion. The detector uses a movable diaphragm that flexes with changing CO_2 concentrations. Light passes through the cell compartment into the detector where the movement of the CO_2 inside the detector flexes the diaphragm, which in turn creates electric signals that reflect to the DOC concentration of the sample. (Schinner et al., 1996)

Due to the low pH values (≤ 4) the presence of carbonates is negligible and therefore total carbon assumed to indicate total dissolved organic carbon (DOC). (Charman, 2002)

2.4.3. Acetate

For measuring acetate concentrations, samples were collected biweekly from 2 different depths – 10cm and 30cm together with samples for DOC, as described in chapter 2.2. When all the planned sampling was finished, samples were sent to Münster, Germany, where Prof. Dr. Klaus-Holger Knorr and his team in Ecohydrology and Biogeochemistry Group were responsible for analysing the samples. Organic acid column on the ion chromatographer (IC) was used. Using this column assures a better separation of organic acids and detects any other low molecular weight organic acid which might be of interest. (Personal communication with prof. Knorr, July 2020)

If the area of the sample peak is lower than the lowest standard, concentrations were displayed as zero, samples shown as zero have an area lower than 0.5 mg. The instrument injects different amounts of sample and depending on concentration the same area is not always the same concentration, although the result displayed is the final values have already been corrected for the injected volume. (Personal communication with prof. Knorr, July 2020)

2.4.4. HWOM

Hot-Water-Organic-Matter Extraction was conducted by an author using the method based on (Heller & Weiß, 2015) and was adjusted according to local possibilities. Firstly, dried peat (105ºC) was milled with the Mixer Mill MM400 with vibrational frequency set at 30 for a period of 60 seconds. 0.5 g of milled peat sample was weighed into 250ml screw-cap bottles (*Schraubglass)* and filled with distilled water (Photo 8).

Photo 8. Preparation for HWOM extraction

All 18 samples (3 per core, 1 for every depth of 10 cm) were placed into water baths and transported into the oven. It was expected that keeping temperature inside the oven around 150ºC (max. for plastic lids) the samples will reach internal temperature of 100ºC needed for the extraction. Measuring after 1h the temperature in the water bath was 96ºC and expected to be the same inside the jar. The samples were left into the oven for 2 hours in total.

After the samples were cooled down, CHROMAFIL® 0.45µm Xtra PET-45/25 disposable syringe filters were used to filter the sample. It was placed in 13ml plastic vials until further analysis, which was carried out with the Shimadzu TOC/TON Analyzer for the measurement of DOC (See 2.4.2 for more detailed description).

2.5. Data analyses statistics

All graphs found in the thesis are made with MS Excel and all statistical analyses were performed in R. The significance levels were determined from Spearman's critical values (p= 0.05). For the comparison of experiment and control group in gas fluxes and isotopes, multilevel mixed-effect regression was used in R (lmer4 package), since the experimental design contains within-subject variables (time) and between-subject variable (clipping). This model is designed to check the interaction of the two variables and assessing whether the clipping had an effect on the gas flux. Random intercepts of plants and time were added to the model. The baseline (before 140h) was accounted for. Furthermore, this kind of design does not have assumptions such as normal distribution or homogeneity of variances, so these do not have to be taken into account.

Other relationships were evaluated with multiple regression models, where all measured variables were checked for significance.

3. RESULTS

This chapter contains all the results from the aforementioned experiments and is the result of the author's own work. Interpretation of the results can be found aligned with statistical analyses and comparison with other similar values found in literature follows in the discussion.

3.1. Physical properties of peat

Water content (WC) values ranged between 88.8% and 93.6% with an average of 91.7%.

Porewater pH values were found to be significantly different at the two depths ($F_{1,46}$=58.31, p<0.001), ranging from 3.93 to 4.45 in upper layer (10cm) and 3.88 to 4.16 in the lower layer (30cm) of the mesocosm and generally decreased with depth. No significant change in pH was observed over time ($F_{1,46}$=1.694, p=0.2) nor between treatments (control vs experiment) ($F_{1,46}$=0.0119, p=0.913). The average values were 4.13 ± 0.12 and 4.14 ±0.14 (at 10 cm) and 3.88 ± 0.07 and 3.88 ± 0.1 (at 30cm) for experiment and control group, respectively. Due to technical issues, there was no pH from the pore water measured before the clipping.

It was observed that the overall share of roots decreased with depth in a significant manner at depth 20 ($F_{2,15}$=17.6, p<0.001) and depth 30 ($F_{2,15}$=17.6 p<0.001) and the share of roots varied between 8.06 and 56.1 ± 13 % in all depths, based on dry mass. More in detail graph can be found as Figure 7, where different shades of blue represent different depth sections of the peat cores. No significant difference in root percentage between the treatment groups was detected ($F_{1,16}$=0.139, p=0.714).

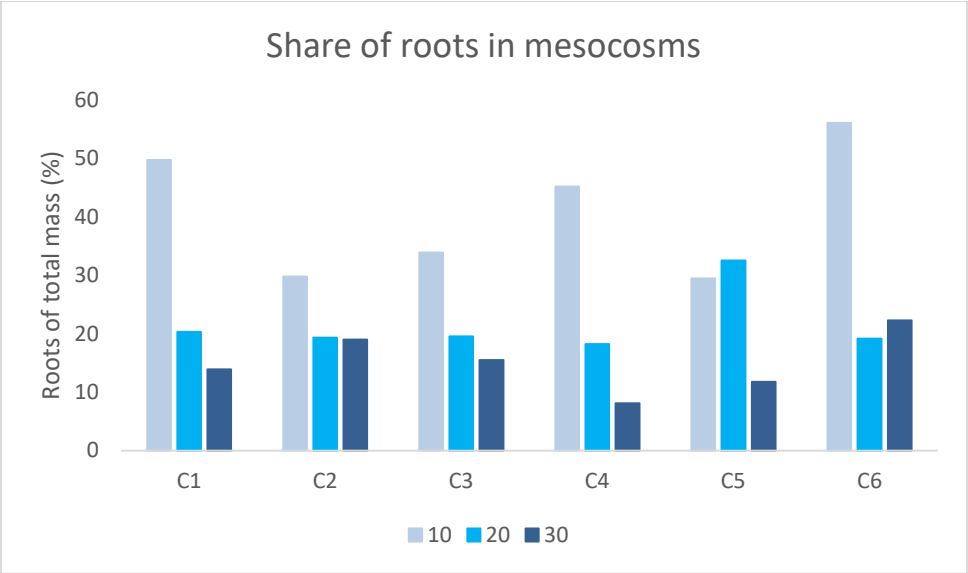

Figure 7. Percentage of root shares in different cores based on dry mass.

3.2. GHG fluxes

Both flux rates were calculated by regressing methane concentration in the mesocosms against time. Each value represents a 4-minute measurement time for each core and was converted to hourly flux. Each mesocosm is represented separately, considered that each core has slightly different properties, and averages of the treatment groups are reported. 25 and 23 measurements were rejected ($r^2 < 0.9$, n=198), for CH_4 and CO_2, respectively.

3.2.1. Methane

Average hourly CH_4 emissions of 0.0234 ± 0.012 g m^{-2} h^{-1} were calculated before the clipping for the experiment group, after clipping there was an increase observed, averaging to 0.0442 ± 0.25 g m^{-2} h^{-1}. In the control group the average hourly CH_4 flux was 0.141 ± 0.12 g m^{-2} h^{-1}. In the experiment group, CH_4 fluxes ranged from 0.058 to 0.16 g m^2 h^{-1} and in the control group, from 0.046 to 0.751 g m^2 h^{-1}.

Appendix 1 – CH_4 flux over time, contains the illustrative changes in CH_4 fluxes over time. From the additional core with only porewater no CH_4 flux was detected and it is therefore excluded from these figures. Figure 8 depicts the averages measured for each core, for cores 1-3 (experiment group) both before and after treatment values are showed separately, indicated by lighter and darker color. The values are expressed in g m^{-2} h^{-1}.

Maximum CH_4 values were detected 362 hours after the clipping in C1 from the experiment group and C5 at the same time in the control group. Average cycles for the fluxes for each treatment group are found to be similar.

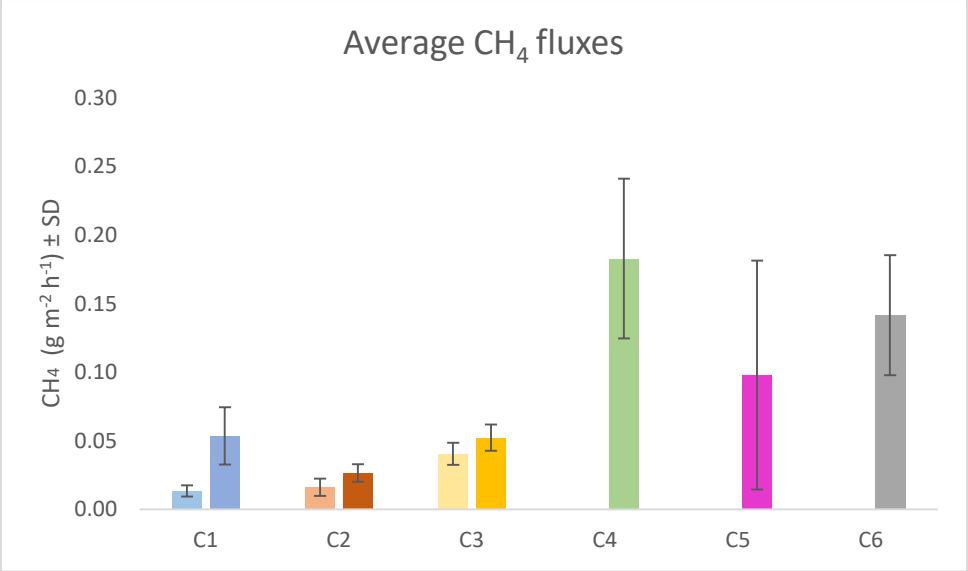

Figure 8. Averages of CH_4 fluxes before and after with standard deviations (SD).

The comparison between experiment and control group revealed that clipping of the *Calluna Vulgaris* had a significant effect on the emissions of CH_4 (ß= -0.106, $t_{137.9}$= -4.09, $p<0.001$) considering the first 140h before clipping as the baseline.

3.2.2. CO_2

Average hourly CO_2 emissions of 6.71 ± 1.97 g m^{-2} were calculated before the clipping for the experiment group. After the clipping there was decrease observed, with average of 5.03 ± 1.82 g m^{-2} h^{-1}. For the control group hourly average was 7.04 ± 1.47 g m^{-2} throughout the experiment.

In the experiment group, CO_2 fluxes ranged from 2.4 to 12.2 g m^2 h^{-1} and in the control group, from 4.13 to 14.6 g m^2 h^{-1} throughout the whole experiment period. The changes in fluxes over time are illustrated in the Appendix 2 – CO_2 flux over time. Figure 9 depicts the averages of corresponding cores. In the experiment group (C1-C3) before and after treatment values are indicated by lighter and darker color.

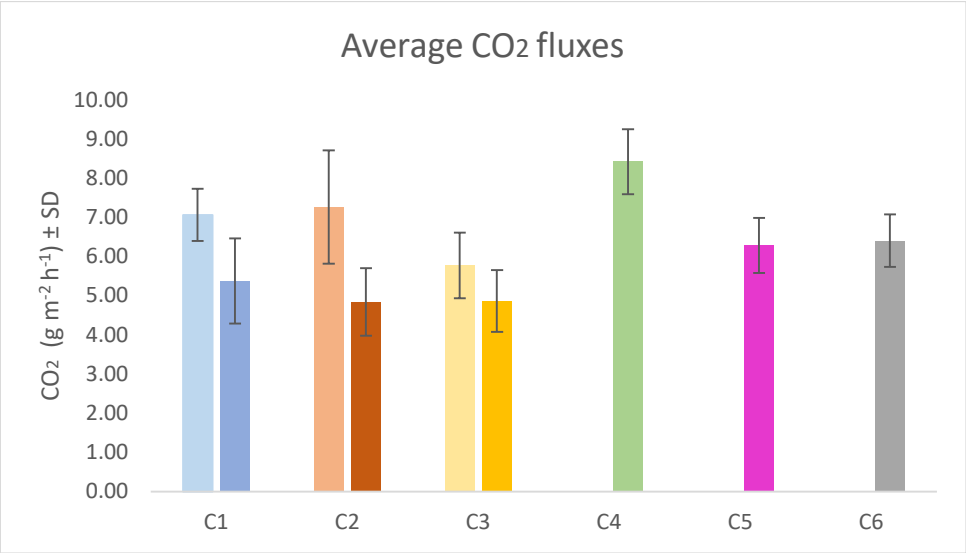

Figure 9. Averages of CO_2 fluxes before and after with standard deviations (SD).

There was statistical significance found between the experiment and control group related to CO_2 emissions (ß= -1.94, $t_{137.9}$= -3.97, $p < 0.001$) considering the first 140h before clipping as the baseline, demonstrating, that clipping had an effect on the CO_2 emissions. Core number 7 with only porewater showed some CO_2 flux, although excluded from the figures.

3.2.3. $\delta^{13}C$

Isotopic signatures were measured simultaneously with the CO_2 and CH_4 fluxes. In the first week of the measurements, $\delta^{13}C$ for CH_4 values in the experiment group (-55.6 ± 2.45‰) were in the same range with the control group, whereas after the clipping the experiment group values were slightly decreasing with the average of -54.1 ± 2.65‰. For the control group, the average values were -55 ± 2.2‰. All averages values can be found on Figure 10. Lighter and darker colours depict before and after treatment values on both graphs, as on previous figures. There was no significant difference found between the experiment and the control group (ß=1.59, $t_{143.7}$=1.64, p=0.104).

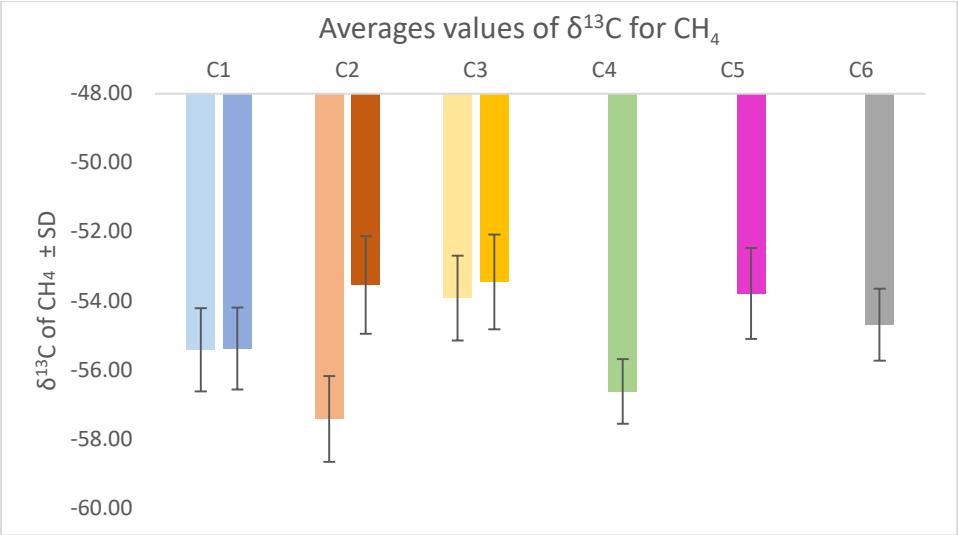

Figure 10. Average values of $\delta^{13}C$ (‰) for CH_4 for all the cores.

$\delta^{13}C$ for CO_2 averaged to -11.2 ± 0.72‰ before and to -10.8 ± 0.67‰ after the clipping in the experiment group. In the control group, the average was 11.2 ± 0.71‰. There was significant difference detected between the experiment and the control group (ß=0.44, $t_{137.9}$=1.99, p=0.0488). Average values are illustrated on Figure 11.

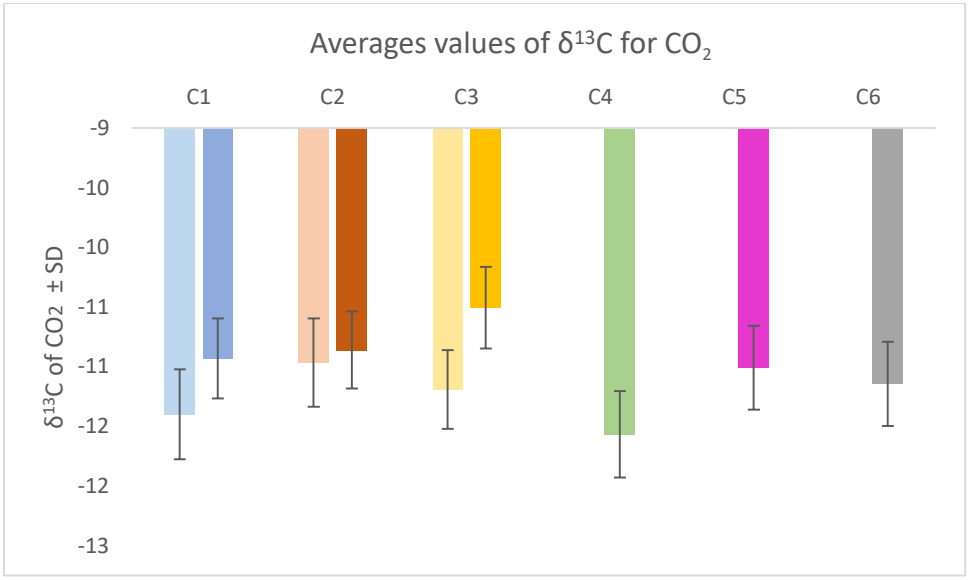

Figure 11. Average values of $\delta^{13}C$ (‰) for CO_2 for all the cores.

3.3. DOC

Average content of DOC in the porewater ranged between 86.6 and 222 mg/L at 10 cm depth and 84.3 and 191.8 mg/L at 30 cm depth, generally decreasing with depth. In the upper layer the concentration averaged to 135.8 ± 6.45 before the clipping and 152 ± 8 mg/L after the clipping in the experiment group. The DOC trend was relatively constant in the control group, with an average of 92.8 ± 4.94 mg/L. Figure 12 & Figure 13 illustrate the DOC concentrations on both treatments groups over time and at different depths. For core 7 (only porewater) DOC concentration was averagely 40.6 ± 2.08, but it is excluded from the graphs.

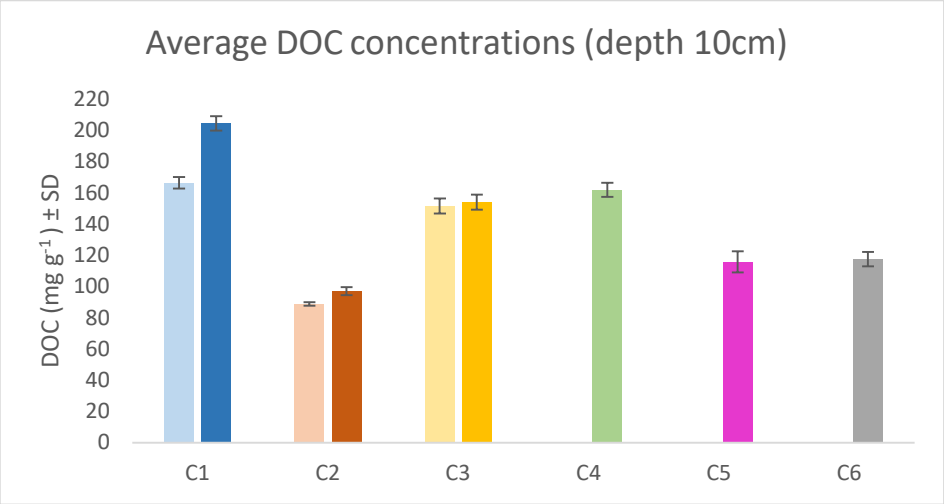

Figure 12. Average DOC (mg g^{-1}) concentration in all the cores in the upper part (10cm depth).

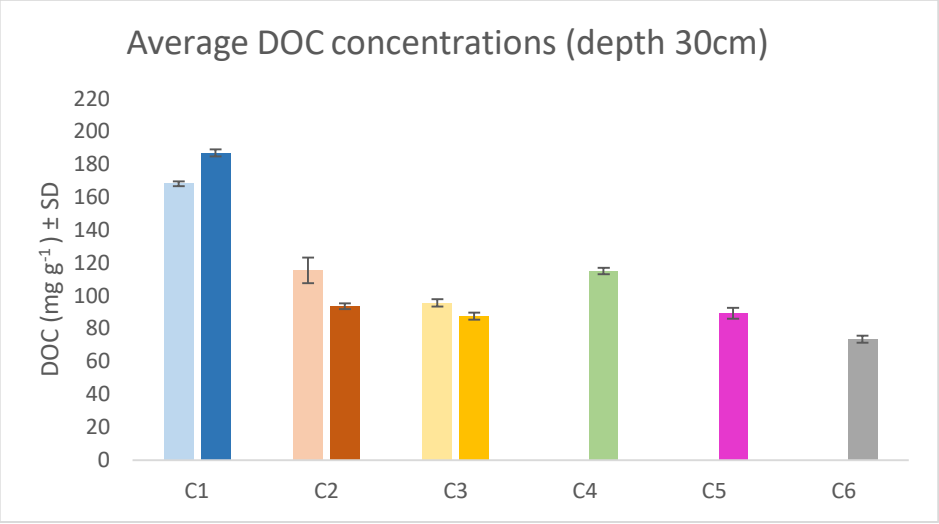

Figure 13. Average DOC concentration (mg g^{-1}) in all the cores in the lower part (30cm depth).

No significant difference was found in DOC concentration over time ($p = 0.98$), but concentrations were found to be significantly different at the two measured depths (10 and 30 cm) ($F_{1,82} = 15.71$, $p < 0.001$), whereas, the treatment (clipping) had a significant effect on the DOC concentrations ($F_{1,82}=8,05$, $p=0.0058$).

3.4. Acetate

Acetate concentrations varied between 0-80.9 mg/L (mean=18.18, median=6.51). Extended data on the acetate concentrations can be found in Appendix 5 – Acetate concentrations. There was no significant difference in acetate concentration over time in any of the mesocosms ($F_{1,65}=0.914$, $p = 0.34$). Although, comparing acetate concentrations between all cores showed a significant difference ($R^2=0.664$, $F_{5,61}=24.12$, $p<0.001$), which are illustrated on the Figure 14 and Figure 15.

Depth played a significant role in acetate concentration ($R^2=0.102$, $F_{1,65}=7.41$, $p=0.008$) with an average of 29.4 ± 2 mg/L for upper layer and 18.04 ± 2.6 mg/L for deeper layer in the experiment group and 24.5 ± 11.8 mg/L and 3.2 ± 1.63 mg/L for the control group, respectively.

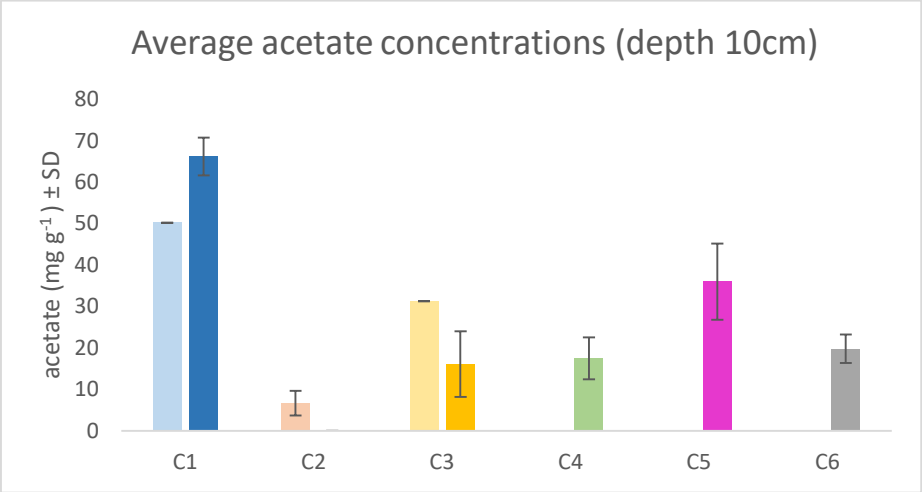

Figure 14. Average acetate concentration (mg g^{-1}) in all the cores in the upper part (10cm depth).

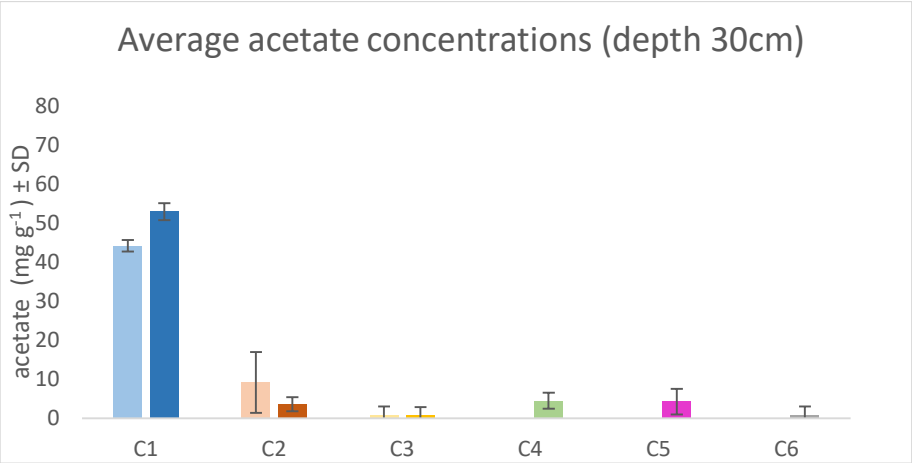

Figure 15. Average acetate concentration (mg g^{-1}) in all the cores in the lower part (30cm depth).

3.5. HWOM

Hot water soluble carbon (C_{HWE}) was measured from the peat, after the draining of the cores, as an indicator of the state of the peat. The carbon content was found to be in the range of 23.5 and 31.2 mg/g and is reported on a dry-mass basis (oven-dried samples at 105ºC). C_{HWE} concentration showed no significant difference between treatment groups (p=0.07), but C_{HWE} content decreased with depth in a significant manner, when comparing depth 10cm and 30cm ($F_{2,15}$=6.21, p=0.046). Figure 16 illustrates depth profiles indicative of hot water extractable carbon for all the mesocosms.

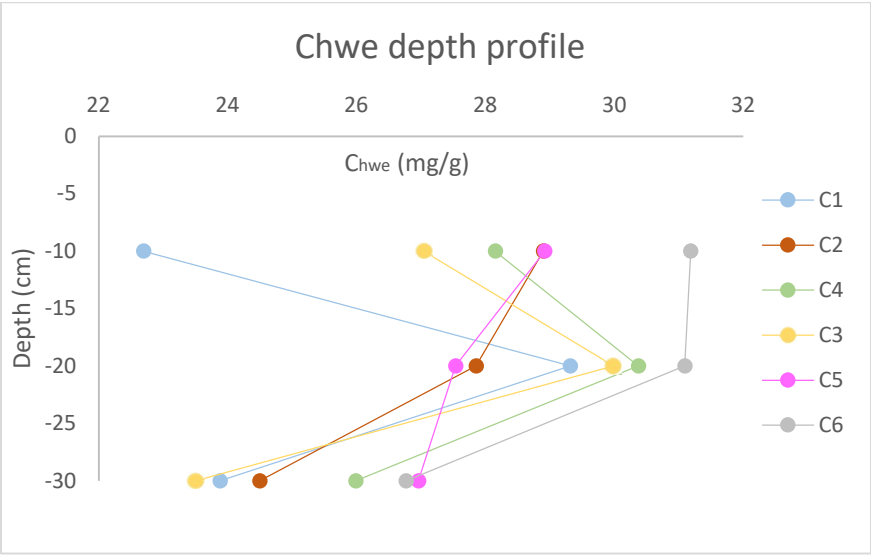

Figure 16. C_{HWE} depth profile for all the cores.

4. DISCUSSION

Several parameters were measured and analyzed within the framework of this experiment. Hereafter the author is relying on the relevant literature to assess and compare the findings of this experiment and discuss the potential reasons behind in the context of an alpine peat bog.

4.1. Peat properties

Water content values found (average 91.7%) is in accordance with frequently reported values, e.g. (Zaccone et al., 2009) reported WC values to be averagely 90.9% and reflects the impressive characteristic of peat to hold immense amount of water.

Ordinarily, pH values decreased with depth, however there was no significant change over time nor the treatments. It is reported, that the acidic conditions inhibit the activity of methanogenic bacteria, who prefer pH range 6-8, opposed to ombrotrophic bogs having a pH of the pore water usually around 4. Latter values is similar to those reported in the present work – at 10cm depth 4.13 ± 0.12 and 4.14 ±0.14 and at 30cm depth, 3.88 ± 0.07 and 3.88 ± 0.1 for experiment and control group, respectively. (Dunfield et al., 1993; LAI, 2009)

There was no significant difference found in the share of roots between the treatment groups. This observation might indicate that the roots did not decay due to the clipping of the plant, as was initially expected and the fine roots are not accounted for in this work. (Shotyk & Steinmann, 1994) Decrease of root proportion in relation to depth was observed and this is reported previously also by (Gill & Burke, 2002) and (Schwieger et al., 2021). |The latter reported decrease of root biomass with depth for finer roots (<2mm) both for drained and rewetted forested peatlands, however observed an increase for 2-5mm roots in depth.

The difference in values of pH at various depths reflect the importance of reactions happening on specific layers of peat. Acidity is dependent on the organic acid formation when the plant matter is decomposing. (Shotyk & Steinmann, 1994) Similar pH values have been reported in many other ombrotrophic bogs.

4.2. Porewater

Regarding the DOC values in the porewater, the values found (86.6 and 222 mg/L at 10 cm depth and 84.3 and 191.8 mg/L at 30 cm depth) are in accordance with findings by (Limpens et al., 2008), who found in their research in an UK blanket bog, that DOC concentration was significantly greater in areas where *Calluna Vulgaris* was dominant, compared to ones dominated by *Eriophorum* or *Sphagnum*. (Waddington et al., 2008) found similar DOC values in cutover and restored peatlands and (Glatzel et al., 2003) reported somewhat lower values in the range from 25 to 110 mg/L. In northern peatlands, DOC concentrations in the range 20-60 mg/L have been detected. (Blodau, 2002)

Values for hot water extractable carbon (between 23.5 and 31.2 mg/g) are similar to those published by (Heller & Weiß, 2015). (Szajdak et al., 2019) reported two times lower values and identified lower decomposition in *Sphagnum* covered peat, compared to one with *Carex*. They furthermore indicated significant drop in relation to depth in C_{HWE} concentrations.

In half of the mesocosms shoot biomass was removed and with it less root-borne acetate is suspected and thus a changed CH_4 source, based on the idea that the ^{13}C isotopy reflects the path that form CH_4. It is not surprising to have higher concentration in upper levels, where roots are present or decomposition of fresh roots may take place due to the clipping of the plants, which was clearly the case in the present experiment. Transition between higher and lower concentrations are sharp, since acetate is the primary intermediate. In some samples no concentration (or lower that of the detection limit) was observed, therefore the high values are not artifacts but rather actual representation of the porewater. (Zhang et al., 2019) reported acetate values of 2.88-7.83 µmol l^{-1} (equivalent to 0.17-0.46 mg l^{-1}), which are noticeably lower than found in the current thesis. However, the values are in accordance with research by (Duddleston et al., 2002), who reported maximum acetate concentrations reaching up to 1.0 mM (59 mg l^{-1}), when saturated with water in upper peat layers. In literature, usually lower values are represented, as it is the case in the current experiment at lower depths, although porewater from the root zone is rarely analysed in order to report such high values. (Personal communication with prof. Knorr, August 2020)

For hot water extractable carbon, the tendency of increased root zone is visible in the mid-depth (20cm), which indicates that this is where most of the roots are found. There is no notable difference between the control and experiment group, which leads to believe, that perhaps the clipping of the plants did not successfully lead to decaying of the roots, as was expected initially. For 30cm depth it can be clearly seen, that the C_{HWE} values drop for all the cores. For cores 5 and 6, however,

the decrease is less defined. These two cores also have lower CH_4 and CO_2 fluxes as well as lower DOC concentration compared to core number 4.

4.3. Importance of plant cover

Plants have a complex role in the production as well as the transport of methane in peatlands, where they live in anoxic waterlogged conditions, hence they need to transport oxygen down to the roots in order to provide roots with oxygen for survival. Production and exudation of organic carbon from roots stimulates methanogenesis, although, vascular plants increase the O_2 in the rhizosphere, therefore suppressing methanogenesis, which requires anaerobic conditions. (Thomas et al., 1996)

Methane emissions differed significantly between the experiment and the control group, showing that the vegetation has an impact on the CH_4 emissions. This distinction between the experiment and the control group CH_4 fluxes highlights the importance of plant cover in CH_4 transport to the atmosphere. It might be indicative of the vegetation's effect on the activity of bacteria – oxygen is transported to the rhizosphere via plants, which may enhance the oxidation of methane therefore reducing the total CH_4 emissions. (Shannon et al., 1996)

(Van Winden et al., 2012) compared mesocosms with and without *Sphagnum* cover and found significant differences – mesocosms with removed *Sphagnum* cover had significantly higher methane fluxes, which furthermore is a sign of the importance of vegetation cover on peat. The values presented in this work are in accordance to those of (LAI, 2009), who reported hourly CH_4 emissions between 0.0002 and 0.0033 g m^{-2}.

A recent peat mesocosm experiment from Pürgschachen Moor with root exudate addition showed increase in CH_4 fluxes after adding the route exudates from below 0.03 up to 0.09 mg m^{-2} h^{-1}. However, there was no noticeable increase in CO_2, which remained below 5 mg m^{-2} h^{-1} (unpublished data, personal communication with Mag. Maier, May 2020).

Furthermore, (Garnett et al., 2020) found in their radiocarbon dating experiment, that clipping the vegetation strongly influences the isotope composition and the methane fluxes from peatlands. They reported 70-94% decrease in emission rates in absence of plants. (Marinier et al., 2004) presented that CH_4 emissions from the areas with clipped plants (*E. vaginatum)* were 79% lower than from peatlands with undisturbed vegetation cover. Moreover, (Shannon et al., 1996) claimed that in case of *S. Palustris* the contribution was 64-90% of all net methane flux. (Fisher et al., 2017) detected differences in $\delta^{13}C$ on different sites, posing that it might be the result of distinctive emission

processes. Sites with *Eriphorum angustifolum* cover released more methane than *Sphagnum*-covered areas.

4.4. Dominant methanogenic pathway

Although (Leroy et al., 2017) found that presence of plants (*Molinia caerulea)*, in addition to *Sphagnum*, increase both CO_2 and CH_4 fluxes, in the present work the CO_2 fluxes were decreasing in the absence of plants. Decreasing emissions of CO_2 in the experiment group might indicate presence of methanogenic bacteria, who are responsible in consuming CO_2 for production of CH_4 via hydrogenotropic pathway (Shannon et al., 1996). As the CH_4 flux is indeed rising in the experiment group, the author acclaims, that it might be due to the dominance of hydrogenotropic pathway in Pürgschachen Moor, as hypothesized. Conversely, (Drollinger, Kuzyakov, et al., 2019) found a diurnal cycle of CH_4 release in the same study site, which is an indicator of an acetoclastic pathway. Acetate can be produced from organic carbon or CO_2 and consumed by conversion to CH_4, CO_2 or biomass (Conrad, 2005). This is explained in more detail previously (See 1.2).

Methane isotopic signature of -71‰ is suggested by (Fisher et al., 2017) to represent boreal wetland emissions and -59‰ is typically used for wetlands globally. (Zhang et al., 2019) analysed stable carbon isotope compositions in an alpine wetland and found that methane produced in methanogenesis was rich in ^{13}C and $\delta^{13}CH_4$, with values ranging between -28 and -20‰. The value of $\delta^{13}CH_4$ from acetate is higher than of CO_2 in hydrogenotrophic pathway, generally varying between -60‰ and -20‰. Moreover, (Oleg R. Kotsyurbenko et al., 2004) demonstrated in his research, that both pathways co-exist and one is not more important than the other, as the values for $\delta^{13}CH_4$ were found in the range between -60 and 55‰, indicating that CH_4 is produced via both pathways in the peat layer with a depth of 30-50 cm. The same findings are supported in the research of (Zhang et al., 2019). However, in the deeper layer (70cm), they found that CH_4 was produced only via hydrogenotrophic pathway, with values lower than -70‰ as the methane production is happening at lower rates (Hornibrook et al., 2000; Keller & Bridgham, 2007; Michael J. Whiticar, 1999). This is in line with the finding in the current work, where the acetate levels were significantly lower in the lower peat layer (See Chapter 3.4).

Furthermore, (Lansdown et al., 1992) reported CH_4 values between -61 to -83% (mean -74 ±5 ‰) and found no $\delta^{13}C$ trend related to season or CH_4 flux rate in mesocosm study of a northern peatland. Same research detected consistent $\delta^{13}C$ of the CO2 flux (mean -24.5±0.8 ‰). (Shannon &

White, 1996) found acetate to consistently and uniformly building up for 4 months, starting from less than 20µM in the beginning of the year and reaching maximum of 600-1200 µM in April. Acetate concentrations went back to below 20 µM by May-June. However, when the water table was above the surface of the peat, no accumulation of acetate was observed. Acetate was constant at 200 µM in the porewater below 15-20cm. Methane values of δ^{13}Cwere higher in July and August due to the OM decomposition into acetate being the dominant pathway for methane production. They further suggested that the accumulation of acetate and its consecutive consumption by acetoclastic methanogens leads to increase in δ^{13}C values. (Shannon & White, 1996) (Zhang et al., 2019) reported acetate concentrations of 2.88–7.83µmol/L and significant decrease over time in an alpine wetland.

All in all, even though in the current experiment there was no significant difference found between the control and the experiment group for $\delta^{13}CH_4$, based on proposed values mentioned by others (Conrad, 2005; Fisher et al., 2017; Zhang et al., 2019), it can be acclaimed that in Pürgschachen Moor the hydrogenotrophic pathway is dominant, with average $\delta^{13}CH_4$ values of -55 ± 2 ‰, although both pathways may coexist.

CONCLUSIONS

Production of methane is result of acetoclastic and hydrogenotrophic methanogenic pathways, which contribute in different rate and the share depends on availability of organic matter. Research has concluded, that acetoclastic methanogenesis is responsible for about 2/3 and hydrogenotrophic for 1/3 of CH_4 production.

During this rather short period of measurements of the peat mesocosms, variations were observed regarding methane and carbon dioxide fluxes as well has DOC and acetate of porewater. Albeit, with the findings discussed in the previous chapter, both of the hypotheses (see Chapter 1.4) can be accepted, therefore concluding that, based on the literature research done, values observed and the analysis made:

- Methane release in the experiment and control group is significantly different (*Sphagnum*-cover vs *Calluna Vulgaris*-cover, see more Chapter 3.2.1)
- In Pürgschachen Moor hydrogenotrophic pathway is dominant, although both pathways coexist (see more Chapter 4.4)

Carbon dynamics in peatlands are complex, with many processes involved. There are many factors involved, which were not all included in the current experiment. Vegetation cover can alter the greenhouse gas emissions from a peatland to some extent, as observed in this study, since CH_4 is transported to the atmosphere both via physical (ebullition, diffusion) and biological (through plants) pathways. Therefore, there lies a need for a more in-depth and detailed studies to reveal the ecological mechanism and complex dynamics of methane production and methanogenic pathways in an alpine peat bog.

Although it was presumed that peat cores were intact and without any additions, there was still a certain distance from the ecological reality at Pürgschachen site, of which the author is aware of. The measurement of gas fluxes using mesocosms can raise some questions. The CH_4 and CO_2 fluxes reported in this study should not be extrapolated to ecosystem levels, as high spatial variability

has been reported by many studies. For this, *in situ* measurements and using eddy covariance towers would be beneficial.

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APPENDICES

Appendix 1 – CH_4 flux over time

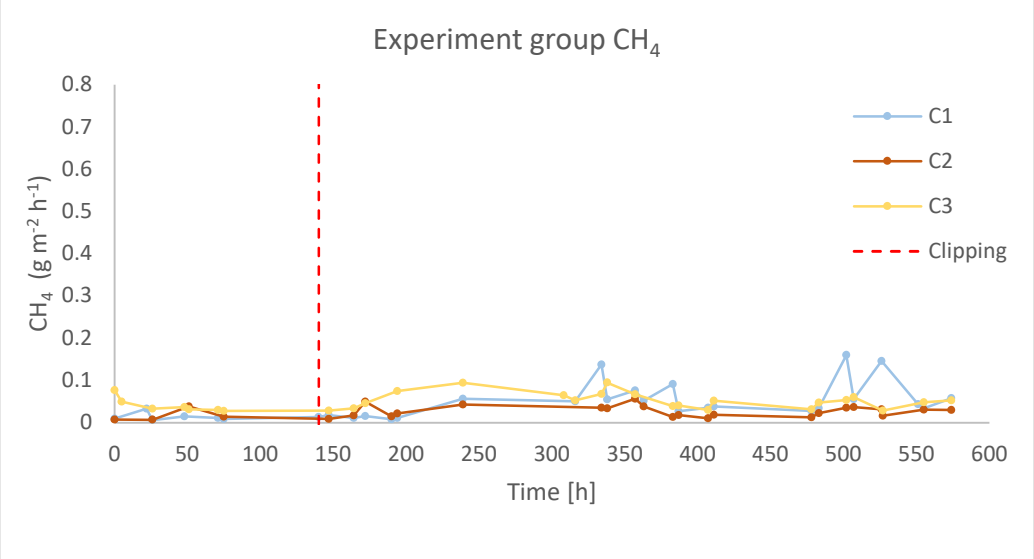

Figure 17. CH_4 flux (g m^{-2} h^{-1}) over time for cores 1-3 in the experiment group.

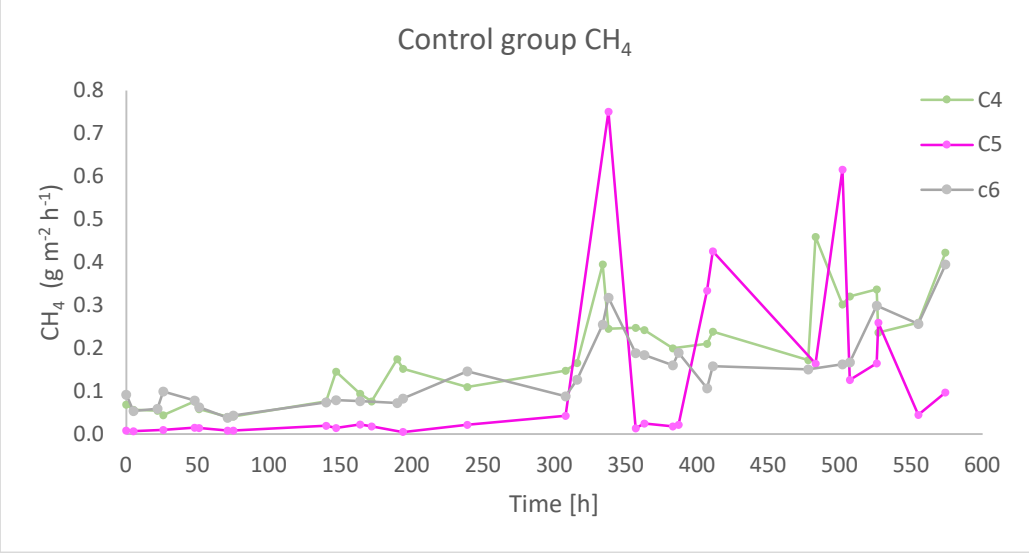

Figure 18. CH_4 flux (g m^{-2} h^{-1}) over time for cores 4-6 in the control group.

Appendix 2 – CO_2 flux over time

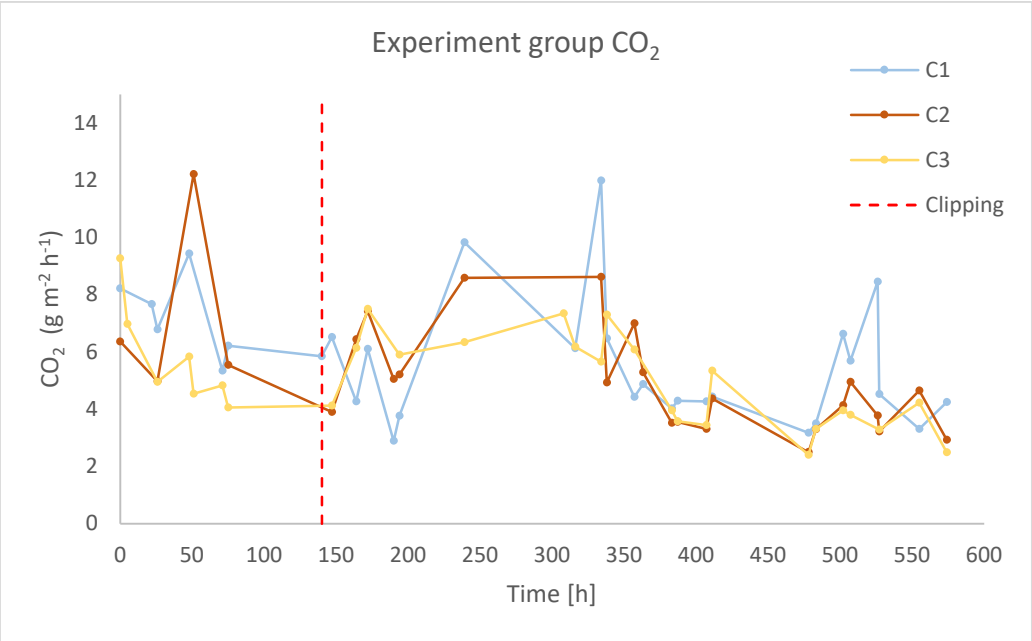

Figure 19. CO_2 flux ($g\ m^{-2}\ h^{-1}$) over time for cores 1-3 in the experiment group

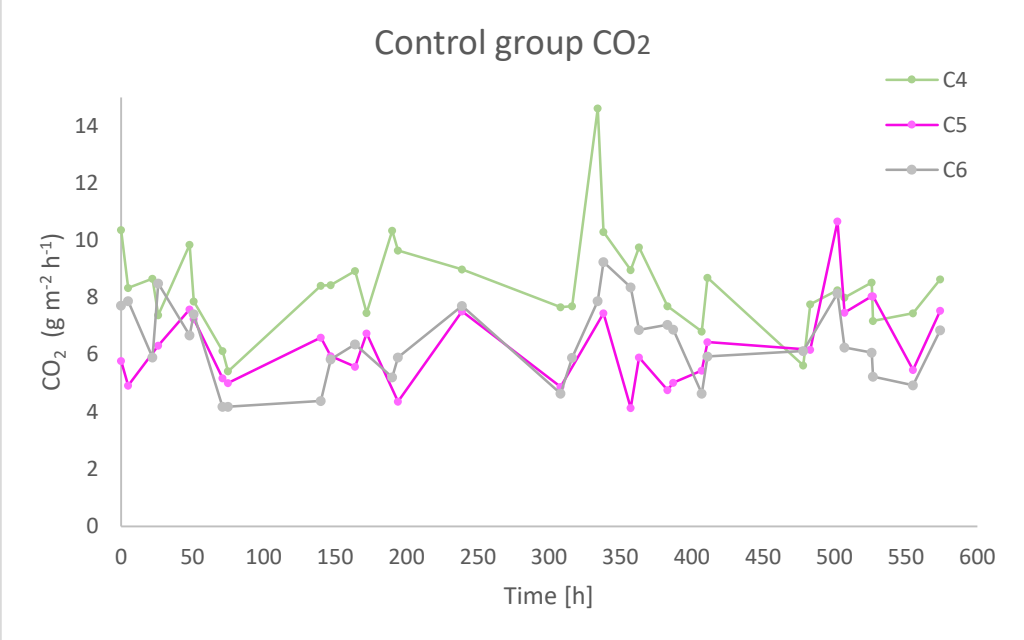

Figure 20. CO_2 ($g\ m^{-2}\ h^{-1}$) flux over time for cores 4-6 in the control group.

Appendix 3 – $\delta^{13}C$

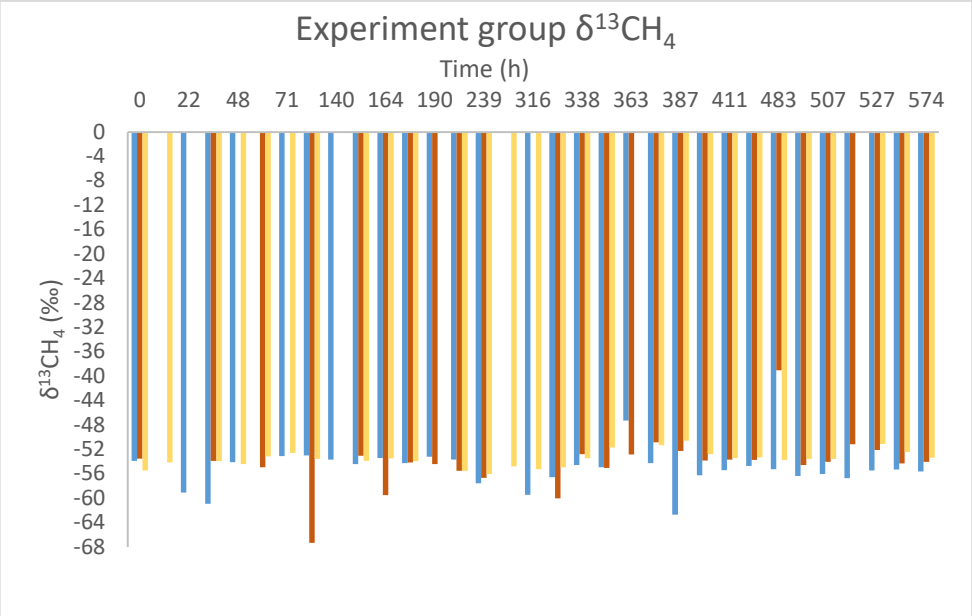

Figure 21. $\delta^{13}CH_4$ values over time in the experiment group.

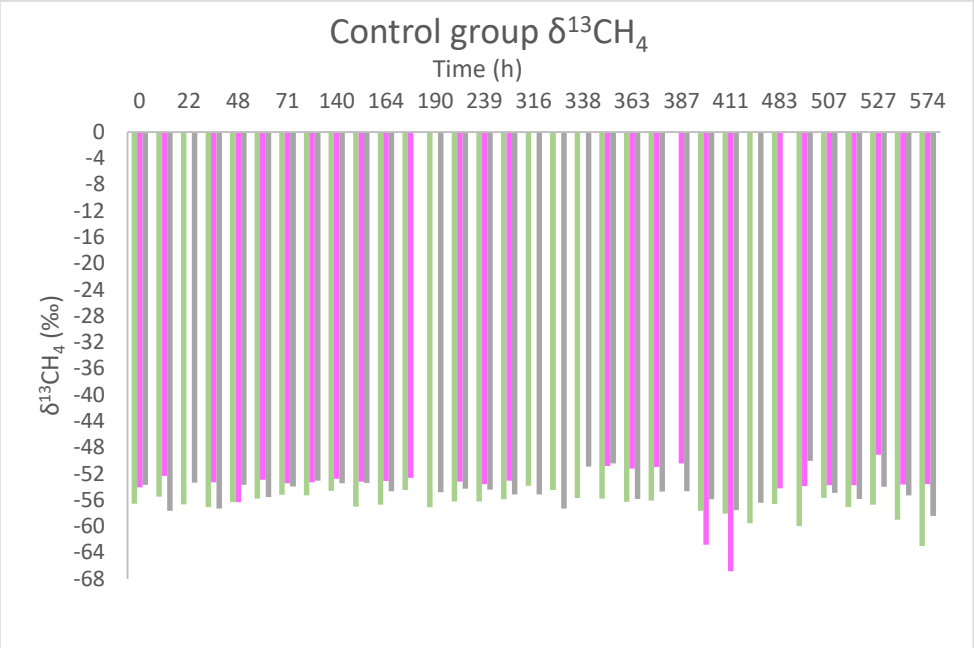

Figure 22. $\delta^{13}CH_4$ values over time in the control group.

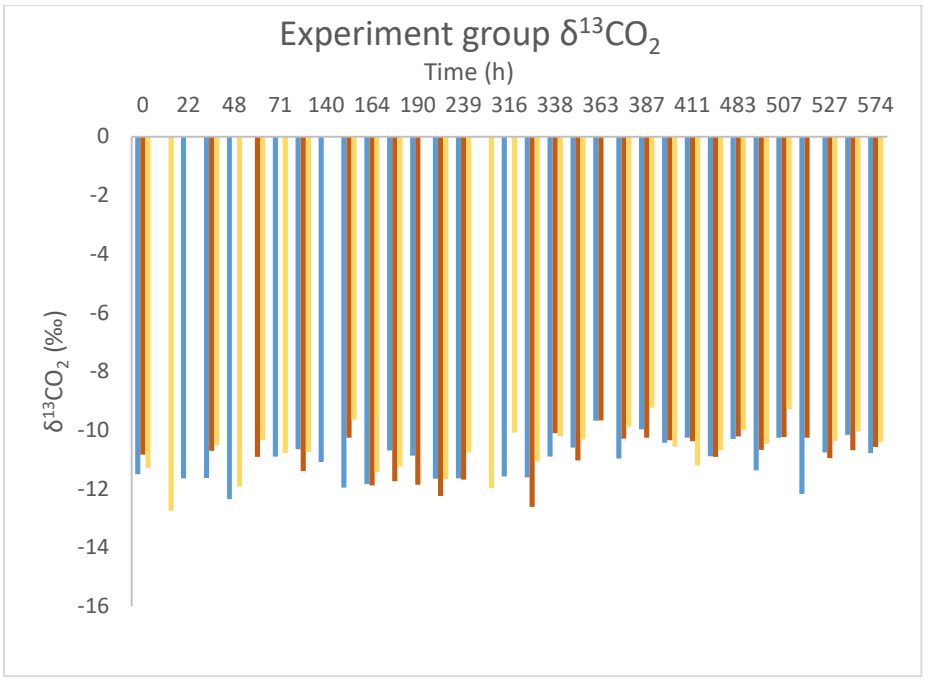

Figure 23. $\delta^{13}CO_2$ values over time in the experiment group.

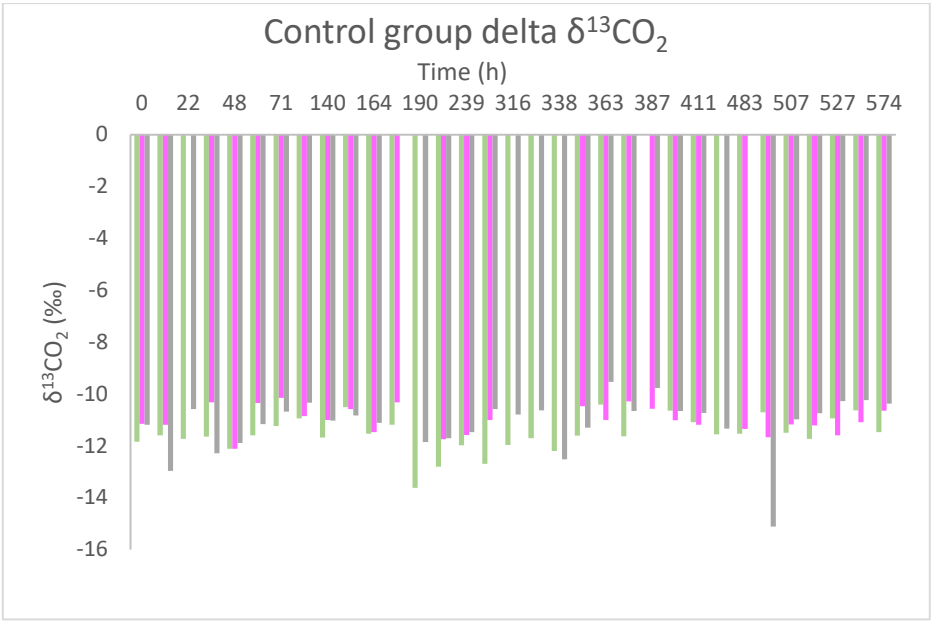

Figure 24. $\delta^{13}CO_2$ values over time in the control group.

Appendix 4 – DOC concentrations

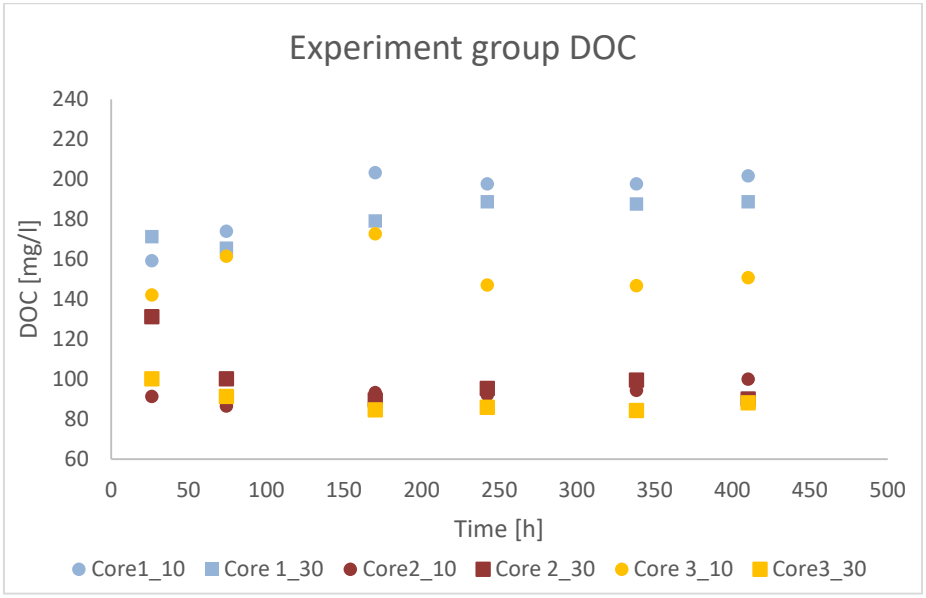

Figure 25. DOC concentrations at different depths in the experiment group (10cm and 30cm).

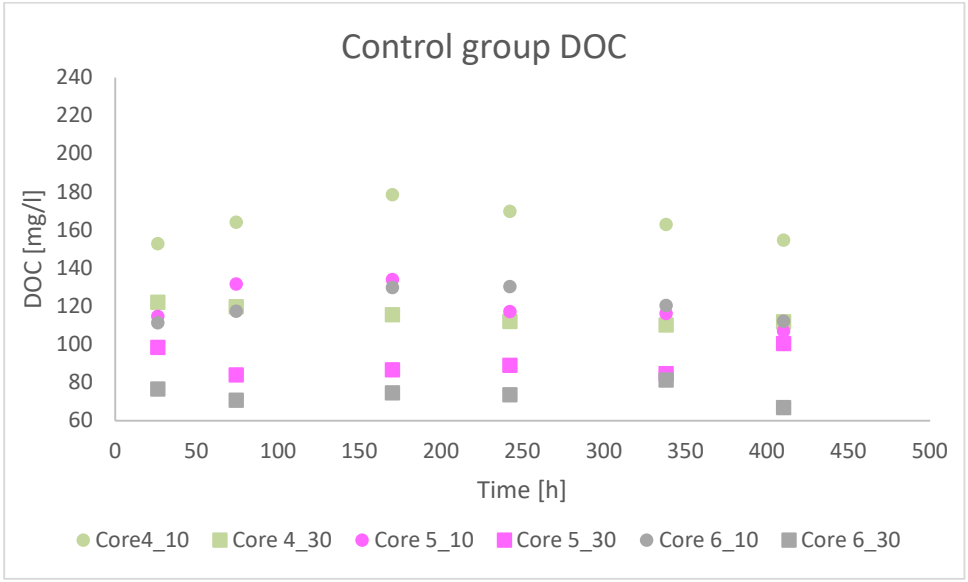

Figure 26. DOC concentrations at different depths in the control group (10cm and 30cm).

Appendix 5 – Acetate concentrations

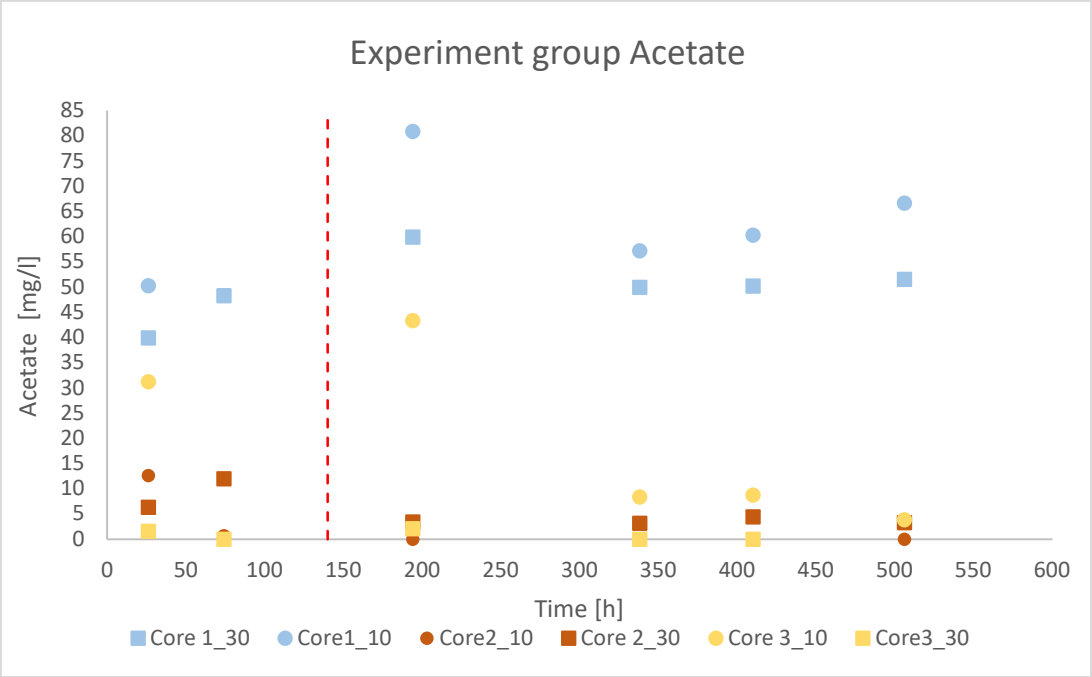

Figure 27. Acetate concentrations in the experiment group at depths 10cm and 30cm.

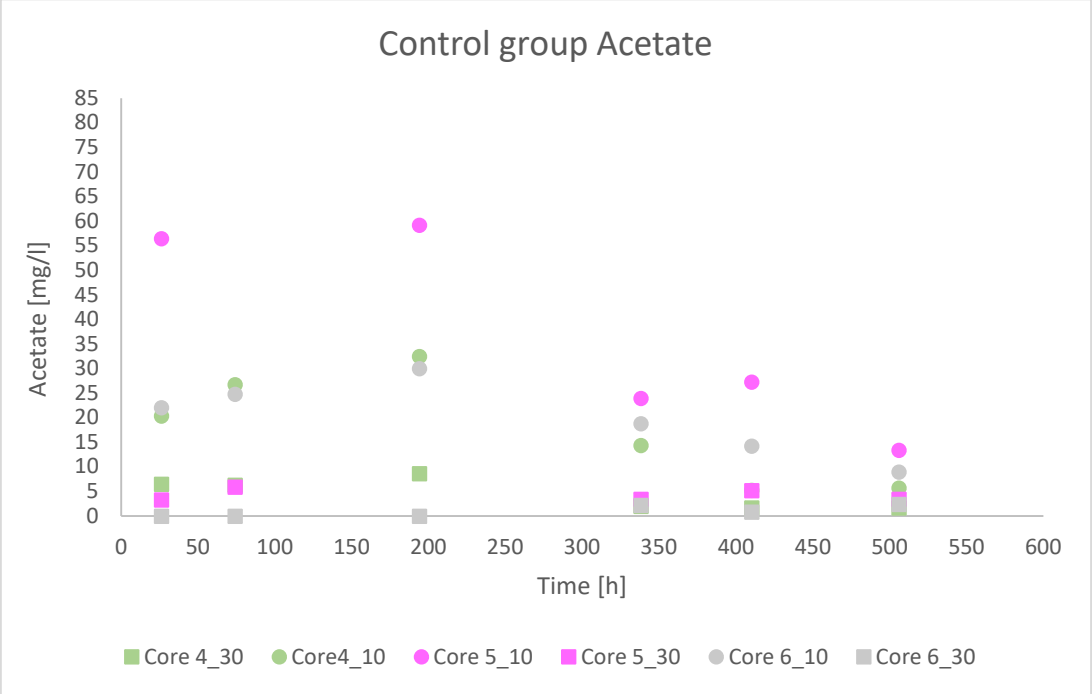

Figure 28. Acetate concentrations in the control group at depths 10cm and 30cm.