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Rapid microbial responses to temperature changes in Arctic anoxic peat soil

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Table of contents

Abbreviations

at% - Atom percent

C – carbon

CF – Chloroform fumigation

CHO-MFR – formylmethanofuran

CH_3CN – Acetonitrile

CH_4 – methane

Cmic – soil microbial biomass carbon

CO_2 – carbon dioxide

DNA – Deoxyribonucleic acid

DW – Dry weight

GC – Gas chromatography

ppm – parts per million

H_2 – Hydrogen

HPLC – High pressure liquid chromatography

H_2SO_4 – Sulfuric acid

H_4MTP – tetrahydromethanopterin

IPCC – Intergovernmental panel on climate change

IRMS - isotope ratio mass spectrometry

kEC number – kEX number for carbon

keN number – kEX number for nitrogen

kEX number - The proportion of carbon or nitrogen that can be extracted form microbial biomass in comparison to actual carbon or nitrogen in microbial biomass

MeOH – Methanol

N – Nitrogen

NA – Natural abundance

NaCl – Natrium chloride

Nmic – soil microbial biomass nitrogen

N_2 – Nitrogen gas

O - oxygen

O DNA – Oxygen from DNA

Abstract

Arctic peatlands act as important sources and sinks of carbon. Microbial decomposition takes place in these soils, producing the greenhouse gasses carbon dioxide and methane as end-products. A variety of aerobic and anaerobic microbial pathways are involved in the decomposition of organic material in peat soil. In anoxic soil layers, methane and carbon dioxide is often produced through syntropic partnerships between several fermenters and methanogens. Changes in soil conditions like temperature and substrate availability affect which methanogenic and fermentative pathways are dominant in the soil, thus affecting the final gas emissions. Due to their size and fast metabolism, microorganisms have the potential to respond rapidly to environmental changes like temperature variation and are constantly exposed to short-time temperature changes on daily and hourly basis in many natural and anthropogenic ecosystems. How short-term temperature variation affect soil microbial communities is yet poorly understood.

In this master thesis I have investigated microbial responses in Arctic peat soil to temperature changes (heating and cooling). A high resolution 9-week incubation experiment with temperature increase from 2 – 10°C followed by cooling from 10 – 2°C was carried out, thus exposing the peat soil to a temperature range and timeframe similar to Arctic summer season temperature shifts. Gas and metabolite accumulation and microbial community growth and biomass was monitored to establish knowledge about the effects. Methane accumulation was rapidly affected by heating and showed increasing accumulation rates at warmer temperatures. However, exposure to cooling did not immediately reduce the accumulation of methane. This delay might be an effect of established high growth rates at higher temperatures that takes longer time to reverse. A change from no net carbon dioxide emission below 6°C to emission rates increasing rapidly due to heating above 6°C was observed. This change occurred at the same time and temperature as radical changes in concentrations of the fermentative metabolites acetate and propionate and more rapid cell growth. A combination of a change in the ratio between different methanogenic pathways, fermentative pathways and rates of carbon dioxide fixation relative to production are proposed as possible explanations to the shift in carbon dioxide emission seen at 6°C.

This master thesis represents a comprehensive study of time-dependent temperature effects on greenhouse gas emissions from anoxic peat soils, an important and understudied topic in literature.

Introduction

Arctic peatlands in a climate perspective

Northern permafrost soils often act as carbon (C) sinks, storing 50% of the global soil organic C (1). Nineteen percent of the land surface in northern permafrost areas is covered by peatlands (1). Peatlands are of particular importance in the global C budget due to their ability to store large amounts of organic C (1) and their role as natural sources of two important greenhouse gases – methane (CH_4) and carbon dioxide (CO_2) (2-4). Due to these properties, peatlands also have a key role in climate change. Levels of CH_4 in the atmosphere have increased from 0.7 to 1.8 parts per million (ppm) since the industrial revolution (5), while CO_2 has increased from 280 ppm to 414 ppm (6). Global increases in atmospheric CH_4 concentrations contribute to 20% of greenhouse gas-driven global warming (7). Methanogenesis, the biological formation of CH_4, is responsible for 70% of the total CH_4 emission, and 33% of these 70% originate from wetlands including peatlands (7). Thawing and temperature increase affect these large C storages and have the potential to make permafrost soils potent greenhouse gas sources in the future (1).

Peat formation in Arctic permafrost soil

Peat is accumulated organic material and forms when the input rate of organic material is higher than the degradation rate, thereby increasing the C content of the soil (8). Under anoxic and cold conditions, the rate of degradation is slow (8). Permafrost soils are defined by temperatures below 0 °C for more than two consecutive years, but often the permafrost surface layer is seasonally thawed and called the active layer, while the permafrost itself is found deeper in the ground (9). Ice in the deeper layers of Arctic peatlands slows water drainage from the soil, thereby increasing the water content (9). Thus, anoxic conditions in the soil are induced due to the limited solubility of oxygen in water, separating the soil active layer into an oxic and an anoxic part that follows the water table (8). Because of its high reaction potential, oxygen is an efficient electron acceptor and aerobic organisms are therefore able to gain more energy and to grow faster than anaerobic organisms that use less efficient electron acceptors with lower redox potential (10) like nitrate or sulfate (4). A combination of anoxic conditions and low temperatures in the soil will therefore lead to lower microbial activity and slower degradation rates, promoting the accumulation of organic matter.

Organic matter degradation in Arctic peat soil

Despite slow degradation in Arctic peat soil, a vast variety of microorganisms participate in the decomposition of organic matter, harvesting energy and nutrients in the process (4). Also, the active layer is characterized by a higher functional diversity and species diversity compared to the frozen soil below (11). When a plant dies, bacteria and fungi decompose the cellulose and other complex plant-derived molecules. This happens both in the oxic and anoxic soil layers. An important initial degradation step is polysaccharide hydrolysis. This hydrolysis leads to release of monosaccharides (4). These monomers are further oxidized in the anoxic part of the soil by for example fermentative microorganisms and anaerobically respiring bacteria, producing CO_2, hydrogen (H_2), acetate, ethanol, propionate and other small products (4, 12, 13) as waste. Such fermentation metabolites can when accumulated in high concentrations reduce the thermodynamic favourability and energy yield of their production, affecting the rates and pathways of decomposition (14). A syntrophic relationship between fermenters and methanogens that consume and utilize these metabolites are therefore important to omit high concentrations of these compounds. This mutually beneficial relationship benefit both the producer (fermenters) and consumer (methanogen) (4, 13).

Propionate oxidation is an important example of such a syntrophic relationship. When there is a low concentration of acetate, the energy consuming (at standard conditions) oxidation of propionate to acetate, CO_2 and H_2 is performed in the soil (see equation 1) (15).

Equation 1:

$$Propionate + 2\ H_2O \rightarrow Acetate + CO_2 + 3\ H_2 \quad \Delta G^{0'} = +76.0\ kJ/reaction \quad (15)$$

Equation 2:

$$Propionate + 2\ H_2O + 2\ CO_2 \rightarrow Acetate + 3\ Formate + 3\ H^+ \quad \Delta G^{0'} = +65.3\ kJ/reaction \quad (15)$$

Methanogenesis using acetate (see next paragraph) is therefore essential for this fermentative process to take place (15). Tveit *et al.* (14) found this fermentation process to be exergonic in artic peat soil in temperatures between 7 and 12°C when acetate levels were low. At lower temperatures, fermenters oxidizing propionate to acetate, formate and H_2 (equation 2) where more active (14).

Ethanol is a common metabolite in peat soils but is usually consumed too fast or with sufficiently high affinity to prevent high concentrations, as shown for example in northern peat soil (14, 16, 17) and in peat soil from Germany (12). Ethanol oxidation to acetate (equation 3) is another common syntropic fermentation process. The unfavourable energetics of this reaction would not allow ethanol consumption at low concentrations unless the syntrophic partners of the ethanol oxidizer would rapidly consume the products.

Equation 3:

$$Ethanol + H_2O \rightarrow Acetate + 2\ H_2 + H^+ \qquad \Delta G^{0'} = +50\ kJ/reaction\ (16)$$

The final step in anaerobic degradation of organic matter is methanogenesis by methanogenic archaea. The three main pathways are hydrogenotrophic, methylotrophic and aceticlastic methanogenesis (13, 14) (Figure 1). Hydrogenotrophic methanogens are reducing CO_2 using H_2, formate or alcohols (like ethanol) as electron donors producing CH_4 and water (13, 18, 19). This process starts with the reduction of CO_2 to formylmethanofuran (CHO-MFR), the formyl group is then transferred and reduced through several steps to methyl-H_4MTP. Finally, the methyl group is transferred to coenzyme M and reduced to CH_4 (Figure 1, green pathway). H_2 or formate is used to reduce the formed heterodisulfide back to coenzyme M and coenzyme B (19). Methylotrophic methanogens on the other hand, uses methanol or other methylated compounds (Figure 1, green pathway). Methyl groups are transferred to coenzyme M through several steps. Methyl-coenzyme M can then be oxidized to CO_2, via reverse hydrogenotrophic methanogenesis, facilitating the reduction of other methyl-coenzyme M molecules to CH_4 (19). Some methylotrophic methanogens however, lack the reverse hydrogenotrophic methanogenesis pathway and use external H_2 as electron donor (20). Methylotrophic methanogenesis is generally regarded as being more important in marine environments (21), but recently discovered H_2-dependent methylotrophic methanogens have been detected in a broad range of anoxic habitats including Arctic and temperate peat soils (20, 22). Aceticlastic methanogens transform acetate with ATP and coenzyme A to acetyl-coenzyme A and further to methyl-H_4MTP, while the carboxyl group of the acetate is oxidized to CO_2 (Figure 1, yellow pathway). The methyl-H_4MTP is reduced to CH_4 like in the hydrogenotrophic methanogenesis and CH_4 and CO_2 are released (13, 18, 19).

In most methanogenic pathways the main substrate(s) are used for both energy conservation and carbon assimilation, but among the methylotrophic methanogens there is some variation in the carbon fixation pathways and CO_2, acetate and other substrates are utilized (13, 18).

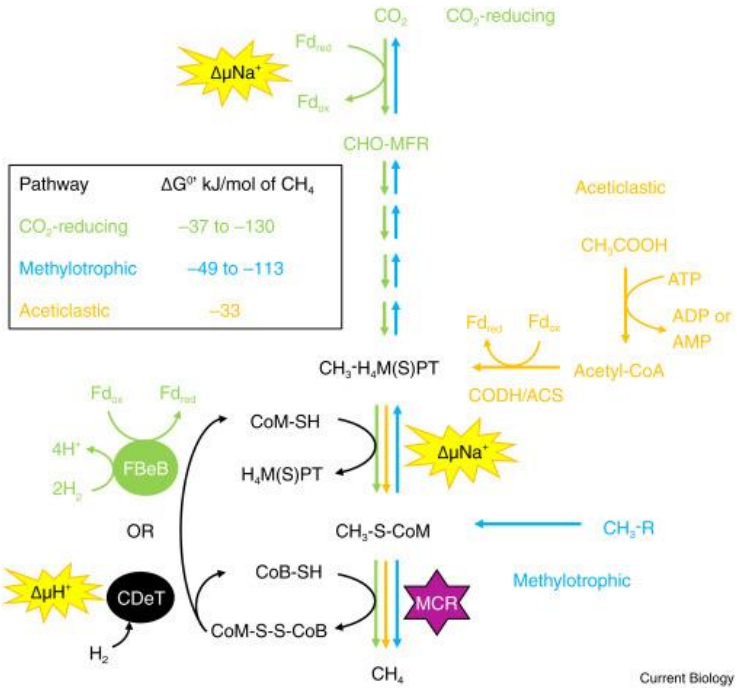

Figure 1: Schematic overview of the three main methanogenic pathways: Hydrogenotrophic (CO_2-reducing, green pathway) methylotrophic (blue pathway) and aceticlastic (yellow pahtway) methanogenesis. Figure from Lyu *et al.* (7).

On a global scale, aceticlastic methanogenesis is the dominant methanogenic pathway in wetlands (7), even though only one aceticlastic methanogen order is known (20). Yet, soil conditions and the selection of syntrophic fermenters in the soil strongly impact to what extent different methanotrophic pathways contribute to the overall CH_4 production in the soil (21). For example, in peat soil form Finland 80% of the CH_4 produced originated form hydrogenotrophic methanogens (17). In Arctic peat soil from Siberia investigated by Metje *et al*. (16), hydrogenotrophic methanogenesis was important in the overall CH_4 production at low temperatures, contributing to 50% of the CH_4 emission below 4°C. However, above this level aceticlastic methanogens became more active, being the source of 70% of the CH_4 release at 25°C (16). Thus, in this study, the hydrogenotrophic activity decreased when exposed to higher temperatures, while aceticlastic methanogenesis became more important. In Svalbard soils investigated by Tveit *et al.* (14), hydrogenotrophic methanogenesis was the source of 35% of the CH_4 while aceticlastic methanogenesis yielded 65% of the CH_4 at all temperatures from 1 – 30 °C.

Tveit *et al.* (14) showed that increasing temperature above 7°C correlated with a decrease in propionate and acetate levels, thus being a potentially important threshold temperature in this soil. Higher temperatures also led to changes in the microbial community from a state where the oxidation of propionate to acetate was thermodynamically limited (due to aceticlastic methanogenesis being too inefficient to reduce acetate concentrations), to a state where polysaccharide hydrolysis alone seemed to be the rate limiting step for organic matter degradation (14). This demonstrates that the decomposition cascade in anoxic peatland soils can be highly affected by the temperature conditions.

CO_2 might either be utilized (hydrogenotrophic) or produced (acetolactic, methylotrophic) during methanogenesis and the emission of CO_2 relative to CH_4 is therefore affected by the CH_4 production pathways (21). In the Siberian soil Metje *et al.* (16) found a coupling between CO_2 and CH_4 production rates, especially at higher temperatures (25°C). They inferred that this was due to aceticlastic methanogenesis being responsible for the production of both gases. Others have also found anaerobic CO_2 production rates to correlate with CH_4 production. A correlation between the two gases was for example found in Canadian peat soil (no experimental temperature treatment) but, in this soil, a variability in the samples from the predicted correlation was seen (R^2 of 0.58) (23) This demonstrates that CO_2 and CH_4 production do not correlate under all circumstances. Interestingly, it has been shown that accumulation of CH_4 and CO_2 does not correlate at all ($r = 0.05$) when emission from both oxic and anoxic soil layers are measured together (peat soil incubations at 12°C) (24). This shows that the correlation between the gasses depend on both the anaerobic and aerobic processes and indicates the potential for a decoupling between CH_4 and CO_2 under anaerobic conditions. Potential reasons for a decoupling might be differences in the organic matter being decomposed, or incomplete degradation (21). However, many anaerobic microorganisms, other than hydrogenotrophic methanogens, fixate CO_2 (25) affecting the balance between CO_2 production and CO_2 fixation. Thus, as long as there are energy sources to support growth, there is potential for CO_2 fixation.

Even though methanogens are responsible for the last step of organic matter degradation in anoxic soils, the CH_4 released by these organisms contain enough energy to be used as a substrate for growth. Methanotrophs are able to oxidize CH_4 to formaldehyde, which they can use for biomass synthesis or oxidize it further to CO_2, thus preventing CH_4 release to the atmosphere. Methanotrophs exist in both oxic and anoxic environments (7, 26). CH_4 oxidation with nitrite reduction and intracellular oxygen production occurs in many freshwater

environments and wetlands (27). Furthermore, *Methylobacter tundripalaudum*, which is an abundant methanotroph in peatlands on Svalbard, express genes for nitrite reduction, indicating the potential for anaerobic CH_4 oxidation in addition to its known ability to oxidize CH_4 aerobically (4, 28). The fraction of CH_4 not captured or oxidized by methanotrophs is released from the soil to the atmosphere (4, 7). Thus, the activity of methanotrophs relative to methanogens is important for the net emissions of CH_4 and CO_2 from the soil. In the end, multiple aerobic and anaerobic metabolisms influence the production and release of gasses such as CO_2 and CH_4 from peat soils.

Temperature & its effect on microorganisms

The cold and heat of a system is measured as temperature, and the energy transfer is in the direction from heat to cold (29). Heat leads to higher motion in atoms, giving higher kinetic energy (30). This energy increases the potential for a reaction (31). Therefore, temperature or the energy of a substrate or a system is important in biological contexts, affecting the potential for reactions and activity. Microorganisms have to be adapted to a certain temperature range to be able to live and perform their activities in their environment. When exposed to temperature changes a period of acclimation is needed for the organisms to be able to perform optimally (32). Typical microbial mechanisms for acclimation to temperature changes are adjustments of the fluidity in the membrane, growth rate, and RNA expression (32).

Temperature changes in Arctic soils

Temperature change can affect microbial communities on many different timescales. Arctic soil microorganisms are exposed to a gradual increase in average temperatures due to global warming, but they are also exposed to seasonal, daily and even hourly changes in temperature which they must adjust to. Before the turn of the millennium the mean temperature on Svalbard was -6 °C but, according to different climate models of emission scenarios from IPCC (2013), we can expect this mean temperature to reach higher than 0°C during the end of this century (33). Along with this increased average temperature, we also expect increased temperature variability (34, 35). Such long-term changes in weather and climate will contribute to changes in soil conditions, including higher and more variable soil temperatures and increasing thickness of active layers. On Svalbard the active layer in the soil is mostly in a range between one and two meters (33). The station Bayelva is located in Ny-Ålesund and is close to Knudsenheia where the soil used in this master thesis was collected. At Bayelva, the Climate service center in Norway has monitored the permafrost and active layers for several

years. The thickness of the active layer has increased 20 cm in ten years; it was measured to be 200 cm in 2016-2017 compared to 180 cm in 2008-2009. They also measured an increase in the permafrost temperature of 0.06 °C per year and in the upper one meter of soil they expect a mean temperature of approximately 1 °C in 2100 (33). This demonstrate that soil microorganisms are already exposed to gradual long-term temperature changes, and this development is highly likely to continue in the future.

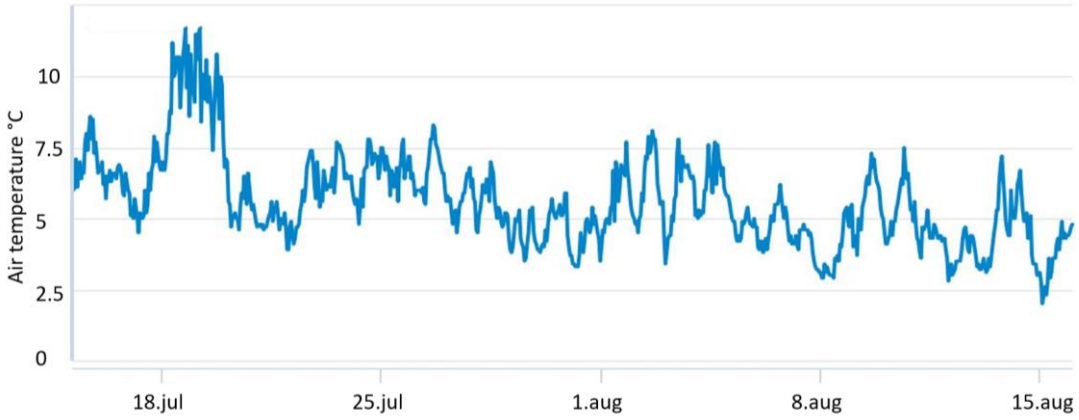

Figure 2: Air temperature (°C) in Ny-Ålesund during July and August 2016. Figure adjusted from Meteorological institute in Norway (MET)*. Sampling day for the soil used in this master thesis was the 7. August.

*Norsk Klimaservice senter, Seklima Observasjoner og værstatistikk [Internet], Meteorologisk Institutt (MET), (CC BY 4.0), [cited 2021, 22. April]. Available from https://seklima.met.no/observations/

Figure 2 shows the daily change in air temperature during July and August in 2016 in Ny-Ålesund and illustrates that temperature changes of several degrees within a day or a few hours are common (the peat soil used in this master thesis was collected on the 7th of August, 2016). Air temperature changes affect the soil temperatures but, temperature changes in the soil are slower. In Figure 3 adjusted from Westermann *et al.* (36), Arctic soil temperatures in June and August 2008, at a depth of 30 cm below the soil surface are shown. Temperature changes of 2 to 3°C were observed within less than a day (36). These rapid temperature shifts seem to be interspersed by periods of stable temperatures. Even in a much warmer future, soil microorganisms will be exposed to a mix of temperature stability and fluctuations underlining the importance of microbial short-term temperature responses.

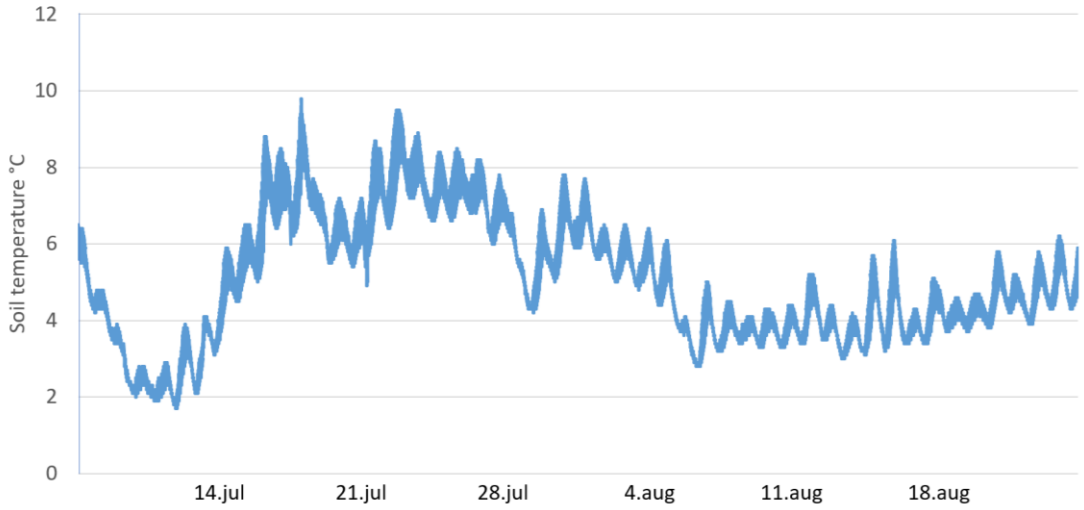

Figure 3: Temperature (°C) measured over time 0.3 meter below soil surface in Arctic peat soil from Ny-Ålesund. Temperature measurements were done in July and August, 2008 by Westermann *et al.* (36).

Temperature effects on soil C stocks, microbial growth and CO_2 emissions

In comparison to other organisms, microorganisms can respond very rapidly to changes in their surroundings. Reasons for that are their potential high cell division rates and short generation times. To be able to benefit from favorable temperature periods, microorganisms adapted to cold climate are also thought to respond faster to temperature change than microorganisms living in warmer climate (37). By this rationale, microbial responses to temperature in general, and particularly in cold ecosystems, may be seen within a short time after the onset of change. For example, CO_2 production increased within minutes after adding additional C to agricultural grassland soils (38) and peat soil from Finland displayed large differences in CO_2 emission rates after one day of incubation at different temperatures (17), illustrating such fast microbial responses. Furthermore, daily or hourly changes in temperature might lead to microbial community responses at several different levels, from cell physiology to community interactions, depending on the response time to temperature change of the microorganisms present.

According to Metje *et al.* (17) the optimum temperature for methanogenesis in northern peat soil is 25°C, which means that global warming has a large potential for increasing CH_4 emissions from Arctic peat (16). However, already at 4°C CH_4 production rates are high, corresponding to 25% of the emission at 25 °C (17). This demonstrate how active Arctic microbial ecosystems are, even at low temperatures (14). However, while both CH_4 and CO_2

production rates are likely to rise due to higher temperatures in peat (14, 16, 39), the mechanisms behind changes in these rates are still not understood

Microbial biomass accumulation and soil respiration rates are major factors contributing to C loss or storage capacity in peat soil. Čapek *et al.* (40) found in 2015 that the C loss in Russian tundra peat soil increased exponentially with increasing soil temperatures and suggest that the loss in soil C is affected by the temperature effect on microbial metabolisms. When investigating anaerobic conditions they concluded that soil methanogenesis contributed little to the C loss while CO_2 production through fermentation and anaerobic respiration was the dominant reason for C loss (40).

Warming has been shown to increase growth and respiration rates in oxic soils. An equation that predict the temperature effect on chemical reaction rates is Arrhenius law. The equation predicts exponential increase in the reaction rates due to temperature increase (39, 41) and has also been used frequently to predict cellular growth and respiration rates (41). This model has both been confirmed (39) and disproved (14) in various experiments in retrospect and different variants of the equation have been suggested to improve the model (41). Its lack of predictive power for biological rates suggests that temperature is not the only factor determining cell growth, and that a large number of reactions with different temperature sensitivities are involved in the temperature responses of cells.

Higher temperatures may also limit the substrate availability due to high microbial activity, thus induce restrictions on the number of microbes that can sustain a living. In this way high activity can be seen in microbial communities without increasing the biomass and result in higher microbial activity per biomass (42). This, so-called, mass specific growth rate is a measure of the daily microbial DNA production per unit of microbial biomass, while produced DNA per gram of soil will tell something about the overall growth and size of the microbial community. Together with gas emission rates, these are important measures of microbial activity.

In 2019 Janette Grunnvåg did her master thesis on "Time dependent temperature effects on methane production in Arctic peat soil" (43). In her work she studied the effects of increasing seasonal temperature change on the CH_4 producing microorganisms in soil from Knudsenheia in Ny-Ålesund, Svalbard. Her results are particularly relevant for this master thesis since the sampling sites for both master projects are the same. Her short-term (one week) peat soil incubations at different temperatures indicated a shift from low mass specific growth of

microorganisms when the soil was exposed to temperatures below 5°C to high mass specific growth at 9°C (Figure 4 A). No significant change in the mass specific growth was observed between 2 and 5 °C (43). Interestingly, even though the growth rate increased she found no change in the total soil microbial biomass indicated by the total amount of DNA in the soil, between temperature treatments. Measurements of the soil microbial biomass C (Cmic) in the soil can also be used as an estimate of biomass (Cmic from intact cells). Cmic measurements performed on the same soil as the growth rate estimates show that there were no significant differences in Cmic between temperature treatments (Figure 4B). However, the amount of Cmic was largest at 10°C, suggesting that the increased growth rate may have had an effect on the amount of microbial biomass in the soil (Grunnvåg, 2019, unpublished).

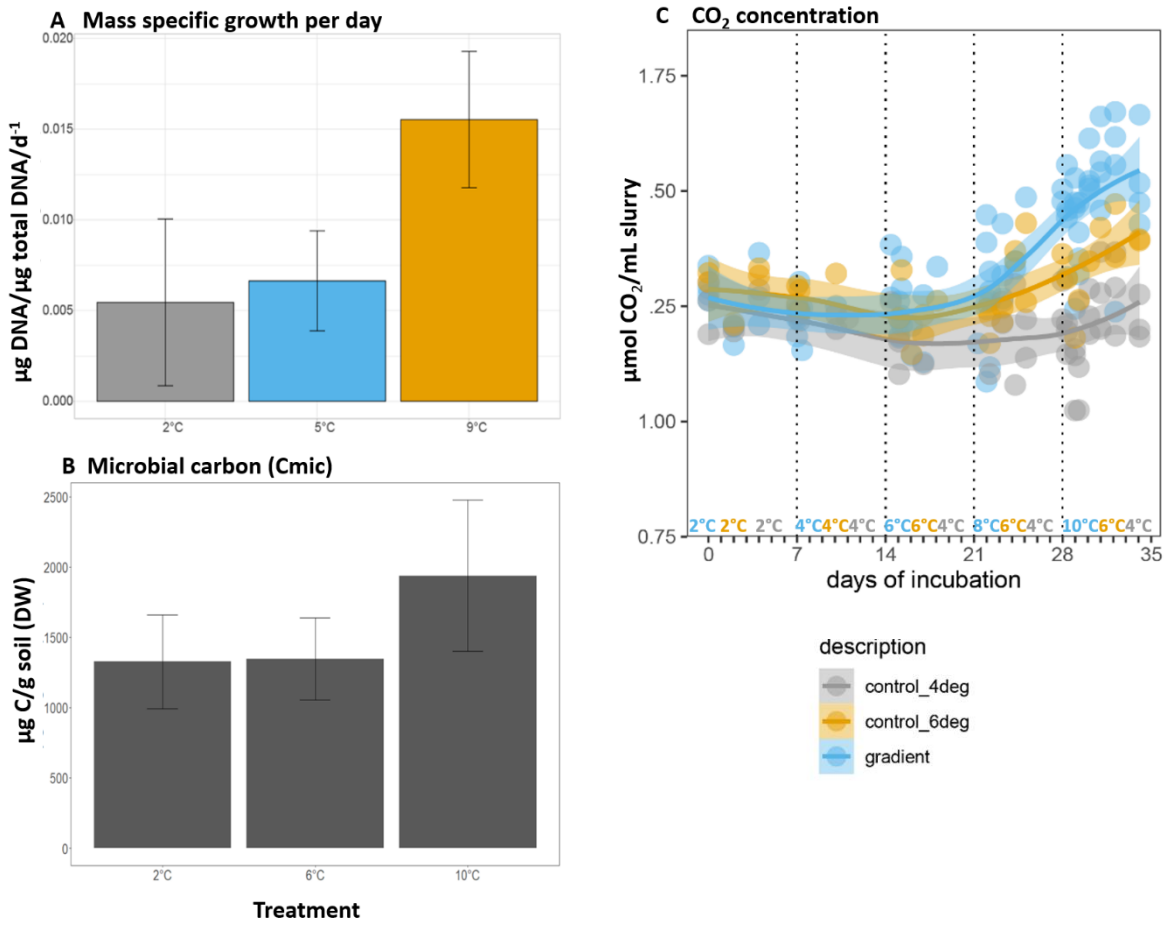

Figure 4: (A) Mass specific growth per day in slurry from Arctic peat soil under different temperature treatments (43). (B) Mean soil microbial biomass C (Cmic) per gram soil (DW) from arctic peat soil incubations under different temperature treatments (data retrieved from Grunvåg, 2019, unpublished). Error bars represent standard deviation from the mean. (C) CO_2 accumulation (µmol gas per mL soil slurry) in Arctic peat soil over a timescale of 34 days under different temperature regimes. The "gradient" corresponds to a temperature increase from 2 to 10 °C increasing 2 °C every week. "control_4 deg" samples underwent a temperature change from 2 to 4 °C the first two weeks and stayed at 4 °C for the rest of the experiment. "control_6deg" samples experienced a temperature increase from 2 to 6 °C the first three weeks and stayed a 6 °C for the rest of the experiment. The weekly temperature is indicated by the colour coded temperature labels above the time scale (Bender *et al.* in prep.).

In a related peat soil incubation experiment CO_2 accumulation over time at different temperatures was measured. A zero net CO_2 accumulation was observed at 6 °C and below, while at 8 and 10 °C, a rapid temperature dependent increase in net CO_2 accumulation occurred (Figure 4 C) (Bender *et al.* in prep.).

The net CO_2 accumulation observed after reaching 6°C correspond with the increase in cell growth between 5 and 9°C. Grunnvåg suggested that temperatures below 5°C do not support sufficient energy conservation and/or microbial biomass synthesis to grow as effectively as at 9°C (43). A key question that arises from this study is whether the shift in CO_2 production seen at approximately 6°C is a reoccurring temperature response that coincides with a more rapid mass specific growth. Another important question is what underlying processes are involved in this rapid and striking response to rather small temperature changes. In my master thesis an important aim was therefore to collect data with a better resolution to quantify these microbial temperature responses over time. With this, I wanted to come closer to an answer to these questions and contribute to a better understanding of how soil microbial communities will react to frequent temperature variations in a warming Arctic.

Objective & research questions

Soil microorganisms have to adjust to rapidly changing temperatures that offer different conditions for energy harvest and growth. In this master thesis the rapid short-term temperature effects on microbial activities in anoxic Arctic peat soil, within the range and timeframe of summer season temperature shifts, were investigated. The study's overall aim was to study temperature effects on microbial CH_4 and CO_2 production across a temperature gradient experiment (from 2 to 10 to 2°C) and to relate this to microbial cell growth and metabolite concentrations to illuminate the potential CO_2 and growth shifts indicated in Grunnvåg's work and other preliminary data. To do this, a high-resolution experiment with frequent soil and gas sampling at each temperature was designed. Within this overall aim, I propose three research questions, each of them matched by a hypothesis:

1. How does short time temperature changes affect CH_4 and CO_2 production in Arctic peat soil?
2. Exactly at what timepoint and temperature is the CO_2 shift occurring, is the shift dependent on temperature or time and what is the magnitude of this CO_2 shift?

3. Is the CO_2 shift related to change in microbial activity (cellular growth and metabolic pathways)?

Hypotheses

1. CH_4 and CO_2 production is temperature dependent and will increase and decrease with rising and declining temperatures. However, CO_2 accumulation will only be seen above 6°C.

This hypothesis is based on previous measurements of gas emission in Arctic peat soil from Metje *at al.* (16), Tveit *et al.* (14), Bender *et al.* (in prep.) and Grunnvåg (43). It has been shown that higher temperatures lead to increase in both CH_4 and CO_2 emissions (14, 16, 43). Hypothesis 1 was formulated with the assumption that cooling will reverse the process and the microorganisms will be able to adjust to decreasing temperatures at the same rate as seen for temperature increase.

2. A temperature increase above 6°C triggers a CO_2 shift from a zero net accumulation to a net accumulation. Above 6°C, the CO_2 accumulation rate will increase with increasing temperatures.

Hypothesis 2 is based on the gas measurements from Bender *et al.* (in prep.) indicating a CO_2 shift at 6°C (Figure 4 C).

3. The CO_2 shift is related to change in microbial activity.

The results from Bender *et al.* (in prep.) and Grunnvåg (43) showed two events (CO_2 increase and higher cell growth, respectively) at approximately the same temperature (6°C). The effects were seen in two different experiments, thus requiring an independent testing of this co-occurrence within a single experiment. Tveit *et al.* (14) also identified a drop in acetate and propionate levels at approximately the same temperature. In my master thesis, I want to look into this by using a high-resolution sampling setup to test whether these events occur at the same temperature. Identifying such co-occurrence patterns would be a strong argument for a biological link between the CO_2 shift, acetate and propionate drop and the change in cellular growth rate at what seems to be a key threshold temperature in these soils, 6 °C.

Material & Methods

Sampling and preparations

Sampling of soil

Figure 5: The Arctic peatland Knudsenheia in Ny-Ålesund on Svalbard. Photo: Alexander Tøsdal Tveit

The peat soil for this experiment was collected from an Arctic peatland at Knudsenheia (Figure 5) in Ny-Ålesund on Svalbard, Norway (78°55' north, 11°56' east). Ny Ålesund has had an annual mean temperature of -1.2 to -3.9°C over the last five years (Data obtained from: https://klimaservicesenter.no/observations/), and a summer mean temperature of 4.7 to 6°C in the same period (Data obtained from: https://klimaservicesenter.no/observations/). Peat soil was sampled from the frequently water saturated and anoxic part of the active layer 10 – 20 cm underground. The soil was stored frozen (-20°C) in a plastic box with peat water until use. Sampling was done by Alexander Tøsdal Tveit on the 7th of August, 2016.

Preparing of soil slurry

All handling of the soil was done as sterile as possible: The equipment was washed, wet and dry autoclaved or baked at 200°C before use. Equipment not fitted for such treatments was washed and then wiped with 70% ethanol. Rubber stoppers used to close airtight bottles were wet-autoclaved 10 times to get rid of any contaminants before use (the water was replaced between each time). Disposable equipment like plastic syringes, needles and filters was ordered sterile.

Artificial peat water was mixed with peat soil to a slurry to simplify the sampling and incubation work, promote anoxic conditions, and help homogenize the soil. The artificial peat water was prepared with distilled water containing NaCl, to avoid too much dilution of the salt concentrations, and then blended with a commercial blender (Waring, CT, USA) to create a soil slurry. NaCl concentrations in the artificial peat water was set to the natural peat water concentration. Based on earlier measurements, NaCl was added to the water to reach

approximately 0.02 g/L. The artificial peat water was poured into glass bottles and autoclaved before mixing with the peat.

Soil preparations were performed in an anoxic chamber from Plas-Labs (Lansing, MI, USA). The chamber was made anoxic by flushing it multiple times with nitrogen gas (N_2) and H_2 (10%) gas mix. Between every flushing a vacuum was created in the chamber. To reach and maintain anoxic conditions, a catalyst (Plas-Labs, Lansing, MI, USA) was used to increase the speed of the reactions between H_2 and oxygen to make water vapor that was removed from the chamber by drying. Once the chamber was anoxic, the peat soil was thawed inside the chamber over two days at a temperature of approximately 7 °C.

After thawing, a slurry was made of the soil with 1:1 ratio of peat soil and artificial peat water (Figure 6). For this experiment 2.1 kg of peat and 2.1 L of artificial peat water was used. The soil and water were mixed to a slurry with a commercial blender (Waring, CT, USA) on high speed for 1.5 minutes before it was poured into a 5 L airtight anoxic glass bottle (main bottle). To be able to mix all the soil and water the mixing was performed in several rounds. After all the slurry had been transferred to the main bottle, the bottle was mixed by shaking. The main bottle was closed with a rubber stopper and a cap and brought out of the chamber. All handling of the soil slurry outside the cooled anoxic chamber and outside incubators was done on ice when possible. The main bottle was then flushed with N_2, 10 times, with vacuum in between, being sure that an overpressure of N_2 was introduced in the bottle between each vacuum. This was done to remove H_2, a potential energy source for the soil microorganisms, from the headspace of the bottle. Afterwards, the slurry was incubated at 2°C for a month to let it stabilize. During this incubation time the bottle was inverted five times per day every weekday to prevent the soil from sedimenting. For the entire experiment (pre-and main-experiment), all handling of the soil and soil slurry was done either inside the anoxic chamber, under a N_2 stream or in airtight anoxic bottles before being transferred to sampling tubes.

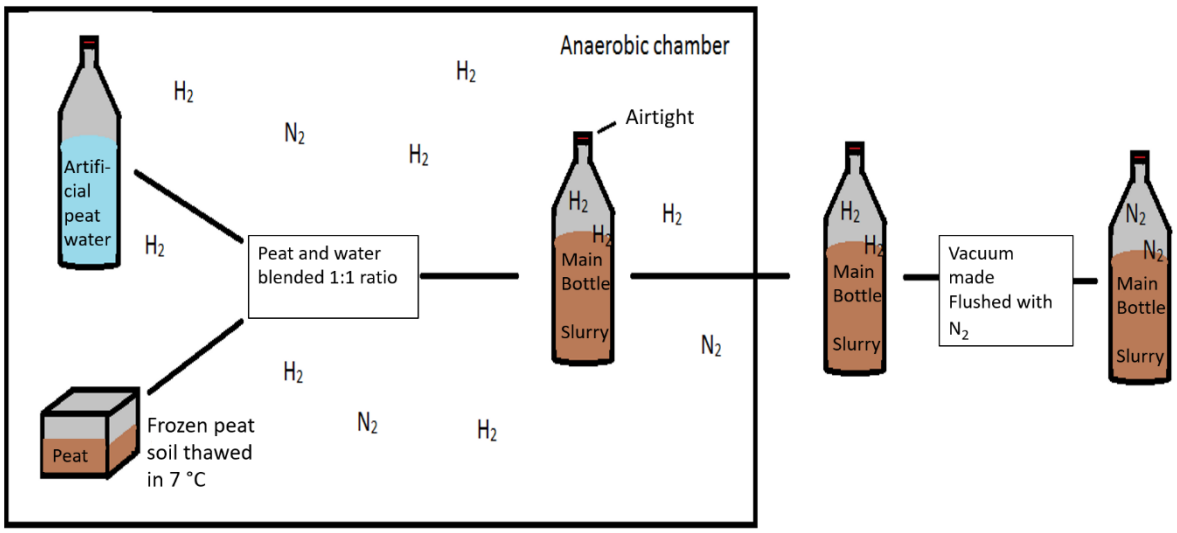

Figure 6: Main steps in the preparation of the peat soil slurry used in this project. All steps were done in an anaerobic chamber with H_2 and N_2 in the atmosphere. In the chamber artificial peat water and thawed peat soil (1:1 ratio) was mixed with a commercial blender to a homogeneous slurry. The slurry was poured into an airtight bottle. Because H_2 is a potential nutrition source for the microorganisms, the H_2 and N_2 atmosphere in the bottle was changed outside the chamber by flushing the headspace of the bottle with N_2. The main bottle was then incubated at 2°C for approximately 1 month before the pre-experiment was initiated.

During the experiment (pre-and main- experiment), flushing of the bottles was done using a gas outlet (Glasgerätebau Ochs, Bovenden/Lenglern, Germany) connected to a rubber hose. At the end of the hose a sterile syringe filter (0.2 µm Cellulose Acetate, VWR, PA, USA) and a hollow sterile disposable needle (0.60 x 30 mm, Sterican®, B.Braun, Melsungen, Hessen, Germany) were connected. The needle was used to penetrate the rubber stoppers on top of the different bottles and then N_2 gas was injected to the bottles. Depending on the size of the bottle, flushing was continuous 10 – 30 minutes, or with application of vacuum between each of several gas injections. Smaller bottles (120 mL) were filled with N_2 until the pressure in the bottle reached 1 – 2 bars and then a vacuum was made to remove gas inside the bottle. This was usually repeated three times. Finally, a last gas injection was performed, and the bottles were left with a tiny overpressure.

Pre-experiment

After one month of incubation, a pre-experiment was started to collect data about the conditions of the system prior to the temperature experiment. The pre-experiment included measurements of gas accumulation and sampling of pore-water. Monitoring of CH_4 and CO_2 concentrations were performed using a gas chromatograph (GC) and pore-water samples were collected and analysed by high pressure liquid chromatography (HPLC) to look at the concentrations of different metabolites like formate, propionate, acetate, butyrate and ethanol.

Preparing for measurements

Slurry from the main bottle was used for the pre-experiment. For GC measurements, 30 mL of slurry was added to five airtight 120 mL serum bottles. For pore-water sampling, 80 mL slurry was added to two 120 mL serum bottles. The distribution of the slurry was done in the anoxic chamber. Before distribution, the main bottle was gently shaken to mix the slurry and opened inside the chamber. Slurry was poured into a beaker and a 60 mL disposable sterile syringe was used to distribute the right amount of slurry to the bottles. Rubber stoppers (Butyl stoppers (Glasgerätebau Ochs, Bovenden/Lenglern, Germany)) were used to close the bottles. Outside the chamber the bottles were immediately closed further with crimp caps. The main bottle and the GC- and the pore-water-bottles were then flushed with N_2 to remove the H_2. The main bottle was placed under continuous flow of N_2 (1 bar) for 10 minutes before it was flushed three times to a pressure of 0.2 bar N_2 in the bottle with vacuum in between. The GC- and pore-water-bottles (sampling bottles) were flushed 5 times with 1 bar N_2 with vacuum in between. The bottles were left at 1 bar overpressure. The sampling bottles and the main bottle were incubated at 2 °C and the slurry was mixed one time per day (five times per week), to keep it homogenised. The pre-experiment lasted for 80 days.

GC measurements

Two times a week (Mondays and Thursdays) the concentration of CH_4 and CO_2 in the five GC-bottles was measured using GC (SRI 8610C gas chromatograph, SRI Instruments, CA, USA with 8600-PKDC 3m 9`Haysep D Column 80/100 mesh, Samsi). H_2 was used as carrier gas and the oven temperature was set to 40°C. An airtight gas syringe (Pressure-Lok® Precision Analytical Syringe, A-2 series, VICI Precicion Sampling, Schenkon, Switzerland) with a needle (Luer Needles A-2, VICI Precicion Sampling, Schenkon, Switzerland) was used to collect 0.5 mL gas sample from the bottles and inject it into the port on the GC. The

ambient pressure (bar) in the room was measured all days of measurement. To obtain the pressure inside the bottles it was measured on the first two days of gas measurements. After this, the daily pressure in the bottles was calculated for the rest of the pre-experiment (equation 4). The pressure was measured using a digital manometer (Leo 1, Keller, Winterthur, Switzerland). The program PeakSimple version 4.88 was used to integrate peak areas for raw data. The concentrations of the standards (ppm) were converted to µmol/mL using equation 5 and a standard curve was made for CH_4 and CO_2 (Figure 7).

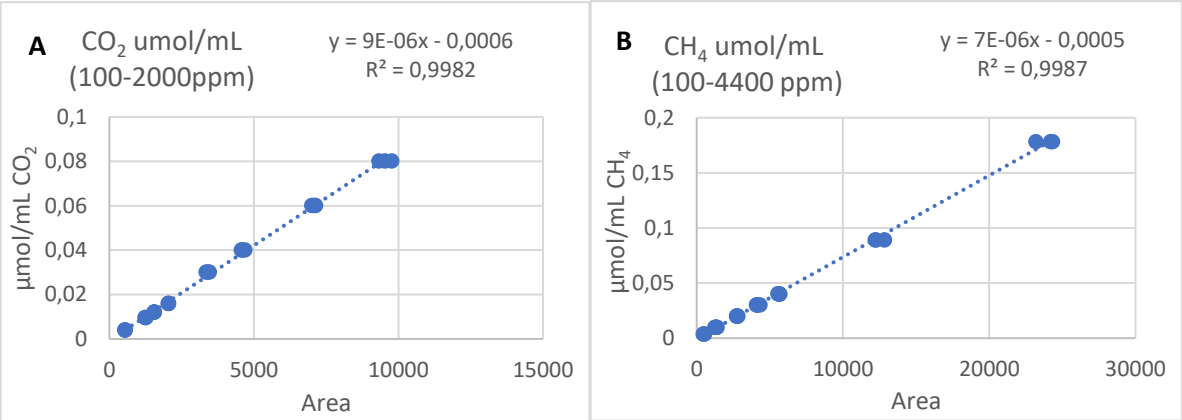

Figure 7: The standard curve (µmol per mL gas compared to peak area) for (A) CO_2 and (B) CH_4 used in the calculations of gas measurements in this thesis.

$$\textit{Equation 4: pressure (bar)} = P_{t\text{-}1} \times \left(\frac{V(removed)}{V(gas\ space)} \times P_{t\text{-}1}\right)$$

Where $P_{t\text{-}1}$ is the pressure (bar) in the bottle the previous day, V(removed) is the volume (mL) gas removed from the bottle the previous day and V(gas space) is the volume (mL) of the gas space in the bottle.

$$\textit{Equation 5: µmol/mL gas} = \frac{P \times V}{R \times T}$$

Where P is the air pressure in bar, V is the volume of gas in L, R is the ideal gas constant and T is the temperature in kelvin.

For all measurements, both standards and samples, the needle was wiped with 70% ethanol after sampling. Before each sampling the syringe was flushed with air three times followed by a flushing with N_2 gas three times. This was done to avoid gas contaminating between samples and protecting the anoxic sampling bottles from introduction of oxygen. Three 0.5 mL samples of the standard gases were measured every measurement day and compared to the standard curve to control the validity of the standard curve each measurement day. The

GC results (areas under the respective gas peak) were compared to the regression models for the CH_4 and CO_2 standard curves and the concentrations of the gases were calculated and normalized to the volume of the bottle headspace to obtain µmol gas per mL headspace in the bottle.

To calculate the total amount of gas produced, the volume of gas was corrected for removal of gas for measurement and gas dissolved in the liquid phase in the GC-bottles. To assess the amount of dissolved gas in the liquid phase, the concentration (ppm) in the bottles was calculated using a concentration to area standard curve for each gas. The dissolved gas was calculated using Henry's law for dilution factors (kH) for different gases at different temperatures. Calculating the dissolved gas in the liquid phase was done using equation 6.

$$\textit{Equation 6: dissolved mol gas per mL slurry} = \left(\frac{ppm}{1000000} \times P \times kH(T)\right)/1000$$

In equation 6 ppm is the gas concentration in the bottle on the day of measurement, P is the pressure (bar) in the bottle at the timepoint off measurement and kH(T) is the dilution factor for the respective gas type at the respective temperature.

To calculate the weekly change in gas rate the "slope" function in Microsoft Excel was used. The calculations were done in Microsoft ® Excel ® for Microsoft 365 -64 bit (version 2008), Microsoft Excel, was used for all further calculations, if not mentioned otherwise.

Pore-water sampling

Samples were collected two times a week (Mondays and Thursdays) from the pore-water-bottles. Approximately 20 minutes before sampling the bottles were carefully shaken. For sampling, a sterile disposable 1 mL syringe and needle (0.80 x 120 mm, Sterican®, B.Braun, Melsungen, Hessen, Germany) was used to penetrate the rubber stopper and sample 0.5 mL of the water in the top phase of the slurry. The water was transferred to a 1.5 mL tube (Eppendorf). This was repeated for each pore-water-bottle on each sampling day. The tubes were centrifugated at 4°C and 10000 rpm for 7.5 minutes to sediment the soil. After centrifugation, 400 µL of supernatant was transferred from each tube into individual filter cups and filtered through 0.2 µm plunge filter (Syringeless filter Devise, Mini-UniPrep™, PVDF filter media with polypropylene housing, Whatman™, Maidstone, UK). The pore-water samples were kept in a -80°C freezer until HPLC measurement. After each sampling, the pore-water sample bottles were flushed with N_2 as described above and left at 1 bar overpressure to ensure anoxic conditions.

HPLC

Accumulation of important microbial metabolites in the slurries was measured by analysing the pore-water (see pore-water sampling described above). The HPLC preparation was done in cooperation with and supervised by Bente Lindegård. Running of the instrument for acetate and propionate measurements was done by Lindegård while running the instrument for ethanol (main-experiment) measurements was carried out by the author. Analysis was done on a Waters 2690 separation module HPLC chromatograph (Waters Alliance, Milford, USA) with Aminex Resin-Baced HPX-87H Column, 300 x 7,8 mm (Bio-Rad, Ca, USA) and Empower 2 software Build 2154, feature release 5 (Waters Alliance, Milford, USA). Before measuring, 200 µL pore-water was transferred into measurement vials with a cap preventing evaporation. Standards for each metabolite of interest were prepared the same way as the samples and used to make a standard curve. The different standards where made using standard stock solutions for ion chromatography (Sigma Aldrich, Munich, Germany) and diluted in Milli-Q (MQ) water. MQ water was used as blank. For the pre-experiment a mobile phase containing 2.5% Acetonitrile (CH_3CN) (HPLC quality; Merck, Hessen, Germany) and 97.5% MQ water with 0.005 M H_2SO_4 (VWR, PA, USA) was used. In combination with a 996 Photodiode Array (PDA) detector (Waters Alliance, Milford, USA) (wavelength: 210 λ) and the used column this mobile phase uncovered the presence and concentration of acetate, propionate, formate and butyrate in the pore-water. To rinse the system before and after measurement, a 10% MeOH washing solution was used.

For the measurements, the column had a temperature of 60°C, the mobile phase had a flow of 0.6 mL per minute through the column and the samples were kept at 10°C in a sample chamber. For each measurement run, 20 µL of a sample was collected from the measurement vial automatically and injected in the column. Every run lasted for 25 minutes.

The peak areas were mostly determined automatically by the software (Empower 2). Small or unclear peaks were integrated manually. The peak areas from the samples were compared to the standard curve and the molecular masses of the compounds were used to calculate the concentrations in µmol/L.

Main- experiment

Preparing for measurement

After 80 days of pre-experiment, monitoring the soil conditions under a stable temperature (2°C), the main-experiment was started. A temperature gradient corresponding to a typical Arctic summer temperature range was introduced (2 – 10°C). For the main-experiment, new sample bottles were prepared and filled with slurry from the main bottle in the anoxic chamber. For preparation of the sampling bottles, the same method and conditions were used as for the pre-experiment. Six 500 mL tap-bottles, (i.e. Airtight glass bottles with a tap; Glasgerätebau Ochs, Bovenden/Lenglern, Germany)) and 13 x 120 mL serum bottles for GC measurements were prepared. The tap bottles were filled with 300 mL of slurry using a beaker. These bottles were used in the main-experiment, allowing anoxic sampling through the tap. The GC-bottles were filled with 30 mL slurry (see pre-experiment). Tap-bottles were flushed with N_2 in continuous flow for 30 min and 1 bar, GC-bottles were flushed as described in the pre-experiment. All bottles were incubated at 2°C until the next day.

Experimental setup for sampling

Figure 8 presents the experimental setup for sampling during the main- experiment. The GC-bottles were used for the measurement of CO_2 and CH_4 accumulation. The tap-bottles were used for sampling of slurry for analysis of cell growth, biomass and concentrations of the fermentation intermediates propionate, acetate, formate, butyrate and ethanol. The temperature experiment was done using ten GC- bottles and three tap-bottles incubated at 2°C the first week and increasing by 2°C every week, up to 10°C. In addition, three GC-bottles and 3 tap-bottles were used as a control and not exposed to higher temperatures than 6°C. These bottles started at a temperature of 2°C increasing by 2°C every week up to 6°C and were kept at that temperature for the rest of the experiment. The ten bottles incubated at temperatures from 2 to 10°C reached 10°C in the fifth week. Five of those GC-bottles were then kept at 10°C for the rest of the experiment while the last five GC-bottles and the three tap-bottles were returned to 2°C by decreasing the temperature by 2°C every week. CH_4 and CO_2 concentrations were measured three times per week in all 13 GC-bottles. Samples for measurement of metabolite concentrations, such as fatty acids, were collected three to four times per week by taking pore-water samples for HPLC from the tap-bottles. The GC

measurements and pore-water sampling of the control bottles kept at 6°C and 10°C ended after 7 weeks, while the sampling and measurements of bottles experiencing the complete

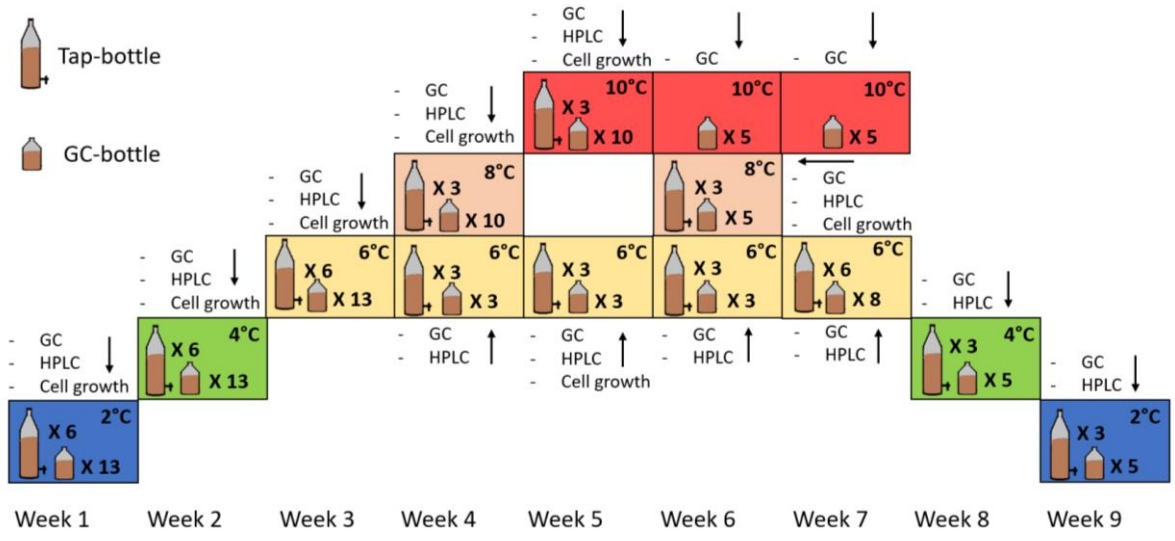

Figure 8: The experimental setup for sampling of gas and slurry. Tap-bottles and GC-bottles (described in the main text) were used for incubation and sampling. Each box represents one week with the week temperature. Numbers of tap-bottles and GC-bottles sampled for the respective week and temperature are indicated in each box. Measurements done are indicated next to each box (arrows indicate the respective week and temperature).

temperature gradient ended after 9 weeks. The microbial cell growth was measured once per week in tap-bottle samples by using ^{18}O enriched water as described below. Measurements of microbial biomass were performed at the same time-points using chloroform fumigation (CF) as described below. Sampling for cell growth and microbial biomass was performed during the temperature increase (until week 5 (10°C)) with an additional measurement in week 6 (8°C). Control measurements at 6°C were performed after 5 weeks, using the three 6°C control tap-bottles. For the slurry experiencing the whole temperature gradient from 2 to 10°C and back to 2°C again, the treatment will be referred to as the "gradient" treatment hereafter. Likewise, the samples incubated in temperatures from 2 to 6°C, with an extended period at 6°C will be referred to as "6°C control" and the samples incubated at 2 to 10°C, with an extended period at 10°C, will be referred to as "10°C control".

GC measurements

The gas chromatograph measurements started on the second day of incubation of the GC-bottles at 2°C. The measurements were done for all 13 GC-bottles and were carried out as described in the pre-experiment section, with some exceptions. Those exceptions were: The

volume of gas collected from each bottle for measurements was reduced from 0.5 mL in the pre-experiment to 0.25 mL and the pressure (bar) in all the bottles was measured on every measurement day. Calculations were done as explained in the section for the pre-experiment.

Pore-water sampling

Samples for pore-water measurements were usually collected every week on Monday, Wednesday, Thursday and Friday. The samples were transferred to 1.5 mL Eppendorf tubes from the tap-bottles. Before collecting the samples, the bottles were shaken to homogenize the slurry. The tap-bottles were kept on ice outside the incubator. A very small overpressure of N_2 (0.2 bar) was made in the bottle by a needle connected to a rubber hose on a gas outlet (see description above). Then the rubber hose was disconnected from the needle and overpressure from the bottle was released partly. This was done to ensure an overpressure to prevent oxygen contamination and to keep the overpressure low enough to avoid uncontrolled flow of slurry from the tap. If any oxygen contamination during the tapping process was suspected, the tap-bottle headspace was evacuated and then flushed three times with N_2. Eppendorf tubes containing the soil slurry samples were kept on ice between sampling and filtering. The tubes were centrifuged 7.5 minutes at 4°C and 10000 rpm. After centrifugation 500 µL water was transferred to a filter cup and filtrated through 0.2µm plunge filter as explained for the pre-experiment, and later kept at -80°C until analysed with HPLC.

HPLC measurement

Most measurements and calculations of pore-water sampled from the main-experiment was done like explained in the section for the pre-experiment. In addition, a second mobile phase and detector was used to determine the presence of ethanol. This mobile phase contained MQ water with 0.005 M sulfuric acid (H_2SO_4). The detector used was a W410 detector. Ethanol standard stock solutions (E-040 and E-032) from Supelco (Merck, Hessen, Germany) were diluted in MQ water to create the standard curve. The run and calculations were done as previously described in the pre-experiment section.

Microbial cell growth estimates

The samples were mixed with water enriched in the stabile isotope ^{18}O ($H_2{}^{18}O$). The stock concentration of the $H_2{}^{18}O$ was 98 at% (atom percent) ^{18}O and by mixing the slurry with an appropriate volume of enriched 98 at% water, an enrichment of approximately 30% $H_2{}^{18}O$ in the slurry samples was achieved. The method is based on the fact that during growth, water is

consumed by the cells through various metabolic reactions and thus the isotope ends up being incorporated into newly synthesised DNA (44). The level of ^{18}O enriched DNA can then be measured and used to provide a basis for estimating the rate of DNA replication which is equivalent to the cell division rate (44). From each tap-bottle six samples were taken, three samples were treated with ^{18}O water and three samples with nuclease free water (negative control, i.e. natural abundance (NA) control).

Before sampling, the tap-bottles were prepared like explained previously for tapping of pore-water samples. The sampling of the slurry for the ^{18}O enrichment experiment was performed under anoxic conditions to avoid damaging or otherwise influencing the anaerobic microorganisms in the soil slurry and ensure further growth as prior to addition of ^{18}O enriched water. Cryovials (1 mL, VWR, PA, USA) without a lid were placed into small glass vials (38 mL serum bottles). In addition, one glass vial without a cryovial was used to collect the slurry from the tap-bottles before distributing the slurry to the cryovials. A 0.2 bar N_2 flow was directed into all glass vials before tapping the slurry. This was done by placing a disposable sterile needle connected to a filter, the rubber hose and the gas outlet in each glass vial, ensuring oxygen-free conditions. Soil slurry was tapped into the empty glass vial and from this, 300 or 350 µL of sample was distributed to the cryovials in the other glass vials using a pipet (the volume of soil in each sample was adjusted to the volume of ^{18}O water). The glass vials with the cryovials inside were then quickly closed with a rubber stopper and crimp cap, followed by continuous flushing with 0.2 bar of N_2 for approximately 3 minutes with a needle outlet. After 3 min the outlet was removed and the N_2 flow was turned off.

For the ^{18}O enrichment experiment and the preparation of NA controls, a bottle with N_2, a N_2 flushed bottle with water enriched with the stabile isotope ^{18}O and a N_2 flushed bottle with nuclease free water were prepared. A gastight syringe and needle (Hamilton, NA, USA) were used to add nuclease free or ^{18}O enriched water to the samples. To rinse the needle and syringe before use it was flushed three times with nuclease free water, then it was flushed three times with N_2 (using the N_2 bottle) to make sure no oxygen was introduced to the glass vials with the slurry. Then 120 or 140 µL of nuclease free or ^{18}O enriched water was added to the cryovials containing the slurry (to vials containing 300 µL slurry, 120 µL of water was added, to vials containing 350 µL slurry, 140µL water was added). Between every injection of water, the needle was wiped with ethanol and dried, and the syringe was flushed tree times with N_2. The samples were incubated for two days at their respective temperatures. At the end of the incubation, the glass vials were opened, and the samples transferred into lysing tubes

from Fast DNA™ SPIN kit for Soil (MP Biomedicals, CA, USA) and weighed. The samples were frozen in liquid N_2 upon transfer. When lysing tubes were not available, the sample were frozen directly in the cryovials. Samples were kept at -80°C until processed further for DNA extraction.

Cell growth- DNA extraction

Extraction of DNA from the samples (from approximately 0.3 g) was done with Fast DNA™ SPIN kit for Soil (MP Biomedicals, CA, USA). The kit uses bead beating in lysing matrix tubes to lyse DNA and silica spin column filtering to extract the DNA. The kit protocol was used with the following adjustments: Samples with slightly higher water content than the others were filtered in three parts instead of two, to be able to filter all the material. The DNA was incubated at 55 degrees for 5 minutes, instead of directly being diluted in distilled water after the DNA cleaning. The DNA pellet was dissolved in 50 µL distilled water.

The concentration of DNA in the samples was measured on a Qubit 2.0 fluorometer using the Qubit™ dsDNA HS Assay Kit (Thermo Fisher, MA, USA), the concentration of DNA was used as an estimate of cell numbers in the soil. A gel electrophoresis (agarose gel with GelRed) was used to confirm the presence and inspect the quality of DNA in the samples. The DNA samples were stored at -20°C.

Cell growth – Isotope ratio mass spectrometry (IRMS)

For measurement of ^{18}O incorporated into the DNA, the samples were sent on dry ice to the Centre for Microbiology and Environmental Systems Science at the University of Vienna, Austria.

In Vienna, IRMS (isotope ratio mass spectrometry) measurements were performed by Margarete Watzka and Victoria Sophie Martin to determine the level of ^{18}O in the DNA extracts. The IRMS system consists of a thermochemical elemental analyser (TC/EA, Thermo Fisher, MA, USA) coupled via a Conflo III open split system (Thermo Fisher, MA, USA) to a Delta V Advantage Isotope Ratio Mass Spectrometer (Thermo Fisher, MA, USA). The level of oxygen (O) in the samples and the amount of ^{18}O isotopes present in the ^{18}O enriched DNA and NA samples was measured and compared to determine the amount of newly incorporated ^{18}O in the DNA from the ^{18}O enriched samples. This was then compared to the enrichment at% (approximately 30%) and the total mass of oxygen from DNA in each sample using equation 7. The result represents the amount of ^{18}O incorporated in each sample (and thereby

the level of new O incorporated in the total DNA (O DNA produced)) during the incubation. The O DNA produced was multiplied with the percent of oxygen in DNA ([weight%], 31.1981%) and the sample volume was corrected for to obtain the level of new DNA in the samples (total DNA produced (µg)). From this result the produced DNA per gram soil dry weight and incubation time was calculated (DNA produced [ng/g/h]) (equation 8).

To express microbial growth in the slurry samples, mass specific growth rates were calculated as the level of DNA produced (ng/g/h) divided by the total measured sample DNA per g dry weight (DW) slurry (µg/g DW). This resulted in a mass specific growth rate expressed in mg DNA produced per g total DNA per hour (mgDNA/gDNA/h). Also, the turnover time was calculated by dividing the total DNA in the sample by produced new DNA per day giving the number of days needed to replace the present DNA.

$$\textit{Equation 7:O DNA produced} = DNA\ O\ (\mu g) \times \frac{new\ 18O\ (\text{at\%})}{\text{enrichment}\ (\text{at\%})}$$

Where the DNA O (µg) is the measured level of oxygen DNA in the sample, new 18O (at%) is the difference between measured ^{18}O (at%) in ^{18}O and NA samples and the enrichment (at%) is the exact enrichment for the respective sample calculated from the level of water in the sample compared to the level of enrichment added (µL enriched water and at%) and the level of ^{18}O in NA samples.

$$\textit{Equation 8: DNA produced (ng/g/h)} = \frac{\frac{Total\ DNA\ produced\ (\mu g)\times 1000}{Dry\ weight\ (g)}}{Time\ (h)}$$

Total DNA produced is the µg produced DNA in the samples for the total incubation. Dry weight is the weight of the dry weight for the samples (g) and time is number of hours of the incubation.

Microbial biomass - Chloroform fumigation

To estimate the microbial biomass (of intact cells) in in the soil slurries at different temperatures a chloroform fumigation (CF) and KCl (potassium chloride) extraction was carried out on the soil. This was done to find the C and nitrogen (N) content in the microbial biomass. The method after Brookes *et al.* (45) and Joergensen (46) was used for the fumigation and KCl extraction. Slurry from the tap-bottles was used for fumigation. For each bottle, three positive (fumigated) and three negative (none fumigated) replicates where made.

The tap-bottles were prepared like explained in previous method sections for tapping. The fumigation slurry was tapped into a small glass vial. From this vial, 2 mL of slurry was distributed in aluminium cups using a pipet and placed in a desiccator. Inside the desiccator, a beaker with 70 – 100 mL of chloroform was placed, making a chloroform atmosphere inside the desiccator when it was closed. This chloroform atmosphere lead to cell destruction and release of microbial cell contents into the soil slurry. The fumigation was performed in the dark and lasted for two days.

When the soil was fumigated, C and N from the fumigated soil samples and a corresponding set of non-fumigated slurry samples was extracted using KCl. For each sample the slurry from the aluminium cups was put into a 50 mL falcon tube and 30 mL of autoclaved 1M KCl was added. The non-fumigated soil slurries were sampled from the tap-bottles as described for the fumigated samples, but the 2 mL of slurry for each sample was directly pipetted into the falcon tubes and then 30 mL of 1M KCl was added. The tubes were shaken horizontally for 30 minutes. After the shaking, the tubes were centrifugated for 5 minutes at 10000 rpm. This was done to remove soil particles before filtering, to avoid clogging of the filter. The samples were filtered using a filtration manifold with stainless steel chimneys (10 × 20 mL) (DHI LAB Products, Hørsholm, Denmark), vacuum and Wathman^{TM} quantitative Ashless filter paper, grade 40. The filtrate was then collected in tubes and frozen. For every day of KCl extraction, a blank sample was made of 1M KCl, that experienced the same treatment as the slurry. The samples were then stored at -80 °C until further use.

Microbial biomass – Analysis of C and N in the soil

The KCl extracts were sent to Vienna, Austria, together with the ^{18}O enriched DNA for further analysis. Analysis of the KCl was also done at the Centre for Microbiology and Environmental Systems Science at the University of Vienna by Ludwig Seidl. The content of total C and N in the KCl extract from fumigated and non-fumigated soil was measured and compared to find the C and N from the microbial biomass. The KCl extracts were diluted to a concentration of 0.2 M KCl. An analyser for dissolved organic carbon and total dissolved nitrogen (TOC-VCPH /CPNTNM-1, Shimadzu, Kyoto, Japan) was used to measure the total soil C and N in the KCl extracts. Results from the blanks were used to remove results coming from impurities in the KCl. The sample results were related to the proportion of C and N that can be extracted form microbial biomass in comparison to actual C and N in microbial biomass (kEX number) (kEC=0.45, kEN=0.45) and normalized to the dry weight of the soil to

determine the soil microbial biomass C (Cmic) and soil microbial biomass N (Nmic) (45, 46). Cmic was used as the estimate for microbial biomass.

Figures and Statistical analysis

Standard curves and standard regression line were made using plot diagrams in Microsoft® Excel® for Microsoft 365 -64 bite (version 2008). Other results were visualized using RStudio and R version 4.0.4 (2021) from R foundation for statistical computing (47). Functions in the library 'tidyverse' (48) were used to reorder and structure data. To generate plots and bar graphs the libraries 'ggplot2' (49) 'plotrix' (50) and 'ggpubr' (51) were used.

All significance testing was done in R using pairwise.t.test() from base R (47) with Benjamini & Hochberg adjustment (52) to adjust for multiple comparisons. Correlation between propionate and acetate concentrations in the soil was tested using cor.test() (Pearson's correlation) and visualized by plotting the acetate and propionate concentrations against each other using plot() function, both functions from base R (47). Also CO_2 and CH_4 accumulation rates were tested for corelation using cor.test().

Results

Soil from Arctic peat has been investigated to get insight into microbial responses to rapid temperature changes in a summer season temperature range (2 to 10°C). In a pre-experiment, incubation of peat soil for 67 days, was used to reveal the behaviour of the system at a stable low temperature of 2°C. Measurements of gas production and microbial metabolites were performed to monitor the system during the pre-experiment incubation. The subsequent main-experiment lasted for 63 days and was designed to provide information on the temperature response of the soil microorganisms during exposure to temperature increases that mimic Artic summer temperatures: A heating period from 2 to 10 °C, followed by cooling from 10 to 2°C. In the main-experiment, gas production, microbial metabolites, microbial cell growth and biomass in the peat soil incubations were monitored.

CH_4 and CO_2 production

Results from the pre-experiment showed a constant CH_4 accumulation from day one (Figure 9 A and B). Later, in the main-experiment, the accumulation of CH_4 increased when exposed to higher temperatures. In both the pre-experiment and main-experiment, the CH_4 accumulation was higher than the CO_2 accumulation rate (Figure 9). While CH_4 accumulated at all temperatures, CO_2 accumulation was not clearly visible at lower temperatures (below 6°C) in the pre- and main-experiments. However, above 6°C the CO_2 production was more similar to what seen for CH_4 and the percentage of CO_2 contributing to the overall gas accumulation increased, however during the prolonged period of 10°C, CH_4 accumulation seemed to speed up even more than CO_2 accumulation and the percentage of CO_2 contribution decreased again (Table 1).

The overall correlation between CH_4 and CO_2 was strong (Pearson correlation: r = 0.740, P= 6.371E-09, n = 45). However, after more careful analysis, differences in correlations below and above 6°C were revealed. CH_4 and CO_2 were not correlating during the first three weeks of gradient treatment (2 – 6°C) (Pearson correlation: r = 0.283, P= 0.306, n = 15) while the two gases showed a strong correlation in week 3 to week 5 (6 – 10°C) (Pearson correlation: r = 0.829, P= 0.00013, n = 15)). Extended exposure to higher temperatures seemed to further strengthen the correlation. In the 10°C control (the treatment having temperatures above 6°C for the longest time), CH_4 and CO_2 were not correlating during the first three weeks (2 – 6°C)

(Pearson correlation: r = 0.0935, P= 0.7402, n = 15) while the two gases showed a very strong correlation in week 3 to week 7 (6 – 10 °C and extended incubation at 10°C) (Pearson correlation: r = 0.958, P= 6.32E-14, n = 25).

Table 1: Contribution (%) of CO_2 and CH_4 to the daily gas accumulation rate in anaerobic Arctic peat soil. The percentage is the mean contribution to the daily accumulation rate from each week in different temperature stages during the experiment. The temperature stages used in the calculation was:2 – 6°C, 8 – 10°C and the prolonged 10°C.

Week 1 to 3 (2 – 6°C)		**Week 4 to 5 (8 – 10°C)**		**2 weeks prolonged 10°C**	
CO_2 prod.	CH_4 prod.	CO_2 prod.	CH_4 prod.	CO_2 prod.	CH_4 prod.
11.07%	88.93%	21.11%	78.89%	16.49%	83.51%

Comparing the daily mean CO_2 accumulation rates at the end of the pre-experiment with the first two weeks for the main-experiment, the rates were similar, being 0.002 µmol/mL per day for the second last week and 0.0003 µmol/mL per day for the last week of the pre-experiment compared to 0.001 µmol/mL per day in week 1 (2°C) and 0.0004 µmol/mL per day in week 2 (4°C) for gradient samples during the main-experiment (Figure 9). These rates did not differ significantly (P > 0.4), indicating constant low or absent accumulation and no significant changes in the CO_2 accumulation rates below 6°C.

In the following total gas accumulation (Figure 10) and daily accumulation rates (Figure 11) from the main-experiment will be presented in detail. For CH_4 in particular, the response to heating was faster than the response to cooling. The CH_4 accumulation rate peaked in week 7, two weeks into the cooling period (gradient treatment), when the temperature had already returned from 10°C back to 6°C (Figure 12 B). However, after week 5, even though the CH_4 accumulation rates still increase until week 7, the increase between weeks were not as large as seen during temperature increase (Figure 11 B and 12 B). This delayed cooling response also resulted in the CH_4 accumulation rate not returning to the rate seen at 2˚C in week 1 of the main-experiment and the pre-incubation. In fact, the daily CH_4 accumulation rate at 2˚C in week 9 (at the end of the main-experiment) was most similar to the rate in week 4, at 8˚C (the rates in these two weeks were not significantly different, P = 0.17).

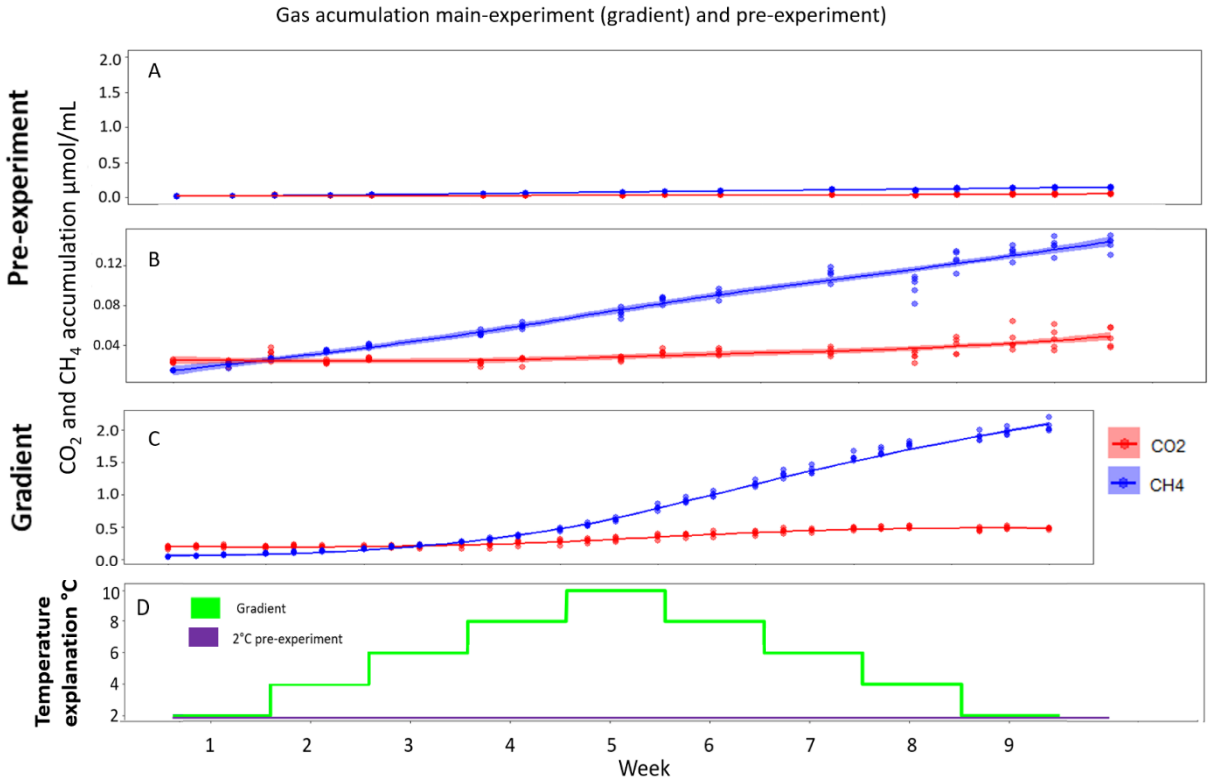

Figure 9: Headspace gas concentrations of CO_2 and CH_4 in Arctic peat soil incubations experiencing different temperature treatments. (A) Concentrations measured during stable 2°C conditions in the pre-experiment, the scale of the y-axis is corresponding to the y-axis for the gradient samples. (B) Concentrations measured during stable 2°C conditions in the pre-experiment (same results as shown in A), the scale of the y-axis being adjusted to bring out the details. (C) The concentrations measured during a gradient experiment. (D) Temperature explanation (°C) for gradient samples (green) and 2°C pre-experiment (purple).

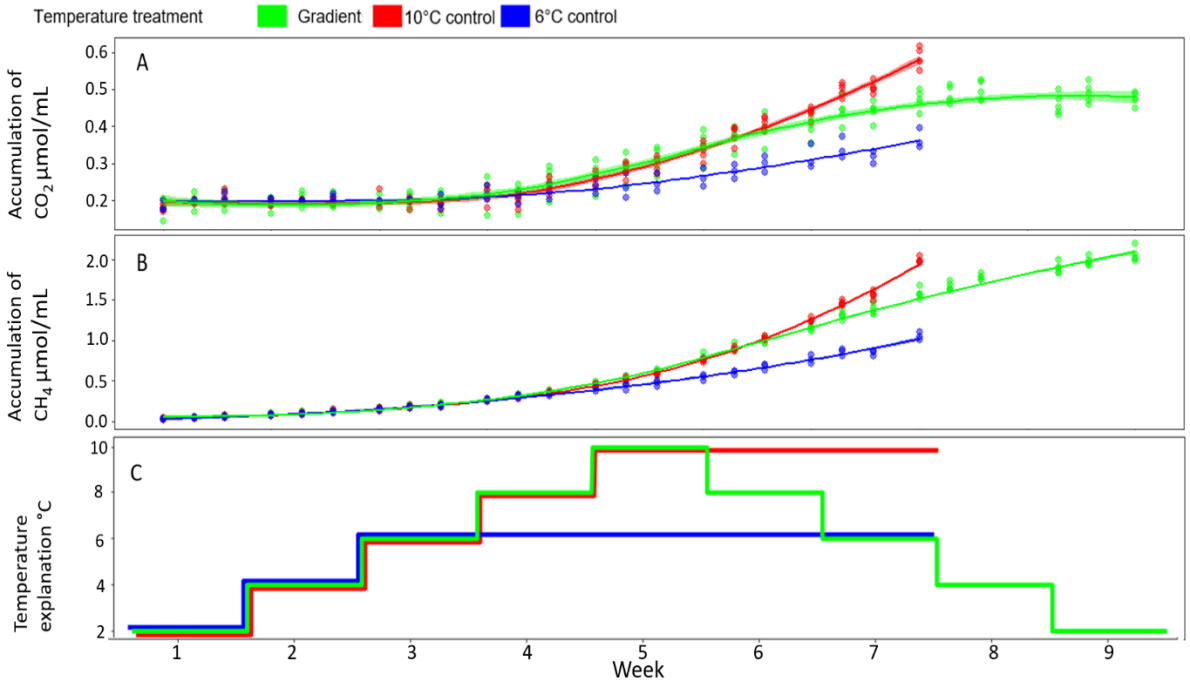

Figure 10: Total headspace gas concentration (µmol/mL) of CO_2 (A) and CH_4 (B) in airtight bottles containing anoxic peat soil slurry under different temperature treatments (C) over time (weeks). The soil in the different bottles origins from the same homogenized soil slurry. The two controls (red and blue) are exposed to prolonged periods of incubation at 10 and 6 °C, respectively.

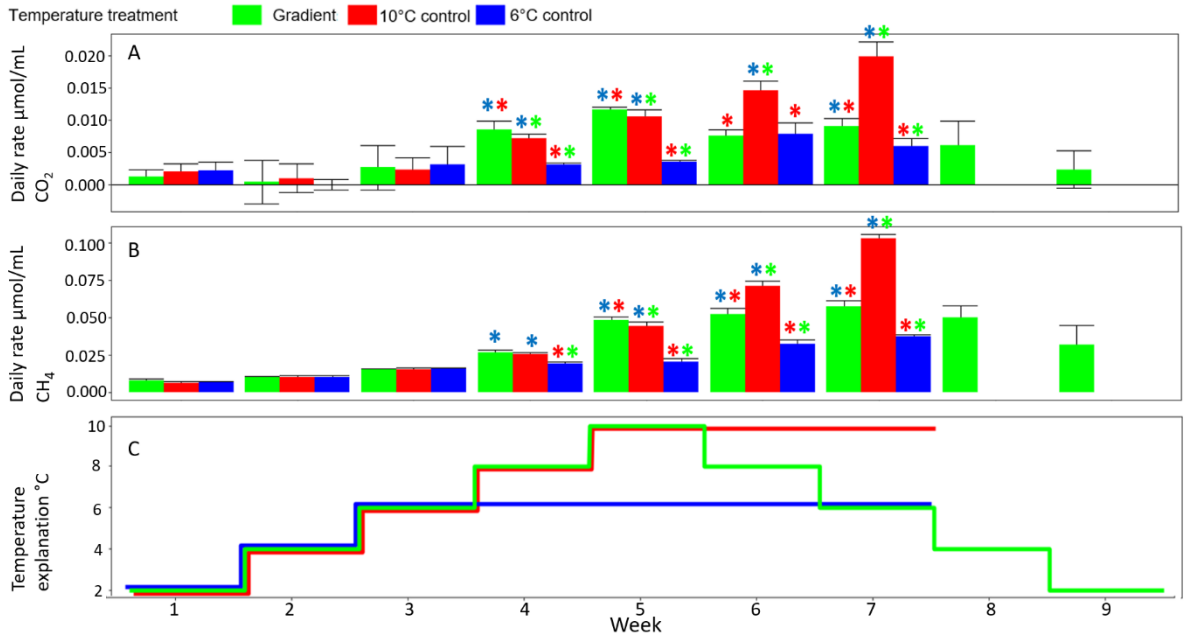

Figure 11: The daily mean headspace CO_2 and CH_4 accumulation rates in µmol/mL for each week with standard deviation (error bars) for CO_2 (A) and CH_4 (B) in anoxic peat soil under different temperature treatments (C). Significant difference (pairwise t test with Benjamini & Hochberg adjustment) between temperature treatments in each week are labelled above the bars with asterisks, the colour indicating the treatment.

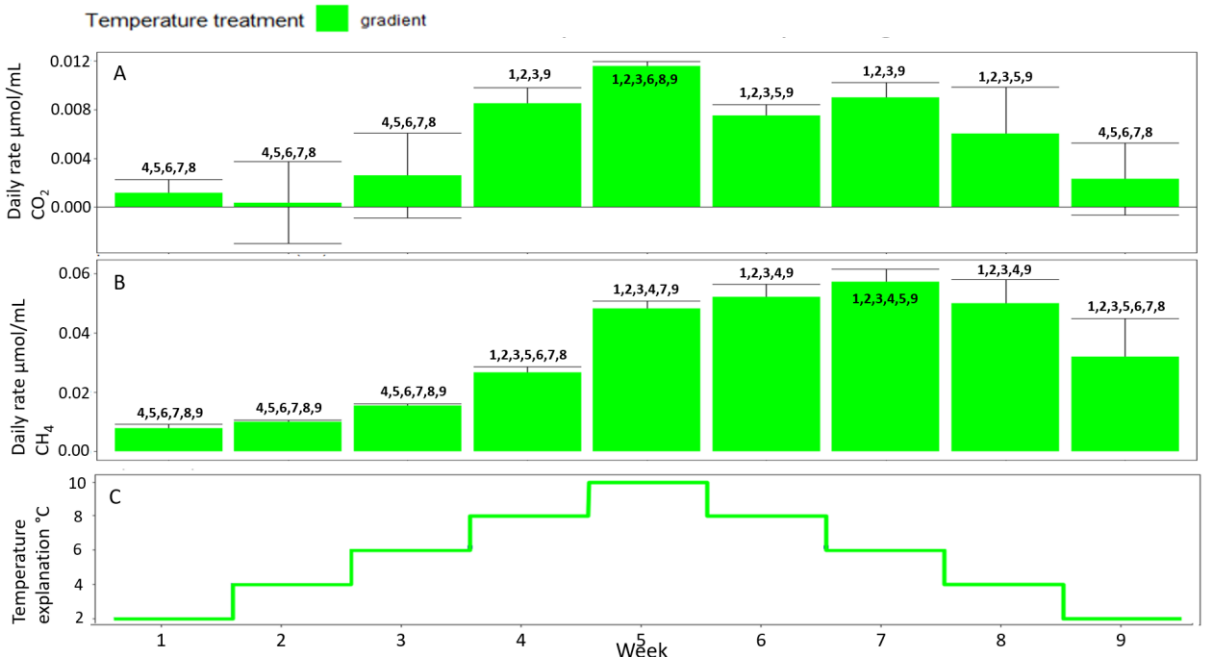

Figure 12: The figure presents the daily mean gas accumulation rate in µmol/mL for each week with standard deviation (error bars) for CO_2 (A) and CH_4 (B) in anoxic Arctic peat soil under a gradient based temperature regime (C). Significant differences (pairwise t test with Benjamini & Hochberg adjustment) between weekly rates are labelled above the bars, numbers indicating which weeks the respective rate is significantly different from, numbers 1 – 9 refer to week 1 to 9.

Indications for a slight increase in CO_2 accumulation was detected at the end of the pre-experiment incubation period. Before this point there was no net CO_2 accumulation occurring (Figure 9 B). During the first three weeks (2 – 6°C) of the main-experiment, accumulation was also slow and close to zero, with a slight increase at 6°C in week 3. (Figure 10 and 12 A). These first three weeks revealed only small and insignificant changes in the CO_2 accumulation rates between weeks (Figure 12 A). In week 4 (8°C), a large and significant increase in the CO_2 accumulation rate was observed (Figure 12 A). The CO_2 accumulation rate peaked at 10°C in week 5, and when the temperature returned to 2°C in week 9, the accumulation rate was back to the same level as observed in the first three weeks ($P > 0.2$) (Figure 12 A).

The variation between the replicates was higher during cooling treatment than during temperature increase, introducing some additional uncertainty to the CH_4 accumulation rates. For CO_2, a rather high variation between replicates was seen in both heating and cooling treatments (Figure 12 A).

Significant differences in accumulation rates for the gradient, 6˚C and 10°C control appeared already the second week after the different treatments were introduced to the soil (Figure 11), demonstrating how fast the soil ecosystem responds to the treatments. The gas accumulation

rates from control treatments substantiates the temperature dependence of the gas accumulation. It shows that prolonged exposure to a temperature after temperature increase leads to sustained increases in CO_2 and CH_4 accumulation rates that differ significantly from the rates observed during the first week of exposure (Figure 11). However, for the 6°C control this response appear more slow and longer time is needed to see a clear response than in the 10°C control (Figure 11). All results from the pairwise t.tests are found in the Appendix (Appendix. Table 1 – 6).

Metabolite accumulations (Acetate, propionate and ethanol)

The concentrations of acetate, propionate, butyrate and formate were measured for the pre-experiment to monitor the state and stability of the system, while the concentrations of acetate, propionate, butyrate, formate and ethanol were measured for the main-experiment to detect changes in system state and function. Only acetate, propionate and ethanol were detected in the samples and will be presented in the following (Figure 13 – 16).

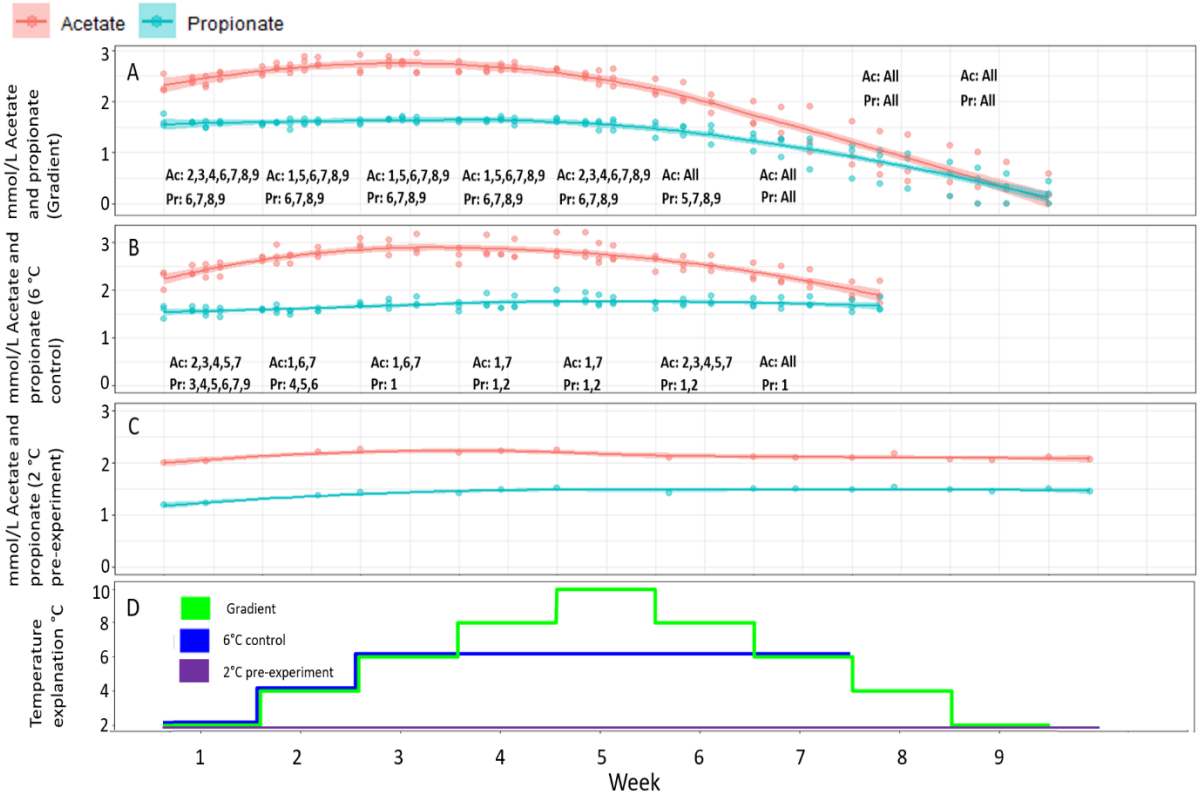

Figure 13: Changes in acetate and propionate concentrations (mmol/L) in anoxic peat soil incubations exposed to temperature change. The incubations experienced different temperature regimes over time. The figure show temperature gradient treatment samples (A), the 6 °C control (B) and the 2 °C pre-experiment control samples(C) with explanation of temperature regimes (D). In panel A and B, significant differences (pairwise t test with Benjamini & Hochberg adjustment) between weeks for acetate (Ac) and propionate (Pr) are presented. For each week, the numbers indicate which of the other weeks have a significantly different concentration. When a week was significantly different from all other weeks, it is labelled with "All". HPLC detection limit = 0.0025 mmol/L, all measurements under this limit are represented as a concentration of 0 mmol/L in the plot.

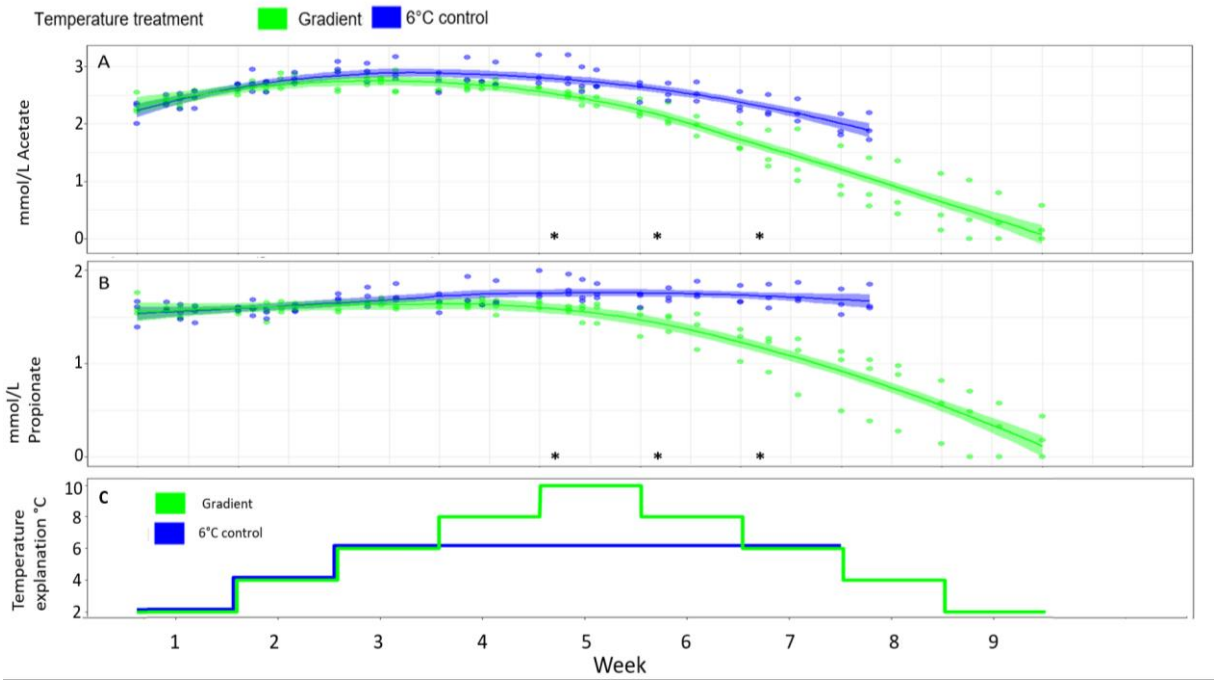

Figure 14: Concentrations (mmol/L) of acetate (A) and propionate (B) in anoxic peat soil incubations at different temperatures (gradient and 6 °C control) over time (C). Significant differences (pairwise t. test with Benjamini & Hochberg adjustment) between the different treatments are presented. The label (*) placed at the beginnings of weeks in the figure indicates significant difference between the gradient and the 6 °C for the respective week.

When exposed to 2°C for a longer period during the pre-experiment the concentrations of acetate appeared to be at a constant level of approximately 2 mmol/L with only small changes in the concentrations over time (Figure 13 C). During the main-experiment the concentrations of acetate increased slightly during temperature increase from 2 to 6°C (Figure 13 A). Acetate concentrations started at a level between approximately 2 and 2.5 mmol/L in week 1 of the main-experiment (2°C), peaking at 2.5 – 3 mmol/L in week 2 (4°C) and 3 (6°C). The concentrations in week 1 (2°C) were significantly lower than week 2 (4°C), 3 (6°C) and 4 (8°C) ($P < 0.02$). In week 4, a beginning decline in acetate concentrations was observed. This decline continued throughout the experiment, including the week of peak temperature at 10°C (week 5) and the cooling during week 6 – 9. At the last day of the experiment, the acetate concentrations were 0.578 mmol/L or lower. In the 6°C control samples, acetate concentrations responded differently (Figure 13 B). During the first three weeks (2 – 6°C), the control behaved like the gradient samples, being exposed to the same temperatures. In week 4 (gradient at 8°C and control at 6°C) a faster decline in concentrations was observed in the gradient samples and already in week 5, the gradient samples (now at 10°C) had significantly

lower concentrations of acetate than the 6°C control (still at 6°C) ($P < 0.001$). Despite the large differences, the control samples did display a similar trend of decreasing acetate concentrations, but at a much slower rate than the gradient (Figure 14 A).

Propionate concentrations followed the same flat trend as the acetate concentrations during the pre-experiment. A small increase in propionate was seen during the first two weeks, before it stabilised around a level of 1.5 mmol/L (Figure 13 C). In the main-experiment, the same concentrations (1.5 mmol/L) were observed during the first five weeks (until reaching 10°C). No significant changes were seen until week 6 (8°C) (week 1 – 5 : $P > 0.11$), but already within week 5 a slight decline in propionate concentrations was observed (Figure 13 A, 14 B). As for acetate, the decline was more apparent during week 7 – 9, propionate concentrations reached 0.43 mmol/L or lower in the last week. For the 6°C control, the propionate levels stayed at 1.5 – 2 mmol/L during the whole experiment. From week 5, (gradient treatment at 10°C) the propionate concentrations in the gradient treatment samples were significantly lower than the 6°C control samples ($P < 0.0004$) (Figure 14 B).

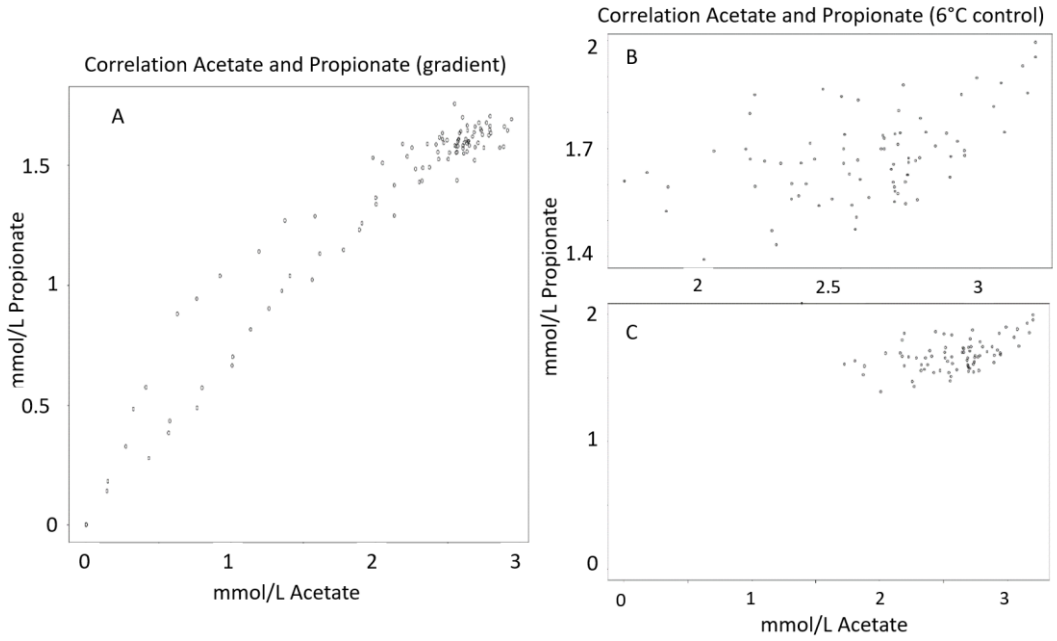

Figure 15: (A) Propionate concentrations (mmol/L) measured during the gradient treatment plotted against acetate concentrations (mmol/L). (B) Propionate concentrations (mmol/L) measured during the 6°C control treatment plotted against acetate concentrations (mmol/L). (C) Propionate concentrations (mmol/L) measured during the 6°C control treatment plotted against the acetate concentrations (mmol/L); the scale is adjusted to the range of the gradient measurement.

For both propionate and acetate, larger differences between replicate measurements of the same treatment were seen in the last weeks of the experiment (gradient treatment) than in the first (Figure 13 A, 14 A and B). However, the acetate and propionate concentrations seemed

to be closely related, prompting a test of the correlation between these two metabolites (Figure 15).

The Pearsons's correlation coefficient in the gradient samples was r = 0.968 (P = < 2.2E-16, n = 102) and for the 6°C control it was r = 0.499 (P = 2.379E-06, n = 81). The very strong correlation seen in gradient samples therefore seem to be linked to the low concentrations of both metabolites in the last weeks of the gradient treatment.

In addition to acetate and propionate, ethanol was also detected in the experiment (Figure 16). The highest concentrations (0.36 – 0.61 mmol/L) of ethanol were observed at the beginning of week 1 (2°C). Ethanol concentrations decreased rapidly already during the first week of incubation for both gradient and 6°C control treatments. During week 2 most replicates reached ethanol concentrations below the detection limit (0.01 mmol/L) and stayed

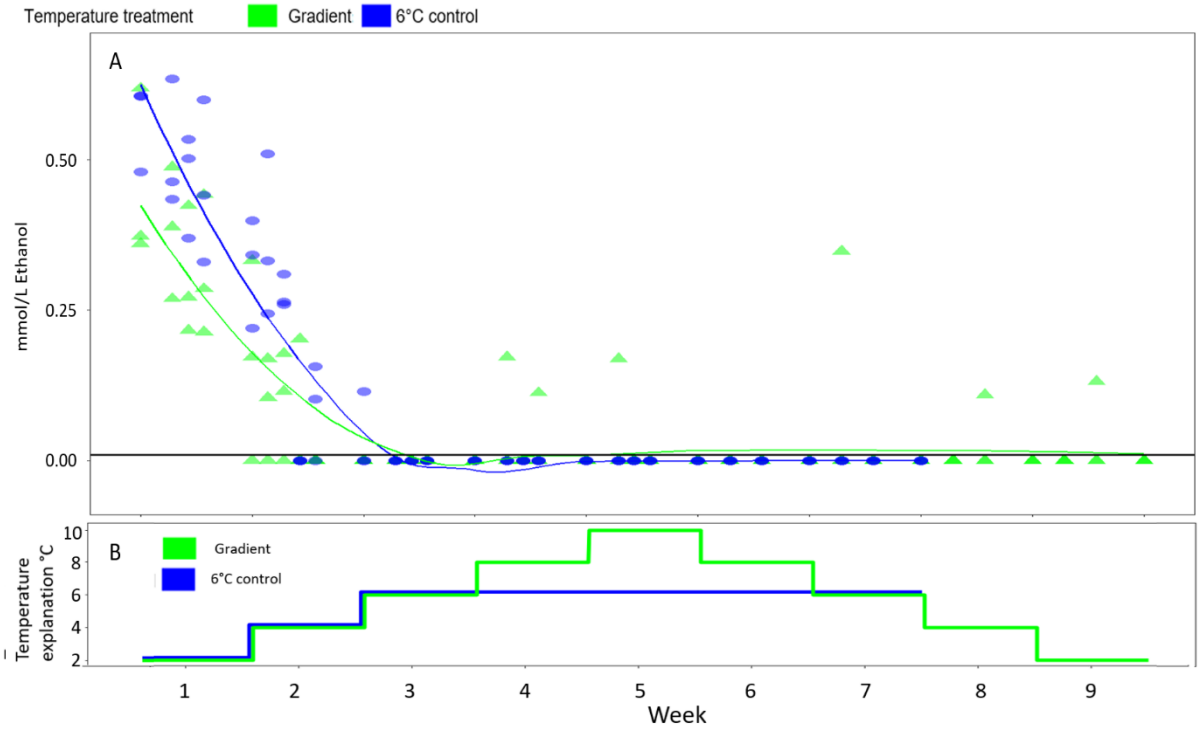

Figure 16: The concentration of ethanol (mmol/L) in anoxic peat soils over time (A) experiencing different temperature treatments (B). The black horizontal line in part A represents the detection limit (0.01 mmol/L) for ethanol, all measurements under this line are treated as a concentration of 0 mmol/L. Stronger colours in the plotted values indicate several replicates with the same result.

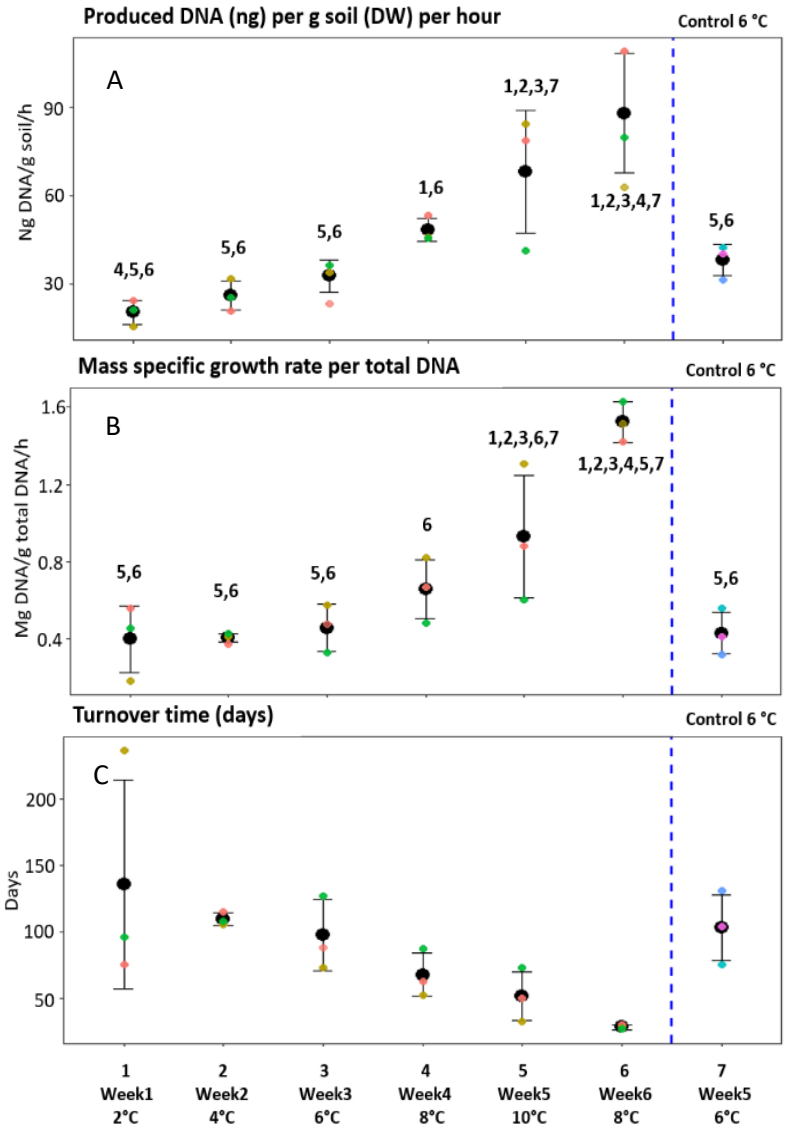

Figure 17: Estimates of growth presented as the mean and standard deviation of three replicates. The experiment was performed every week at different temperatures following the "gradient" temperature regime until week 6 (left of the blue dotted line). Measurements for a 6 °C control was performed in week 5 (right of blue dotted line). The figure shows: ng DNA produced per g dry mass of slurry per hour (A), mg DNA produced per g total DNA per hour (mass specific growth) (B), and turnover time in days (C). Significant differences between different week and temperature treatments are indicated by numbers (1 to 7) corresponding to the numbers above week numbers on the x-axis; If the measures in a week is significantly different from another (pairwise t test with Benjamini & Hochberg adjustment), the number of the significantly different week and treatment is above the plotted estimate.

at this level for the rest of the experiment. Some replicates from the gradient treatment had temporally higher concentrations at a few timepoints during the remaining incubation period.

Microbial growth and biomass estimates

Microbial growth & turnover. Incorporation of ^{18}O from enriched water in DNA was monitored in short-term incubation experiments; from this and the total DNA content in the soil, hourly production rates of DNA, mass specific growth rate and turnover time was estimated, enabling a study of cell division in the soil at different temperatures (Figure 17).

For soil sampled during the 6 first weeks of the gradient treatment (tap-bottle samples). No significant differences in DNA production were observed for the first 3 weeks ($P > 0.42$). In week 4 (8°C), 5 (10°C) and 6 (8°C), significantly higher DNA

production rates were observed (Figure 17 A) (week 1 vs week 4: $P = 0.047$, week 1 – 3 vs 5: $P < 0.013$, week 1 – 3 vs 6: $P < 0.0018$). Furthermore, although the difference was not significant, the mean DNA production was higher in week 6 (8°C) than week 5 (10°C). The 6°C control in week 5 had approximately the same values as week 3 (6°C) and only a slight non-significant ($P = 0.6$) increase in DNA production was seen over time from a week mean at 32.6 to 37.9 mg DNA/g total DNA/h (Figure 17 A). The estimated mass specific growth rates (Figure 17 B) followed the pattern of DNA production (Figure 17 A). Only minor changes in growth rates were observed in the first three weeks, followed by significant increases in week 5 (10°C) ($P < 0.014$) and 6 (8°C) ($P < 0.000027$) (Figure 17 B). For the turnover time (Figure 17 C), no significant differences were seen between any of the temperatures or weeks ($P > 0.08$), but a very clear trend of gradual decrease in mean turnover time with increasing temperature was observed. The turnover time decreased from a week mean of 136 days in week 1 (2°C) to a week mean below 52 days in week 6 (8°C). The 6°C control in week 5 also had approximately the same values as seen in week 3 (6°C). The lack of significant differences between the weeks for the estimated turnover time was due to the high variation between replicates in week 1. When this week was removed from

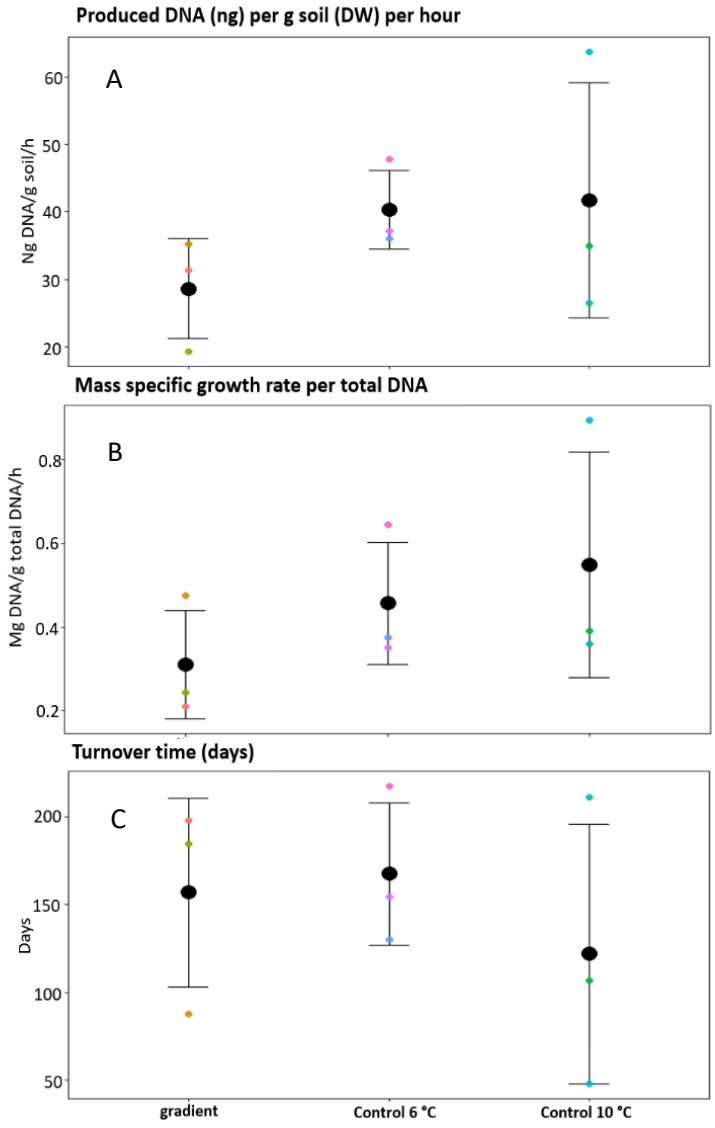

Figure 18: Growth presented as the mean and standard deviation of three replicates. The estimates are measured at the endpoint of the different temperature treatments used in the GC experiment. Gradient samples from week 9 (2°C), 6°C control from week 7 and 10°C control samples from week 7. Growth is estimate as ng DNA produced pr g dry mass of slurry per hour (A), mg DNA produced per g total DNA per hour (mass specific growth) (B) and turnover tidme in days (C). No significant differences (pairwise t test with Benjamini & Hochberg adjustment) were uncovered between the different treatments.

the test, significant differences between treatments were found (Appendix, Table 7).

Endpoint samples from the GC bottles were measured after exposure to the total period of the different temperature regimes (gradient samples from week 9 (2°C), 6°C control from week 7 and 10°C control samples from week 7). No significant differences in produced DNA, mass specific growth rate, and turnover time were seen between the treatments (Figure 18). However, the mean DNA production and mass specific growth was highest in the 10°C control and lowest in the gradient samples. For the turnover time, the 6°C control had the longest mean turnover time, but the gradient samples had approximately the same mean value (Figure 18). Higher levels of produced DNA was seen at 2°C in the gradient endpoint samples (week 9) from the GC bottles (Figure 18 A) compared to the produced DNA at 2°C in week 1 from the weekly monitored gradient samples from tap-bottles (Figure 17 A). These data represent respectively the DNA production at 2°C before and after a temperature rise to 10°C.

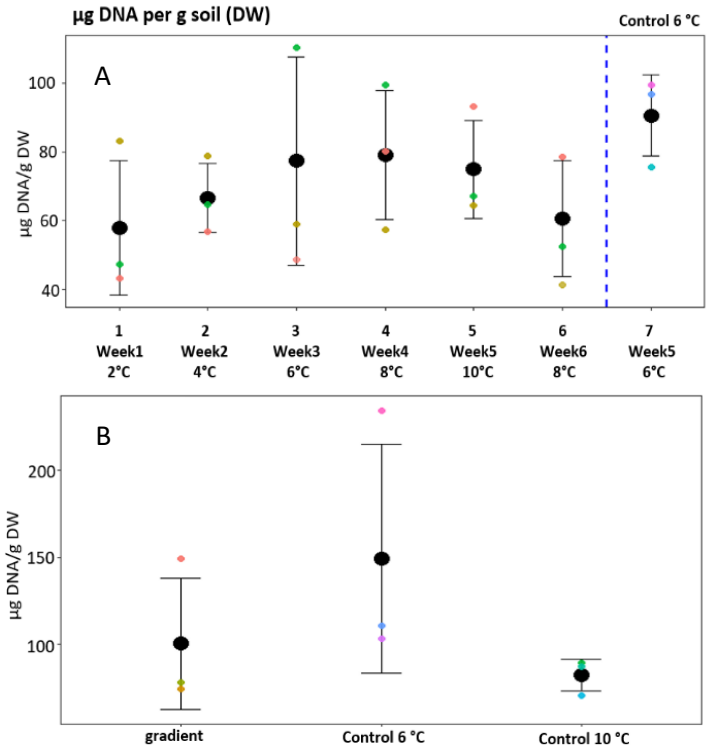

Figure 19: Indications of microbial cell numbers estimated by µg DNA per g dry mass of slurry. The estimates are presented as the mean and standard deviation of three replicates. (A) Estimation were done on gradient samples and performed every week at different temperatures following the "gradient" temperature regime until week 6 (left of the blue dotted line). Measurements for a 6 °C control were performed in week 5 (right of blue dotted line). (B) Estimates were done at the endpoint of the different temperature treatments used in the GC experiment. Gradient (2°C) samples from week 9, 6°C control from week 7 and 10°C control samples from week 7. No significant differences (pairwise t test with Benjamini & Hochberg adjustment) between the different treatments were uncovered.

Cell number and biomass. Two approaches were used to obtain estimates of microbial community size in the samples; i.e. quantification of microbial C (Cmic) (per dry weight of soil (biomass estimates) and total microbial DNA per dry weight of soil (indicative of cell number) (see Material and Methods). These measurements were performed every week of the main-experiment until week 6 (8°C). Additionally, one measurement was conducted in the last week of the 6°C control

treatment (week 5). When sacrificing the endpoint samples from the bottles used for GC measurements, measurements were performed at the end of the gradient treatment, 6°C control and 10°C control. No significant changes within the gradient treatment ($P > 0.74$) or within end-point samples from the GC-bottles ($P > 0.41$) were observed (Figure 19). This means that no temperature-driven changes in mass of DNA per gram of dry soil occurred between different temperatures and weeks. (Figure 19).

The measurements of Cmic did not work for the majority of the samples and most of the results were therefore excluded from this master thesis. For the GC-bottles, the Cmic biomass estimation worked, providing endpoint measures presented in Figure 20. The biomass estimates show that after 9 weeks of incubation, being heated to 10 °C and then cooled back to 2°C the last week, the microbial biomass was averagely larger than at the end of the 10 and 6°C control incubations. The biomass estimated of the control incubations were obtained after 3.5 weeks at 10°C and 5.5 weeks at 6°C, respectively. The results also showed a substantial variation within the same treatments and there were no significant difference between the different endpoints ($P > 0.3$).

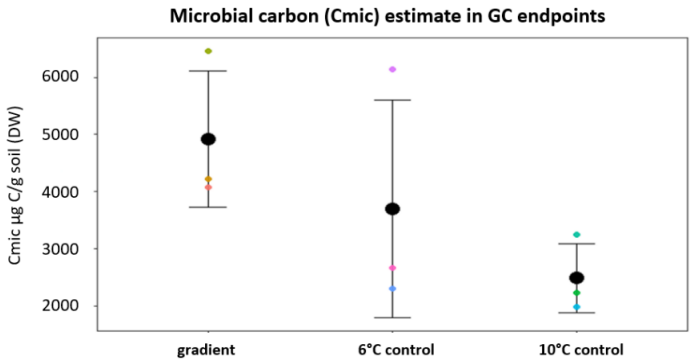

Figure 20: Microbial carbon (µg C/g soil (DW)) in anoxic peat soils under three different conditions: "gradient" are samples from bottles exposed to temperature increase from 2 to 10 °C and colling back to 2 °C before being harvested for various measurements, including microbial C (Cmic). "6°C control" are exposed to temperature increase from 2 to 6°C with a prolonged period of incubation at 6°C. "10°C control" are exposed to temperature increase from 2 to 10°C with a prolonged period of incubation at 10°C The estimates are presented as the mean and standard deviation of three replicates. No significant differences (pairwise t test with Benjamini & Hochberg adjustment) between the different treatments were uncovered.

Discussion

Temperature effects on peat gas emissions in the Arctic

Coupling between CO_2 and CH_4 emissions

CH_4 and CO_2 are two major end-products of organic matter decomposition in anoxic peat soil. An overall higher production of CH_4 than CO_2 was observed at the low temperatures applied in this experiment (2 – 10°C). Interestingly, while a net accumulation of CH_4 was observed at all temperatures, including 2°C, substantial CO_2 accumulation only occurred at temperatures above 6°C. However, exposure to higher temperatures seemed to have a strong effect on the CO_2 accumulation rate and the mean CO_2 contribution to the total daily gas accumulation rate increased from 11.07% of the rate below 6°C to 21.11% above 6°C. The highest CO_2 accumulation rates and concentrations were observed in the last measurement week of the 10°C control samples, reaching the same rate level as for CH_4 at temperatures between 6 and 8°C (during temperature increase). Even though prolonged periods at high temperature led to higher CO_2 accumulation rates, CH_4 accumulation also speeded up, leading to a decrease in the percentage of CO_2 contribution to the total gas accumulation after exposure to 10°C for a longer period. These results are not in line with the theoretical methanogenic degradation pathways reviewed by Conrad (21). Theory argues for an equal emission of CH_4 and CO_2 during complete methanogenic degradation from cellulose (21). The large difference between the accumulation rate of the two gases in my experiment might indicate incomplete degradation, as suggested by Conrad (21) or a higher consumption via anaerobic CO_2 fixation pathways at low temperatures.

An overall corelation test of CH_4 and CO_2 accumulation rates revealed a positive correlation between the two gases throughout the entire experiment. Metje *et al.* (16) also reported strong correlations between the two gasses at 25°C and an overall connection between CH_4 and CO_2 at all temperatures in Siberian peat soil. However, a careful review of the correlation between CH_4 and CO_2 rates observed during this master thesis demonstrated a decoupling between the accumulation rates of the two gases below 6°C and strong correlation above 6°C. Longer periods at high temperatures further strengthened the correlation. Therefore, at temperatures

above 6°C the Arctic peat system also approached towards the equal CH_4 to CO_2 production ratio presented by Conrad (21). Metje *et al.* (16) explained the correlation between the two gases with aceticlastic methanogenesis producing both CO_2 and CH_4, and reported that these gases was produced in equal amounts when only considering aceticlastic methanogens at 25°C. Thus, the poor correlation between the two gases at temperatures below 6°C indicates that hydrogenotrophic or methylotrophic methanotrophs may also influence the accumulation rates at the lowest temperatures. Metje *et al.* (16) also reported differences between the overall CO_2 and CH_4 emitted from the soil at 25°C. Methane was produced both via aceticlastic and hydrogenotrophic methanogenesis and the CO_2 emission from the soil was lower than the amount of CO_2 actually originating from aceticlastic methanogens. The difference in produced and emitted CO_2 from the soil was explained with hydrogenotrophic methanogens consuming some of the CO_2 produced by aceticlastic methanogens (16). Thus, another possible explanation might be that consumption of CO_2 (methanogenic, fermentative or CO_2 fixation pathways) is relatively more effective at low temperatures, "hiding" the C in the CO_2 produced by methanogens within microbial biomass.

Resource availability – a possible time dependent factor

The pre-experiment revealed that the change from zero net CO_2 accumulation to net accumulation above 6°C does not depend on time on a short scale. However, a slight increase in CO_2 production was seen at the end of the pre-experiment, possibly indicating a change in the microbial community due to the length of the incubation. However, these might have been fluctuations in the measurements as no significant differences were revealed. The pre-experiment might also have been too short to properly investigate this issue. A new experiment should be performed to further investigate the effect of time at constant conditions on the accumulation rates. In a long-time perspective microbial adjustments or changes in resource availability might affect the microbial community sufficiently to alter gas emission rates. In nature a rapid fluctuation between different temperatures is common and more likely than stable conditions at 2°C as in the pre-experiment. However, if a temperature of 6°C is the threshold for change in the microbial community, fluctuations between 0 and 4°C over a long period might also lead to the same trend as seen in the pre-experiment. To confirm this another experiment at lower temperatures (0–4°C) has to be performed.

Delayed response of CH_4 emissions to cooling

The initial 5 weeks of temperature increase from 2 to 10°C led to constant accumulation of CH_4. With every temperature increase, the rate of CH_4 accumulation also increased. This corresponds to the frequently observed event of higher temperatures leading to higher rates of methanogenesis, until reaching the temperature optimum of methanogenesis (14, 17, 39).

Interestingly, when the temperature was decreased from 10 to 8°C and subsequently to 6°C in week 6 and 7, the rate of CH_4 accumulation still continued rising, reaching the maximum rate in week 7. During the cooling from 6 to 4°C and subsequently to 2°C in week 8 and 9, the CH_4 accumulation rate dropped, but the rate did not decrease to the same low level as seen at 2°C in the initial week of the gradient experiment. This argues that adjustment to lower temperatures is more time-consuming than the response to higher temperatures. Changes driven by temperature increase, such as upregulation of growth, altering population sizes and cellular activities (42, 53), might take time to reverse. In a climate perspective, slower adjustment to cooling means that the effect of high temperatures last longer than the exposure to high temperatures, thus showing that microbial short-term physiological responses are key to predict the temperature effect on greenhouse gas emission rates. It is not simply a question of temperature effects on chemical reactions, as predicted by the Arrhenius equation (39).

6°C – the temperature threshold for changes in CO_2 accumulation

Below 6°C, the net accumulation of CO_2 emitted from the soil was close to zero. Close to zero accumulation of CO_2 was also seen for the pre-experiment period. When the temperature was kept at 6°C for longer time a tiny increase in CO_2 accumulation was seen, meaning that at approximately 6°C a temperature threshold was found for one or more processes related to the CO_2 accumulation rate. Below 6°C the production of CO_2 was equal to the consumption. When the temperature increased above 6°C, the CO_2 emission also increased. In contrast to CH_4, the CO_2 accumulation rate peaked at the temperature peak (10°C). The response time to decreasing temperatures was also much shorter than for CH_4 and the cooling back to 2°C resulted in approximately the same CO_2 accumulation rates as seen at 2°C prior to the temperature increase. In contrast to the CH_4 accumulation, the CO_2 varies more between replicates, possibly pointing out that the observed concentrations of CO_2 are influenced by multiple processes of production and consumption, like propionate and ethanol oxidation, iron reduction and methanogenesis (17, 21, 54), and anaerobic CO_2 fixation pathways like the Wood Ljungdahl pathway (25, 54) and the reverse citric acid cycle (25).

Temperature effects on microbial metabolisms and growth in Arctic peatlands

Drop in acetate and propionate levels at higher temperatures – possible indications of pathway shifts

Being potential substrates for direct use by methanogens or utilization in syntrophic partnerships, fermentation intermediates and knowledge of their concentrations are important to understand microbial activity in anoxic soils (21). In my experiment the level of acetate increased until 6°C, but after reaching 8°C, the concentration of this metabolite started declining. This may indicate that a change in the microbial community physiology occurs around 6°C. Drop in acetate concentrations was also seen in the 6°C control but it appeared slower compared to the soil samples reaching higher temperatures (8 – 10°C). This indicates that temperatures above 6°C accelerate the drop in acetate concentrations. Herby, the observations argue for a temperature dependent effect on acetate dependent pathways. The slow drop in the 6°C control could also be part of a stabilization of the concentration at a new temperature. The same drop was seen in the propionate concentrations, and although the effect seemed delayed relative to acetate, the concentrations did correlate, a correlation that seemed enhanced by low acetate levels.

The drop in acetate and propionate concentrations at temperatures above 6°C correspond to the result presented by Tveit *et al.* in 2015 (14). The drop in acetate levels could be related to increased activity of aceticlastic methanogens, but Tveit *et al.* (14) did not see any overall changes in the overall level of transcripts for aceticlastic methanogenesis at the same time as the drop. However, they saw a shift in the dominating aceticlastic family from *Methanosarcinaceae* to *Methanosaetaceae*, and also a shift in the composition of taxa associated with major fermentative pathways (14). While these shifts cannot unequivocally explain the observed decline in acetate levels, the shift in aceticlastic methanogens is interesting, as members of the family *Methanosaetaceae* (currently renamed to *Methanotrichaceae)* are believed to have a higher affinity for acetate than *Methanosarcinaceae* (14, 55). The later drop in the propionate level might in turn be a result of efficient propionate oxidation being dependent of low acetate levels (15). The dependency between the two metabolites was also confirmed looking at their correlation, particularly for the gradient treatment, confirming that when acetate levels drop, so do propionate. Interestingly, even during cooling from 10 °C to 2°C, the levels of acetate and propionate

continued to fall, suggesting that this is not merely a thermokinetic effect, but rather the result of a change in the overall microbial community physiology or a shift in the responsible species.

Ethanol - rapidly consumed

Ethanol levels declined rapidly during the first two weeks and stayed at low levels throughout the rest of the experiment with temporally higher concentrations at a few timepoints during the remaining weeks. This shows that ethanol is rapidly consumed throughout the entire experiment, but it is also regularly produced in the soil and probably a highly desired substrate. Metje *et al.* (17) observed that oxidation of ethanol to H_2 and acetate was important for the hydrogenotrophic methanogens. The rapid decline in ethanol levels the two first weeks of the experiment, possibly consumed by syntropic ethanol oxidizers in a relationship with hydrogenotrophic methanogens, at low temperatures, could therefore be a plausible source for the increased concentrations of acetate observed in the first weeks. If hydrogenotrophic methanogens are outcompeted at higher temperatures this might also affect the rate of ethanol oxidation and thus the rate at which acetate is supplied to the community, perhaps contributing to the decreasing acetate levels. Since the ethanol concentrations measured after week two were low it cannot be confirmed to what extent ethanol is produced or oxidized during the rest of the experiment. Earlier studies with Svalbard peat soil did show that by inhibiting aceticlastic methanogenesis, ethanol accumulates at all temperatures between 1 and 30°C, confirming that it is a major intermediate also at temperatures between 2 and 10°C (14). Also, in the study by Tveit *et al.* (14) no accumulation of H_2 was observed at any temperatures, thus in combination with the above indicating that no major change in the rate or affinity of H_2 utilization or ethanol oxidation occurred due to temperature change.

Temperature induced shift in cellular growth

The differences in DNA production and growth rates between 2, 4 and 6°C was small. Therefore, the strong increase in DNA production seen between 6 and 8°C, and between 8 and 10°C indicates that substantial changes in the community physiology at 6°C are leading to higher production of DNA, more rapid cell division and growth. Thus, temperature changes do not only have a purely thermokinetic effect on the rate of biochemical reactions. This results corresponds to the results from Grunnvåg's master thesis (43). She observed that temperature did not affect the growth at 5°C relative to 2 °C, while growth increased at 9°C within the timescale of her experiment.

Prolonged incubation at 6°C did not change the growth rate for the soil microorganisms in my experiment. Thus, time to acclimate to the temperature does not change the growth at this temperature level. However, looking at the result for the 8°C samples in week 6 of the experiment, the length of the exposure to a higher temperature can have a substantial effect on the growth, as the cooling from 10 to 8°C further increases the growth rate relative to that observed at 10°C, likely due to some physiological lag. This might indicate that after a certain threshold (probably at 6°C) the cell division speeds up, but the physiological acclimation need time to take effect at the new temperature. The increase in cell growth rate seen after cooling in this master thesis did correspond to the lag effect seen in the CH_4 accumulation rate. High growth rates even at a declining temperature gradient might be the reason for the delayed reduction in CH_4 accumulation rates. Yvon-Durocher et al. (2014) investigated the effect of temperature on pure culture methanogens and reveal that both cell growth and methanogenetic activity (CH_4 accumulation) respond similarly and correlated when exposed to temperature increase (39). Thus, the delay in CH_4 response to cooling might be induced by continuous high cellular activity. However, at temperatures below 6°C the minor change in cell growth when the soil was exposed to rising temperatures were more similar to the rate of the low accumulation rate seen for the CO_2 at temperatures below 6°C. This suggests that the observed community growth is not only due to changes in the methanogens but, also linked to non-methanogenic organisms altering the CO_2 accumulation in the soil.

During this experiment no significant changes in the microbial biomass (GC bottle Cmic) was observed due to increased cell growth. However, since the chloroform fumigation-based biomass estimation for the samples from the tap-bottles did not work, this approach should be tested again, especially as the GC bottles only represent the endpoints of the experiment. Unfortunately, the failed measurement of microbial C during the temperature increase on the gradient complicates the biomass estimates for these samples. However, measurement of total DNA concentration in the soil was provided for all timepoints as an indication of cell number, showing no significant change in the community size due to temperature change. Tveit *et al.* (14) estimated variation in biomass in the same type of soil from total nucleic acid concentration, Grunnvåg (43) estimated soil microbial biomass from total DNA concentrations. Both approaches indicated no changes in microbial biomass due to temperature change (14, 43). Also, the Cmic data from Grunnvåg performed on the same type of soil as the DNA extraction revealed no significant changes in biomass concentrations. Because the sum of these results from Tveit *et al.* and Grunnvåg all indicates the same

constant biomass, independent of temperature change, and this also is seen in the total DNA concentrations from my experiment, total DNA concentrations were also used as biomass estimates in my thesis to replace the missing Cmic values.

Because of the lack in temperature effect on the microbial biomass, it seems that higher cellular growth also leads to higher death rates in the peat soil and in this way prevents biomass accumulation (42). Viruses, predation and limited nutrient availability are important factors preventing biomass increase (56). Predatory eukaryotic protists have also been shown to be more abundant when temperature increases in arctic peat soil, thus increasing the predation pressure on the microbial community (14). In this way the total living biomass might be kept at a low level even though the rate of cell growth increases. Unless all constituents of microbial necromass are rapidly consumed in the system, estimating the size of the microbial necromass pool could help to clarify where the organic matter from the rapidly growing microbial cells is channelled.

While the CF based Cmic measurements did not work for most of the samples in this experiment, the estimates from the GC bottles seemed to have worked better. One possible explanation for the problems with fumigation of the slurry is the high water content in the samples. Possibly the water might have led to slow diffusion of chloroform and more time would be needed for the chloroform to access and destroy the cells in the soil, making the fumigation proses harder to perform. However, this explanation will not clarify why Cmic measurements of soil from the GC bottles worked. Another experiment with less water content or longer fumigation time might be tested later.

The 6°C shift

A 6°C warming shift in CO_2 accumulation, growth and fermentation

Interestingly, many of the major changes observed in this master thesis occurred during warming, between 6 and 8°C. The system shifted from zero net CO_2 accumulation at temperatures below 6°C to much higher rates, within days. Cellular DNA production increased significantly within the same temperature-time window, indicating higher replication levels and mass specific growth. In addition, levels of acetate and propionate started dropping at this temperature. The fact that all these events happened within a short temperature range indicates that they are connected. Metje *et al.* (16) revealed that hydrogenotrophic and aceticlastic methanogenesis contributed equally to methanogenic degradation at 4°C, while aceticlastic methanogenesis contributed to 70% of the methane

when the temperature increased in arctic peat. Higher aceticlastic methanogenesis increases the consumption of acetate, in addition it is the source to production of both CO_2 and CH_4, while hydrogenotrophic methanogenesis consumes CO_2 to produce CH_4. A CO_2 producing metabolic pathway replacing a CO_2 consuming metabolic pathway might be one contributing factor to the accumulation of CO_2 above 6°C. However, Tveit *et al.* (14) did not find changes in the level of the aceticlastic pathway with temperature and concluded that the aceticlastic contribution to methane production in peat soil from Svalbard was not affected by temperatures. Nevertheless, in that soil a transcriptional shift in the dominating aceticlastic family was seen, possibly explaining the drop in acetate (14). Corresponding to the results from Metje *et al.* (16) from Siberia, Tveit *et al.* (14) also detected changes in the activity of hydrogenotrophic methanogens due to rising temperatures. Declining hydrogenotrophic activity can be a part of the explanation of the change in CO_2 emission rates seen at 6°C. The changes in aceticlastic activity cannot be investigated further without more information about the abundances and activities of the methanogens in the soil.

Chemolithotrophic acetogenesis is performed by bacteria which consume CO_2 and H_2 and produce acetate (21). Conrad *et al.* (57) saw that these bacteria performed better at low temperatures in paddy soil and lake sediments than hydrogenotrophic methanogens and herby limited methanogenesis. Active acetogenesis at low temperatures could help explain the increase in acetate before 6°C and be part of the explanation for low CO_2 emissions at low temperatures. Reduced acetogenic activity at higher temperatures could also partly explain the decline in acetate concentrations in the soil. There are indications from Tveit *et al.* that acetogenesis is not of high importance in peat soil from Svalbard, thus not competing with the hydrogenotrophic methanogens at low temperatures (14). However, this conclusion was based on poor-resolution transcriptional data, and the experiment lacked the temporal resolution of the new experiment presented here. Thus, these statements need further validations.

The increase in cell growth at 6°C indicated higher microbial activity. This activity might be explained by increased methanogenic activity (see previous paragraph) but could also be influenced by altered activity patterns for several metabolisms related to CO_2 consumption and production. As already explained, a large variety of pathways are involved processes linked to CO_2 emissions. Therefore, I propose that a combination of a decline in hydrogenotrophic activity, a change in the fermentative pathways, changes in the rates of CO_2 fixation relative to CO_2 production and increased growth, together, result in the CO_2 shift seen at 6°C. To reveal the exact source of the effects seen in this experiment molecular analysis of

the soil at the different temperatures should be performed. One could also imagine doing isotope tracer experiments to identify the fate of decomposed organic carbon and CO_2 in the system. This was not possible to do during this master thesis because of limited time and an extensive workload, but soil for molecular analyses was sampled for all timepoints and stored at -80°C for further investigations.

Is there a 6°C shift in CO_2 production during cooling?

The delayed decrease in CH_4 accumulation rates compared to the instant decrease in CO_2 rates after temperature drop co-occurred with the constant decline in acetate and propionate concentrations. This suggested that other factors than temperature-effects on enzyme rates are affecting the system during the cooling period. CO_2 production rates did drop during cooling, but indications for a delay in the CO_2 accumulation rate drop was also seen during cooling. The larger variation within the CO_2 measurements might mask the actual CO_2 production rates derived from the aceticlastic pathway. The accumulation rates for 8°C to 4°C during cooling showed no significant differences, possibly also due to the high variation within the different temperatures (Figure 13). Aceticlastic methanogenesis is a possible main source of CH_4 emissions from this soil, leading to emissions of both CO_2 and CH_4. It is therefore expected that both gases should respond in similar ways to the same temperature decrease if aceticlastic methanogenesis is affected by a temperature change. The potential decline in CO_2 accumulation rates, interrupted by a stable period at 8 to 4 °C, might therefore be a result of several sources of production and consumption of CO_2. Like hydrogenotrophic methanogenesis (21) or CO_2 oxidation or fixation pathways like mentioned in the previous paragraph about CO_2 accumulation (6°C – the temperature threshold for changes in CO_2 accumulation) (17, 21, 54). These processes might be triggered due to time, temperature or changes in substrate concentrations in the soil. The large variation in CO_2 linked pathways might also be the reason for the large variation within replicates for CO_2 measurements which is not seen in the CH_4 measurements.

Only metabolites and gas accumulation were monitored during cooling. To get even more insights, a growth rate experiment should also be performed for all temperatures during cooling. The fact that the community does not behave exactly reversibly during cooling, indicates major physiological changes in the community that might take longer time to reverse. However, in the context of an Arctic summer, the length of time provided at each temperature during cooling does reflect the exposure time experienced *in situ*, and thus the reversal of these processes during late summer and autumn might frequently be incomplete,

significantly affecting emissions rates. More frequent and longer periods with temperatures above 6°C during the summer are also likely to occur during the next decades. This is likely to affect these systems, leading to altered microbial physiologies and higher emission of both CO_2 and CH_4. However, in a different way than previous knowledge might have informed us.

Conclusion

The aim of this thesis was to establish detailed knowledge about short-term temperature responses in anaerobic Arctic peat microbial communities. Very low net accumulation of CO_2 below 6°C was replaced by rapid increases in CO_2 accumulation above 6°C leading to a shift in CO_2 emission rates from the soil. In contrast, rapid temperature responses ensured high CH_4 accumulation rates at all temperatures. Thus, CO_2 and CH_4 accumulation rates were highly affected by temperature confirming parts of the first hypothesis, that expected an increase in gas accumulation due to warming, but minor temperature responses in the CO_2 accumulation below 6°C. However, when exposed to cooling, slow responses in the microbial community were observed, especially for CH_4 production, contradicting the part of the hypothesis expecting the temperature response to be immediately reversable when the soil was cooled. The slow response resulted in a delayed decrease in gas accumulation rates with decreasing temperatures. This might be due to accumulated enzymes or cells at temperatures above 6°C, and longer time required to reverse this.

The CO_2 shift was temperature dependent and enabled a fast accumulation of CO_2 when the soil was exposed to higher temperatures, confirming hypothesis 2 that assumed that the shift in CO_2 production would increase the potential for high CO_2 accumulation rates. Increased microbial growth coincided with the increased CO_2 accumulation rate at 6°C, as well as changes in concentration of acetate and propionate. This is in line with the last hypothesis that connected the CO_2 shift to change in microbial activity. I propose that a combination of a decline in hydrogenotrophic activity, changes in the fermentative pathways and rates of CO_2 fixation relative to production can help to explain the CO_2 and acetate/propionate shifts seen at 6°C. Faster growth may also be an overall triggering factor for the shift and the delayed responses observed when cooling was introduce to the system. However, these aspects need to be investigated further by follow-up studies. I conclude that the effect of short-term temperature increases and decreases on microbial activities and greenhouse gas emissions are stronger and much more complex than reflected in the literature. Such responses are highly important in the context of climate change and this study and other similar studies are likely to allow a much deeper understanding of how and how much microbial communities actually respond to temperature changes.

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Appendix

Table 1: Pairwise t.test with p-value for differences between daily CO_2 accumulation rates in anoxic Arctic peat soil during a gradient temperature treatment from week 1 to 9. Weeks are labelled with the respective week number.

Pairwise t.test: weekly CO_2 gradient accumulation rates

	1	2	3	4	5	6	7	8
2	0.63469	-	-	-	-	-	-	-
3	0.41600	0.20374	-	-	-	-	-	-
4	0.00012	3.2e-05	0.00112	-	-	-	-	-
5	1.0e-06	4.1e-07	8.8e-06	0.08029	-	-	-	-
6	0.00057	0.00015	0.00530	0.57102	0.02084	-	-	-
7	5.0e-05	1.4e-05	0.00053	0.75737	0.14724	0.41194	-	-
8	0.00544	0.00158	0.04807	0.16248	0.00202	0.41207	0.08965	-
9	0.51497	0.26813	0.85813	0.00071	6.7e-06	0.00343	0.00034	0.03314

Table 2: Pairwise t.test with p-value for differences between daily CO_2 accumulation rates in anoxic Arctic peat soil during a 10°C temperature treatment from week 1 to 7. Weeks are labelled with the respective week number.

Pairwise t.test: weekly CO_2 10°C control accumulation rates

	1	2	3	4	5	6
2	0.33194	-	-	-	-	-
3	0.82920	0.24960	-	-	-	-
4	5.2e-05	3.7e-06	8.8e-05	-	-	-
5	1.5e-08	1.7e-09	2.4e-08	0.00354	-	-
6	5.1e-12	1.1e-12	6.7e-12	1.7e-07	0.00074	-
7	2.2e-15	1.1e-15	2.2e-15	4.0e-12	2.3e-09	3.0e-05

Table 3: Pairwise t.test with p-value for differences between daily CO_2 accumulation rates in anoxic Arctic peat soil during a 6°C temperature treatment from week 1 to 7. Weeks are labelled with the respective week number.

Pairwise t.test: weekly CO_2 6°C control accumulation rates

	1	2	3	4	5	6
2	0.1240	-	-	-	-	-
3	0.5153	0.0391	-	-	-	-
4	0.5109	0.0391	0.9602	-	-	-
5	0.3772	0.0264	0.8361	0.8361	-	-
6	0.0018	0.0002	0.0053	0.0053	0.0074	-
7	0.0170	0.0016	0.0513	0.0519	0.0788	0.1847

Table 4: Pairwise t.test with p-value for differences between daily CO_4 accumulation rates in anaoxic Arctic peat soil during a gradient treatment from week 1 to. Weeks are labelled with the respective week number.

Pairwise t.test: weekly CH_4 gradient accumulation rates

	1	2	3	4	5	6	7	8
2	0.570	-	-	-	-	-	-	-
3	0.050	0.156	-	-	-	-	-	-
4	9.0e-06	5.1e-05	0.004	-	-	-	-	-
5	8.5e-13	3.0e-12	1.4e-10	1.1e-06	-	-	-	-
6	1.2e-13	3.2e-13	7.6e-12	4.5e-08	0.282	-	-	-
7	1.5e-14	2.8e-14	3.5e-13	7.6e-10	0.019	0.186	-	-
8	3.2e-13	8.6e-13	3.5e-11	2.4e-07	0.595	0.570	0.062	-
9	1.2e-07	6.8e-07	6.0e-05	0.171	7.8e-05	2.9e-06	5.1e-08	1.7e-05

Table 5: Pairwise t.test with p-value for differences between daily CO_4 accumulation rates in anoxic Arctic peat soil during a 10°C temperature treatment from week 1 to 7. Weeks are labelled with the respective week number.

Pairwise t.test: weekly CH_4 10°C control accumulation rates

	1	2	3	4	5	6
2	0.00519	-	-	-	-	-
3	2.0e-07	0.00067	-	-	-	-
4	1.5e-14	3.5e-12	1.8e-08	-	-	-
5	< 2e-16	< 2e-16	< 2e-16	3.7e-14	-	-
6	< 2e-16	< 2e-16	< 2e-16	< 2e-16	< 2e-16	-
7	< 2e-16	< 2e-16	< 2e-16	< 2e-16	< 2e-16	< 2e-16

Table 5: Pairwise t.test with p-value for differences between daily CO_4 accumulation rates in anoxic Arctic peat soil during a 6°C temperature treatment from week 1 to 7. Weeks are labelled with the respective week number.

Pairwise t.test: weekly CH_4 6°C control accumulation rates

	1	2	3	4	5	6
2	0.01055	-	-	-	-	-
3	4.1e-06	0.00061	-	-	-	-
4	1.7e-07	8.0e-06	0.02598	-	-	-
5	6.4e-08	2.1e-06	0.00418	0.34443	-	-
6	5.0e-11	3.1e-10	8.2e-09	1.0e-07	2.8e-07	-
7	1.2e-11	3.3e-11	3.1e-10	2.1e-09	4.4e-09	0.00096

Table 7: Pairwise t.test with p-value of cell turnover time in anoxic Arctic peat soil. Treatment week 1 (2°C) is removed to look at the effect of a week with high variation between replicates.

Pairwise t.test Turnover time

	week 2 (4°C)	week 3 (6°C)	week 4 (8°C)	week 5 (10°C)	week 6 (8°C)
week 3 (6°C)	0.4741	-	-	-	-
week 4 (8°C)	0.0471	0.1510	-	-	-
week 5 (10°C)	0.0134	0.0432	0.4317	-	-
week 6 (8°C)	0.0039	0.0058	0.0566	0.2263	-
week 5 (6°C)	0.6996	0.6996	0.0753	0.0222	0.0039