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Increased Methane Uptake Under Long-Term Warming in Well-Drained Sub-Arctic Soil

A Field Study of Gas Fluxes at Two Icelandic Sites Using Open-top Chambers and Methanotrophy Inhibition

Master thesis in Industrial Ecology

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Cover picture: Open-top chambers at one Icelandic site (the Heath site) at dusk.

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Abstract

Methane (CH_4) is the green house gas with the second largest contribution to global warming. Well-drained soils make up 87% of the Arctic and have been observed to be a CH_4 sink. There exist several CH_4 sources and sinks in the Arctic. One of the CH_4 sinks in the Arctic is CH_4 oxidation by soil microorganism. Soil CH_4 fluxes are depending on two microorganisms; methanotrophs which oxidize CH_4 and methanogens which produces CH_4. However, there exists knowledge gaps about the size of Arctic CH_4 soil uptake, the drivers of the activity level of CH_4 consuming and producing microbes along with how the CH_4 fluxes will change with global warming. This study measured CH_4 fluxes at two sub-Arctic Icelandic sites with well-drained soil under ambient and passively heated conditions. The passive heating was obtained through open-top chambers (OTC's). CH_4 fluxes were measured at one moss-dominated site (Moss site), and one colder, more species rich heath site (Heath site).

The results showed a 31,7% increase in CH_4 uptake at the Moss site (p<0,05), where the average uptake shifted from 0,790 ± 0,422 in Control plots to 1,04 ± 0,472 $mg[CH_4]m^{-2}d^{-1}$ within the OTC plots. The size of these fluxes is in line with previous studies of CH_4 sinks in the Arctic during the growing season. Soil moisture and soil temperature were measured, but neither factor could be determined as a driver behind the increased CH_4 uptake.

At the Heath site, no change in CH_4 uptake was observed when comparing the OTC and Control plots (p=0,84). Further more, the Heath site had a lower CH_4 uptake than the Moss site with an average uptake of around 0,037 ± 0,03 $mg[CH_4]m^{-2}d^{-1}$, about a tenth of what previous studies observed in well-drained Arctic soils during the growing season. The small CH_4 sink as well as no significant CH_4 flux change between the treatments is believed to be due to a late snow melt, leading to higher soil moisture, lower soil temperature and a lower microbial activity.

Inhibitions of methanotrophic activity were conducted at the Heath site with acetylene gas to separate the soil's cooccuring production and consumption of CH_4. A slight CH_4 production was observed within the soil for some of the inhibitions, indicating that well-drained Arctic soils have active methanogens. However, too few inhibition experiments could be completed for statistical analysis.

Key words: Methane uptake, methane sink, acetylene, passive warming, long-term warming, Arctic, sub-Arctic, open-top chambers, methanotrophs, methanogens

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Acronyms

Below is the list of acronyms that have been used throughout this thesis listed in alphabetical order:

CH_4	Methane
CO_2	Carbon dioxide
Cu	Copper
GHG	Green house gas
GPP	Gross primary production
IPCC	Intergovernmental Panel on Climate Change
ITEX	International Tundra Experiment
m.a.s.l.	Meters above sea level
NEE	Net Ecosystem Exchange
OTC	Open-top chamber
PAR	Photosynthetically active radiation
R_{eco}	Ecosystem Respiration
WFPS	Water filled pore space

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Introduction

Green house gas (GHG) emissions are the driving factor behind climate change and currently more GHGs are emitted than what is taken up at a global scale, leading to an increase concentration GHGs in the atmosphere (IPCC, 2023). There are mechanisms which remove GHGs from the atmosphere, here after called sinks, but knowledge about the size of them and how they will be affected by future global warming is limited (IPCC, 2023). One sink that is believed to be underestimated is the uptake of methane (CH_4) by soil microorganisms in well-drained mineral soils (D'Imperio et al., 2017; Juncher Jørgensen et al., 2015; Keuschnig et al., 2022). This study aims to identify and close some of these knowledge gaps by measuring CH_4 fluxes under warmed conditions at two well-drained sub-Arctic sites in Iceland.

1.1 Background

The Earth is currently experiencing a trend of rising temperatures (IPCC, 2023). 2023 was the hottest year in modern history, with a global annual average of 1,18°C higher temperatures compared to pre-industrial levels (based on the years 1850 to 1900) (IPCC, 2023; NOAA National Centers for Environmental Information, 2024). Climate change is believed to be the cause of several global trends (Papalexiou & Montanari, 2019). In the last 50 years, climate zones have moved poleward (García Criado et al., 2023; IPCC, 2023). Changes in the water cycle and precipitation patterns have been observed, with more dry periods but also more heavy precipitation events (Papalexiou & Montanari, 2019). Further more, extreme weather events have increased in frequency, a trend predicted to continue (Rahmstorf & Coumou, 2011).

Anthropogenic increases of GHGs is driving global warming, and the main contributes are carbon dioxide (CO_2), CH_4 and nitrogen dioxide. The increases are caused by human activities such as burning of fossil fuels, transportation, agriculture and other land use changes (IPCC, 2023; Nazaries et al., 2013). During the last century the GHG sources have been larger than the sinks, leading to an imbalance in the global GHG budget (IPCC, 2023). Thus, understanding the drivers of the GHG sources and sinks and how they will be affected by global warming is important to increase our understanding of future climate scenarios.

The Arctic is one area where regional warming is larger than the global average (IPCC, 2023). Currently, the Arctic is experiencing warming at up to four times higher rate compared to the global average, a term often referred to as Arctic amplification (Huang et al., 2017; Rantanen et al., 2022; Vose et al., 2021). In 2023, an average temperature increase of 2,55°C was observed in the Arctic compared to the pre-industrial levels, which was 1,37°C higher than global averages (NOAA National Centers for Environmental Information, 2024). 19 models over Arctic warming saw a range of predictions between 4 to 13°C increase by the year 2100 compared to 1961-1990 (Chylek et al., 2024). The degree of heating is also seasonally bound, with a higher increase of temperature seen in autumn and winter compared to spring and summer (Zona et al., 2016). The increased rate of global warming in the Arctic is believed to be a combination of several different feedback systems reacting to increased GHG levels and higher air and soil temperatures (IPCC, 2023).

The second largest contributor to global warming after CO_2 is the powerful GHG gas CH_4 (Conrad, 2009) and 0,5°C of global warming to date is estimated to be a result of CH_4 emissions (Jackson et al., 2024). The global warming potential for CH_4 at a 100 year time scale is 28 times higher compared to CO_2 (Balcombe et al., 2018). However, CH_4 has a steady-state lifetime at 9,1 ± 0,9 years in the atmosphere (IPCC, 2023), which leads to the short term global warming effect being much higher. Thus, a decrease in net CH_4 emissions could potentially yield lower global warming over the next few decades (Jackson et al., 2024).

The change in atmospheric CH_4 concentration depends on the sum of the CH_4 sinks and sources. There are both natural and anthropogenic CH_4 sources, while CH_4 sinks are dominated by natural processes (Jackson et al., 2024). CH_4 sinks consist mainly of abiotic processes in the atmosphere and biotic reactions in the form of microbial oxidation in soil (D'Imperio et al., 2017).

One crucial piece to predicting future CH_4 budgets is to understand how the sources and sinks will be affected by climate change and global warming. The International Tundra Experiment (ITEX) is a global research network with an aim to study the impact of global warming in Arctic and Alpine tundra biomes (G. H. Henry et al., 2022). ITEX was founded in 1990 when a group of ecologists met to discuss ways to study the anticipated changes in tundra vegetation and especially major circumpolar vascular plant species' responses to warming conditions (G. Henry & Molau, 1997). In the initial stage of the ITEX network it was decided to use a simple, inexpensive and passive warming experiment, more specifically open-top chambers (OTC's). The OTC's function as greenhouses without roofs and on average the air temperatures inside OTC's increase with 1-3°C (Hollister et al., 2023). By comparing plots with OTC's with paired Controls, one can preform experiments like measuring effects of warming on plants and observe shifts in soil CH_4 uptake and emission during warmed conditions.

Soil CH_4 fluxes depend on two types of microorganisms, methanogens and methanotrophs (Guerrero-Cruz et al., 2021; Keuschnig et al., 2022). Methanogens are archaea which produce CH_4 under mainly anaerobic conditions, while methanotrophs are bacteria and archaea that oxidizes, i.e. consumes, atmospheric or biologically produced CH_4, under aerobic or anaerobic conditions (Guerrero-Cruz et al., 2021; Nazaries et al., 2013). The CH_4 flux from soils is thus the sum of the CH_4 production and CH_4 uptake by these microorganisms. The methanogens and methanotrophs are affected by a large variety of environmental conditions, for example soil moisture and soil temperature (Guerrero-Cruz et al., 2021; Semrau et al., 2010).

The current Arctic CH_4 budget is biased towards the carbon-rich wetlands, which act as CH_4 emitting hot-spots (Bao et al., 2021; van Huissteden et al., 2011), while less attention has been given to the carbon-poor, CH_4 consuming mineral soils that cover 87% of the region (Oh et al., 2020). Well-drained mineral soil within Arctic tundra has been shown to be a CH_4 sink, but in recent years it has been hypothesized that the size of the soil CH_4 sinks have been underestimated (D'Imperio et al., 2017; Juncher Jørgensen et al., 2015). Many studies about CH_4 soil fluxes have been performed recently, but studies about Arctic soil CH_4 uptakes are underrepresented (Christiansen et al., 2015), with large knowledge gaps. The knowledge gaps include how CH_4 oxidation is affected by factors such as temperature and soil moisture and how ecosystem types and vegetation may impact the size of Arctic soil CH_4 sources and sinks (Christiansen et al., 2015; Juncher Jørgensen et al., 2015; Parmentier et al., 2024; Poppeliers et al., 2022).

Iceland is located in sub-Arctic and is dominated by a dry tundra biome with mineral soil (Jónsdóttir et al., 2005), and this study will analyze two well drained ITEX sites in Iceland with different vegetation types. We hypothesize that there will be a net CH_4 uptake at both sites, and that both methanogenic and methanotrophic activity will be observed. Further more, we hypothesize that the warmed OTC plots will cause an increase in CH_4 sinks, by increasing the activity of methanotrophs more than the activity of methanogens.

1.2 Aim

The aim of the project was to increase the understanding of the current CH_4 uptake under warmed conditions in the sub-Arctic, more specifically at two ITEX sites in Iceland. Then, the results of this study were compared and discussed in relation to previous studies over CH_4 uptake in the Arctic during the growing season.

The project was divided into two parts. The first part consisted of a field trip where *in situ* measurements at two sites in Iceland, both in Control plots and in plots under a passive warming treatment. Measurements included CH_4 and CO_2 fluxes as well as soil sampling. The second part of the project was a literature study, where any knowledge gaps seen in previous studies concerning methanotrophs, methanogens, CH_4 budgets and CH_4 fluxes in the Arctic was collected, along with previous studies of CH_4 uptakes in well-drained Arctic soils. The findings of the literature study were then discussed and compared to the field study's observations.

The aim was explored with the following research questions:

- Does the magnitude of CH_4 fluxes between ambient and warmed conditions change at two different Icelandic sites with different vegetation types?
- Which biotic and abiotic drivers that are important for CH_4 consumption can be identified within the OTC plots?
- What future trends in CH_4 fluxes are expected at the two ITEX sites?
- Are our findings of CH_4 consumption rates in line with similar studies?
- What knowledge gaps relating to tundra soil CH_4 fluxes can be identified and what are our recommendations to improve future CH_4 budgets and models?

1.3 Limitations

This study stretched between June and December 2024. The progress was continuously evaluated to increase or decrease the scope of the study to fit within the time frame.

The field portion of the study was limited to June and measured GHG fluxes at two Icelandic ITEX sites with different vegetation types. The study focused as such on well-drained mineral soil in the sub-Arctic. CO_2 and CH_4 flux measurements, soil temperature and soil moisture were taken from the two sites. Weather data was retrieved from nearby weather stations, while soil characteristic along with vegetation shifts was based on previous studies. No further soil characteristic like carbon and nitrogen content or pH were measured in this study to limit the impact on the ITEX sites, which focus areas are vegetation shifts.

Microbial activity would be based on RNA transcripts of the genes *pmoA* and *mcrA* (Nazaries et al., 2013). *pmoA* encoding for the enzyme which is used by most aerobic methanotrophs, and *mcrA* encodes for the enzyme used by most methanogens (Guerrero-Cruz et al., 2021). These enzymatic activity values would however be a proxy, and not include all methanotrophic or methanogenic activity (Guerrero-Cruz et al., 2021).

The literature study focused on the abiotic and biotic factors temperature, soil moisture, vegetation height, vegetation, electron acceptors, and soil pH. These factors are believed to influence methanotrophic and methanogenic activity and could differ between sites and treatments (OTC's and Controls) and are as such relevant measurements for the two study sites (Hollister et al., 2023).

2

Theory

This chapter will present Arctic CH_4 sinks and sources, methanotrophs and methanogens along with environmental factors relevant for their activity level and OTC's.

2.1 Methane sources and sinks

The global CH_4 budget is dependent on sources and sinks from both anthropogenic and natural systems (IPCC, 2023). Around two thirds of CH_4 emissions stems from anthropogenic sources (Jackson et al., 2024), and these sources are dominated by fossil fuels burning, agricultural, rice cultivation, landfills and biomass burning (IPCC, 2023; Nazaries et al., 2013) . Wetlands account for around 20-31% of the total CH_4 emission, and is the largest natural source of CH_4 emissions (Saunois et al., 2024). Other natural sources are emissions from plants, inland freshwater, termites, oceans and hydrates (Jackson et al., 2024; Nazaries et al., 2013). The main natural CH_4 sinks consists of three processes; around 90% is broken down in the atmosphere due to reacting with hydroxyl radicals, around 5% due to chemically reacting with hydroxyl in the excited state and the final 4,8-6,8% is taken up by microbial oxidation in soil (IPCC, 2023; Jackson et al., 2024; Saunois et al., 2024).

Currently, CH_4 emissions are growing at a higher rate than CH_4 sinks (Jackson et al., 2024), while previously CH_4 sinks and sources have grown in similar magnitudes (Conrad, 2009). According to IPCC, 2023 microbial CH_4 uptake from soil is expected to increase with global warming, but the degree of increase is uncertain. Methanotrophs and methanogens are affected by environmental factors, some of which are expected to be impacted by global warming and thus impact the activity of methanogens and methanotrophs (IPCC, 2023; Jackson et al., 2024; Nazaries et al., 2013). Overall, there are still uncertainties related to the size of CH_4 sources and sinks and how they will be impacted by global warming, especially in the Arctic (Juncher Jørgensen et al., 2024). Currently, most large scale climate models do not include CH_4 oxidation in mineral soils within the tundra environment (Zona et al., 2016). Considering that CH_4 consuming mineral soil is believed to make up 87% of the Arctic (Oh et al., 2020), the inclusion of microbial CH_4 oxidation and how they are affected by global warming could be included to improve local and regional climate models (Christiansen et al., 2015).

2.2 Microbial methane within soils

The net CH_4 flux from soil is dependent on methanogenic and methanotrophic activity, where methanogens produce CH_4 and methanotrophs consumes it, at either atmospheric levels or higher. Methanogens are present in soil and especially in more anaerobic (for example deeper or wetter) soil levels (Oh et al., 2020; Zheng et al., 2018). For subsurface soils, a majority of the produced CH_4 will be consumed before reaching the atmosphere (Liu et al., 2016; Zheng et al., 2018).

CH_4 is consumed by methanotrophs, either high or low-affinity. Methanotrophs are mostly aerobic organisms, where low-affinity methanotrophs oxidize CH_4 into CO_2 at relatively high concentration of CH_4 (above 100 ppm), and high-affinity methanotrophs does the same at atmospheric concentrations (about 1.8 ppm) (Guerrero-Cruz et al., 2021; Nazaries et al., 2013). Low-affinity methanotrophs are often active at oxic-anoxic interphases, where methanogens increase the concentration of methanotrophs' substrate, i.e. methane, but enough oxygen is present (Nazaries et al., 2013; Oh et al., 2020). High-affinity methanotrophs can oxidize CH_4 as it diffuses into the soil and the activity of these organisms is as such less dependent on methanogenic activity for a sufficiently high concentration of CH_4, but rather they depend on the diffusion and concentration gradient of methane into the soil (Guerrero-Cruz et al., 2021; Oh et al., 2020). High-affinity methanotrophs have been estimated to account for approximately 90% of all CH_4 oxidation in soils (Topp & Pattey, 1997), and dominate upland tundra soils (Belova et al., 2020). An overview of CH_4 production and consumption can be seen in Figure 2.1.

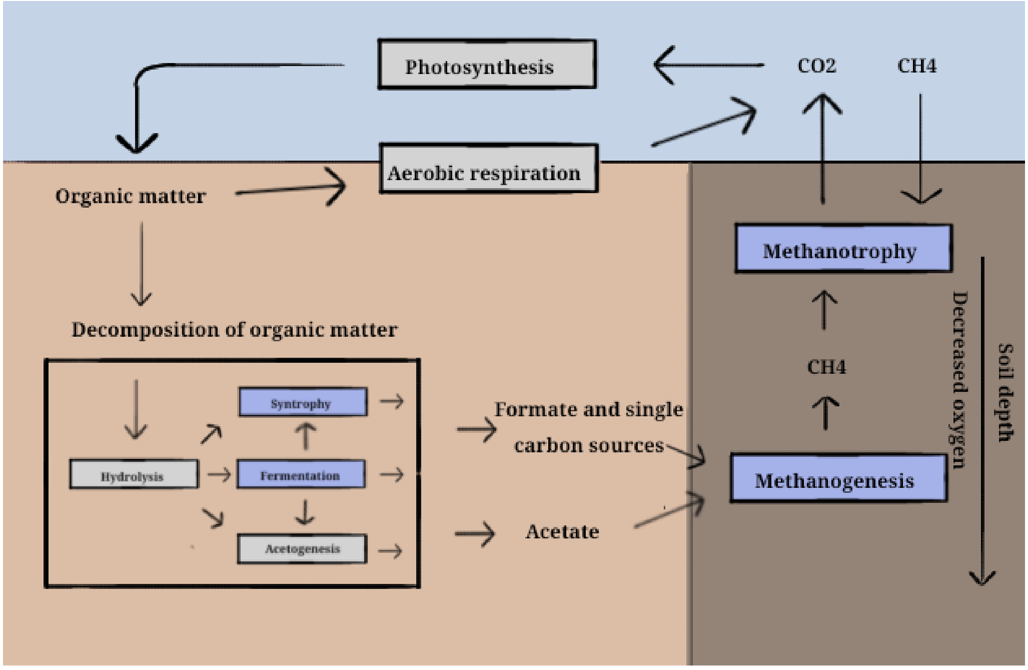


Figure 2.1: An overview of the connection between the CH_4 cycle in soil and the carbon cycle, adapted from Nazaries et al., 2013.

2.2.1 Methanogens

Methanogens are a group of archaea which mainly use single carbon compounds, acetate, formate and methylated organic carbons to drive their metabolism and produce CH_4 (Buan, 2018; Nazaries et al., 2013). Methanogens conduct the final step of anaerobic degradation of organic matter into CH_4 and are active under mostly anaerobic conditions (Guerrero-Cruz et al., 2021), see Figure 2.1. The group of methanogens is diverse with many classes and families that are active in different environments, and which uses varying pathways to produce CH_4 (Nazaries et al., 2013). However, all of the methanogenic pathways have a common final step in the metabolism, catalyzed by the enzyme methyl-coenzyme M reductase (MCR) or its isoenzymes (Gendron & Allen, 2022). The gene which produce the MCR enzyme, *mcrA*, is therefore often used as an activity proxy of methanogenesis (Gendron & Allen, 2022).

2.2.2 Methanotrophs

Methanotrophs are a large and diverse group of bacteria and archaea (Nazaries et al., 2013). Most methanotrophs oxidize CH_4 under aerobic conditions, but there is a large diversity of anaerobic methanotrophs (Buan, 2018; Guerrero-Cruz et al., 2021). Aerobic methanotrophs are often found at oxic-anoxic interfaces in soils where the reactants oxygen and CH_4 are of sufficient concentration and availability (Guerrero-Cruz et al., 2021). These oxic-anoxic interfaces are widened, for example, by increased root systems (De Boeck et al., 2012). High-affinity methanotrophs are dependent on the concentration of CH_4 in their surroundings, of which the primary controlling factors are soil texture, bulk density and water content, as well as nutrient availability (Hansen et al., 2024). Methanogenic activity also affects the concentration of CH_4, and may increase the activity of even high-affinity methanotrophs (E. A. Davidson et al., 2024).

Aerobic oxidation of CH_4 is catalyzed by the enzyme CH_4 monooxygenase (MMO) in either particulate (pMMO) or soluble (sMMO) form (Guerrero-Cruz et al., 2021). pMMO is used, alone or in tandem with sMMO, in all aerobic methanotrophs except two, *Methylocella* and *Methyloferula* (Nazaries et al., 2013). pMMO is encoded by the highly conserved gene *pmoA* (Guerrero-Cruz et al., 2021), and the activity of this gene is often used as a proxy for methanotrophic activity. The three methanotrophic pathways not included by this proxy are anaerobic oxidation, aerobic oxidation using sMMO and CH_4 oxidation in verrucomicrobial methanotrophs (Guerrero-Cruz et al., 2021). There are however relatively few methanotrophic bacteria that live in temperatures below 15°C, and those which are found to date are all classified as Gammaproteobacteria (Nazaries et al., 2013; Patil et al., 2024). This yields that to use the expression of pMMO as a proxy of methanotrophic acticity is closer to the real value in these cold ecosystems.

2.2.3 Environmental factors impacting soil methane fluxes

There are multiple factors that can affect the activity of methanotrophs and methanogens, and thus the net CH_4 flux (Poppeliers et al., 2022; Serrano-Silva et al., 2014). Known factors may be grouped as; (I) Factors impacting general microbial activity, for example soil moisture, temperature, pH, nutrient density, and electron acceptor concentrations (Nazaries et al., 2013; Poppeliers et al., 2022), (II) The amount of available substrates, affected for example by soil moisture, soil depth, soil type, micronutrient concentration (such as copper (Cu), other metals, nitrate and ammonium) (Guerrero-Cruz et al., 2021), decomposition rate, substrate competition and vegetational interactions (Poppeliers et al., 2022) and (III) Top down controls of methanogens and methanotrophs for example impacted by the amount of fungi, bacteriovores and viruses within the soil (Poppeliers et al., 2022). To understand the relationship between these many influencing factors, multivariate studies are sometimes done to facilitate upscaling with less bias, and with respect to many interacting factors. However, these upscaling studies necessitate large datasets over a large variety of spatial and temporal measurements, which is often difficult to obtain, and additionally, overfitting of the models is a common problem (Virkkala et al., 2024). The main environmental factors of interest for this study and its' sites will be presented in the following sections.

2.2.3.1 Soil moisture

Soil moisture has been observed to impact microbial activity within the soil and affects both methanogens and methanotrophs, with an increased moisture observed to decrease net CH_4 uptake in Arctic dry environments (Nazaries et al., 2013; Voigt et al., 2023). The net CH_4 uptake can decrease by both an increase in methanotrophic activity, a decrease in methanogenic activity or a combination of the two. One way of measuring soil moisture is with water filled pore space (WFPS), i.e. a volumetric percentage of water within the soil's pores. A decrease of methanotrophic activity has been observed at WFPS above 60% and the decrease is more pronounced at higher moisture levels (Nazaries et al., 2013). The highest CH_4 uptake in soils is seen at moderate moisture (Kharitonov et al., 2021; Poppeliers et al., 2022), where a mean optimum has been observed at 34% water holding capacity (Gulledge & Schimel, 1998). An increase in soil moisture leads to a more anaerobic environment, which generally increases methanogenesis and decreases methanotrophy in the soil (Serrano-Silva et al., 2014). The more anaerobic environment is due to a diminished transportation of gaseous substrates (such as oxygen and CH_4) in water compared to air. For example, CH_4 diffuses 10 000 times faster in air than in water (Nazaries et al., 2013). An effect is also seen when the soil moisture is too low (WFPS <12%), where both methanogenic and methanotrophic activity is diminished (Kharitonov et al., 2021), probably due to an increased osmotic stress (Nazaries et al., 2013; Semrau et al., 2010).

The soil moisture is affected by the general soil texture, which may create a more or less well drained soil. Small particles such as clay and silt yields smaller pores within the soil, which can become blocked if wet (Abichou et al., 2015). A high amount of clay can thus diminish the diffusion of gasses, such as oxygen, which may cause an increase in anaerobic methanogenic activity and a decrease in aerobic methanotrophy (Abichou et al., 2015; Nazaries et al., 2013).

2.2.3.2 Soil temperature

Soil temperature is a factor which has been shown to impact methanogens and methanotrophs. Methanogenic activity have optimal temperatures between 19-38°C and in general have higher temperature optimums further south according to Kharitonov et al., 2021. Methanogenic activity increases with moderate temperature increases in most Arctic ecosystems. This may in part be due to an increased decomposition rate, leading to higher concentration of available substrate in the form of soil organic carbon for methanogens (Kharitonov et al., 2021; Oh et al., 2020). Methanotrophs have an even wider range of temperature optima, between 55 and below 10°C, with most known methanotrophs having optimal conditions at moderate (20-40°C) temperatures (Nazaries et al., 2013; Semrau et al., 2010). Temperature changes typically causes a smaller effect on overall methanotrophic activity in soil compared to methanogenic (Semrau et al., 2010). However, the observed shifts differ, with an increased temperature causing both increased and decreased effects in methanotrophic activity in Arctic environments (Knoblauch et al., 2008; Voigt et al., 2023).

2.2.3.3 Vegetation

Vegetation shifts have been observed to impact soil CH_4 fluxes. The vegetation type has been seen to impact methanotrophs and methanogens, and is connected to pH, soil temperature, soil moisture and nutrient availability within the soil. For example *Sphagnum* mosses, which often are abundant in wetlands, have an inhibitory effect on methanogens (Rooney-Varga et al., 2007). Plants such as grasses and sedges may increase methanogenesis through release of simple carbon to the soil via root exudates (Liebner et al., 2015). Further more, roots have been observed to increase aeration of soil, causing a wider area where aerobic CH_4 oxidation is possible (Abichou et al., 2015). A higher abundance of vegetation often implies an increased amount of litter to be decomposed, which supplies the methanogens with necessary substrates (Oh et al., 2020), and may impact nutrient availability of for example nitrite and nitrate which impact the activity of methanotrophs (Abichou et al., 2015).

Vegetation height has been observed to correlate with increased CH_4 emissions (von Fischer et al., 2010). One contributing factor may be that higher canopy heights correlates with an increased root system (De Boeck et al., 2012). Plants have been seen to mediate transportation of high concentration CH_4 up through the soil, bypassing methanotrophic zones within the soil (Topp & Pattey, 1997). The rate of CH_4 transport dependent on the plant biomass, phenology, age and to some extent species (Abichou et al., 2015). Aerenchyma tissue transports CH_4 through the plant and has been observed to deposit oxygen to the soil in anaerobic environments, which could widen the area of possible aerobic CH_4 oxidation (Turner et al., 2020). Increased CH_4 emissions have been observed to correlate with increased root growth, which is often connected to vegetation height and the combined ecosystem wide rate of photosynthesis and respiration (Parmentier et al., 2024; Topp & Pattey, 1997). Changes to plant composition due to warmed conditions and/or ecosystem shifts may thus have an effect on both soil CH_4 emissions and uptake (Hough et al., 2020).

2.2.3.4 Soil pH

Soil pH affects the composition of microbes which exist at a site and their activity level (D'Imperio et al., 2023). Generally, soil pH increases with increasing depth in Arctic tundra soils (Bliss et al., 1973). The highest CH_4 production in soil is mostly happening at more neutral pH values close to 7 (Nazaries et al., 2013; Wagner et al., 2017), which also see the highest degree of CH_4 oxidation (Serrano-Silva et al., 2014). A higher pH has been observed to decrease CH_4 uptake in Arctic environments (Voigt et al., 2023). Most methanogens has their optimum pH at a neutral to slightly alkaline range, but there are types with activity optimums at a pH range of between 5,6 and 9,6 (Le Mer & Roger, 2001). On the other hand, methanotrophs have a broader range with optimas both at below 3,5 and above 9,5 (Serrano-Silva et al., 2014). This range of pH for different methanogenic and methanotrophic organisms indicates that microbial composition is affected by pH (D'Imperio et al., 2023; Voigt et al., 2023)

Soil pH has an effect on the availability of electron acceptors and micronutrients within the soil, which in turn affects CH_4 fluxes (D'Imperio et al., 2023). A low pH causes a more reduced environment, and thus affects the soil's concentration and availability of metals (Marquart et al., 2019). For example, Cu is an essential micronutrient for many microbes, often aerobic ones, and is a regulatory substance for the enzyme MMO in methanotrophs (D'Imperio et al., 2023).

Methanotrophs utilize MMO enzymatic systems, which have metallic compounds and are of high importance for methanotrophic activity (Nazaries et al., 2013). The hydrogen bonding active site of sMMO is impacted by pH, while pMMO uses Cu to both regulate and catalyze the reaction of interest (Yao et al., 2023). A change in pH could mediate a switchover between which of the enzymes is most active (sMMO or pMMO) by changing the metal ion availability (Yao et al., 2023).

2.2.3.5 Soil composition and electron acceptors

The activity and diversity of methanotrophs and methanogens are dependent on the environmental concentration of various compounds such as electron acceptors like Cu, sulphate, nitrite and iron within the soil. (Nazaries et al., 2013). When the oxygen level is too low for oxygen to be used as an electron acceptor for methanotrophy, other substances such as sulphate, nitrate, and nitrite are commonly used as electron acceptors by anaerobic methanotrophs (Dean et al., 2018). Iron and manganese oxides can also be used as electron acceptors for oxidation of CH_4 (Dean et al., 2018; Nazaries et al., 2013). However, it is unclear whether these pathways are used for microbial growth, and they are considered small contributors to microbial CH_4 uptake (Dean et al., 2018). More over, Cu is a regulating factor of both methanotrophic enzymes pMMO and sMMO, affecting both expression and activity (Semrau et al., 2010). CH_4 uptake has been observed to increase in Arctic upland soils with increased concentration of dissolved nitrogen, higher turnover rate of sulphate, increased ecosystem respiration and higher soil temperature (Voigt et al., 2023).

2.3 Carbon dioxide fluxes

The flux of CO_2 in an ecosystem can be measured in many ways, with different parts of production and consumption of CO_2 included. The measurement of all CO_2 produced within an ecosystem is known as ecosystem respiration (R_{eco}) and is a net emission into the atmosphere. In this study a proxy of R_{eco} was measured by measuring the CO_2 flux within a ecosystem where no photosynthetically active radiation (PAR) enters, and thus no photosynthesis is occurring. The flux of CO_2 over the ecosystem when both respiration and photosynthesis is occuring is known as Net Ecosystem Exchange (NEE) and may be both a net emission or net uptake of CO_2. NEE was in this study measured as the CO_2 flux when PAR was allowed to enter the chamber. If only the photosynthetic rate is of interest one often uses Gross Primary Production (GPP) (S. J. Davidson et al., 2016) which is the difference between NEE and R_{eco} according (Falk et al., 2015) seen in the following equation:

$$GPP = NEE - R_{eco} \tag{2.1}$$

and since only photosynthesis is included, GPP is always a net uptake of CO_2 from the atmosphere.

2.4 Open-top chambers

This study compares CH_4 fluxes in Controls paired with passively warmed plots, where the warming is achieved using OTC's. If changes are seen between OTC's and Controls in i.e. vegetation community or scale of GHG fluxes this is refered to as an "OTC effect". OTC's function as small roofless greenhouses that passively heats the interior air when hit by daylight, i.e. mostly during daytime and in the summer months, see Figure 2.2 (Hollister et al., 2023). OTC's are made out of transparent plexiglass, and have been seen to affect factors such as temperature, soil moisture and vegetation (De Boeck et al., 2012).

The passive heating achieved by the OTC's is a combination of reduced wind and increased net radiation absorbed (Björkman, 2013; De Boeck et al., 2012). During daylight hours, shortwave solar radiation is transmitted through the walls, heating the plot. Part of the outgoing long-wave radiation from the ground is reflected back by the OTC's walls, resulting in an increased air temperature (De Boeck et al., 2012;

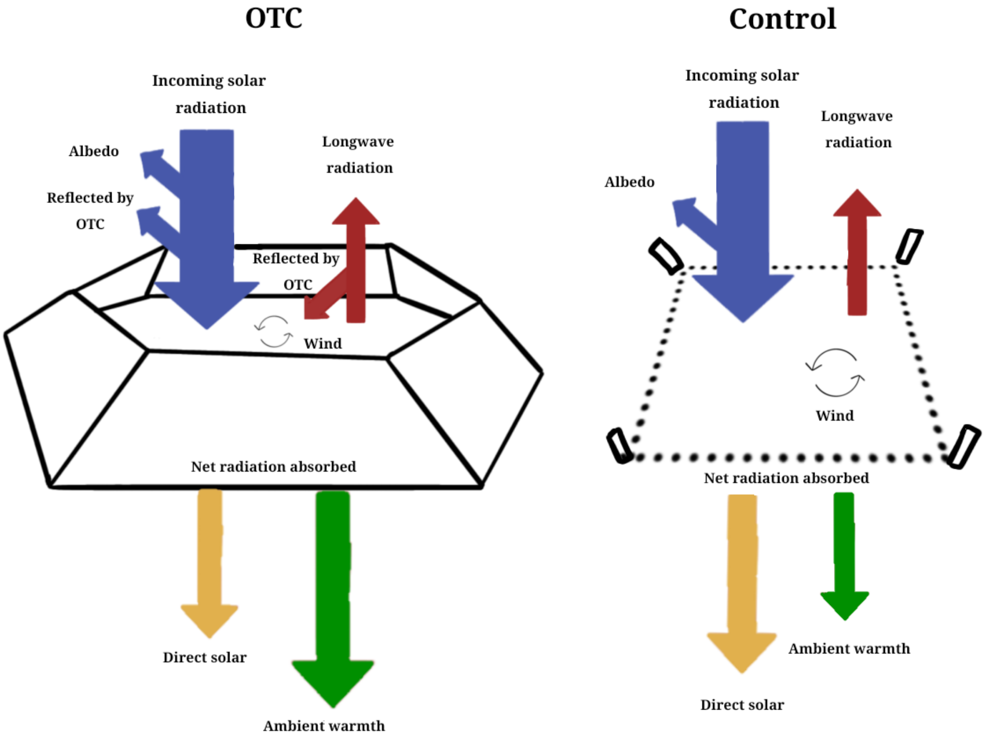


Figure 2.2: A schematic overview over the incoming and outgoing radiation and net radiation absorbed in OTC and Control plots, adapted from Hollister et al., 2023

Hollister et al., 2023). This yields an increase in temperature range over the course of a day and also a higher frequency of freeze-thaw events (De Boeck et al., 2012; Hollister et al., 2023; Welshofer et al., 2018). The highest warming effect is seen close to the middle of the plot, near the soil's surface and the effect is lowered closer to edges (Hollister et al., 2023).

Further more, the OTC's have an effect on air flow and turbulence (De Boeck et al., 2012; Hollister et al., 2023). The shape is designed to block out wind and increase the air's boundary layer by creating a "bubble" of stable air above the ground (De Boeck et al., 2012; Hollister et al., 2023). Wind leads to energy loss through advection and convection, and since the walls of the OTC's partially block wind this leads to less energy loss and a warming effect (Hollister et al., 2023).

The temperature increase in the OTC's depends on several factors, which makes specific heating at each site hard to pinpoint unless measurements are taken. These factors include the amount of incoming radiation, wind, snow, vegetation and vegetation type, the plot's ability to absorb and reflect heat and the coarseness in the plot, vegetational or surface based (Hollister et al., 2023). The average warming in OTC's is between 1-3°C, but the warming effect can vary in individual sites and plots (Björkman, 2013; Hollister et al., 2023; Jónsdóttir et al., 2005).

Several ITEX experiments have been established over the past 30 years (G. H. Henry et al., 2022). Over time, shifts have been observed in the vegetation community and structure, such as increased vegetation height and density (García Criado et al., 2023; Hollister et al., 2023). Vegetation shifts like these can yield both cooling and warming effects, due to factors such as shading, insulation, a more reflecting surface and further diminished winds (García Criado et al., 2023; Gornall et al., 2007; Hollister et al., 2023).

Soil moisture is mainly affected by lateral movements of water within the soil and the location within the landscape (Lindwall et al., 2016). Lateral movements of water within the soil effects factors such as soil moisture, soil temperature, soil properties and nutrient transport (Hollister et al., 2023).

Vegetation also impacts the soil moisture (Lindwall et al., 2016). Plant surfaces, such as leafs and moss, will be warmed more than the air, which affects the leaf to air vapor pressure (Björnsdóttir et al., 2022; De Boeck et al., 2012). This often cause a slight decrease in soil moisture at dry sites, especially near the soils surface (Gornall et al., 2007; Hollister et al., 2023). A slight change of soil moisture at dry sites may cause closure of the plant's stomatas which conducts gas exchange at the leafs, and the closure affects photosynthesis (Venkat & Muneer, 2022). Further more a slight decrease in soil moisture may constrain plant growth and affect microbial activity at dryer sites, while being negligible at moist to flooded sites (Hollister et al., 2023).

Although OTC's effectively warm the tundra, some drawbacks also exist. Increased snow cover and temperature within the OTC's affects nutrient concentration, timing of spring thaws and lengths of the growing season (Hollister et al., 2023; Lindwall et al., 2016). The OTC's accumulate snow within the walls when their height is lower than the snow cover (Drescher, 2014). During snow melt, the availability of water and nutrients in OTC's can be increased compared to surrounding areas (Hollister et al., 2023). Further more, the snow usually insulates the soil during winter, yielding a warming effect, while a cooling effect is seen during spring. (Bokhorst et al., 2013). Since the OTC's effect on snow cover and temperature varies between seasons and years, quantifing and generalizing the effect of the OTC's is difficult (Bokhorst et al., 2013). One general effect of the OTC's is however an increased length of the growing season (Hollister et al., 2023).

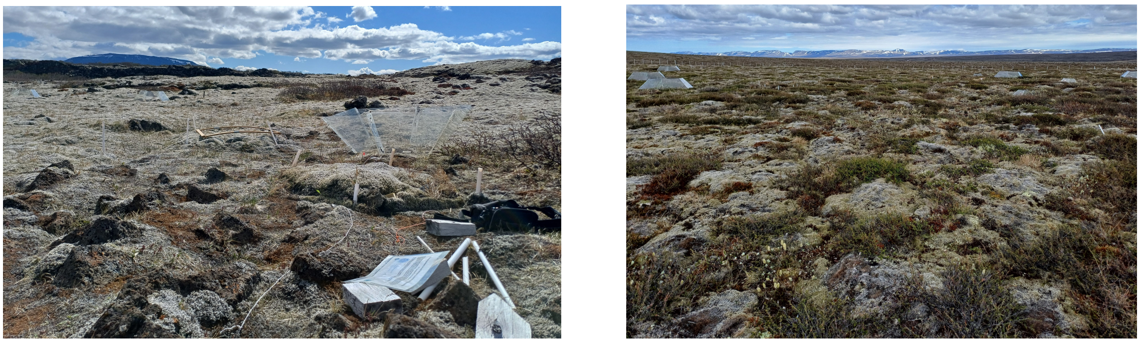
The OTC setup has limitations and should not be used directly as a prediction and generalization of global warming (De Boeck et al., 2012). OTC's are commonly used due to the comparatively affordable experimental setup and passive heating, which often causes vegetational shifts (Hollister et al., 2023). The OTC's in their basic setup do not include other effects of global warming, such as increased atmospheric CO_2 levels and changed precipitation patterns (Hollister et al., 2023; IPCC, 2023; Papalexiou & Montanari, 2019). As such, OTC's are tools that can be used to study warming effects and help identify further trends *in situ*, but should not be the only tool in predicting the effect of global warming in the Arctic.

3

Method

3.1 Study sites

In this study, two ITEX sites in Iceland were visited. The first site, refered to as the Moss site, is classified as a sub-Arctic dry moss tundra (Jónsdóttir et al., 2005). The Moss site is located inside Þingvellir national park at 64°29' N, -21°07' W (Figure 3.1 a)). The second site, the Heath site, is a sub-Arctic alpine tundra (Jónsdóttir et al., 2005) and is located at a higher elevation in northern Iceland, 65°22'N, -19°70'W (Figure 3.1 b)).



(a) Picture of the Moss sites' vegetation, trampled in the foreground.

(b) Picture of the Heath sites' vegetation.

Figure 3.1: Overview of vegetation differences at the two ITEX sites visited in the study.

3.1.1 The Moss site

The Moss site is located in the south-west of Iceland, see Figure 3.2. The site is located at 120 meters above sea level (m.a.s.l.), which is below the potential treeline (Björnsdóttir et al., 2022). The Moss site experiences a mean annual air temperature of 4,1 ± 0,3°C and a mean annual precipitation of 1242 ± 37 mm between 1996-2017 ((Björnsdóttir et al., 2022). Further characteristic of the soil type at the site includes an average soil thickness of one meter, low clay content, high susceptibility to wind and water erosion, homogeneous soil profile, high soil carbon content of 11,12 ± 0,79% and a soil pH of 5,12 ± 0,06 (Jónsdóttir et al., 2005; Thorsteinsson

Figure 3.2: Map over Iceland with the two study sites marked with dots.

& Arnalds, 1992).

Standing on top of a glacial deposit and the over 9000-year old lava field Eldborgier, the landscape is dominated by an uneven bedrock surface covered in a moss carpet (Jägerbrand, 2005; Tuviala & Isotalo, 2023). The uneven lava field bedrocks creates a well-draining soil (Jägerbrand, 2005). Moreover, the soil type is relatively nutrient poor with a high organic matter content and is classified as Brown Andosols (Björnsdóttir et al., 2022).

The flora at the Moss site is dominated by the moss *Racomitrium lanuginosum*, also known as Wooly Fringe-moss (Tuviala & Isotalo, 2023). The moss creates a continuous carpet that can be up to 40 cm thick (Jägerbrand, 2005; Tuviala & Isotalo, 2023). By covering the soil, the moss carpet works as an isolating layer. This leads to the soil remaining frozen longer during spring thaw compared to non-moss covered ground, as well as leading to lower soil temperatures during the growth season (Thorsteinsson & Arnalds, 1992). *Racomitrium lanuginosum* has an optimal growing temperature between 5-13°C and grows 1,6-1,7 mm/year in optimal conditions (Tuviala & Isotalo, 2023). Around 13% of Iceland is classified as moss dominated ecosystems (Náttúrufræðistofnunar Íslands, 2024). While *R. lanuginosum* is the dominant species, the Moss site has a vascular plant cover of under 10% (Björnsdóttir et al., 2022). Twelve different species of vascular plants have been observed at the Moss site, where the dominant vascular plant species is the sedge *Carex bigelowii* followed by the grass *Festuca richardsonii* and the flowering plant *Galium boreale* (Björnsdóttir et al., 2022; Jónsdóttir et al., 2005).

The Moss site is part of the ITEX network and the first OTC's were placed at the site 1995, thus the warming experiment has been running for 29 years (Björnsdóttir et al., 2022). To date, no vegetation shifts have been observed between OTC and Control plots (Jónsdóttir et al., 2005). During the years 1999-2002 the OTC's caused the air temperature to increase 1-2 °C while the soil temperature decreased 1-2 °C compared to the Controls (Jónsdóttir et al., 2005).

3.1.2 The Heath Site

The Heath site is located in the north-western highlands of Iceland at an elevation of 480 m.a.s.l., which is above the potential treeline (Jónsdóttir et al., 2005). Between the years 1996-2017, a low annual average precipitation at 357 ± 14 mm/year and air temperature of 0,7 ± 0,3°C was observed at the Heath site (Björnsdóttir et al., 2022). Soil pH at the Heath site is 6,48 ± 0,06, and soil carbon content is low at 4,89 ± 0,24%, which makes it favorable for a large variety of plants (Jónsdóttir et al., 2005).

The ground consists of well-drained Brown Andosol soil, with high amounts of metal complexes and sits on basaltic bedrock (Jónsdóttir et al., 2005). This type of soil often have a high water retention and cation exchange capacity (Björnsdóttir et al., 2022; Jónsdóttir et al., 2005).

The site is defined as sub-Arctic alpine tundra and is dominated by the dwarf birch *Betula nana*, and the moss *R. lanuginosum* (Jónsdóttir et al., 2005). The site has a relatively high diversity of vegetation, with 49 different flora species present (Jónsdóttir et al., 2005). The Icelandic highlands, including this site, have been seasonally grazed by sheep for centuries (Akello, 2019). However, the plots in which the experiments were carried out were fenced in 1996 to prevent sheep-induced experimental disturbances (Jónsdóttir et al., 2005).

The ITEX OTC warming experiments at the Heath site was established in 1996 and have been ongoing for 28 years (Björnsdóttir et al., 2022). The OTC's were, between 1999-2002, seen to increase air temperature 0,7-1 °C (Jónsdóttir et al., 2005). The soil temperature was however seen to decrease 1-2 °C in the OTC plots compared to Control plots in both 1999-2002 and 2016 (Björnsdóttir et al., 2022; Jónsdóttir et al., 2005). Furthermore, the OTC's has yielded an increase of evergreen dwarf shrubs, a decrease of bryophytes and an increase of canopy height (Jónsdóttir et al., 2005). An example of the vegetation in paired OTC and Control plots is shown in Figure 3.3 below.

3.2 Field work

The field work took place between the 10th and 31st of June 2024. At each of the sites, ten passively warmed OTC plots and ten Control plots were used (n=20[1]). Both OTC and Control plots were set up in a paired factorial design. Each plot mea-

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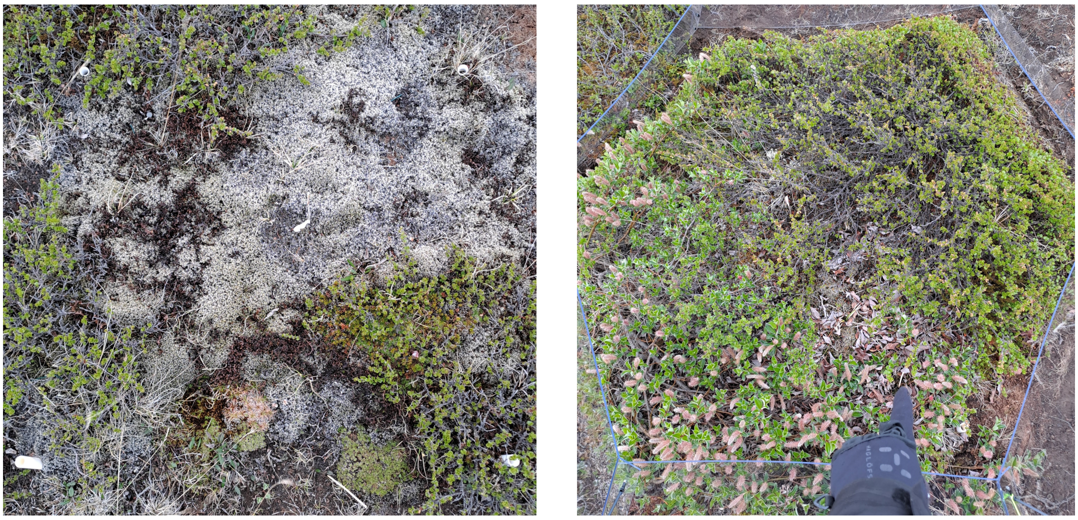
[1] 1 n = 1 pair of OTC and Control plots. Thus there is 10 n per site. Not all plots were not used in the inhibition experiment.



(a) Picture over the vegetation within the Control at plot 5 at the Heath site.

(b) Picture over the vegetation within the OTC at plot 5 at the Heath site.

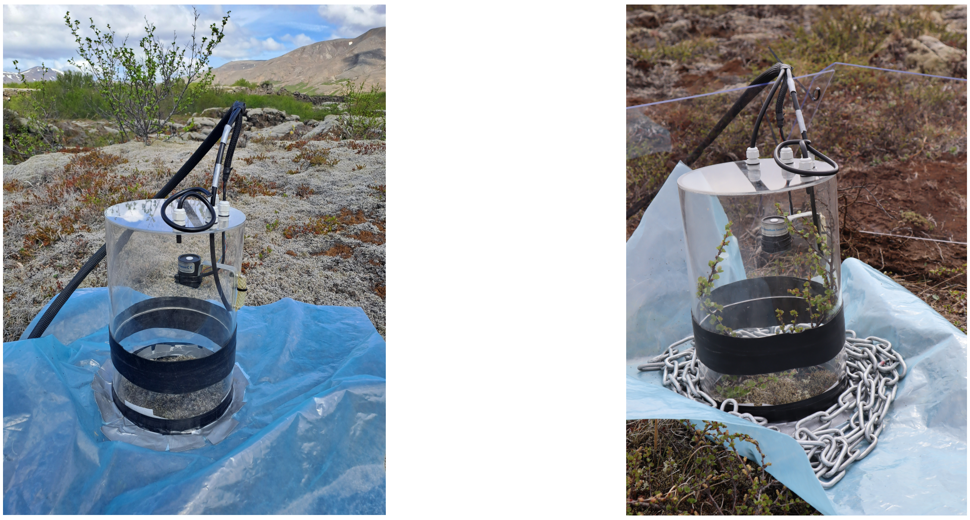
Figure 3.3: Example of vegetation shifts observed at the Heath site.

sured 1 m^2 and at each plot measurements of GHG fluxes where made, soil samples removed for microbial analysis and environmental metadata such as weather data, soil moisture and soil temperature were collected. In total, GHG fluxes were measured 216 times at the Moss site, and 193 times at the Heath site. Own weather observations from each flux measurements were noted down. Further more, data from previous published papers and weather stations regarding topology, flora, soil and weather data were collected.

3.2.1 Methane and carbon dioxide flux measurements

Gas flux measurements, of CH_4 and CO_2, were measured using a LI-7810 Trace Gas Analyzer (LI-COR Biosciences, Lincoln, U.S.), hereafter called a Licor. The Licor measured CO_2 and CH_4 concentrations at one measurement per second, 1 Hz. The measurements were done using a transparent cylindrical chamber with an attached collar (height 30 cm, diameter 25 cm), hereafter called the chamber. The chamber was connected to the Licor via non-transparent 4 mm tubing, approximately 5 m long. A non-permeable skirt made out of a plastic sheet was attached to the collar and extended out at least 15 cm from the chambers walls, see Figure 3.4 (a).

For each gas flux measurement, the Licor were first acclimatized to the atmospheric concentration by holding the chamber up above ground level and placing the opening toward the wind. Thereafter, the chamber was placed inside the plot and chains were put on the surrounding plastic skirt, see Figure 3.4 (b). The plastic skirt is thus weighed down with the intent of sealing the chamber off from the outside air. At each plot, two GHG flux measurements were conducted per sampling occasion. First a light measurement was done, where light could enter the chamber, a proxy for NEE. This was followed by a dark gas flux measurements, a proxy for R_{eco} where



(a) Chamber with plastic skirt attached.

(b) Chamber with chains placed around it, on the plastic skirt.

(c) Lycor instrument in the foreground and a blackout bag on top of the chamber placed in an OTC in the background.

Figure 3.4: Overview of placement of the chamber in the plots for the gas fluxes measurements.

a blackout bag was put over the chamber to block out any PAR from entering, see Figure 3.4 (c). Each measurement was around three to six minutes long. Each measurement's start and end times, along with measured PAR, and chamber temperature were noted down. To reduce wind disturbances during measurement an OTC was temporarily placed around the Control plot.

Specific weather conditions were needed to take gas flux measurements. The Licor instrument was sensitive to water, and as such no measurements were taken in rain. In addition to this, wind speeds over about 5 m/s at the Moss site and 8 m/s at the

Heath site resulted in unstable readings, presumably due to wind entering the chamber through the porous vegetation. To observe potential wind related disturbances, the gas concentrations were followed in real time on a tablet. If wind disturbances were observed, the measurement was redone. A measurement was classified as stable and acceptable when no large slope changes or spikes/dips occurred in the gas concentration gradient during the measurement. Measurements were between three to six minutes long, with the aim of obtaining at least three minutes of stable readings. The goal was to obtain five light and five dark measurements at each plot of sufficient quality. Preferably, repeating measurements of a plot should be done on different days. Due to weather conditions some exceptions to this was made.

A logger (CR 300 series, Campell Scientific) was connected to the Licor. Real time data of inside chamber temperature and PAR was observed using the Logger Link app. The chamber temperature and PAR values were noted down about two minutes into each measurement. PAR was used to verify comparable levels of incoming lights in light and dark measurements respectively. Causes of non-comparable PAR levels could be a gap in the blackout bag and letting in light during dark measurements, and also due to measurements being taken early in the morning and late at night. The wide range of when measurements were taken was due to few and short good weather windows when measuring fluxes was possible during the field study.

3.2.2 Weather data

Air pressure data for each study site were used to calculate gas fluxes. Weather data was retrieved from Iceland Meteorological Office's weather stations, where wind speed, air temperature and air pressure observations were given as averages for each hour (Veðurstofa Íslands, 2024). Weather stations close to each site were used; Kolka weather station for the Heath site, while Þingvallir and Þyrill weather stations were used for the Moss site.

Air pressure data for the Moss site were calculated using weather observations from two weather stations since the nearest weather station (Þingvallir weather station located 1,2 km away from the Moss site and at 110 m.a.s.l.) did not measure air pressure. The air pressure data from Þyrill weather station (located 20,2 km from the Moss site and standing at 61,5 m.a.s.l.) was recalculated to retrieve air pressure at the Moss site's elevation using equation 3.1 (CalcTool, 2024).

$$p = p_0 e^{-\frac{gM(h-h_0)}{RT}} \tag{3.1}$$

Equation 3.1 calculates the air pressure p at the nearby Þingvallir weather station using the known air pressure p_0 and height h_0 of the Þyrill weather station. This is done by using the elevation h and air temperature T in Kelvins at the Þingvallir weather station. Further more, the following constant are used; gravitational force in Iceland g at $9,81 m/s^2$, the molar mass of dry air M standing at $0,028970 kg/mol$, and R which is the universal gas constant at $8,3143 N \cdot m/(mol \cdot K)$ (CalcTool, 2024).

3.2.3 Soil temperature and moisture

For each pair of light and dark gas flux measurements, soil moisture and soil temperature were measured. Soil temperature was measured with a household thermometer, while two values for soil moisture were obtained using a ThetaProbe Soil Moisture Sensor, one in soil water potential (mV) and one in v/v % (WFPS). The measurements for both soil moisture and soil temperature were taken approximately 5 cm into the soil, below the soil surface. The small area of the moss carpet was carefully lifted and the temperature and moisture probes were submerged around 5 cm into the soil. Thereafter, the moss carpet was put back. The same spot in each plot was used throughout the study.

3.2.4 Moss height

The moss height at the Moss site was measured using a 25 cm long straight wooden skewer. The stick was inserted into the moss until rocks or soil were hit. The moss height used was an average of five locations in each plot, where measurements were spread out in the plot. For plots where the moss was higher than the skewer a measurement of 25 cm was used for further analysis.

3.2.5 Methane production inhibition

To be able to separate the CH_4 flux into consumption and production in the soil, an inhibition of methanotrophs by acetylene gas was performed, in accordance to the methodology of Pedersen et al., 2018 and King, 1996. Before the inhibition, a light and dark gas flux measurement was taken according to section 3.2.1 to act as a baseline flux, and the location of these measurement was marked with wooden skewers. Thereafter, a dry glass jar, approximately 200 ml, was placed in the middle of the marked inhibition area. 2 ml of calcium carbide, approximately 1,5 g, was poured into the glass jar. A lidded inhibition collar with a 15 cm wide plastic skirt attached was placed over the jar, and chains and stones were placed around it, intended to create a seal from outside air. With a syringe, 10 ml of water was injected through a rubber septum in the lid of the inhibition collar and into the glass jar, initiating a reaction forming about 10 v/v % acetylene gas within the chamber, to diffuse down into the soil (Pedersen et al., 2018). The inhibition collars were left to incubate for two hours, see Figure 3.5. Thereafter, the inhibition collar was removed and the area was aired for five minutes before another light gas flux measurement was conducted.

Figure 3.5: Incubating inhibition outside of the exclosure at the Heath site.

Inhibitions were only preformed at the Heath site, due to a too large impact on the vegetation within the Moss site. Two inhibitions were performed outside the exclosure at the Heath site and the rest in the exclosure, on the OTC and Control, with in total seven successful inhibition attempts.

3.2.6 Gas flux calculations

The measured GHG fluxes were calculated using the programming language R (version 4.3.0, R Core Team, 2021) via the FluxCalR package (version 0.2.2, Zhao, 2019). Parameters, including start and end times, chamber temperature and air pressure, were imported as well as the Licor files of gas concentrations of CH_4 and CO_2 over the measurement period. Disturbances in the concentrations changes, which may be due to delays in the instrument, wind or other external factors, were manually trimmed away to retrieve the longest possible window of stable measurements.

For measurements where the chamber temperature was not noted down, the measured chamber temperature closest in time was used. No chamber temperatures were measured during the first two days of measurements at the Moss site. Instead the hourly mean air temperature from Þingvallir weather station was used as the chamber temperature for these flux calculations.

The quality of the data was controlled. If the R2 value for both the CO_2 and CH_4 were below 0,6, the measurement was excluded from the study. If only one of the R2 values were below 0,6 more analysis were done. Low fluxes close to zero, defined as a flux within 0,25 standard deviations of 0, were visually examined to ensure a stable reading. If the reading was deemed stable the measurement was included in the study, but high fluxes with low R2 values were excluded.

Using FluxCalR, the CO_2 and CH_4 gas flux outputs and R2 values were exported and collected in an excel document. The gas fluxes were thereafter visualized in box plots using ggplot2 in R (version 3.5.1, Wickham, 2016). The Hedge's g' effect size package (version 0.8.0, Torchiano, 2016) was used to calculate the Hedge's g' effect size, and to visualize the results. The Hedge's g' effect size is a measure of the effects of different treatments, in this study comparing gas fluxes at OTC and Control plots. The common praxis is that a small effect size is defined as 0,2, medium as 0,5 and large effect as 0,8 (Statistics How To, 2016). A repeat measure ANOVA was performed to retrieve p values comparing the different sites and treatments. The pairwise difference of the GHG flux measurements were compared within a site, while the measured environmental factors were compared both within and between sites and treatments.

Since the rate of photosynthesis and thus CO_2 fluxes change with the incoming PAR, one light and one dark measurement were taken for each plot. The CO_2 fluxes measured during light measurement represented NEE, and the dark measurements as a proxy for R_{eco}. For each measurement, where both light and dark measurements were of sufficient quality, the difference between the measurements of NEE and R_{eco} were calculated to yield GPP (S. J. Davidson et al., 2016), in accordance with equation 2.1 (Falk et al., 2015).

3.3 Soil samples

Soil samples were taken from each measured plot, along with soil temperature and soil moisture values at the time of sampling, as described in Section 3.2.3 above. A make-shift soil sampling probe was constructed by attaching a 90°angular metallic shaft to a handle. The sampling was done by inserting the probe into the soil, twisting it and extracting a soil core with intact soil profile visible. The obtained core depth was then measured, noted down and the soil was put into sterile 15 ml falcon tubes. Approximately 5 g of soil was taken from each plot and immediately submerged in $DNA/RNAshield^{TM}$ (ZymoBIOMICS, 2024) to preserve DNA and RNA genetic integrity at the point of collection by inhibiting pathogenic activity and slowing down DNA and RNA breakdown. Samples were protected from heat and light by insertion into the soil while the remaining samples were collected. The probe was thoroughly cleaned with soapy water and dried off between each sampling to reduce the risk of contamination. The samples were then brought inside and refrigerated for around one week before being transported and frozen at -80°C at the University of Gothenburg in Sweden.

Soil samples were retrieved from the top organic soil layer at a depth between 6 to 28 cm at the Heath site. Due to the shallow organic soil horizon at the Moss site, samples were collected at a depth between 3 and 15 cm. Both between and within the sites, the soil samples had varying moisture, degree of vegetation and visible sediment, see Figure 3.6.

Figure 3.6: Example of two soil samples taken at the same day from different plots with visually differing soil moisture.

3.4 Literature study

A semi-structured literature study was conducted to understand both the general CH_4 budget within the Arctic and also to understand the microbial functioning of methanotrophs and methanogens and how they are affected by a range of different environmental factors. This was done with an iterative approach to better include new information and perspective in the process of gathering information.

The search engine used was Google scholar and search terms included: Arctic, methane flux, definition Arctic, permafrost, methane uptake Arctic, methane sink, methane source, methanogens, methanotrophs, methanogenic activity arctic, methane oxidating bacteria. The relevance of the studies was checked through reading the abstracts and conclusions and cross referencing the studies if possible.

4

Literature study

This chapter presents what knowledge is missing in the understanding of CH_4 fluxes at the two Icelandic sites, as well as our recommendations for future studies. The results of the literature analysis of previously done studies on upland (sub-)Arctic uptake of CH_4 is also presented in this section.

4.1 What knowledge gaps exist?

To more accurately predict future climate scenarios it is crucial to improve the knowledge of the mechanisms and feedbacks behind climate change, and their effect on net CH_4 fluxes. However, there still exist several knowledge gaps on the processes behind climate change, especially in the Arctic and sub-Arctic (Schmale et al., 2021). Furthermore, the understanding of which climate feedbacks are the driver of climate change, at both a local and regional level, needs to be improved to better simulate future climate scenarios (IPCC, 2023; Juncher Jørgensen et al., 2024). We have divided the knowledge gaps into four categories: definition of the Arctic, sampling, vegetation feedbacks and microbial unknowns.

4.1.1 Defining the Arctic

There is no common definition of what geographic areas are considered part of the Arctic and sub-Arctic (Federov et al., 2019). Instead several definitions are used in Arctic studies, some are strictly geographical, while others are based on traits like average temperatures or Arctic flora (Federov et al., 2019; Walker et al., 2005). Overall, there is no standard practice to which definition should be used in different fields and situations (Federov et al., 2019; Polunin, 1951). Further more, we observed that studies do not always state which definition of the Arctic they have used. The lack of uniformity and statements over how the Arctic is defined can make research data and models over Arctic environments difficult to compare, since the results may depend on which definition is used. We believe that a singular universally accepted definition of the Arctic and sub-Arctic should be decided, which could increased the replicability and comparability of Arctic studies, and could make modeling of the Arctic environments easier.

4.1.2 Sampling

One of the main barriers of understanding the driving processes in Arctic climate change and it's effect on CH_4 fluxes is the relatively small amount of studies and sampling done on CH_4 fluxes in the Arctic dry tundra (Christiansen et al., 2015; Juncher Jørgensen et al., 2024). The Arctic consists of a large geographical area and a large percentage of it is far away from civilization and is experiencing harsh conditions, making parts of the Arctic inaccessible during parts of the year (Juncher Jørgensen et al., 2015). To date, a limited amount of studies in the Arctic and sub-Arctic has been done and with a focus on relatively few places and seasons (Juncher Jørgensen et al., 2024; Metcalfe et al., 2018). More measurements are taken in high emitting and accessible areas which are relatively easy to reach with transport of resources and staff, rather than focusing on the wide spread CH_4 uptaking areas with relatively low fluxes (Juncher Jørgensen et al., 2015). Previous measurement equipment has also not have the precession to be able to measure the low CH_4 fluxes areas (Ueyama et al., 2023).

The majority of field studies in the Arctic are seasonally bound, and only performed during a short time window in the peak growing season, during summer (Juncher Jørgensen et al., 2015). The cold season is however important, since 54-81% of the total annual CH_4 fluxes are occurring during the 9 month long cold season (September to May) (Zona et al., 2016). There are many factors playing into how CH_4 consumption and production could be affected by seasonality such as changing moisture levels, temperatures, vegetation cover, geomorphological factors and biological processes such as decomposition and competition over substrates (Voigt et al., 2023).

Why, when and where CH_4 fluxes changes occurs is poorly understood, in part due to the complex mechanisms behind emissions and uptakes (Howard et al., 2020; Zona et al., 2016). A broader variety of sampling with regards to seasons, locations, vegetation types and time of day would help in broadening the picture of Arctic soil CH_4 fluxes, the CH_4 budget over all along with its variability between seasons (Juncher Jørgensen et al., 2024; Saunois et al., 2024; Voigt et al., 2023; von Fischer et al., 2010). This could also lead to increased understanding of the mechanism behind the changing methanogenic and methanotrophic activity due to global warming, which could improve future estimates of the CH_4 budget (Juncher Jørgensen et al., 2024; Voigt et al., 2023).

4.1.3 Vegetation feedback

Over 90% of global plant trait observations are on tropical and temporal traits (Thomas et al., 2020). Thus, less studies have been preformed in the Arctic leading to a less comprehensive understanding of plant traits. Some studies on Arctic vegetation shifts have observed changes in the distribution of species and how the vegetation grows, which may impact the nutrient cycling in the soil itself, creating competition over substrates and therefore also affecting CH_4 fluxes (García Criado et al., 2023; Poppeliers et al., 2022; Voigt et al., 2023).

The distribution of species in the Arctic has shifted, where species and climate zones have moved polewards (García Criado et al., 2023; IPCC, 2023). A so called shrubification is seen, where shrub species are growing faster and higher and global warming is the expected driving cause (García Criado et al., 2023). An increased vegetation height has been observed to increase CH_4 emissions in Arctic wetland species, which is belived to be due to an increased root system (von Fischer et al., 2010). This allows for more methanogenic activity within the soil and more CH_4 transport from the soil through some wetland plants' highly porous, air-filled aerenchyma tissue (von Fischer et al., 2010). Furthermore, the type of vegetation within a ecosystem affects the CH_4 fluxes. For example *Sphagnum* mosses in wetlands has an inhibitory effect on methanogens (Rooney-Varga et al., 2007) causing a lower CH_4 flux, while vascular plants like grass and sedges may release simple carbon to the soil via root exudates, causing an potential increase in methanogenesis (Laiho et al., 2024; Liebner et al., 2015). However, vascular plats also create a more well-drained and aerated soil that favors methanotrophic activity over methanogenic activity (Keuschnig et al., 2022). The information about upland Arctic vegetation's effects on CH_4 emission is more sparse, but it is known that plant species plays a role in CH_4 fluxes and impacts the regional GHG budget (Virkkala et al., 2024).

4.1.4 Microorganisms

Changes in CH_4 fluxes are not necessary a result of changing abiotic soil properties such as soil temperature and soil moisture, but could be due to biotic properties such as vegetation shifts and/or a changed microbial community (Nazaries et al., 2013; Poppeliers et al., 2022). Furthermore, the IPCC predicts that air temperatures will increase and expects precipitation patterns to be altered in different regions (IPCC, 2023). Current models have predicted the Arctic to be between 4 to 13°C warmer by the year 2100 compared to 1961-1990 (Chylek et al., 2024) Additionally, precipitation is estimated to increase between 30 and 60% by the year 2100 (Bintanja & Andry, 2017; McCrystall et al., 2021). These predicted changes combined are expected to influence the activity of both methanotrophs and methanogens (IPCC, 2023). Therefore, including microbial processes can help improving climate predictions and models (Nazaries et al., 2013). However, then there is a need to further improve the knowledge of microbial CH_4 fluxes in the Arctic, their drivers and feedbacks to be able to include these processes in future climate models. Factors that we recommend should be included in future research are how methanogens and methanotrophs will be affected by global warming, increased atmospheric CO_2 levels, vegetation shifts, changed precipitation patterns and soil moisture.

Some smaller and basic climate models include CH_4 fluxes from methanogens and methanotrophs, with mostly abiotic factors such as temperature being taken into account (Bridgham et al., 2013; Parmentier et al., 2024; Xu et al., 2016). Previously, most global or regional climate models did not include soil microbial CH_4 processes, due to the complexity of these processes and their interactions various enviornmental factors (IPCC, 2023; Nazaries et al., 2013). However, some regional models are now including microbial processes (Xu et al., 2016), and larger regional models have also started to try to include them (Parmentier et al., 2024).

The microbial community and it's functioning is complex and affected by many environmental factors, such as temperature, soil moisture, vegetation, pH, nutrient density and bacterial consortiums (Nazaries et al., 2013; Poppeliers et al., 2022). However, any of these factors is expected to shift with global warming, which can lead to a change in microbial activity levels (IPCC, 2023). Higher temperatures are observed to increase both methanogenic and methanotrophic activity (Parmentier et al., 2024), but to varying degrees (Oh et al., 2020). The changes in functional microbial community are often seen in global warming studies and has been strongly linked to the soil CH_4 flux, but the rate of change is still unclear (Nazaries et al., 2013; Oh et al., 2020). Uncertainties in how, for example, the changing weather and precipitation patterns will affect CH_4 sinks increase the over all uncertainties regarding future CH_4 sink trends (Nazaries et al., 2013).

One challenge in understanding the mechanisms of CH_4 producers is the difficulty to growing methanogens and analyzing the methanogenic enzyme MCR in a lab setting (Lyu et al., 2018; Nazaries et al., 2013). The methanogens are often in synthrophic relationships with bacteria within the soil and often do not function in the same way outside of it (Keuschnig et al., 2022; Nazaries et al., 2013). Instead analysis is currently done on a ecosystem level rather than in labs, which makes analysis of specific mechanisms of a microbial type and the network of drivers behind their activity more difficult to discern from the many interactions taking place at once (Nazaries et al., 2013). Ecosystem wide analysis of which methanotrophs and methanogens are active and to what extent is made possible by the technological steps behind Next Generation Sequencing of DNA and RNA, but the pathways driving the observed shifts are still largely unknown (Poppeliers et al., 2022).

One way to try to discern effects on a ecosystem level is to use a so called phylogenetic molecular ecological network over methanogenic and methanotrophic activation, which can be used to analyze what specific structures, species and interactions are prevalent occurs under certain conditions (Wang et al., 2022; Zhou et al., 2011). These networks has been successfully used in predicting CH_4 fluxes, and need high-throughput metagenic sequencing data of the assembly (Wang et al., 2022). Further more, there are gaps of knowledge within the workings of the enzyme MCR, such as when it assemble, mature and activates due to its instability *in vitro* (Lyu et al., 2018).

To link a shift in CH_4 flux with the community structures of methanogens and methanotrophs is difficult due to complex biotic systems of the microbes, and is seen as a problem within microbial ecology as a whole (Nazaries et al., 2013; Poppeliers et al., 2022). The community structures and their shifts is also difficult to generalize as they are dependent on many factors, which varies both seasonally and between years, within and between sites (Oh et al., 2020; Poppeliers et al., 2022). The complexity of the system could be reduced with more knowledge of what are the main drivers of these shifts, the interaction between them and knowledge of whether or not they are generalizable (Poppeliers et al., 2022). This could yield a more focused research and the ability to include a more accurate microbial effect in Arctic climate models.

4.2 Previous studies

A collection of studies done on soil CH_4 sinks in Arctic environments is presented in Table 4.1. No distinction was done with regards to Sub-Arctic or Arctic environments since the definitions are varying between the studies of what is defined as High, Low, or Sub-Arctic. The collection is primarily based on studies from Emmerton et al., 2014 and Lau et al., 2015. These studies did not include plots with passive warming treatments, such as OTC's.

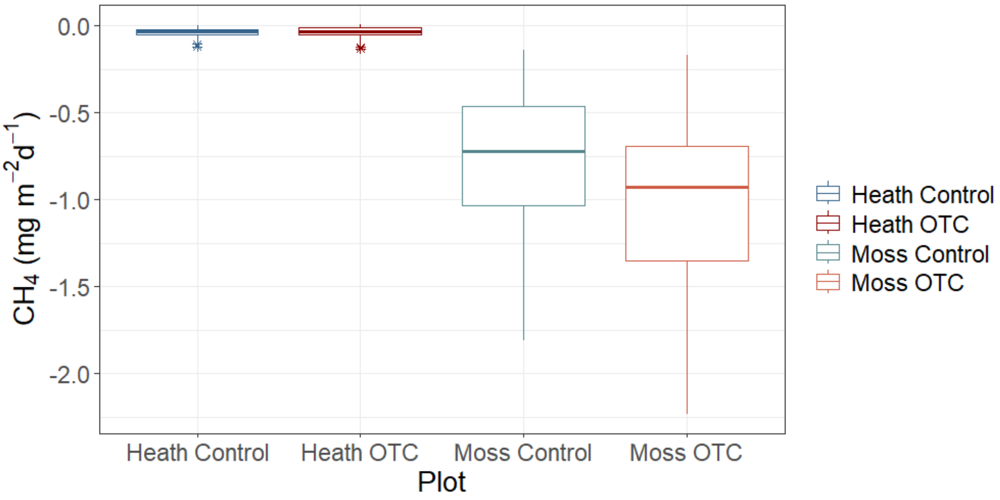
Table 4.1: Summary of multiple previous CH_4 flux studies in the Arctic, with CH_4 fluxes along with environmental type, location, primary vegetation, soil moisture at 0-5 cm depth, when the measurements were taken and sources.

Environmental type	**Location**	**Primary vegetation**	**Soil moisture at 0-5 cm depth**	**Year of measurement**	CH_4 **atmospheric uptake** $mg[CH_4]m^{-2}d^{-1}$	**Source**
Mildly acidic, dry upland soil	Russia, Siberia, Nadym town	Lichen dominated pine forest tundra	Not disclosed	July 2014	-0,4 to -0,6	Belova et al., 2020
Arctic polar desert	Canada, Ellesmere	Patchy herb barren	<24 v/v % at all sites	July-August 2010	-0,9 to -0,3	Stewart et al., 2012
Arctic valley	Greenland, Zackenberg	*Salix Arctica* snowbed and *Cassiope* heath	Not disclosed	Summer 1997	-0,3	Christensen et al., 2000
Low tundra, moist tundra meadow	USA, Skan Bay	Moss dominated with lichens, ferns, dwarf shrubs and grasses	186-223 w/w %	October 1987	-2,7	Whalen and Reeburgh, 1990
Arctic cold deserts	Canada, Novanut	No vegetation	15,1 ± 1,0 v/v %	Summers 2008-2012	-1,37 ± 0,06	Emmerton et al., 2014
Dry Arctic tundra	Greenland, Disko Bay	No vegetation, mosses, sparse grass and lichens	Ranged between 0-27 v/v %	July-August 2015	-2,834 ± 0,138	Juncher Jørgensen et al., 2024
Proglacial tundra	Greenland, Kangerlussuaq	Dwarf willows, grasses, and no vegetation	Ranged between 0-16 v/v %	August 2016	-1,793 ± 0,1152	Juncher Jørgensen et al., 2024
Alpine and grassland tundra, boreal forest	Canada, Yukon, Kluane Lake	Grass, shrubs, pines, aspen	Ranged between 0-27 v/v %	August 2017	-3,59 ± 0,250	Juncher Jørgensen et al., 2024

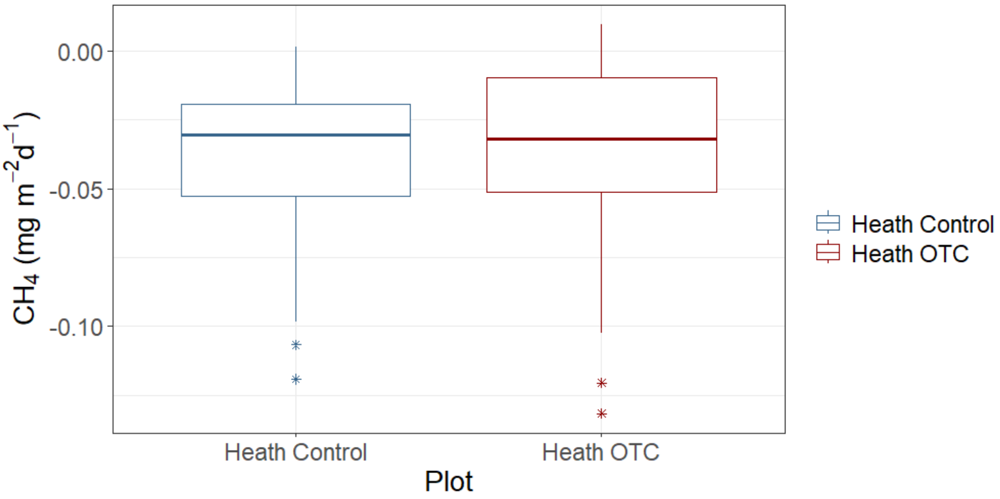
5

Results

5.1 Methane gas fluxes



(a) Box plots over CH_4 fluxes in OTC and Control plots at both sites.



(b) Box plots over CH_4 fluxes in OTC and Control plots at the Heath site.

Figure 5.1: Box plots of CH_4 fluxes for OTC and Control treatments at both sites. The box includes the middle 50% of the data points, making up the interquartile range (IQR), the thick horizontal line represents the average value, and the vertical line spans no further than 1,5 IQR from the edge of the box. Values not covered by the box or the lines are outliers, visualized with a *.

A CH_4 uptake is observed in both Control and OTC plots at the Heath and Moss site, see Figure 5.1. The Moss site sees a 32% higher CH_4 uptake within the OTC plots compared to the Control plots (p<0,001) with a mean CH_4 uptake of -1,04 ± 0,472 $mg[CH_4]m^{-2}d^{-1}$ in the OTC plots and -0,790 ± 0,422 $mg[CH_4]m^{-2}d^{-1}$ in the Control plots, see Table 5.1 and Table 5.2. The $CH4$ fluxes at the Heath site is smaller and a 4,2% lower CH_4 uptake is seen in the OTC plots compared to the Control plots (p=0,836), see Figure 5.1 (b) and Table 5.2. At the Heath site, no significant different between the two treatments could be observed (p=0,836), where the average flux was -0,0361 ± 0,0336 $mg[CH_4]m^{-2}d^{-1}$ in OTC plots and -0,0377 ± 0,0259 $mg[CH_4]m^{-2}d^{-1}$ in Control plots, see Table 5.1.

The CH_4 fluxes are differing between the two sites, see Figure 5.1 (a), and the Heath site has an about 95% lower flux than the Moss site. A summary of the measured CH_4 fluxes including means, medians, number of observations (Obs) along with standard deviations (Std dev) can be seen in Table 5.1, and the pairwise differences between treatments (OTC or Control plots) is seen in Table 5.2, wherein estimate is the estimated difference between the pair, SE stands for standard error and df for degrees of freedom. A higher variation of the measured CH_4 fluxes was observed at the Moss site compared to the Heath site, for both Control or OTC plots.

Table 5.1: Summary statistics of CH_4 fluxes ($mg[CH_4]m^{-2}d^{-1}$) on both sites' OTC plots and Control plots, which gives the number of measurements in each category (Obs), the mean and median fluxes along with the standard deviation (Std dev).

Plot	Obs	Mean	Std dev	Median
Heath Control	83	-0,0377	0,0259	-0,0305
Heath OTC	73	-0,0361	0,0336	-0,0319
Moss Control	77	-0,790	0,422	-0,723
Moss OTC	73	-1,041	0,472	-0,926

Table 5.2: Pairwise differences between the treatments' CH_4 uptake ($mg[CH_4]m^{-2}d^{-1}$) at both sites, which gives the estimated difference between the two treatments, the estimated standard error between them, the number of degrees of freedom along with the obtained p value.

Plots	Estimate	SE	df	p value
Heath Control - Heath OTC	-0,000963	0,00463	146	0,836
Moss Control - Moss OTC	0,254	0,0689	140	<0,05

A Hedge's g' effect size is a test to compared the effect of different treatments, in this study between the OTC and Control plots, see Figure 5.2. At the Moss site, a medium negative effect (-0,558) is observed, which indicates an increased CH_4 sink in the OTC plots. For the Heath site, a slight positive effect (0,0521) was observed. This effect is smaller than a small effect at 0,2, and indicated a negligible effect on CH_4 uptake within the OTC plots compared to the Control plots. The neglible effect is also strengthened by the high, non-significant p value (p=0,836), see Table 5.2.

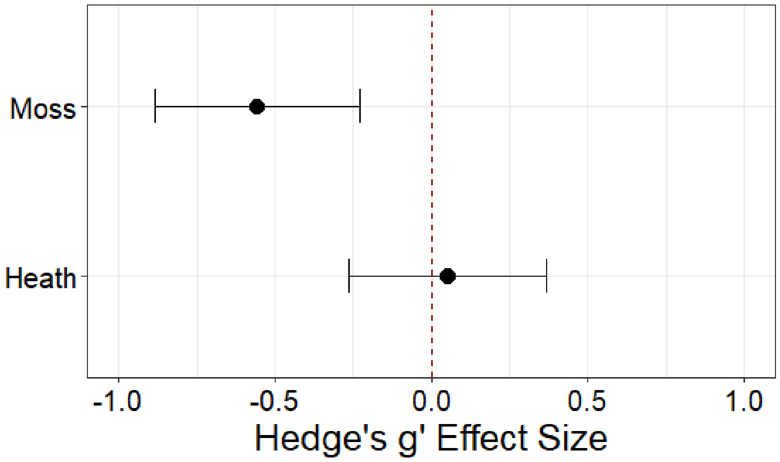
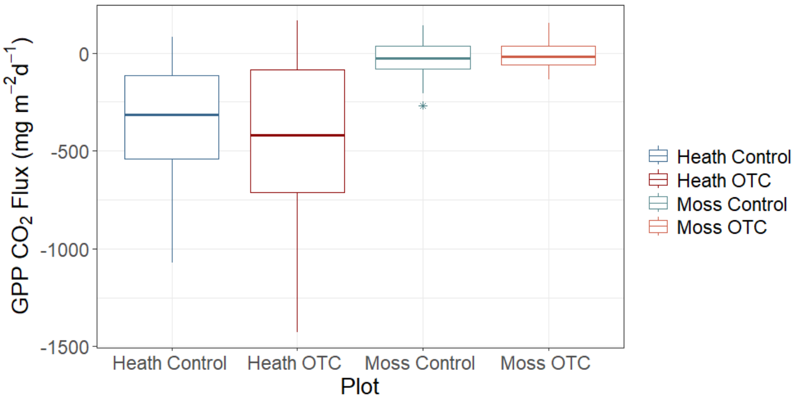


Figure 5.2: Hedge's g' effect size between the OTC treatment and Control plots for CH_4 fluxes at both sites. The point represents the Hedge's g' effect size, while the line reaches out to cover a 95% confidence interval.

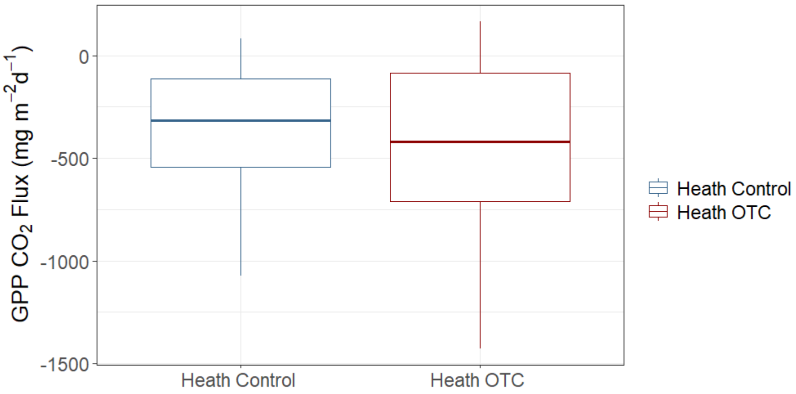
5.2 Carbon dioxide fluxes

Three types of measurements of CO_2 were analyzed: GPP, R_{eco} and NEE. The measured CO_2 fluxes were overall varied and about three to six times larger at the Heath site compared to the Moss site. Between treatments, the Heath site saw a larger variation of CO_2 in the OTC plots compared to the Control plots, while the Moss site saw a smaller variation within the OTC's. The large variability between measurements is shown in both GPP seen in Figure 5.3 and NEE seen in Figure 5.5 (b).

5.2.1 Gross Primary Production



(a) Box plots over GPP for both the Heath and Moss sites, divided into OTC and Control plots.



(b) Box plots over GPP for the Heath site, divided into OTC and Control plots.

Figure 5.3: Box plots over GPP for both the Heath and Moss sites, divided into OTC and Control plots. In (a) both sites are visualized, while (b) shows only the Heath site's fluxes. The box includes the middle 50% of the data points, making up the IQR, the thick horizontal line represents the average value and the vertical line spans no further than 1,5 IQR from the edge of the box. Values not covered by the box or the lines are outliers, visualized with a *.

The GPP was not significantly changed by the OTC's at either site, see Figure 5.3. Within the OTC plots at the Moss site, the GPP sink decreased 73,5% compared to the Control plots (p=0,272). An average GPP shifting from -32,1 ± 98,2 $mg[CO_2]m^{-2}d^{-1}$ in the Control plots to -8,57 ± 68,9 $mg[CO_2]m^{-2}d^{-1}$ in the OTC plots was observed. The Heath site's OTC plots saw a 22% larger GPP sink compared to the Control plots (p=0,250), -357 ± 290 $mg[CO_2]m^{-2}d^{-1}$ in the Control plots to -459 ± 429 $mg[CO_2]m^{-2}d^{-1}$ in the OTC plots, see Table 5.3. The percentual GPP change between OTC and Control plots is larger at the Moss site than at the Heath site. The actual change CH_4 uptake between treatments is larger at the Heath site, while the Moss site saw a lower average GPP flux than the Heath site. However, the p values are non significant.

Table 5.3: Summary statistics over GPP ($mg[CO_2]m^{-2}d^{-1}$) on both sites' OTC plots and Control plots, which gives the number of measurements in each category (Obs), the mean and median fluxes along with the standard deviation (Std dev).

Plot	**Obs**	**Mean**	**Std dev**	**Median**
Heath Control	37	-357	290	-316
Heath OTC	31	-459	429	-419
Moss Control	32	-32,1	98,2	-28,5
Moss OTC	33	-8,57	68,9	-17,8

Table 5.4: Pairwise differences between the treatments' GPP ($mg[CO_2]m^{-2}d^{-1}$) at both sites which gives the estimated difference between the two treatments, the estimated standard error between them, the number of degrees of freedom along with the obtained p value of the difference.

Plots	**Estimate**	**SE**	**df**	**p value**
Moss Control - Moss OTC	-20,7	18,7	55,3	0,272
Heath Control - Heath OTC	102	88,1	60,4	0,250

The Hedge's g' effect size for GPP is presented in Figure 5.4, and a large standard deviation can be seen. At the Moss site a small positive effect of 0,27 is seen, implying a decreased CO_2 sink in the OTC's compared to the Control plots. The Heath site saw a small negative effect at -0,28, implying a small increase of the GPP sink in the OTC plots compared to the Control plots.

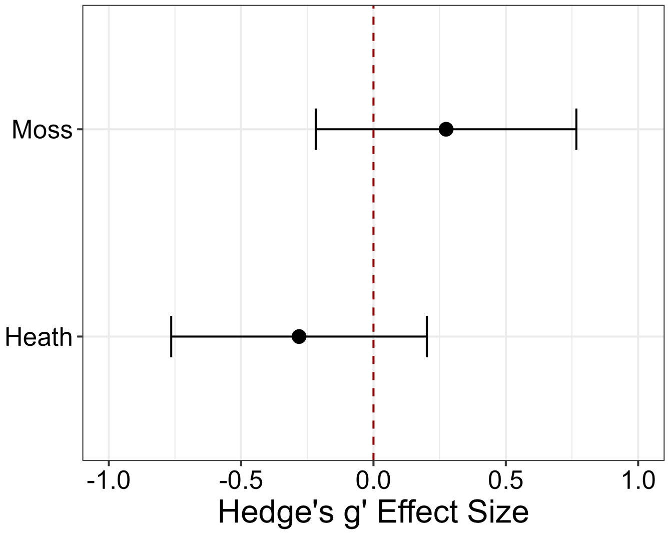
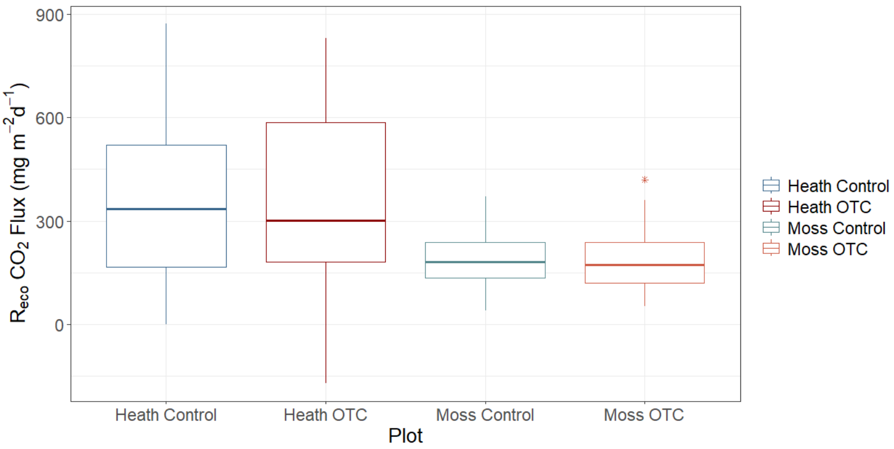
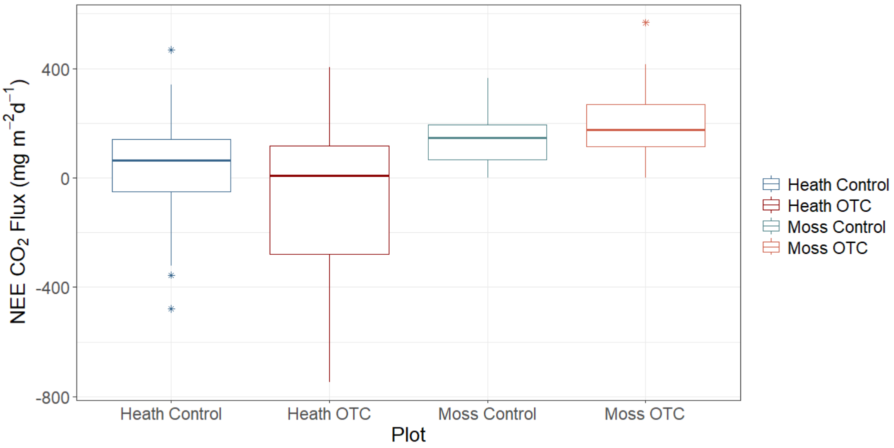


Figure 5.4: Hedge's g' effect size between the OTC treatment and Control plots for GPP at both sites. The point represents the Hedge's g' effect size, while the line reaches out to cover a 95% confidence interval.

5.2.2 Ecosystem respiration and Net Ecosystem Exchange



(a) Box plot over R_{eco} at the Moss and Heath site, with and without warming treatment.



(b) Box plot over NEE at both Moss and Heath sites, with and without warming treatment.

Figure 5.5: Box plots over NEE and R_{eco} at the two sites' OTC and Control plots. The box includes the middle 50% of the data which makes up the IQR, the thick horizontal line represents the average value, and the vertical line spans no further than 1,5 IQR from the edge of the box. Values not covered by the box or the lines are outliers, visualized with a *.

R_{eco} was not significantly impacted by the OTC's at either site (p=0,587 at the Heath site and p=0,801 at the Moss site). The mean R_{eco} was about two times higher at the Heath site than the Moss site, visualized in Figure 5.5 (a). Both sites saw a very small effect on R_{eco} caused by the OTC's, where the mean R_{eco} changed less than 8% between OTC and Control plots (p>0,58 for both sites), see Table 5.5 and Table 5.6. Further more, Hedge's g' effect size is close to zero for both sites (Moss 0,0491, Heath -0,0893), indicating that the OTC's effect on R_{eco} was negligible, see Figure 5.6 (a).

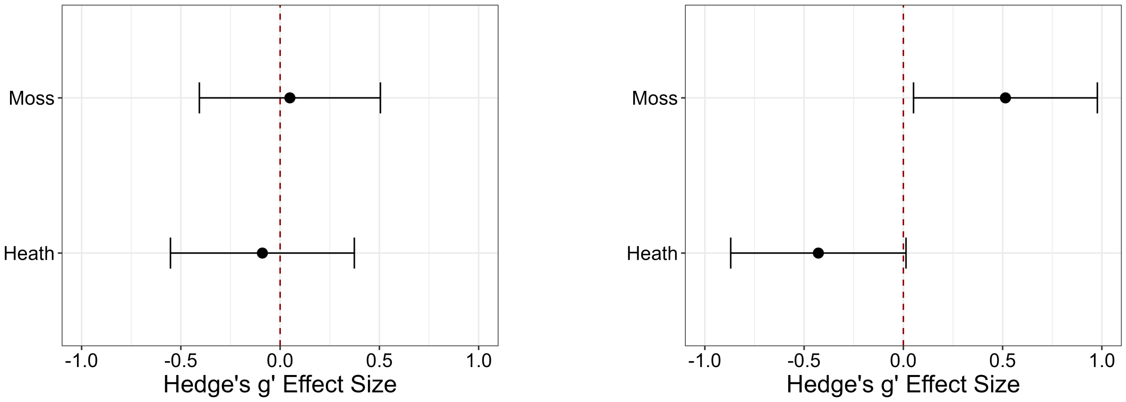
An effect caused by the OTC's was however observed for NEE, see Figure 5.6 (b). The exact changes can be seen in Table 5.5 and Table 5.6. The Heath site saw a 254% reduction of the NEE flux within the OTC plots compared to the Control plots (p=0,055), a 102± 52,5 $mg[CO_2]m^{-2}d^{-1}$ reduction where the NEE flux went from a mean emission of 40,2 ± 186 $mg[CO_2]m^{-2}d^{-1}$ in Controls to a mean uptake of 60,7 ± 278 $mg[CO_2]m^{-2}d^{-1}$ within the OTC plot. The Moss site saw 28% increase of the NEE uptake within the OTC's compared to the Control plots (p=0,0698), corresponding to a 39,9 ± 21,6 $mg[CO_2]m^{-2}d^{-1}$ increase. The mean emission is 142 ± 98 $mg[CO_2]m^{-2}d^{-1}$ in Moss site's Control plots and 197 ± 116 $mg[CO_2]m^{-2}d^{-1}$ in the Moss site's OTC plots. Hedge's g' effect size over NEE, see Figure 5.6b, shows a positive medium sized effect at 0,514 at the Moss site, indicating a lower NEE uptake in the OTC plots compared to the Control plots. At the Heath site, a small to medium effect size is seen at -0,428, which indicates a higher NEE uptake in the OTC plots compared to the Control plots. Between the sites NEE differs, with a around 3,5 times higher mean flux at the Moss site's Control plots compared to the Heath site's Control plots, see Figure 5.6 (b).

Table 5.5: Summary statistics over NEE and R_{eco} ($mg[CO_2]m^{-2}d^{-1}$) on both sites' OTC plots and Control plots, which gives the number of measurements in each category (Obs), the mean and median fluxes along with the standard deviation (Std dev).

R_{eco}				
Plot	**Obs**	**Mean**	**Std dev**	**Median**
Heath Control	39	377	246	336
Heath OTC	34	355	251	301
Moss Control	39	186	74,0	182
Moss OTC	36	190	92,6	173
NEE				
Plot	**Obs**	**Mean**	**Std dev**	**Median**
Heath Control	44	40,2	186	65,0
Heath OTC	38	-60,7	278	8,55
Moss Control	38	142	98,0	145
Moss OTC	37	197	116	177

Table 5.6: Pairwise differences between the treatments' NEE and R_{eco} ($mg[CO_2]m^{-2}d^{-1}$) which gives the estimated difference between the two treatments, the estimated standard error between them, the number of degrees of freedom along with the obtained p value.

R_{eco}				
Plots	**Estimate**	**SE**	**df**	**p value**
Heath Control - Heath OTC	31,6	57,9	63,1	0,587
Moss Control - Moss OTC	-4,46	17,6	64,7	0,801
NEE				
Plots	**Estimate**	**SE**	**df**	**p value**
Heath Control - Heath OTC	102	52,5	72,1	0,0550
Moss Control - Moss OTC	-39,9	21,6	57,6	0,0698



(a) Hedge's g' effect size between the OTC treatment and Control plots for R_{eco} at both sites.

(b) Hedge's g' effect size between the OTC treatment and Control plots for NEE at both sites.

Figure 5.6: Hedge's g' effect size between the OTC treatment and Control plots for R_{eco} and NEE at both sites. The point represents the Hedge's g' effect size, while the line reaches out to cover a 95% confidence interval.

5.3 Inhibition

The result from the successful inhibitions is presented in Figure 5.7. For all successful inhibitions, the CH_4 flux after inhibition is close to zero. Further more, a slightly positive CH_4 flux is observed in some cases, indicating CH_4 production in the soil.

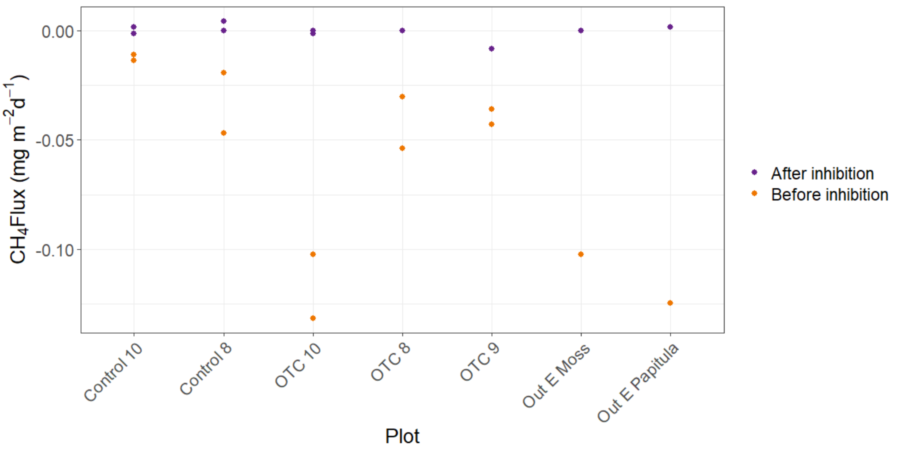


Figure 5.7: Successful inhibition attempts done at the Heath site. Two inhibitions were performed outside exclosure with two different primary vegetation types included. Three inhibitions were done in OTC plots and two in Control plots.

5.4 Other measured conditions

Other environmental factors were measured and soil moisture, soil temperature, wind speed and moss height are presented below. The pairwise comparison of moisture, soil temperature, and moss height is summarized in Table 5.7 below.

Table 5.7: Pairwise differences between the sites' and treatments' soil moisture, soil temperature and moss height, which gives the estimated difference between the pair, the estimated standard error between them, the number of degrees of freedom along with the obtained p value.

Soil moisture (v/v %)				
Plots	**Estimate**	**SE**	**df**	**p value**
Heath Control - Heath OTC	4,42	2,38	285	0,0638
Heath Control - Moss Control	27,3	4,66	23,2	<0,0001
Heath OTC - Moss OTC	21,5	4,72	24,4	0,0001
Moss Control - Moss OTC	-1,35	2,42	285	0,578
Soil temperature (°C)				
Plots	**Estimate**	**SE**	**df**	**p value**
Heath Control - Heath OTC	-0,343	0,195	285	0,0799
Heath Control - Moss Control	-1,74	0,354	24,3	0,0001
Heath OTC - Moss OTC	-1,64	0,360	25,7	0,0001
Moss Control - Moss OTC	-0,246	0,199	285	0,217
Moss height (cm)				
Plots	**Estimate**	**SE**	**df**	**p value**
Moss Control - Moss OTC	1,63	0,803	89	0,0453

5.4.1 Soil moisture

Soil moisture is shown in percentages in Figure 5.8 and Table 5.8 for each treatment at the two sites. The average soil moisture was more than 20 v/v % higher at the Heath site than at the Moss site ($p<0,001$), with a higher degree of soil moisture variability within the Heath site compared to the Moss site. When comparing the soil moisture between OTC and Control plots at each site, the Heath sites soil moisture decreased with 4,4 v/v % in the OTC plots compared to the Control plots ($p=0,0638$), and the Moss sites soil moisture non-significantly increased by 1,3 v/v % in OTC plots compared to the Control plots ($p=0,578$). Additionally, one can see that the variance at the Moss site's OTCs is larger than it's Controls, a trend not seen at the Heath site.

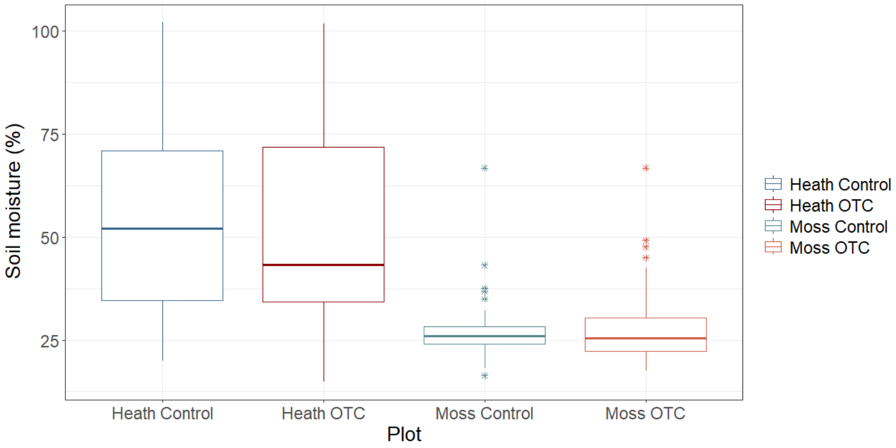


Figure 5.8: Box plots over soil moisture percentage for each plot type at both sites. The box includes the middle 50% of the data which makes up the IQR, the thick horizontal line represents the average value, and the vertical line spans no further than 1,5 IQR from the edge of the box. Values not covered by the box or the lines are outliers, visualized with a *.

Table 5.8: Summary statistics over soil moisture percentage (v/v %) on both sites' OTC plots and Control plots, which gives the number of measurements in each category (Obs), the mean and median moistures along with the standard deviation (Std dev).

Plot	Obs	Mean	Std dev	Median
Heath Control	83	54,3	21,8	52,1
Heath OTC	73	50,5	23,1	43,3
Moss Control	77	27,1	6,86	25,9
Moss OTC	73	28,5	9,39	25,5

5.4.2 Soil temperature

The Moss site's soil temperature in the Control plots was about 1,7 °C higher than the Heath sites' ($p<0,001$), see Figure 5.9 and Table 5.9. Between treatments, a slightly higher soil temperature was seen in the OTC plots compared to the Control plots. At the Heath site, an increase of 0,34°C was observed in the OTC plots compared to the Control plots (p=0,0799). The increase was lower at the Moss site, where the soil temperature was 0,25°C (p=0,216). higher in the OTC plots compared to the Control plots.

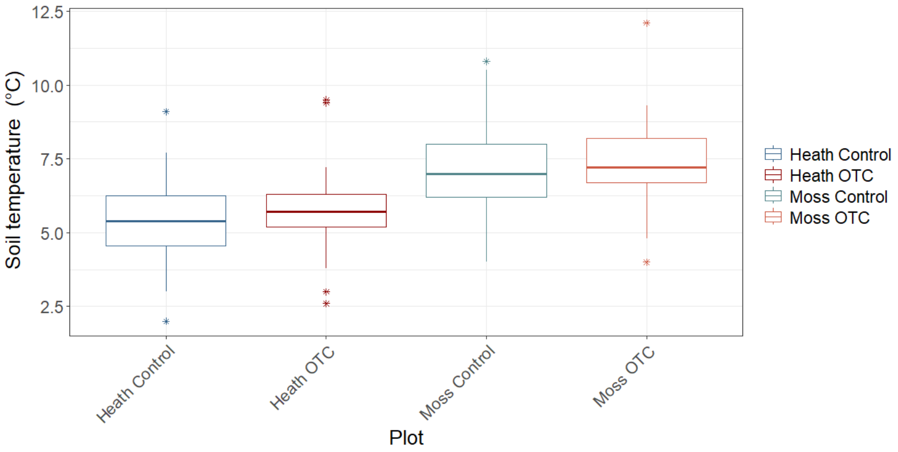


Figure 5.9: Box plots over soil temperature at both sites and treatments. The box includes the middle 50% of the data which makes up the IQR, the thick horizontal line represents the average value, and the vertical line spans no further than 1,5 IQR from the edge of the box. Values not covered by the box or the lines are outliers, visualized with a *.

Table 5.9: Summary statistics over soil temperature (°C) on both sites' OTC plots and Control plots, which gives the number of measurements in each category (Obs), the mean and median temperature along with the standard deviation (Std dev).

Plot	Obs	Mean	Std dev	Median
Heath Control	83	5,42	1,41	5,4
Heath OTC	73	5,73	1,26	5,7
Moss Control	77	7,13	1,34	7,0
Moss OTC	73	7,37	1,45	7,2

5.4.3 Wind speed

The average wind speed was similar for both sites and treatments. However, a larger variety of wind speeds was observed at the Heath site compared to the Moss site, see Figure 5.10 below.

5.4.4 Moss height

The moss height at the Moss site is presented in Figure 5.11 below. A small difference is seen, with a slightly lower average moss height, around 1,6 cm, was observed in the OTC plots compared to the Control plots ($p<0,05$).

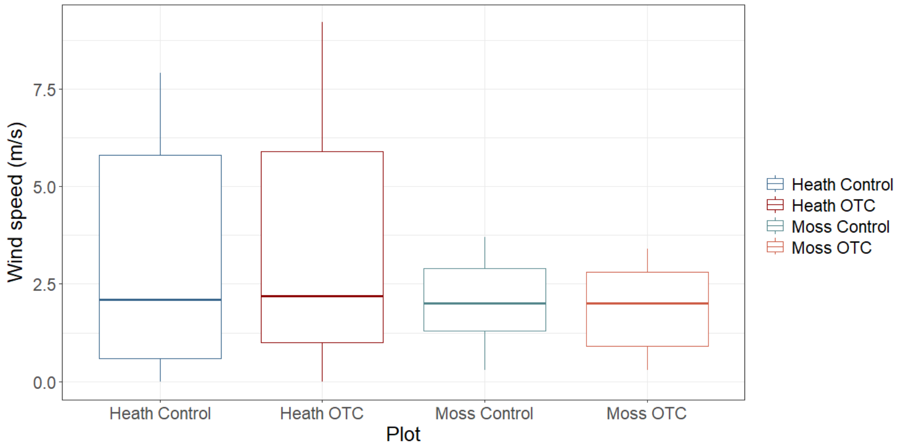


Figure 5.10: Box plots over wind speed for both sites' OTC plots and Control plots. The box includes the middle 50% of the data points, making up the interquartile range (IQR), the thick horizontal line represents the average value, and the vertical line spans no further than 1,5 IQR from the edge of the box.

Table 5.10: Summary statistics over wind speed (m/s) on both sites' OTC plots and Control plots, which gives the number of measurements in each category (Obs), the mean and median wind speed along with the standard deviation (Std dev).

Plot	Obs	Mean	Std dev	Median
Heath Control	83	3,0	2,6	2,1
Heath OTC	73	3,3	2,8	2,2
Moss Control	77	2,0	1,0	2
Moss OTC	73	1,9	1,0	2

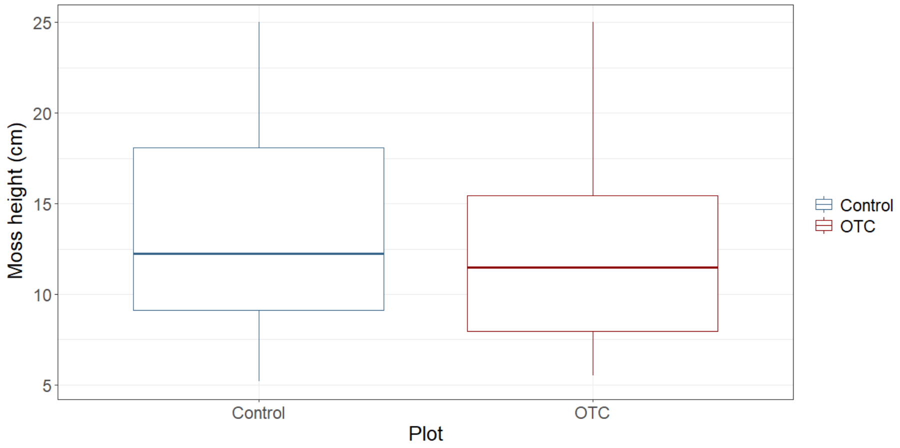


Figure 5.11: Box plot over moss height for OTC and Control plots at the Moss site. The box includes the middle 50% of the data which makes up the IQR, the thick horizontal line represents the average value, and the vertical line spans no further than 1,5 IQR from the edge of the box.

Table 5.11: Summary statistics over moss canopy height (cm) on the Moss site's OTC plots and Control plots, which gives the number of measurements in each category (Obs), the mean and median height along with the standard deviation (Std dev).

Plot	**Obs**	**Mean**	**Std dev**	**Median**
Moss Control	50	13,7	5,70	12,2
Moss OTC	50	12,1	4,85	11,5

6

Discussion

First site specific discussions will be presented for each CH_4 followed by CO_2. This is followed by a comparison between the two sites, and previous studies done in similar conditions and the inhibition experiments.

6.1 The Moss site

6.1.1 Methane

An OTC effect is observed at the Moss site, with an increased CH_4 uptake in the OTC plots compared to the Control plots. The difference is statistically significant (p < 0,05), see Table 5.2. Further more, the Hedge's g' effect size showed a medium negative effect. The size of the fluxes are comparable to other CH_4 flux studies at Arctic CH_4 sink sites, see Section 4.2. Thus, the results indicates that there is a trend of increased soil CH_4 uptake during warming conditions in the growing season at the Moss site.

At the Moss site, no shift in vegetational species composition has been observed, and thus a changing vegetation community in OTC plots is not believed to be a factor in increasing CH_4 uptake. However, the existing vegetation was affected and the moss was on average 1,6 cm lower (p<0,05) in the OTC plots then the Control plots, see Figure 5.11. Vegetation height is seen to have an impact on CH_4 fluxes, with higher vegetation causing higher emissions of CH_4 in wet soils (von Fischer et al., 2010). The slightly higher moss in Control plots could be part of the explanation to why a smaller CH_4 uptake is seen in these plots. However, the underlying lava field crates an uneven ground and the moss depth as such varies. To verify that a correct average moss depth was calculated, more measurements in each plots should be preformed in future studies with a longer measurement probe. No shift in vegetational species composition has been observed to date (Björnsdóttir et al., 2022), but could occur under increased warming conditions based on observations at other ITEX sites (Hollister et al., 2023). If shifts of the plant community structure would occur, we recommend observing how this shift could affect the soil CH_4 fluxes, since the vegetation type influences methane production and consumption (Keuschnig et al., 2022; Nazaries et al., 2013; Poppeliers et al., 2022). Since around 13% of Iceland is covered by moss dominated vegetation, (Náttúrufræðistofnunar Íslands, 2024) increasing the understanding of how these ecosystems will be affected by global warming is important to understand how Iceland as a whole will be affected.

The soil moisture percentage and the soil temperature did not significantly change between the OTC and Control plots at the Moss site (p=0,578 and p=0,217 respectively). Thus, soil moisture and soil temperature are not believed to be factors which are driving the observed effect of the OTC's within this study.

R. lanuginosum ecosystems in Iceland are fragile and prone to soil erosion, especially from sheep grazing (Thorsteinsson & Arnalds, 1992). Þingvellir national park has been fenced of since 1928, which has prevented grazing since then, thus leading to less soil erosion inside the national park compared to outside of it (Jägerbrand, 2007). Since a lot of Moss heath ecosystems in Iceland is grazed by sheep (Akello, 2019), it would be interesting for future studies to also include flux measurement at sheep grazed plots.

In an attempt to upscale the results within the study, the following assumptions were made; (1) all moss dominated ecosystem in Iceland take up on average -1,04 $mg[CO_2]m^{-2}d^{-1}$ CH_4 (as was observed in the OTC plots at the Moss site) during the growing season and (2) that 13 % of Iceland is covered by moss dominated ecosystems as reported by Náttúrufræðistofnunar Íslands, 2024. This thought experiment then implies that the Icelandic moss dominated ecosystems would take up 13,9 ton CH_4 per day and 5090 ton per year. The real CH_4 uptake in these areas will differ depending on location, season, moisture, erosion, temperature and other factors. This thought experiment does, however, put our results into perspective. In relation to Iceland's CH_4 emissions of 160 000 tons CH_4 per year (Climate and Clean Air Coalition, 2023) , this makes up for a small part of the budget, and even less on a global scale, where the CH_4 budget is described in the order of millions of tons (Saunois et al., 2024). Even if the CH_4 uptake increases shown in this study would be assumed to be applicable for all Dry Moss Tundra Ecosystems in the growing season the changes in the global or regional CH_4 budget is very small.

6.1.2 Carbon dioxide

The GPP at the Moss site is low for both treatments with an average flux of -32,1 ± 98,2 $mg[CO_2]m^{-2}d^{-1}$ in the Control plots and -8,57 ± 68,9 $mg[CO_2]m^{-2}d^{-1}$ in the OTC plots. A low value of GPP is expected since the moss grows very slowly (1,6-1,7 mm/year on lava if the conditions are good), and thus also stores carbon slowly (Tuviala & Isotalo, 2023).

The GPP uptake at the Moss site did not see any significant shifts based on the OTC treatment (p=0,272), see Figure 5.4. GP is based on both R_{eco} and NEE fluxes and as such both the light and dark measurement of a single plot and time had to be used and be of good quality. As such a smaller number of GPP observations was able to be analyzed compared to NEE and R_{eco}, which may be one reason for the higher p value with these measurements. Additionally, some of the observed NEE flux changes could be hidden by the natural variance within the R_{eco} measurements and vice versa, leading to a GPP with higher p values. OTC's did not yield a significant effect on R_{eco} (p=0,801), but an OTC effect was seen on NEE (p=0,0698) at the Moss site, see Figure 5.6. One possible reason for why an increased NEE is seen is a high temperature yielding a diminished growth within the OTC plots. The OTC's could increase the temperature in the surface of the vegetation compared to the air, causing an increased leaf-air vapor pressure (Hollister et al., 2023). This could cause the moss' stomata to close in order to reduce water loss, resulting in a lower rate of photosynthesis (Venkat & Muneer, 2022). The optimal temperature for Wooly-fringe moss is 5°C (Jägerbrand, 2005), which the Moss site's soil temperature exceeds in both plot types, and is yet higher in the OTC's than in Controls. A growth limiting temperature was theorized by Jägerbrand, 2005 to be a reason for a future potential lower moss height within the OTC's. The moss is today slightly lower, by 1,6 cm (p=0,0453), and a decreased rate of photosynthesis is seen within the OTC's compared to the Control plots.

6.2 The Heath site

6.2.1 Methane

At the Heath site the observed CH_4 fluxes were low, about a tenth of the smallest uptakes seen in the literature analysis, see Table 4.1. The observed CH_4 uptake was about 0,03 ± 0,030 $mg[CH_4]m^{-2}d^{-1}$ for both OTC and Control plots, and no significant difference between treatments (p=0,836). The low uptake could be due to the late spring and snow melt, which could impact environmental factors and yield the observed higher soil moisture, and lower soil temperatures.

Over all, the low CH_4 flux made it difficult to discover changing fluxes, due to them being close to the limit of what the instrument could detect with high precision. A high percentage change could in low flux observations be covered by the inherent noise of the machine, and/or small leaks which could lead to unrecoverable deviations. To be able to observe the soil methanogenic and methanotrophic communities and their activities with a higher degree of certainty a microbial analysis would be required.

The late snow melt could affect the activity of microbes, both methanogenic and methanotrophic. The soil moisture is relatively high, probably due to the late snow melt, which could yield a lowered methanotrophic activity and increase any potential methanogenic within the soil. The cold soil decreases the activity of both types of microorganism. That both methanotrophs and methanogens have low activities at the Heath site is a hypothesis which is strengthened by the very low CH_4 emissions seen during the inhibition experiments. If we would return to the site later in the season, the increased leaf area, temperature, nutrient accessibility within the soil, decomposition rates and lowered moisture content would probably lead to larger CH_4 sinks, based on previous studies (Hollister et al., 2023; Ueyama et al., 2023; Zona et al., 2016). Seasonal shifts could also be the determining factor of the lower

CH_4 sink at the Heath site compared to the ones seen in the literature study (see Table 4.1). All included previous studies were conducted during the growing season (July-October), making fair comparisons difficult. According to Poppeliers et al., 2022 the sampling occasion explains most of the differences in microbial community structures, more than soil temperature, soil moisture, ecosystem type, vegetation type or snow cover, and the winter-spring transition sees the most abrupt change in CH_4 flux.

The soil temperatures were increased slightly by 0,31 degrees in the OTC plots at the Heath site (p=0,0799).An increase in soil temperature has been shown to increase both methanogenic (Poppeliers et al., 2022; van Hulzen et al., 1999), and methanotrophic activity (Poppeliers et al., 2022; Semrau et al., 2010). To pinpoint the impact that an increased temperature has on the CH_4 flux itself is difficult due to the low fluxes over all, and convoluting factors such as vegetational impacts. The soil temperature increase in the OTC plots was around 0,3 ± 1,33 °C (p=0,0799). This is less than the predicted Arctic warming, with 19 models (based on CMIP6) having predicted between 3-13 °C increase in 2100 compared to the years 1961-1990, with a mean predicted increase of 7,4°C (Chylek et al., 2024). More feedbacks and shifts may as such occur at these sites in the future than what is seen in this study. These possible missed shifts include both vegetation, moisture, microbial community changes, length of growing season, cold season heating, and nutrient competitions. All of these factor could impact the CH_4 flux.

6.2.2 Carbon dioxide

The vegetation in the OTC plots at the Heath site saw a shift of vegetation community composition, with more vascular plants and less moss. Further more, an over all increase of vegetation biomass with increased density and canopy height was observed (Björnsdóttir et al., 2022; Jónsdóttir et al., 2005). The shift could be driven by the site's increased temperature, both in air (Jónsdóttir et al., 2005) and in soil, as observed in this study. The increased vegetation density and height in the OTC's also cause the plot to experience lower average wind speeds (De Boeck et al., 2012; Hollister et al., 2023) and a lower average soil moisture level (García Criado et al., 2023), which in the Heath site was measured to a decrease of 3,8 ± 2,38 v/v % (p=0,0638). The increased vegetation density also leads to an increase in photosynthetically active material, and as such causes a larger, photosynthetically driven, uptake of CO_2 in the ecosystem (Falk et al., 2015). This effect is shown at the Heath site by GPP and NEE, where a larger sink was seen in the OTC's, with small negative Hedge's g' effect size, see Figure 5.4.

A large variation between the measurements is seen, further increased within the OTC plots. A large variation in CO_2 fluxes could be due to varying amount of PAR hitting the plots during light measurements, due to cloud coverage and a varying time of day when the measurements were taken. Another factor which could play into the large variation of all CO_2 fluxes is the inclusion of different amounts and types of vegetation within the chamber during measurements. To include a varying amount of vegetation in the chamber during measurements was a choice to try to obtain means which are more representative for the whole plot, where both spots with and without dense vegetation coexisted. The increased variation within OTC plots compared to the Control plots are probably due the increased density of vegetation, which causes both a higher variation over all and a more difficult terrain to get an air tight seal over. The large variation of fluxes within each plot leads to a setup where more measurements per plot are needed to obtain statistically significant results than was able to be completed within the temporal scope of the field study.

6.3 Comparison between the two sites

The CH_4 fluxes was lower at the Heath site compared to the Moss site. This is believed to be due to that the Moss site was further along in growing season compared to the Heath site, which was seen both by the Moss site's 2°C warmer soil temperature, a 20 v/v % drier soil and visually seen through the amounts of bursted buds in the vegetation. Factors including leaf area, temperature and moisture are known to have an effect on methanogenic and methanotrophic activity (Nazaries et al., 2013). Poppeliers et al., 2022 saw that seasonal changes had a large impact on community composition even though the drivers were unclear, and that the most abrupt change were during the winter-spring transition. Thus, it is believed that a higher CH_4 uptake would be observed at the Heath site if measurements were done later in the growing season. For future similar studies, we propose to take measurements over comparable seasons and/or obtain full season measurements for each site to get accurate comparisons between the sites.

Both analyzed sites had vegetation with relatively high amounts of moss, but both the density and diversity of other species differ, as well as how the vegetation is impacted by the OTC's. Only the Heath site saw a shift in the distribution of vegetational species between OTC and Control plots, with a decrease of mosses and increase of vascular plants. The different vegetational types as well as species impacts the degree of methanogenic and methanotrophic activity within the soil, see Section 2.2.3.3, and also impacts the seasonality of the methane uptake (Voigt et al., 2023). However, the effect on CH_4 fluxes that the specific plants species has at the Heath site is not known, making analysis with regards to the community shifts difficult. Rather, the general types vascular plants and mosses were used for analysis, and at the Heath site this would probably cause an increase of methanotrophic activity within the OTC plots, seen later in the season. The Heath site also saw an increase in vegetational density and canopy height within the OTC plots compared to the Control plots, which most likely increases the amount of roots present in the soil. Roots aerate the soil, leading to a higher nutrient availability and increases the methanotrophic activity over all (von Fischer et al., 2010). The Moss site saw a slightly decreased moss canopy height within the OTC plots. An increased moss canopy has been seen to insulate the soil, delay the thaw and decrease soil temperature and moisture levels in the soil (Gornall et al., 2007). The vegetation shifts both above and below the surface may have an impact on the methanotrophic and methanogenic activity (García Criado et al., 2023; Topp & Pattey, 1997), and it would be interesting to analyze the site's phylogenetic molecular ecological network within the OTC and Control plots to try to pinpoint the interactions between specific plant and microbial species and how they are affected by temperature increases. This would however require more extensive and high-throughput analysis which was not possible within the scope of this study.

This study did not measure soil pH, but previous studies showed that the Moss site have a more acidic pH at 5,12 ± 0,06, while the Heath site have a pH of 6,48 ± 0,06 (Jónsdóttir et al., 2005). Neutral soils has shown to have the highest methanotrophic and methanogenic activity (Nazaries et al., 2013; Serrano-Silva et al., 2014), and to have a larger diversity of methanogens and methanotrophs (Li et al., 2021; Seppey et al., 2023). The Moss site is more acidic, which could lead to a possible decline in methanogenic and methanotrophic activity compared to the Heath site, which is more neutral. We recommend future studies to measure soil pH as this could shift due to potential vegetation shifts in the OTC's. Further more, it would be interesting to measure the soil's different micronutrient contents and their availability of these within soils to see if they are changed due to vegetational shifts, since they could have a significant impact on both methanogenic and methanotrophic activity (Voigt et al., 2023).

6.4 Comparison to previous studies

The observed magnitude of CH_4 fluxes in the Control plots at the Moss site of about -0,8 $mg[CH_4]m^{-2}d^{-1}$ is in line with previous studies of CH_4 uptakes measured in the Arctic, of between -0,3 and -3 $mg[CH_4]m^{-2}d^{-1}$ (see Table 4.1). The Heath site's flux is however about a tenth of the lowest flux included in the literature study. However, the CH_4 fluxes in the literature study are all taken during the growing season, while the CH_4 flux at the Heath site is taken just after snow melt. The Moss site's CH_4 flux is most similar to the polar desert in Ellesmere, Canada, slightly higher than the slightly acidic upland soil in Nadym, Sibiria and slightly lower than the cold desert in Novanut, Canada. All of these measurements are taken in dry areas during the growing season and has a soil moisture of below 24 %. They are mineral soils which are well drained, but their vegetation types, temperatures, latitudes and nutrient availability are however all different, showcasing how even different environments could cause the approximately same rate of uptake.

6.5 Inhibition

The inhibitions presented in Figure 5.7 indicates that there are active methanogens in the soil with small positive CH_4 fluxes observed. This strengthens the hypothesis that both methanogens and methanotrophs are active in Arctic upland soil, and illustrates the need to better understand these processes. However, due to methodological challenges, too few successful inhibitions were done to statistically verify the results of this study.

An unsuccessful inhibition meant that there are still active methanotrophs, shown by a negative CH_4 flux. This was true for several inhibition attempts, indicating that not enough acetylene gas had diffused down to achieve full inhibition of methanotrophs. This means that most likely no significant inhibition of the methanogens, most often active in the deeper, wetter, more anaerobic part of the soil, is occurring, since they require ten times the acetylene concentration compared to methanotrophs (Chan & Parkin, 2000; Urmann et al., 2008). For the successful inhibitions, there were no way to verify that no methanotrophic activity was still happening, but covered by the methanogenic activity. One way to verify the results is to analyze the activity level of methanotrophs and methanogens through quantifying RNA transcripts of the enzymes pMMO and MCR.

The challenges of creating successful inhibitions were due to several factors. The chamber was placed on the ground, making a tight air seal difficult to obtain in comparison to using collars submerged into the ground, the standard method in measuring gas fluxes (Parkin & Venterea, 2010). The obtained air seal were good enough for short gas flux measurements of three to six minutes, but not for the two hour inhibition period. These longer time periods increases the probability of wind gusts penetrating the air seal, removing the acetylene gas horizontally instead of it diffusing down vertically into the soil. The two hours incubation time may also have been too short for the gas to diffuse downwards due to the relatively high soil moisture contents at the Heath site. Since gases diffuse slower in liquids, the high soil moisture could lead to a slower diffusion of acetylene gas and be a contributing factor for the relatively few successful inhibitions.

Inhibitions could only be done at the Heath site due to the very fragile nature of the Moss site and its dry, slowly growing moss. Conducting the inhibitions would cause too big of a disruption to the ongoing vegetation research.

We recommend further inhibition experiments to be performed in Arctic environments. However, for further experiments we recommend changes in the method to improve the chances of success inhibitions. We recommend to use submerged collars in the ground that the inhibition collar and measurement chamber can be attached to. This should increase the chances of successful inhibition by creating a better air seal with less lateral movements of the gas. Additionally, to use a submerged collar during the entire experiment removes the risk of the flux measurement not being done at the exact same place as the inhibition. A submerged collar could however affect the ecosystem for a long time, and affect the ecosystem functioning as a whole (Parkin & Venterea, 2010). To reduce these problems a collar could be installed which is in the soil of the plot for the duration of the measurements and inhibitions on a timescale of days, weeks or months instead of years, with the collar being as flush to the soil as possible to minimize the microclimate effects (Parkin & Venterea, 2010).

6.6 Sources of error

Some plots within the Heath site had high and dense vegetation. This caused issues in finding several good enough placements of the chamber for gas flux measurements. Thus fluxes from approximately the same location were measured multiple times in some plots, which may lead to a higher degree of dependence and replication within our results. A preference was also seen for lower vegetation in order to obtain the air seals. This could lead to a smaller CO_2 sink than what the real ecosystem has.

The soil moisture and soil temperature values were taken in the same spot within each plot each time to lessen the destructive effect on the fragile vegetation and to reduce the impact on ongoing experiments. The soil moisture and soil temperature does however vary within plots due to the porous and rough terrain, causing pockets of moister or warmer soils. This variability is not captured in full by one single measurement spot. Further more, by measuring multiple times in the same spot, there is a risk of increased aeration, causing more moisture to escape and thus lower soil moisture. Warmer air may also go deeper into the soil than what the site in general experiences, which could impact the measurements. The exact depth of which moisture and temperature was measured was difficult to know, especially since the thermometer stick was much shorter than the moss in some spots, with only a thin layer of actual soil to measure in.

The levels of incoming PAR was variable within the plots and measurements, and some measurements were taken late at night to exploit all good weather windows. This could have an impact on measured CO_2 fluxes due to the plants' circadian rhythm (Venkat & Muneer, 2022), and due to different amount of incoming light.

The OTC setup present challenges. Firstly, the OTC's passive warming is variable over days and between years. OTC's heat mostly during the summer (Hollister et al., 2023), even though most of the global warming effect is seen in the winters in the Arctic (Zona et al., 2016), which may yield skewed results when the obtained GHG fluxes is used for whole year models. The surrounding environment may dilute the OTC's effects on for example soil temperature, moisture and nutrient availability, causing a smaller effect to be observed than what would be otherwise. There is also the convoluting effect of blocked wind yielding higher plants in the OTC plots, than the paired Controls. The higher vegetation could in turn have an effect on the soil in both moisture level, nutrient density, porosity and transport of methane from and into the soil.

7

Conclusions

This study observed increased CH_4 uptake within OTC plots, with a 31,7 % larger CH_4 sink compared to Control plots ($p < 0,05$) at the Moss site. However, no significant effect ($p = 0,8355$) on the CH_4 fluxes between the OTC and Control plots was observed at the Heath site. The average CH_4 uptake in Control plots at the Moss site of -0,790 ± 0,422 $mg[CH_4]m^{-2}d^{-1}$ is in line with previously CH_4 uptake measurements in the Arctic during the growing season (July-October). The CH_4 uptake at the Heath site was low and observed CH_4 fluxes are about a tenth of the previously done studies within the growing season. The low uptake is believed to be due the spring and a late snow melt as the GHG flux measurements were done early in the growing season at the Heath site. We recommend that future studies of similar scopes as this are done later during the growing season to increase comparability of the results within one study, and avoid that the differences between the measured sites are dominated by seasonal shifts.

Our results indicated that a higher CH_4 uptake can be expected in upland moss dominated ecosystems in Iceland with rising temperatures. However, global warming is also expected to increase the atmospheric CO_2 level and change precipitation patterns, which are further factors that should be taken into account when modeling future CH_4 fluxes in the Arctic, both at a local, regional and global level.

There were challenges in identifying biotic and abiotic shifts at the two sites that could explain the observed CH_4 fluxes. At the Heath site, no significant CH_4 flux changes occurred, even though a vegetation shift was observed within the OTC plots with higher and denser vegetaion and a shifted distribution of species. An increase of soil temperatures of 0,34°C ($p = 0,0799$) and a slight drying of the soil, 4,42 v/v % lower soil moisture was observed in the Heath site's OTC plots compared to Controls. The main differences between the sites was theorized to be the seasonal shifts, which may make the shifted uptakes too small, close to the instrument's limit of detection.

At the Moss site, a significant ($p < 0,05$) increase of CH_4 uptake of 31,7% was observed within the OTC's. The only measured factor which was significantly different between the OTC and Control plots was moss height, which were 1,6 cm lower within the OTC plots compared to the Control plots ($p < 0,05$). However, this increase is thought to be too low to be the driving factor behind the CH_4 flux change. In future studies we recommend to measure more possible shifts caused by the OTC's such as soil organic carbon, nutrient availability of nitrogen, phosphate, sulfate and metals, and soil pH. Further more, we recommend to as well study how upland mosses might impact the soil's methanotrophs and methanogens.

Our recommendations for future studies about Arctic soil CH_4 uptake is to focus on obtaining a better temporal and geographical distribution. More over, the driving factors between increased methanogenic and methanotrophic fluxes should be further analyzed, both *in* and *ex situ.* The ecosystem and landscape level needs to be included for a comprehensive understating of current and future CH_4 fluxes. Thus, care must be taken to design studies that measure CH_4 fluxes in a variety of places and ecosystems and at different time of day and growing season.

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