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The functional potential of methane producing and consuming microorganisms in a changing world

Den funktionella potentialen hos mikroorganismer som producerar och förbrukar metan i en föränderlig värld.

White, Joel

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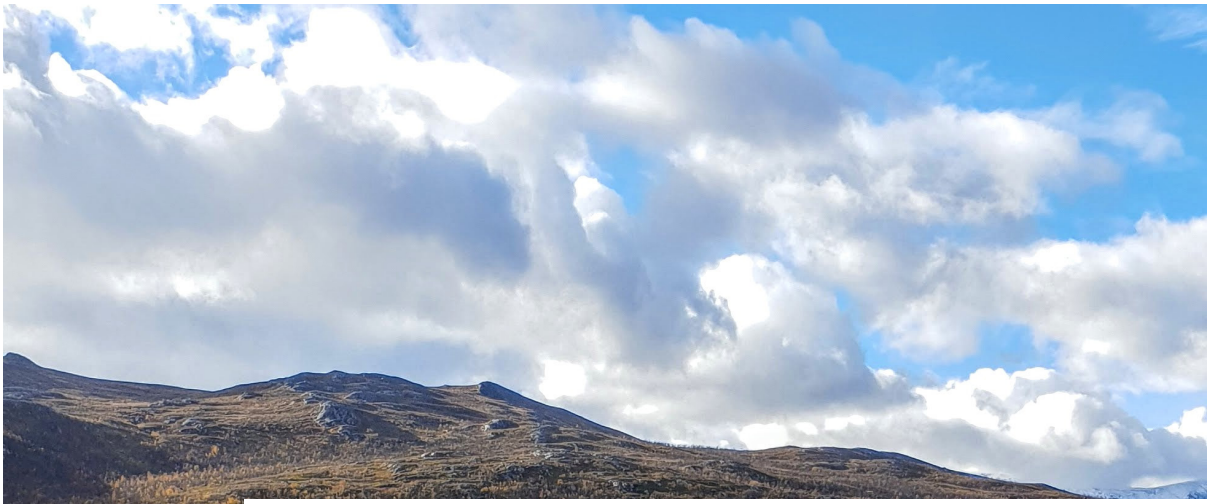

The functional potential of methane producing and consuming microorganisms in a changing world

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DOCTORAL DISSERTATION

Doctoral dissertation for the degree of Doctor of Philosophy (PhD) at the Faculty of Science at Lund University to be publicly defended on 24th of February at 13.00 in Pangea Hall, Department of Physical Geography and Ecosystem Science, Sölvegatan 12, Lund

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Prof. Mette Marianne Svenning

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Title and subtitle: The functional potential of methane producing and consuming microorganisms in a changing world

Abstract: It's clear that human activities have heated our climate via the release of anthropogenic greenhouse gases. Recent changes within the earth climate are rapid, intensifying, and unprecedented. With each additional increment of warming, climate change is impacting ecosystems through changes in average conditions, climate variability, coupled with other associated changes such as biodiversity loss and changes in elemental cycles. One ecosystem of particular importance are peatlands.

Despite covering only ~3% of the terrestrial environment, northern peatlands are estimated to store 415 ± 150 Pg C, while permafrost peatlands are estimated to store 185 ± 66 Pg of soil organic carbon and 7.1 ± 4.7 PG of total nitrogen, thus acting as a large carbon sink. The highly concentrated carbon is maintained by the waterlogged anaerobic conditions that limit oxygen and bacterial decomposition; however, these anaerobic conditions favour methanogenesis, i.e. the formation of the strong greenhouse gas methane.

During the thesis, one laboratory and three in-situ field studies were conducted. In **paper I**, we studied variation in methane fluxes with the structure and function of methane producing and consuming communities. In **paper II**, we performed an in-situ drought experiment across two years where we identified the effects of drought on the functional potential of methane producing and consuming communities. While working on **paper II**, we conducted a temporal experiment (**paper IV**) to intemperate the variation in methane emission rates and their $\delta^{13}C\text{-}CH_4$ values. Finally, **paper III** addresses how sub-arctic peatland microbial communities respond to permafrost thaw at different degradation rates and whether this change is reflected in greenhouse gas emissions.

We found that the structure and composition of the whole methane producing, and consuming community has minor effect on predicting the magnitude methane fluxes (**paper I**), however, during drought the structure and functional composition significantly changed in favor of more methane oxidation despite the lower methane emissions (**paper II**). In the sub arctic, **paper III** concluded that peatlands with fast permafrost degradation yielded the most differences in functional potential between thaw categories, indicating that the microbial community may be responding to newly available substrate previously inaccessible to microbial degradation. Finally, **paper IV** suggested that the substrate availability for methanogenesis is a major factor in explaining the spatial variation, but not the temporal variation in methane fluxes. Combined, these results suggest that the methane producing, and consuming community hold a high functional potential and can produce and consume methane in many different ways despite disturbances such as drought and permafrost loss. This highlights a resilient community with the functional ability to adapt to future climate conditions.

Key words:

Methane, metagenomics, peatland, arctic,

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The hardest thing to do is to simplify your life
~ Yvon Chouinard

Table of Contents

Abstract

It's clear that human activities have heated our climate via the release of anthropogenic greenhouse gases. Recent changes within the earth climate are rapid, intensifying, and unprecedented. With each additional increment of warming, climate change is impacting ecosystems through changes in average conditions, climate variability, coupled with other associated changes such as biodiversity loss and changes in elemental cycles.

One ecosystem of particular importance are peatlands. Despite covering only ~3% of the terrestrial environment, northern peatlands are estimated to store 415 ± 150 Pg carbon, while permafrost peatlands are estimated to store 185 ± 66 Pg of soil organic carbon soil, thus acting as a large carbon sink. The highly concentrated carbon is maintained by the waterlogged anaerobic conditions that limit oxygen and bacterial decomposition. However, these anaerobic conditions favour methanogenesis, i.e. the formation of the strong greenhouse gas methane.

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Populärvetenskaplig sammanfattning

Det är uppenbart att mänsklig verksamhet har uppvärmt vårt klimat genom utsläpp av antropogena växthusgaser. De senaste förändringarna i jordens klimat är snabba, intensiva och saknar motstycke. Med varje ytterligare steg av uppvärmning påverkar klimatförändringarna ekosystemen genom förändringar i de genomsnittliga förhållandena, klimatvariationer, i kombination med andra associerade förändringar såsom förlust av biologisk mångfald och förändringar i elementära cykler.

Ett ekosystem av särskild betydelse är torvmarker. Trots att de täcker endast ~3 % av den terrestra miljön (Minasny et al., 2019) beräknas nordliga torvmarker lagra 415 ± 150 Pg C, medan permafrosttorvmarker beräknas lagra 185 ± 66 Pg organiskt kol i marken och 7,1 ± 4,7 PG totalkväve i marken och fungerar därmed som en stor kolsänka. Det högkoncentrerade kolet upprätthålls av de vattenmättade anaeroba förhållandena som begränsar syre och bakteriell nedbrytning. Dessa anaeroba förhållanden gynnar dock metanogenes, dvs. bildandet av den starka växthusgasen metan.

Under en fyraårsperiod genomfördes en laboratorieundersökning och tre fältstudier på plats. I **artikel I** studerade vi variationen i metanflöden med strukturen och funktionen hos metanproducerande och metankonsumerande samhällen. I **artikel II** utförde vi ett torkexperiment på plats under två år där vi identifierade effekterna av torka på den funktionella potentialen hos metanproducerande och metankonsumerande samhällen. Samtidigt som vi arbetade med **artikel II** utförde vi ett tidsmässigt experiment (**artikel IV**) för att intemera variationen i metanutsläppshastigheterna och deras $\delta^{13}C\text{-}CH_4$-värden. Slutligen behandlar **artikel III** hur mikrobiella samhällen i subarktiska torvmarker reagerar på permafrostens upptining med olika nedbrytningshastigheter.

Vi fann att strukturen och sammansättningen av hela det metanproducerande och metankonsumerande samhället har en liten effekt på förutsägelsen av de stora metanflödena (**artikel I**), men under torka förändrades strukturen och den funktionella sammansättningen avsevärt till förmån för mer metanoxidation trots de lägre metanutsläppen (**artikel II**). I den subarktiska delen av Arktis konstaterades i **artikel III** att torvmarker med snabb permafrostnedbrytning gav de största skillnaderna i funktionell potential, vilket tyder på att det mikrobiella samhället kan reagera på nytillgängligt substrat som tidigare varit otillgängligt för mikrobiell nedbrytning. I **artikel IV** slutligen föreslås att substrattillgången för metanogenes är en viktig faktor för att förklara den rumsliga variationen, men inte den tidsmässiga variationen i metanflödena. Sammantaget tyder dessa resultat på att det metanproducerande och metankonsumerande samhället har en hög funktionell potential och kan producera och konsumera metan på många olika sätt trots störningar som torka och förlust av permafrost. Detta visar på ett motståndskraftigt samhälle med funktionell förmåga att anpassa sig till framtida klimatförhållanden.

List of Papers

Paper I

White, J. D., Ström, L., Lehsten, V., Rinne, J., and Ahrén, D.: Genetic functional potential displays minor importance in explaining spatial variability of methane fluxes within a Eriophorum vaginatum dominated Swedish peatland. *Manuscript*

Paper II

White J.D., Ahrén D., Ström L., Kelly J., Klemedtsson L., Keane B., Parmentier F-J.W. Methane producing and reducing microorganisms display a high resilience to drought in a Swedish hemi boreal fen. *Submitted to JGR: Biogeosciences*

Paper III

White, J.D., Pascual, D., Lakomiec, P., Parmentier, F.J.W., Ahrén, D., Johansson, M., Ström, L. Three peatlands under different stages of permafrost thaw; a metagenomic approach to investigate carbon exchanges from thawing peatlands in the sub-arctic. *Manuscript*

Paper IV

Rinne, J., Łakomiec, P., Vestin, P., **White, J. D.**, Weslien, P., Kelly, J., Kljun, N., Ström, L., and Klemedtsson, L.: Spatial and temporal variation in $\delta^{13}C$ values of methane emitted from a hemiboreal mire: methanogenesis, methanotrophy, and hysteresis, Biogeosciences, 19, 4331-4349, 10.5194/bg-19-4331-2022, 2022.

Author's contribution to the papers

Paper I

JDW, LS and DA conceptualized and designed the study. **JDW** performed the laboratory work with contributions from LS and JR. **JDW** and DA contributed to the bioinformatics analysis while **JDW** and VL were responsible for the statistical analyses. **JDW** outlined and wrote the manuscript with inputs from all authors.

Paper II

JDW and LS conceptualized and designed the study. **JDW** performed the field work with contributions from LS, LK and JK. **JDW** and DA contributed to the bioinformatics analysis while **JDW**, BK and FJP were responsible for the data and statistical analyses. **JDW** outlined and wrote the manuscript with inputs from FJP and DA.

Paper III

JDW and LS conceptualized and designed the study. **JDW**, DP and PL performed the field work. **JDW** and DA contributed to the bioinformatics analysis while **JDW**, DP, PL and FJP were responsible for the data and statistical analyses. **JDW**, DP and LK outlined and wrote the manuscript with inputs from the remaining authors.

Paper IV

JR and PV conceptualized and designed the study. **All authors** performed the field work and data collection while **JDW** performed the laboratory work. **JDW** performed the bioinformatics while **all authors** were responsible for the statistical analyses. JR, PV and **JDW** outlined and wrote the manuscript with inputs from the remaining authors.

Abbreviations

CH_4	Methane
CO_2	Carbon Dioxide
H_2	Hydrogen
O_2	Oxygen
$\delta^{13}C\text{-}CH_4$	Isotopic signature of emitted methane
GHG	Greenhouse gases
Tg	Tera gram
WMG	Whole Metagenome
DNA	Deoxyribonucleic acid
RNA	ribonucleic acid
rRNA	ribosomal RNA
WTD	Water table depth
GPP	Gross primary production
NEE	Net ecosystem exchange
R_{eco}	Ecosystem respiration

Foreword

Rational and thesis structure

Methane (CH_4) cycling in peatlands is an evolving field over many decades. The overall aim of the research is to understand what drives the magnitude of CH_4 fluxes from peatland environments. In attempt to understand this, research has traditionally been conducted in many areas including measurements of greenhouse gas (GHG) fluxes both spatially (closed chamber method) and temporally (eddy co-variance and automated chambers). Once these studies determined the magnitude of CH_4 fluxes, the identification of major drivers was required. This included analysing the effect of vegetation type, substrate availability, hydrology, and temperature dependence. However, until relatively recently, the analysis of the microbes that produce and consume the CH_4 gas has been relatively ignored.

In this thesis you will find an introduction to the importance of CH_4 in a changing climate followed by microbial controls on CH_4 fluxes. This is followed by an in-depth description of the methods used throughout the study and finally, the results and discussion. The results and discussion of this thesis have been divided into two sections. In **part I**, we look at the spatial variability of CH_4 fluxes from different plots within peatlands. We start with CH_4 emissions from natural wetlands as they are known to exhibit high spatial variability. Traditional drivers of CH_4 emissions often ignore the composition and function of the microbial community, therefore in **paper I** we determine if analysing the whole metagenome of CH_4 producing and consuming community acts as a good proxy for predicting CH_4 fluxes in *Eriophorum vaginatum* dominated mesocosms. However, vegetation is not the only factor contributing to spatial variability in CH_4 emissions. In **paper III** we then expand our spatial variability further to address how sub-arctic peatland microbial communities respond to permafrost thaw at different degradation rates (slow, medium, fast) and whether this change is reflected in GHG emissions.

In **part II** we leave spatial variability and move towards temporal variability in peatland CH_4 emissions. The magnitude of CH_4 emissions are controlled by the microbial production (methanogenesis) and oxidation (methanotrophy) of CH_4, and the transport of CH_4 from peat to the atmosphere. Stable isotope signatures of emitted CH_4 can give us clues to the causes of variation in temporal emissions, therefore in **paper IV** we investigate whether temporal variation in CH_4 emission rates and their associated $\delta^{13}C$-CH_4 values reflect the composition of the CH_4

producing and consuming microbial community. In addition to traditional drivers of temporal variability in CH_4 emissions, disturbance is known to affect emissions in short- and long-term time frames. We know that drought conditions alter the GHG balance in peatlands: CH_4 emissions decrease due to the drop in water table while bacterial decomposition increase in the new oxic zone resulting in higher heterotrophic respiration. However, we have little knowledge on the temporal response in the microbial community. Therefore, in **paper II**, we address whether drought disturbance impacts the CH_4 producing and consuming community and link it to the temporal differences in CH_4 flux between drought years.

Introduction

The importance of methane in a changing climate

The International Panel on Climate Change (IPCC) stated that the widespread rapid warming observed in the atmosphere, ocean, cryosphere and biosphere are unequivocally caused by human activities (IPCC, 2021). Each of the last four decades has been successively warmer than the last and is leading to more frequent and intense drought, storms, heat waves, rising sea levels, melting glaciers and warming oceans that can directly harm animals, destroy the places they live, and affect people's livelihoods.

The main driver of climate change is the increase of well mixed greenhouse gases (GHG) in the atmosphere (IPCC, 2021). Since 2011, concentrations have continued to increase in the atmosphere, reaching annual averages of 410 parts per million (ppm) for carbon dioxide (CO_2), 1866 parts per billion (ppb) for CH_4, and 332 ppb for nitrous oxide (N_2O) (IPCC, 2021). These increases in CO_2, CH_4 and N_2O are a result of many sources, but primarily from fossil fuel burning, agriculture, land use change and forestry (IPCC, 2021). One particularly important GHG is CH_4, due to rapid rise in atmospheric concentrations and its global warming potential is 28 times stronger over a 100-year time-period (Dlugokencky et al., 2009, Saunois et al., 2020). While the atmospheric concentration of CH_4 is 200 times lower than CO_2, the increase in atmospheric CH_4 concentrations has contributed $\sim 23\,\%$ ($\sim 0.62\,W\,m^{-2}$) to the additional radiative forcing accumulated in the lower atmosphere since 1750 (Etminan et al., 2016). Therefore, drivers of CH_4 emissions lay the foundation of this thesis.

The relevance of methane to global climate change

Climate change has the potential to increase CH_4 emissions to the atmosphere from critical environments including peatlands, marine and freshwater systems, permafrost, and CH_4 hydrates, through shifts in temperature, hydrology, vegetation and landscape disturbance. Of particular interest is knowing if an increase in CH_4 emissions from these systems could occur in response to climate change, as increases in these emissions would result a in a further positive climate feedback loop.

Methodologies for researching CH_4 emissions from both natural and anthropogenic sources have primarily focused upon in-situ observations. These measurements are traditionally conducted using cavity ring-down spectroscopy combined with metadata from environmental variables which are known to affect the magnitude of the CH_4 flux to the atmosphere. However, the source of the CH_4 emission from microbial activity are often treated as a "black box" and has been identified by Dean et al. (2018) as a substantial research gap. Therefore, a deeper focus on the role of the microbial processes governing CH_4 production and consumption are needed within the research field with special attention paid to the degree these microbial processes drive key global CH_4 feedbacks at present, and under future climate scenarios.

Recent trends and sources of atmospheric methane

During 2008–2017, global CH_4 emissions from both natural and anthropogenic sources were estimated at 576 (range 550–594) Tg CH_4 yr^{-1} (Saunois et al., 2020), ~2.6 times higher than the pre-industrial equilibrium (Dean et al., 2018, Saunois et al., 2020). The spatial and temporal trends of atmospheric CH_4 have been researched in great detail over the past decades using in-situ and modelling approaches (Dlugokencky et al., 2009). The mean growth rate of atmospheric CH_4 decreased from 15 ± 5 ppb yr^{-1} in the 1980's to 0.48 ± 3.2 ppb yr^{-1} during 2000–2006, better known as the equilibrium phase (Dlugokencky et al., 2003). However, following a low growth period, growth rates quickly returned to an average of 7.6 ± 2.7 ppb yr^{-1} during 2010–2019 and higher again, over the last six years of the 2010 – 2019 period (9.3 ± 2.4 ppb yr^{-1}) (Dlugokencky et al., 2003, Dlugokencky et al., 2009). The IPCC (2021) highlighted that if CH_4 were to continue growing at such rates, it would contribute to decadal scale climate change and hinder any long term temperature goal set by the Paris Agreement in 2015. Therefore, planned mitigation targets focused on CH_4 emissions are underway. However, it is critical to understand if the changes in emission rates are caused by human activities or by natural processes responding to changing climate. Therefore, robust estimations of each major CH_4 source is essential.

CH_4 is emitted from several different sectors and is usually reported as natural or anthropogenic sources (figure 1). The contribution of natural sources to global emissions since 1980 is estimated to be between 33 and 54%, while anthropogenic sources accounted for between 46 and 67% (Kirschke et al., 2013). For the decade between 2008–2017, anthropogenic sources contributed a total of 366 [349–393] Tg CH_4 yr^{-1}, while natural sources represent a lower contribution of 232 [194–276] Tg CH_4 yr^{-1} (Saunois et al., 2020). Natural CH_4 sources are represented by environments including vegetated wetlands and inland water systems, land geological sources, wild animals, termites, thawing terrestrial and marine permafrost, and oceanic sources. Within these natural sources, wetlands are the

largest contributor to the global CH_4 budget emitting 194 [155-217] Tg CH_4 yr^{-1} (Saunois et al., 2020).

Among natural sources, wetlands are the largest contributor and the most uncertain in the global CH_4 budget (Salmon et al., 2022). Peatlands are of particular interests because peat is composed of organic matter that has a high carbon content. Consequently, peatlands are large soil organic carbon reservoirs that have been functioning as a source of CH_4, but also a sink of CO_2 to the atmosphere. They cover around 3 % of the surface of continental lands but store approximately one-third of the global soil carbon (Minasny et al., 2019, Salmon et al., 2022). They are located in boreal and sub-arctic regions with small areas around the tropics. Due to their high carbon content the need to understand what drives CH_4 emissions from high-latitude peatlands is important given the high distribution (figure 2) and concerns on their sensitivity to a warming climate.

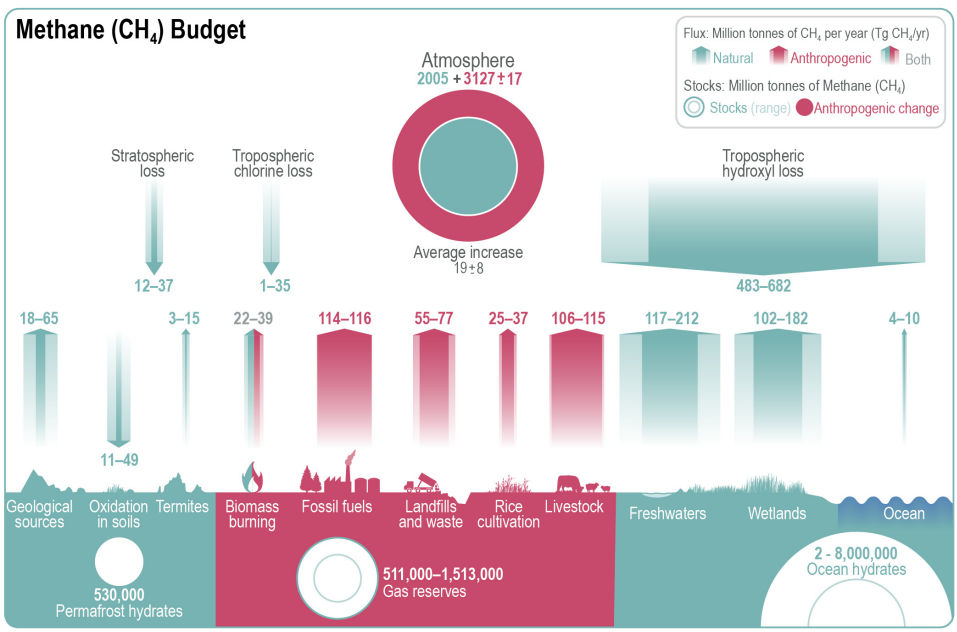

Figure 1: Global methane (CH_4) budget (2008–2017). The atmospheric stock is calculated from mean CH_4 concentration, multiplying a factor of 2.75 ± 0.015 Tg ppb^{-1}, which accounts for the uncertainties in global mean CH_4 (adapted from Chandra et al., 2021).

Peatland carbon cycling under a changing climate

Northern peatland ecosystems have cycled and stored substantial amounts of carbon over the last 11,700 years. Permafrost peatlands, a subsection of natural wetlands, occupy more than 1.7 ± 0.5 million km^2 and are an important component of the permafrost carbon feedback through the release of CH_4 to the atmosphere (Hugelius et al., 2020). Northern peatlands are estimated to store 415 ± 150 Pg C, while permafrost peatlands are estimated to store 185 ± 66 Pg of soil organic carbon and 7.1 ± 4.7 PG of total nitrogen (Hugelius et al., 2020)(figure 2). One third of all terrestrial carbon exists in the form of partially decomposed organic matter, known as peat (Turunen et al., 2002, Vitt et al., 2000).

Northern peatlands occur mostly in boreal and subarctic regions in the northern hemisphere (figure 2). The cold climate, low evaporation rates, and high effective moisture are essential for the formation and development of northern peatlands (Yu et al., 2009). Despite the generally short summer season at high latitudes and the moderate NPP of peatland vegetation, peatlands accumulate excess organic matter as peat owing to depressed decomposition in waterlogged and anoxic conditions (Finlayson and Milton, 2018). Northern peatlands typically establish where mean annual temperatures are between -12°C and 5°C and mean annual precipitation is between 200 and 1000 mm.

Despite overall net uptake of atmospheric C, these saturated, anoxic, organic-rich peatlands are also conducive to CH_4 production, the terminal step in anaerobic decomposition. The initial degradation (hydrolysis) yields a variety of organic molecules (monomers and oligomers), which are then further reduced into carboxylic acids, hydrogen (H_2), and CO_2 (Galand et al., 2005). Generally, methanogenesis is the final reaction in anaerobic degradation and often dominates carbon turnover in peatland environments, see following sections for more details (Dean et al., 2018).

Studies have shown that peatlands respond differently to climate change because of either different hydrological and ecological characteristics in addition to different developmental states (Belyea and Malmer, 2004, Bridgham et al., 2008). Under future climate scenarios we can expect the increased CO_2 uptake by plants, which is likely to be offset by increased soil respiration rates in response to warmer soils and lowered water tables (Moore et al., 1998, Rinne et al., 2020).

CH_4 emissions are likely to decrease in most peatlands because of lowered water tables, and in permafrost areas, the collapse of dry palsa's has lead to increase CH_4 emissions from new anoxic areas and the establishment of sedge vegetation, but this is uncertain (Ström et al., 2003, Woodcroft et al., 2018). The storage of carbon in peatlands is sensitive to all carbon cycle components and is difficult to predict, but the CH_4 cycle is particularly difficult due to the number of drivers affecting the magnitude of flux.

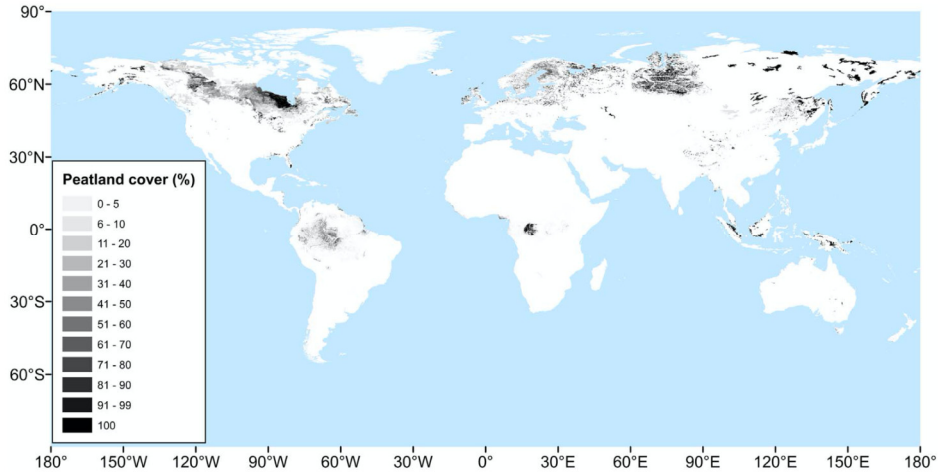

Figure 2: Global peatland distribution map according to PEATMAP (adapted from Xu et al., 2018)

Spatial variability of peatland methane emissions

CH_4 emissions from natural wetlands exhibit both spatial and temporal variability (Sun et al., 2013, Rinne et al., 2022) The spatial variability makes wetland CH_4 emissions difficult to predict, as CH_4 emission within a distance of less than a meter can vary by several orders of magnitude (Bridgham et al., 2013). Traditionally, both production and consumption of CH_4 is influenced by (i) the water table depth, which determines the level of anoxia; (ii) the plant species composition, which provides a transport pathway of CH_4 to the atmosphere; (iii) the temperature of the soil, which affects the rate of microbiological metabolism; and (iv) substrate availability (Ström et al., 2012, Korrensalo et al., 2018, Juottonen et al., 2008, Joabsson et al., 1999).

The recent advancements in molecular techniques have allowed researchers to reveal further complexities in community composition and function, by which microbial processes contribute to spatial variability of CH_4 fluxes in peatlands (Bridgham et al., 2013, Dean et al., 2018). CH_4 production and consumption genes such as methyl coenzyme M reductase (*mcrA*) and methane monooxygenase component A alpha chain (*mmoX*) are commonly targeted to determine the production and consumption of CH_4 (Liebner et al., 2012). However, due to the complexity and large number of genes involved with CH_4 production and consumption, larger sets of genes need to be targeted to address the functional potential of methanogenic communities. Therefore, addressing whether the whole structure of the CH_4 producing and consuming community and their functional genes can explain CH_4 variability has become more important.

Pathways to the atmosphere

CH_4 emissions are not uniform across peatlands due to their heterogeneous nature. Once CH_4 is produced in the anaerobic peat layers it is transported to the atmosphere via three pathways: Diffusion through the water column, ebullition in the form of bubbles and finally via plant mediated transport through specialist vegetation which contains aerenchyma tissue. The variability in research amongst these individual transport pathways is contradicting, making peatland CH_4 emissions difficult to predict.

Diffusion through water is caused by the low solubility of CH_4 (23-40mg l^{-1}), combined with the large difference in the concentration within the soil pore space. Diffusion is believed to contribute least to the flux of CH_4 emissions due to difficulty passing the water-atmosphere boundary (Lai, 2009, Schlesinger and Bernhardt, 2013). However, as more open water areas increase due to permafrost loss in arctic regions, additional attention must be paid to these areas. One recent research article conducted over one year studied the hydrodynamic transport of CH_4 and concluded that diffusion was responsible for 32% of the annual CH_4 emissions (Poindexter et al., 2016).

Within peatland emissions, the largest uncertainty is related to the magnitude of ebullition. Ebullition (the action of bubbling) occurs when the partial pressure of the CH_4 gas in the water surpasses the hydrostatic pressure in the soil (Baird et al., 2004). This pressure increase only takes place when the production of CH_4 is higher than the diffusion potential of the gas (Lai, 2009, Schlesinger and Bernhardt, 2013). The ebullition process bypasses the oxic layer without exposure to oxidation due to the low solubility of CH_4 and rapid transfer from the anaerobic zone to the atmosphere (Lai, 2009). Ebullition events can occur either by natural or artificial disturbance. The proportion of ebullition in the total emission varies from 3 % (Green and Baird, 2013) to 50 % (Christensen et al., 2003).

Some wetland plant species have developed phenological responses to soil anoxia in a process known as plant-mediated transport. This strategy involves developing air-filled channels within their tissues, named aerenchyma (Greenup et al., 2000). The aerenchyma tissue allows plants to survive for long periods of time inundated under water by facilitating the transport of oxygen (O_2) to the water-logged roots (Greenup et al., 2000). However, at the same time, aerenchyma tissue acts as a conduit, facilitating the transport of CH_4 to the atmosphere. During the peak growing season it is believed to be responsible for the majority of CH_4 flux to the atmosphere from wetland ecosystems (Christensen, 2010). Waddington et al. (1996) estimated in boreal peatlands that 55-85% of CH_4 emissions are the result of plant mediated transport.

The contribution of each pathway to the magnitude of CH_4 emissions varies. One potential research gap is how much CH_4 is oxidised due to the presence or absence

of methanotrophs. Tveit et al. (2020) recently identified endo- and epiphytic microbial communities of natural northern peatland mosses relating to peatland type. They concluded that microbial diversity and community structure were distinctly different between *Amblystegiaceae* and *Sphagnum* peatlands (Tveit et al., 2020). These results highlight the need for further genomic research in peatland transport pathways. We may not be aware of potential microbial processes occurring in new areas, thus run the risk of under or over estimating CH_4 emissions.

Microbial controls on methane fluxes in peatlands

Microbial drivers of peatland methane climate feedbacks

CH_4 producing and consuming microorganisms are sensitive to climate change in peatlands due to their susceptibility to peat temperature fluctuations, altered hydrology and drought (Brandt et al., 2015, Potter et al., 2017). Increased peat temperature as a response to a warmer climate is known to increase CH_4 production and consumption (van Winden et al., 2012). In a global meta-analysis, Yvon-Durocher et al. (2014) concluded that peatland CH_4 emissions are likely to increase relative to projected CO_2 emissions due to climate warming. Their results indicated that as peat temperatures increase with climate change, a corresponding 57-fold increase in CH_4 emissions would follow. However, these results are dependent on the temperature optima of different microbes and their associated enzymes, indicating that the community cannot be looked at as a whole but individually according to their species dependant metabolic potential. This becomes important from a microbial modelling perspective where we want to know which functional group dominates under increased peat temperature. This will allow us to determine the size the carbon pools they consume. i.e. if acetoclastic methanogenesis becomes dominant under increased temperature we can then use a mechanistic approach to determine the size of the acetate pool in the model.

The influence of temperature on the production of CH_4 from microbes is also tightly linked to hydrologic conditions. While climate change models are forecasting increased precipitation at northern latitudes, these events are predicted to be more concentrated and less frequent in time with longer periods of dryer warm weather in between (IPCC, 2021). Increased precipitation will raise water tables and even expand wetland areas, thus promoting carbon sequestration and CH_4 emissions, resulting in greater pressure on the oxidative capacity of the methanotroph community due to increase anoxia. It is well established that droughts will lower water tables in peatlands and decrease CH_4 emissions but increase bacterial CO_2 respiration (Rinne et al., 2020). What is not understood well is the resilience of the microbial community to such disturbances.

Polar ecosystems are warming faster than anywhere else on the globe in a phenomenon known as arctic amplification (Rantanen et al., 2022). Permafrost regions including wetlands >60°N are currently experiencing high degradation rates and are expected to contribute ~ 9 (2-18) Tg CH_4 yr^{-1} to the global CH_4 budget (Saunois et al., 2020). These values represent 1–7% of total annual natural CH_4 emissions and are expected to increase under future climate scenarios (Kirschke et al., 2013). At present, these emissions are low in comparison to other CH_4 sources, but they are predicted to rise due to continual permafrost thaw, that leads to increased soil moisture and substrate availability for microbial decomposition (Fofana et al., 2022). As permafrost peatlands thaw, temperature, soil moisture, and substrate availability will on average increase, resulting in the potential to enhance microbial activity. A large body of work has been done on responses of microbial communities to permafrost thaw gradient, primarily at Stordalen mire in Sweden (Fofana et al., 2022, Mondav et al., 2017, Woodcroft et al., 2018). However, Åkerman and Johansson (2008) identified that not all sites lose permafrost at the same rate, despite similar meteorological values. Therefore, if we wish to extrapolate CH_4 emissions across regions such as the sub-arctic, we need to understand how microbial processes may vary under different rates of permafrost degradation.

The balance between peat temperature fluctuations, altered hydrology and drought is critical in determining whether future changes in peatlands are contributing as a positive or negative climate feedback. Protective restoration and sustainable management of peatlands can provide potential climate change mitigation benefits, but a better understanding of the mechanisms controlling the magnitude of CH_4 emissions under a shifting climate are needed to determine the best policies.

Common peatland methanogens

Methanogens, the umbrella term for microorganisms that produce CH_4 as a by-product of cellular metabolism, display high phylogenic diversity spanning Euryarchaeota, Halobacterota and Thermoplasmatota phyla (Bräuer et al., 2020). In total, five orders and two candidate taxa are commonly discovered in peat: Methanomicrobiales, Methanocellales and Methanosarcinales of the phylum Halobacterota; Methanobacteriales of the phylum Euryarchaeota; Methanomassiliicoccales of the phylum Thermoplasmatota, and finally candidate family Methanoflorentaceae and candidate phylum Bathyarchaeota (Bräuer et al., 2020).

Despite the expansive phylogenetic diversity, methanogen's metabolic pathways are extremely limited and include: hydrogenotrophic methanogenesis, the CO_2 reducing methanogens, using H_2, formate, ethanol or isopropanol as electron donors; acetoclastic methanogenesis (acetate splitting) and methylotrophic methanogenesis,

using methyl compounds including methanol or methylamines (Andersen et al., 2013, Bräuer et al., 2020)

Hydrogenotrophic methanogenesis is carried out by microorganisms from the order Methanobacteriales, Methanomicrobiales and Methanococcales. While acetoclastic methanogenesis is carried out by Methanosarcinales (order) within the family Methanosaetaceae and Methanosarcinaceae (Bräuer et al., 2020). Finally, methanogenesis via methanol and methylamines is carried out by members of the Methanosarcinaceae and Methanoplasmatales order (Liu and Whitman, 2008). All pathways hold unique functional genes to process substrates but commonly share the final three genes (mtrA, hdrA and mcrA) used in the formation of CH_4 (figure 3) (Lambie et al., 2015).

Methanogenesis in peatlands: two dominant metabolic pathways

Hydrogenotrophic methanogens utilize H_2 as an electron donor to reduce CO_2 to CH_4 (figure 3). The oxidation of H_2 is achieved using two main hydrogenases: the soluble F420-reducing hydrogenase (Frh) reduces the methanogenic cofactor F420 to $F420H_2$ which is then re-oxidized through the reduction of CO_2 to CH_4. In addition, soluble Mvh hydrogenase forms a complex with a heterodisulfide reductase (HdrABC) that couples the oxidation of H_2 to the reduction of ferredoxin and the heterodisulfide CoM-S-S-CoB in a process called flavin-based electron bifurcation (Kaster et al., 2011). This reduced ferredoxin is essential for the first step in methanogenesis, and ultimately the reduction of CO_2 to a cofactor-bound formyl group. The CoM functions as a methyl carrier and forms the heterodisulfide together with CoB in the last step of methanogenesis (Conrad, 2020). To replenish the cell with reduced ferredoxin, methanogens use energy-converting hydrogenases Eha that catalyzes the sodium motive force driven reduction of ferredoxin with H_2 (Conrad, 2020, Thauer et al., 2008). The energy conservation during hydrogenotrophic methanogenesis happens exclusively during a methyl transfer reaction that is part of the core pathway shared by all methanogenesis pathways. The membrane-bound methyltransferase Mtr, translocate sodium ions across the cell membrane leading to the accumulation of a sodium that is subsequently used by an ATP synthase (Thauer et al., 2008).

During acetoclastic methanogenesis, acetate is activated to Acetyl-CoA within the cell (figure 3). The Acetyl-CoA molecule is dismutated via Acetyl-CoA decarbonylase/synthase. This carbonyl group is oxidized to CO_2 while the methyl group is channelled into the central methanogenic pathway to be reduced to CH_4. The energy conservation happens at the membrane-bound methyltransferase Mtr as observed in hydrogenotrophic pathway. In addition, a membrane-bound electron transport chain utilizes reduced ferredoxin and the heterodisulfide which both are produced during methanogenesis (Ferry, 2011, Welte and Deppenmeier, 2014). During acetoclastic methanogenesis, more Na+/H+ ions translocate during a single

round of methanogenesis compared with hydrogenotrophic methanogenesis, yet the former pathway of methanogenesis also requires an initial ATP investment during the activation of acetate to Acetyl-CoA (Welte and Deppenmeier, 2014).

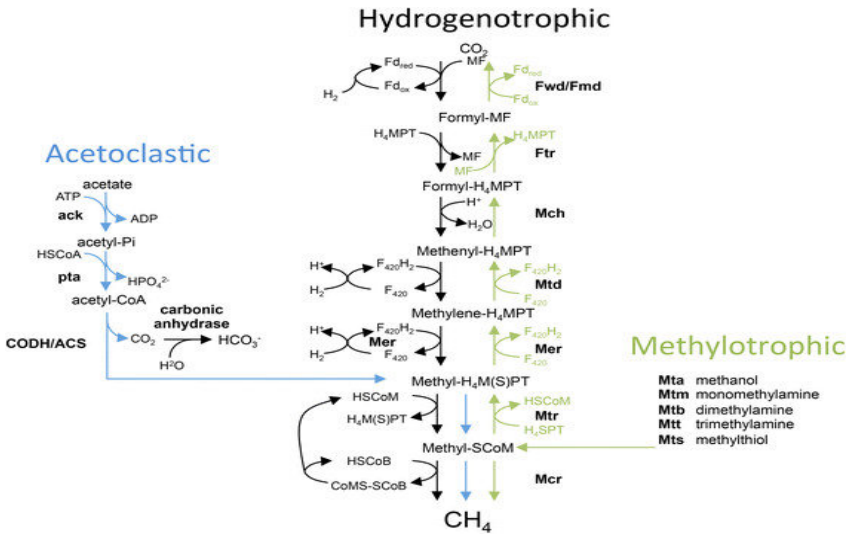

Figure 3: Schematic diagram of the acetoclastic (blue), hydrogenotrophic (black) and methylotrophic (green) methanogenesis pathways. Genes are written in bold black text while compounds are in standard black text. Adapted from (Lambie et al., 2015)

Methanogenesis during drought

During drought, i.e. high air temperatures and reduced precipitation, peatland water tables and soil moisture decrease, aerating previously anoxic peat layers. This leads to increased heterotrophic respiration, and consequently a higher release of CO_2 to the atmosphere (Keane et al., 2021, Rinne et al., 2020). Concurrently, CH_4 emissions are reduced since oxygen (O_2) inhibits CH_4 production upon exposure to methanogen cells combined with increased methanotrophic CH_4 oxidization (Miller et al., 2019, Thauer et al., 2008). In addition, inhibitory phenolic compounds are built-up, these compounds prevent the activity of polyphenolic carbon degrading aerobes, which enable greater conversion of peat organic carbon into smaller substrates such as sugars, organic acids, H_2, and CO_2 that are more bioavailable for the anaerobic methanogens (Wilmoth et al., 2021, Fenner and Freeman, 2011). However, if O_2 is introduced through a drop in water table depth, phenol oxidase can remove phenolic inhibitors, enabling hydrolases to resume normal mineralization of organic matter that subsequently provide additional substrates for

methanogenesis upon the return to anoxic conditions (Fenner and Freeman, 2011, Wilmoth et al., 2021).

Methanotrophy: the biological sink of methane

When CH_4 produced by methanogens reaches the atmosphere it is mitigated by microorganisms called methanotrophs. Methanotrophs oxidize CH_4 to obtain energy under oxic and anoxic conditions using a range of diverse electron acceptors including sulphate, nitrite, nitrate, iron, and manganese. Methanotrophs occur in a wide range of habitats where both CH_4 and oxygen are available. Within peatlands, methanotrophs primarily exist at the interface between oxic and anoxic conditions such as peat layers close to the surface, root adjacent and on the surface of specialty vegetation such a sphagnum mosses (Wendlandt et al., 2010, Tveit et al., 2020). A defining characteristic of these organisms is the use of methane monooxygenase (MMO) enzymes to catalyse the oxidation of CH_4 to methanol (Dedysh and Knief, 2018).

Methanotrophy is performed by bacteria traditionally thought to belong strictly to the phylum Proteobacteria. However, discoveries in the last two decades have broadened the view of methanotrophy with the identification of microorganisms outside the Proteobacteria phylum and archaea domain capable of oxidizing methane anaerobically and aerobically (Guerrero-Cruz et al., 2021). Based on physiological, morphological, ultrastructural, and chemotaxonomic traits, all aerobic methanotrophs can be divided into three major groups, type I and type II and Verrucomicrobia. Aerobic methanotrophs belong to the Gammaproteobacteria (Type I, with families Methylococcaceae and Methylothermaceae), Alphaproteobacteria (type II, with families Methylocystaceae and Beijerinckiaceae), and the Verrucomicrobia phyla (of family Methylacidiphilaceae) (Guerrero-Cruz et al., 2021).

Aerobic CH_4 oxidation is catalysed by particulate and soluble CH_4 monooxygenases. Both enzymes oxidize CH_4 to methanol where then methanol dehydrogenases oxidize methanol to formaldehyde. Finally two more oxidation steps are involved and the intermediates are used for carbon assimilation from formaldehyde (Guerrero-Cruz et al., 2021). In addition to aerobic oxidation of CH_4, a large body of literature on microbial anaerobic oxidation of CH_4 in marine sediments exists. However, its importance in peatlands to the global CH_4 budget, has scarcely been addressed (Smemo and Yavitt, 2011). Anaerobic oxidation of CH_4 was thought to be unimportant in peatlands; however, Gupta et al. (2013) suggest that this process is important in peatlands, but report the primary mechanism of carbon assimilation remains uncertain. The known microbes responsible for this anerobic oxidation of CH_4 are *Candidatus* Methylomirabilis oxyfera and *Candidatus* Methanoperedens nitroreducens. *Candidatus* Methylomirabilis oxyfera belongs to the NC10 bacteria. While *M. nitroreducens*, which is affiliated with

ANME-2d archaea, may be able to catalyse AOM through the reverse methanogenesis pathway (Cui et al., 2015).

More recent evidence indicates that aerobic methanotrophs can thrive under oxygen-limitation, and even under anoxic conditions challenging the conventional view of aerobic methanotrophy (Guerrero-Cruz et al., 2021). Thus, Methanotrophs determine the fate of CH_4 in both natural and human-impacted ecosystems, where the mitigation of greenhouse gas emissions is recognized as one of the most important environmental development goals.

Thesis aims

The aim of the research project is to identify the functional potential of CH_4 producing and consuming microbes in response to different environmental conditions and disturbances in temperate and sub-arctic peatlands. As described above, the production of CH_4 in peatland environments is complex and requires many different methodologies to address the biogeochemical elements that vary both spatially and temporally. Exploring these elements requires new genomic methodologies combined with established closed chamber flux and stable isotope analysis to bring light on previously unknown processes. This was achieved across four individual projects that were conducted during 2017 to 2020. **Paper I** was a laboratory experiment conducted at Lund Universities growth laboratory while **papers II, III** and **IV** were in-situ conducted at Myckelmossen mire and sub-arctic Abisko.

Having data from spatial and temporal sources in temperate to sub-arctic mires is particularly valuable as it allows us to identify variability in processes from within and across different sites. Specifically, this project combines different methodologies with spatial and temporal data to address research gaps within the peatland CH_4 emissions, therefore this thesis aims to:

1) Determine if analysing the whole metagenome of CH_4 producing and consuming community acts as a good proxy for predicting CH_4 fluxes
2) Identify whether the CH_4 producing or consuming community are resilient to drought
3) Address how sub-arctic peatland microbial communities respond to permafrost thaw at different degradation rates (slow, medium, fast) and whether this change is reflected in GHG emissions.
4) Investigate whether temporal variation in CH_4 emission rates and their associated $\delta^{13}C$ values reflect the composition of the CH_4 producing and consuming microbial community.

Methods

The following section outlines the experimental set-ups and methodological approaches used in this thesis. A combination of laboratory (**paper I**) and field experiments (**papers II, III and IV**) were used in combination with metagenomics, GHG flux and stable isotopes (**papers I and IV**) methodologies to address the **research aims I, II, III and IV**.

Site descriptions

Paper I was conducted in a growth laboratory where 9 peat-plant mesocosms were collected from Fäjemyr, an ombrotrophic bog located in Skåne, southern Sweden (56°15'53.3"N 13°33'14.1"E). The peatland is classified as an eccentric bog and is dominated by semi-forested areas alternating between raised hummocks, hollows and moss lawns (Lund et al., 2007). Long-term (1961-1990) mean annual temperature and precipitation are 6.2°C and 700mm respectively (SMHI, 2006). The peat depth ranges between 4-5m, while the peat water pH is generally below 4 throughout the entirety of the growing season (Lund et al., 2007). Vegetation composition at Fäjemyr is diverse including hummocks dominated by dwarf shrubs such as *Calluna vulgaris* and *Erica tetralix*. The moss lawns are carpeted with *Sphagnum*-mosses including *S. magellanicum* and *S. rubellum*, while the raised drier hummocks are dominated by dwarf Scots pine (*Pinus sylvestris*). The dominant sedge species within the site is *Eriophorum vaginatum* (Lonnstad and Löfroth, 1994, Lund et al., 2007)

Paper II and IV were conducted at Mycklemossen peatland, a hemi-boreal mire with bog-like vegetation, located in Southern Sweden (58◦21'N, 12◦10'E). Mycklemossen is a sub-section of the Skogaryd Research Catchment and Swedish Infrastructure for Ecosystem Science network. Common to hemi-boreal fens, the peatland consists of wet low areas dominated by *Sphagnum rubellum* and *Rhynchospora alba*, while the raised intermediate areas are a result of the tussock building sedge *Eriophorum vaginatum*. Once the tussocks are established, soil conditions become drier and are no longer anoxic. This allows for the establishment of small shrubs such as *Calluna vulgaris*, *Erica tetralix* and *Andromeda polifolia*. The long-term (1990–2019) mean annual air temperature and total precipitation were 6.7°C and 1021 mm respectively, as measured by the closest national

monitoring station (Vänersborg, 10 km to the east and at a 30 m lower elevation to Mycklemossen).

P**aper III** was conducted in the Torneträsk region, located in northern Sweden, and extends along a strong West-East oceanic-continental climatic gradient, with precipitation and winter air temperature decreasing towards the East due to the greater distance from the Atlantic Ocean and the rain-shadow effect exerted by the Scandes Mountains. For this study, we selected three sites which present different rates of permafrost degradation: Katterjåkk (68°25'N, 18°10'E) that thawed completely in 2010, Kursflaket (68°21'N, 18°52'E) which has been degrading at a rate of 1.3 cm y^{-1} over 1978-2020, and finally Storflaket (68°20'N, 18°58'E) which is degrading at 1.0 cm y^{-1}. The climate differs considerably among these sites, contributing to the observed differences in permafrost dynamics: mean winter (DJF) air temperature is -10.0 °C in the westernmost site at the Katterjåkk mire (515 m a.s.l; SMHI), -9.7 °C at the Abisko naturvetenskapliga station (~385 m a.s.l; ANS 2020), which represents Kursflaket mire, and -11.1 °C at the easternmost site Storflaket (350 m a.s.l.; M. Johansson, not published). Total annual precipitation between 2010 and 2019 ranged from 848 mm at the Katterjåkk mire (SMHI) to only 358 mm at the ANS (ANS 2020). The more maritime climate at the Katterjåkk mire results in a much thicker mean winter snowpack (c. 80 cm, 1972-2019; SMHI), compared to the continental climate at the Kursflaket and Storflaket mires (c. 10 cm at Storflaket; Johansson et al. 2013).

Measurement techniques

Metagenomics

Historically, methanogens have been difficult to study using culture dependent methods (Brumfield et al., 2020). However, the field of metagenomics developed rapidly by utilizing the genetic material of an environmental sample to identify accurately the functional gene composition (Brumfield et al., 2020, Smith and Osborn, 2009). Techniques included the establishment of polymerase chain reaction (PCR) based studies, where marker genes are used to evaluate microbial community composition via employing amplification of conserved regions by targeting the marker gene 16S in ribosomal ribonucleic acid (rRNA).

In addition to the analyses of taxonomic composition via 16S rRNA, researchers have also targeted functional genes via quantitative PCR (qPCR) to obtain measurable levels of activity in microbial groups. In peatland research, methane production and consumption genes such as mcrA and pmoA are commonly targeted to determine the production and consumption of CH_4 (Liebner et al., 2012). However, due to the complexity and large number of genes involved with

methanogenesis, larger sets of genes need to be targeted to address the functional potential of methanogenic communities.

Many studies in peatland environments have used the techniques described above to establish knowledge of microbial community structure (Chroňáková et al., 2019, Freitag et al., 2010, Galand et al., 2002). Studies have identified how community change from hydrogenotrophic to acetoclastic methanogenesis with increasing depth, changes in community composition and how communities respond to vegetated upper layers in peatland environments (Cadillo-Quiroz et al., 2006, Galand et al., 2002, Galand et al., 2003, Merilä et al., 2006, Chroňáková et al., 2019).

Recently it has been suggested that studying the entire metagenome is a better prediction of soil functional potential (Gravel et al., 2012, Kushwaha et al., 2015, Manoharan et al., 2017). To attain the necessary depth of coverage required to analyse the functional potential of soil microbial communities, whole metagenomic sequencing is required (Dinsdale et al., 2008, Fierer et al., 2014, Manoharan et al., 2017). Though, even with the constant advancements in sequencing technology, metagenomics studies require large computational data and financial resources to obtain the necessary coverage depth to ensure small microbial communities such as Archaea, are detected (Escobar-Zepeda et al., 2015, Pereira-Marques et al., 2019). In response to this issue, we choose to use the technique "captured metagenomics" to target key genes related to the metabolism of both methanogenic Archaea and methanotrophic Bacteria (Kushwaha et al., 2015, Manoharan et al., 2015).

Captured Metagenomics

Whole metagenome (WMG) sequencing is the process of obtaining information on the functional potential of all microorganisms present within the sample. **Paper III** used WMG sequencing to obtain information on the WMG community while **papers I, II** and **IV** used the novel molecular technique named captured metagenomics. Captured metagenomics uses custom-designed, hybridization-based oligonucleotide probes to target functional genes of interest in WMG libraries, where only probe-bound DNA fragments of interest are sequenced (figure 4) (Manoharan et al., 2015). In this thesis, we targeted CH_4 producing and consuming sequences to obtain the functional potential of these communities in peatland environments.

To design the hybridization-based oligonucleotide probes for the captured metagenomics technique we used the Kyoto Encyclopedia of Genes and Genomes database (KEGG). KEGG is an integrated database resource for biological genome sequences and was used for developing a local CH_4 database which contains the nucleotide sequences coding for CH_4 taxonomy and function. Within the KEGG database, functional genes are linked to ortholog groups and are stored under KEGG

Orthology (KO) numbers. Using the KEGG map for methane metabolism (map 00680), we selected KO's of interest and downloaded associated genes through a custom R script. In total 564 055 gene sequences were downloaded to create a local CH_4 database.

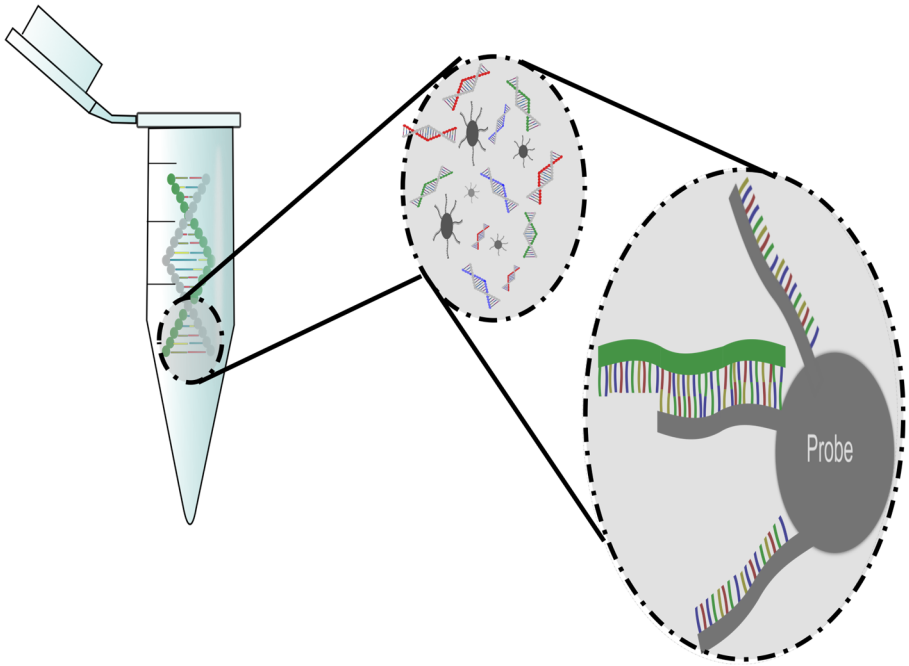

Figure 4: Captured metagenomics visualisation where we can see the custom-designed, hybridization-based oligonucleotide probes hybridizing to target sequences (Green sequences in this exaple).

The nucleotide coding sequences of the CH_4 database were used to design probes for sequence capture. From the local database, 564 055 sequences were obtained. Using the MetCap pipeline developed by Kushwaha et al. (2015). The probe design was based on sequence similarity of 90% with an average 4 probes per cluster. In total, 193,386 individual probes were generated after clustering. They were generated with a melting temperature of 55°C and probe length 40mer which is suitable for use with our protocol that is based on NimbleGen SeqCap EZ (Roche NimbleGen Inc., Madison, USA).

DNA from samples obtained in the field are extracted using DNeasy Power Soil DNA isolation kit (Qiagen, Venlo, Netherlands). The extractions were carried out according to the manufacturers protocol with 0.25 g of peat as the starting material. The extracted DNA was tested for quality (A260/280) and concentration using

NanoDrop 2000 spectrophotometer (NanoDrop Technologies, Wilmington, NC, USA).

Sequence capture process is carried out following the SeqCap Hypercap protocol (Roche Nimblegen, Basel, Switzerland). The University of Liverpool's Center for Genomic Research performed the protocol and sequenced the libraries on the Illumina HiSeq 4000 (**Papers I, II** and **IV**) (Illumina Inc., California, USA). Samples are processed with paired end reads and with 2x150 base pair sequencing, generating >280 million data clusters per lane.

Greenhouse gas flux measurements

The static chamber technique

The closed chamber method was used in **papers I, III** and **IV**. Flux measurements of CO_2 and CH_4 were made using the static chamber technique (Crill et al., 1988, Livingston and Hutchinson, 1995). For each replicate, 6-minute-long measurements in both light and dark conditions were conducted to establish Net Ecosystem Exchange (NEE) and Ecosystem Respiration (R_{eco}). We used a negative sign convention where negative values indicate an uptake of CO_2 from the atmosphere and positive a release. Gross Primary Production (GPP) was calculated according to the relationship GPP = NEE - R_{eco}. Measurements were performed using a transparent 5-liter cylindrical polycarbonate chamber that was covered with a dark hood for R_{eco} measurements (figure 5). The chamber was equipped with a rubber list to ensure an airtight seal and a fan to circulate air. Both CO_2 and CH_4 concentrations were measured with a LGR Fast Greenhouse Gas analyser (model 9110010, Los Gatos Research, CA USA). The CO_2 and CH_4 fluxes were calculated via changes in gas concentration as a function of time using linear fitting over 6-minute measurement periods. Data was corrected for both air pressure, volume of the chamber and ambient air temperature.

Figure 5: The static chamber method at Myckelmossen mire during the 2018 drought.

Automated greenhouse gas flux measurements

Surface GHG fluxes were measured using the SkyLine2D system in **paper II**. The SkyLine2D is an automated chamber system designed and built at the University of York to measure greenhouse gas exchange. For a full description of the SkyLine2D system, we refer to Keane et al. (2018). In short, the flux chamber comprised of a translucent Perplex cylindrical chamber (inner diameter 20 cm, height 40 cm), which was suspended from a motorized trolley and programmed to traverse ca. 2 m above the transect. The system was pre-set to visit the pre-selected plots along the transect, where the chamber was lowered onto pre-installed collars for a measurement period of 4 minutes. Following the 4-minute measurement period, the system raised the chamber and moved to the next plot. The time taken to complete a full cycle was approximately 2.5 h, which allowed each chamber to be measured ca. 10 times per day. The headspace gas from within the sealed chamber was circulated through a Los Gatos cavity ring-down laser (CRD, LGR U-GGA-91, Los Gatos Research, CA United States) to measure the change in concentration of CH_4. Fluxes were calculated as the increase in headspace concentration over time, determined by linear regression, and adjusted for temperature and area of the chamber.

Stable isotope analysis

The CH_4 emission and its $\delta^{13}C$ signature were determined using a cavity ring-down laser absorption spectrometer (CRDLAS) (**paper I** and **IV**) with the closed chamber technique described above (G2201i, Picarro, Santa Clara, USA). The surface of each peat mesocosm was covered with a transparent cylindrical chamber for 25-30 minutes while the CH_4 mixing ratio and $\delta^{13}C$-CH_4 was recorded with 1 second intervals. Data was averaged over one minute and the δ13C signature of emitted CH_4 was determined with a Keeling plot intercept approach (Keeling, 1958, Thom et al., 1993). We compared values from the CRDLAS instrument with an isotope ratio mass spectrometer (IRMS) by taking air samples from the flux chamber during measurements from the CDRLAS and analyzing these with the IRMS (Rinne et al., 2022). The values from the IRMS indicated a bias of -3.4 ‰ on the CRDLAS, thus we have corrected the values of the $\delta^{13}C$ signature by adding 3.4 ‰.

In **paper III**, Using the same chamber described above, $\delta^{13}C$-CH^4 and δD_{CH4} were measured. Air was sampled to the supleco Supel™-Inert Foil Gas Sampling Bags through a three-way valve that was installed onto the original tubing system. A separate line was connected to the external electric pump to take a small portion of air, to avoid disturbing the flux measurement system. The sample filled bags were sent to Utrecht University in the Netherlands, where $\delta^{13}C$-CH^4 and δD_{CH4} were measured.

Results and discussion

Part I: Spatial variability of methane fluxes and their relationship to the microbial functional potential

Large spatial variability in CH_4 fluxes has been observed in many natural peatlands and CH_4 emissions can vary by several orders of magnitude within a distance of less than a meter (Bridgham et al., 2013). It is traditionally thought that CH_4 fluxes are driven by (i) the water table depth, (ii) the plant species composition (iii), the temperature of the soil and (iv) substrate availability for microbes (Joabsson et al., 1999, Juottonen et al., 2008, Korrensalo et al., 2018, Ström et al., 2012). However, these traditional drivers often do not explain spatial variability. Therefore, in **paper I** we explored whether the functional potential of the whole CH_4 producing and consuming community could explain the variability of CH_4 fluxes in temperate peatlands (**thesis aim I**).

In **paper I**, we observed significant variability in CH_4 fluxes that ranged between 152 (SE ±22.2) to 371 (SE ±9.6) µmol of $CH_4\, m^{-2}\, h^{-1}$ despite the mesocosms being dominated by one singular vascular plant and the same growth conditions (figure 6). Surprisingly, we observed no significant differences in the composition and functional potential of the methanogen and methanotroph community in relation to low, medium, and high emitting mesocosms. We believed that the lack of structural difference observed in **paper I** may have been in response to the dominant vegetation present in all the mesocosms. In a similar study, Wu et al. (2021) concluded that the vegetation type considerably influenced the methanogenic community composition. Therefore, an expanded study to include all vegetative types (**paper III**) present in peatlands was needed to fully determine if analysing the functional potential of the microbial community acts as a good proxy for predicting CH_4 fluxes.

The results in **paper I** become relevant in the terms of ecosystem models, where microbial processes are assumed as spatially homogenous within peatlands (Chadburn et al., 2020). Our results confirm this model assumption as correct, but only in terms of peatlands dominated by one vegetative ecotype. Due to the spatial heterogeneity of vegetation in different peatlands, this conclusion may not be robust.

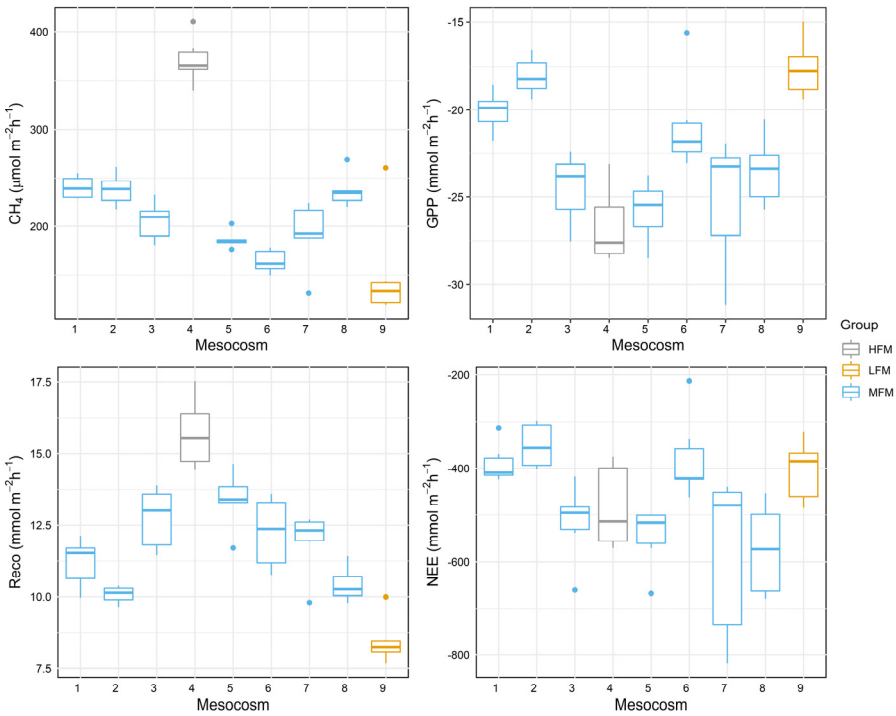

Figure 6: Boxplots of all carbon fluxes according to individual mesocosms. Fluxes measured during the peak growing season. CH_4 µmol m^{-2} hr^{-1}, gross primary production (GPP) mmol m^{-2} hr^{-1}, ecosystem respiration (R_{eco}) mmol m^{-2} hr^{-1}, net ecosystem exchange (NEE) mmol m^{-2} hr^{-1} (**paper I**).

In **paper III**, we expanded our aims to include all vegetative types. In addition, three peatlands, including drained, mesic, and wet ecotypes with different rates of permafrost degradation were added to further explore spatial variability across and within sites (**thesis aim III).**

In **paper III,** we observed the same spatial variability of GHG's as in **paper I**. Variability occurred both between sites and within. The same variability was also observed within the relative abundance of microbial taxa, where significant variation in the structure and composition of the microbial community was observed between Storflaket, Kursflaket and Katterjåkk peatlands. Our results suggest that shifts in microbial communities are time dependent, indicating that mires with faster permafrost degradation (i.e. Kursflaket) require time to reach a new microbial steady state, such as communities observed at Katterjåkk. In addition to spatial variability observed between sites, we also observed large spatial variability between ecotypes within and across sites. We believe this variation was driven by the dominant vegetation found in the plots and this is in line with previous research. Galand et al. (2003) observed that dry ecotypes were dominated by members belonging to Methanomicrobiales, and sequences dominating the upper part of the

wet ecotypes were related to members of the order Methanosarcinales. This result confirms our conclusions from **paper I**, where the lack of structural difference observed in the microbial community may have been in response to the vegetation present in the mesocosms.

After upscaling the C fluxes, it became apparent that the most degraded mire, Katterjåkk, has the highest area-weighted emission of CH_4 (4.72 mg CH_4 m^{-2} h^{-1}) compared to Kursflaket (4.40 mg CH_4 m^{-2} h^{-1}) and Storflaket (1.68 mg CH_4 m^{-2} h^{-1}). Of the three peatlands, Katterjåkk has a larger proportion of wet areas (100%), while Kursflaket and Storflaket have 71% and 21% respectively. The high levels of wet areas lead to anoxic conditions where methanogenesis carbon cycling prevails, and thus it becomes reasonable that Katterjåkk is the overall largest emitter of CH_4 when averaged across the site.

Although Katterjåkk held the highest total CH_4 emissions when summed across the entire site, it held the lowest per plot average in CH_4 flux for the wet category. One reason for the observed low fluxes could be the availability of usable substrates for methanogenesis. As the permafrost thawed quickly at Katterjåkk, organic material previously held in permafrost became available for microbial decomposition. This may have resulted in a sudden burst of microbial activity with associated larger GHG fluxes, like results seen at Storflaket and Kursflaket mires. Once this newly available organic material was consumed by microbes, the only new carbon input was through litterfall and plant exudation. This, in combination with increased anoxia from permafrost subsidence led to a new microbial community steady state with lower associated R_{eco}. This shift is reflected in the microbial community, where we observed significantly different communities between Katterjåkk and Storflaket mires. In addition, no statistical difference in the structure of the microbial community was observed between Katterjåkk and Kursflaket plots suggesting that mires with slower permafrost degradation (i.e., Storflaket) hold the potential for larger GHG fluxes during thaw.

Our results suggest that shifts in microbial communities are time dependent, indicating that peatlands with faster permafrost degradation (i.e., Kursflaket) require time to reach the new microbial steady state observed at peatlands that have completely thawed (i.e. Katterjåkk). This result is also reflected in the GHG emission of peatlands where rapidly thawing sites emit larger amounts of CH_4, while GPP increased with thawing wet locations, presumably from the availability of new substrates injected into the system through via GPP and plant exudation. This is an important result as we can no longer assume that the highest CH_4 in sub-arctic peatlands is driven solely by wetness, but rather the combination of wet areas and rapid permafrost degradation that contribute to larger CH_4 fluxes in sub-arctic mires (Cooper et al., 2017).

Interestingly, Katterjåkk was the only peatland where acetoclastic methanogenesis was present according to the $\delta^{13}C$ CH_4 signal, despite fen like environments in all

sites. As this metabolic pathway in methanogenesis is common in fen environments, the lack of any isotopic signal indicating acetoclastic methanogenesis was surprising. This result is supported by McCalley et al. (2014) where in nearby Stordalen mire they suggested that changes in vegetation and increasing methane emissions with permafrost thaw are associated with a switch from hydrogenotrophic to partly acetoclastic methanogenesis. This was also observed in the functional methanogenesis genes where 3 hydrogenotrophic sequences coding for mch, fwdA and mer were significantly lower at Katterjåkk than Kursflaket, while the acetoclastic gene, pta, significantly increased. This result is in agreement with **paper IV's** conclusions, where we observed hysteresis like behaviour in the $\delta^{13}C$ signal of emitted CH_4. Thus, indicating the potential to produce CH_4 via multiple metabolic pathways despite the dominant environmental conditions.

Part II: Temporal variability in methane fluxes and metabolic pathways

Drivers of temporal variation in CH_4 emissions from mire ecosystems are not fully understood (Rinne et al., 2022). Temporal data in metagenomic studies are limited and most often studies focus upon the peak growing season rather than the entire year, especially in hard to sample arctic locations (Metcalfe et al., 2018). Here, we present two temporal studies. First, **paper IV** where we investigate whether temporal variation in CH_4 emission rates and their associated $\delta^{13}C$-CH_4 values reflect the composition of the CH_4 producing and consuming microbial community. And secondly, in **paper II**, we address whether drought disturbance impacts the CH_4 producing and consuming community and link it to the temporal differences in CH_4 flux.

In **paper IV** we observed the time series of CH_4 emission rates and display a typical seasonal cycle, with the highest emission rates in late summer and lower emissions in winter. The highest emission rates were observed in chambers containing abundant sedges such as *E. vaginatum* and *R. alba*. While chambers dominated by *Erica tetralix* held the lowest values and displayed a less pronounced annual cycle. These results are consistent with previous research that addressed plant species composition and the functioning of wetland ecosystems that indicated changes in the species composition alter CH_4 emissions (Ström et al., 2005). However, the results of **paper IV** showed that large and systematic spatial variations in $\delta^{13}C$-CH_4 of up to 15‰, within one plot, indicating not only variability in the magnitude of CH_4 emissions but also shifts in metabolic production pathways.

The temporal relation of $\delta^{13}C$-CH_4 values and CH_4 emission rates showed a hysteresis-like behaviour in three of the measurement chambers. They displayed lower $\delta^{13}C$-CH_4 values in the early part of the growing season than during a period

with similar emission rates later in the season. This hysteresis like behaviour indicates that the temporal variation in CH_4 emission rates from this mire could be a result of two-time lagged compounding effects. First, the increasing CH_4 emissions during the first half of the growing season could be caused by increasing peat temperature enhancing the activity of methanogens, and secondly, later in the growing season, the increased input of root exudates from vascular plants would increase the substrate availability, resulting in less depleted $\delta^{13}C\text{-}CH_4$ values than in the early season. In addition, the genomic analysis revealed that the microbial community hold the functional potential to produce CH_4 via the hydrogenotrophic and acetoclastic pathways, thus confirming the ability of shifts in $\delta^{13}C\text{-}CH_4$ to reflect both spatial and seasonal changes. This temporal variability in $\delta^{13}C\text{-}CH_4$ combined with the genomic analysis suggests a dynamic microbial community with specific functional groups responding to environmental stimulants at different times of the year.

We concluded that the seasonal variation in CH_4 emissions is likely controlled by both temperature (beginning of season) and substrate availability (end of season). The microbial community composition has the functional potential to produce CH_4 via multiple metabolic pathways (i.e. hydrogenotrophic and acetoclastic methanogenesis), enabling shifts depending upon changes in the substrate availability. However, the highly depleted $\delta^{13}C\text{-}CH_4$ values observed indicate the dominance of hydrogenotrophic methanogenesis, and thus the variation in $\delta^{13}C\text{-}CH_4$ may be due to the energetics of this process.

Paper II built upon the functional potential of the microbial community at Myckelmossen mire (**paper IV**), but under drought conditions. Here we measured the functional potential of the methane producing and reducing community combined with environmental parameters, including soil and air temperature, precipitation, water table depth, as well as CH_4 fluxes during the summer of 2017 under typical growing conditions – and in 2018 during a drought to address **thesis aim III**.

During the 2018 drought, we observed a substantial increase in air and soil temperature, reduced precipitation, and a lower water table depth. The increased air temperature resulted in higher peat temperatures, a common driver of microbial activity. In addition, the reduced precipitation, primarily observed in June and July, drastically lowered the water table depth resulting in oxidative stress on the methanogen community. These factors led to a significant reduction in CH_4 emissions from plots dominated by *C. vulgaris* and *R. alba*, however the same reduction was not observed in *E. vaginatum* plots (figure 7).

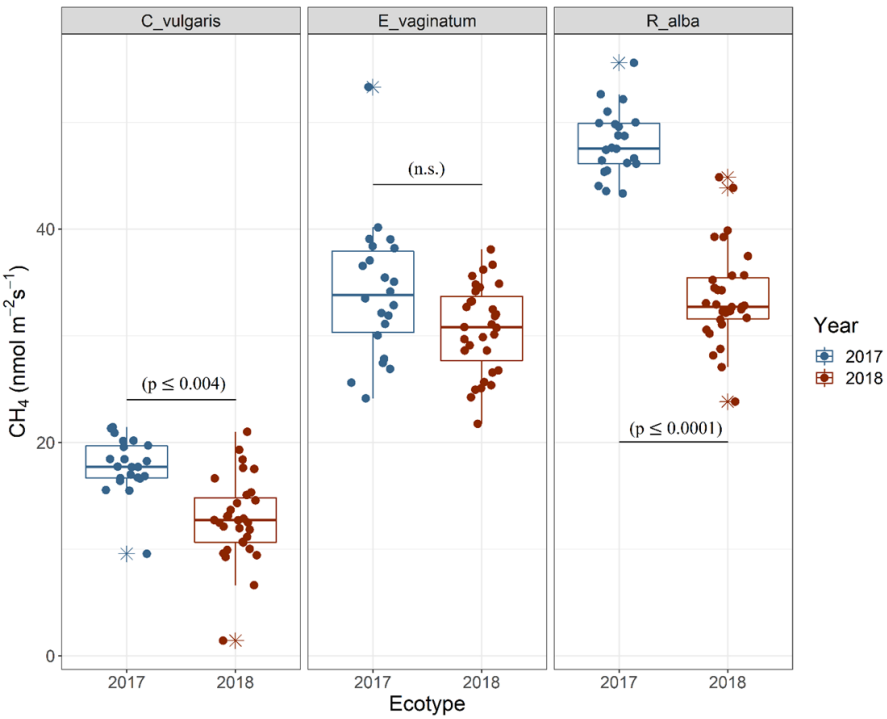

Figure 7: Boxplots of daily mean CH_4 flux measured during the growing season (May to October) in 2017 (blue) and 2018 (red) at Mycklemossen mire. The boxes show quartiles and the median, the whiskers denote data within 1.5 times of the interquartile range. Colored stars denote outliers while significant differences using linear mixed effects models are labelled with the p value and "n.s." indicates a non-significant result (**Paper II**) .

Under drought conditions, the taxonomic and functional gene composition significantly shifted in favour of a higher methanotroph community. The relative abundance of methanotrophs increased by 8% and the shift in dissimilarity between genera was driven by type II methanotroph *Methylocella*, *Methylosinus* and type I: *Methylococcus*. Interestingly, the relative abundance of methanogens also increased, primarily from genera *Methanoregula*, *Methanosarcina* and *Methanosphaerula* that possess expanded genomic features that enable better adaptation to oxidative environments using oxygen-detoxifying enzymes such as catalase, superoxide dismutase, superoxide reductase. These genomic traits not found in all methanogens gave these genera an eco-physiological advantage that allows for growth during desiccation.

Our results suggest that specific functional groups respond differently to drought events due advantageous genomic traits, giving a competitive edge when under oxidative stress. To be able to predict more accurately the effect of anthropogenic climate change, including drought events, on methanogen and methanotroph

communities, additional attention should be paid towards the frequency and length of drought events. In peatland environments we observe a highly resilient methanogen community, which surprisingly expanded in relative abundance during drought conditions, but this increase is not reflected in CH_4 emissions, presumably due to the even higher increase in methanotrophy. This high abundance of both communities indicates that the severe drought from 2018 did not deteriorate the functional potential of the peatland microbial community.

Consistencies across all peatland environments

Interestingly, the methanogen and methanotroph community were consistent in **papers I, II, III** and **IV** and held all the necessary functional groups to produce CH_4 via all metabolic pathways (i.e., hydrogenotrophic, acetoclastic, methylotrophic), despite differences in site characteristics. This consistency suggests an adaptive community that can be found from temperate bogs to sub-arctic peatlands. In **paper I, III and IV** we observed that the $\delta^{13}C\text{-}CH_4$ signal of emitted CH_4 indicated the dominant pathway was hydrogenotrophic methanogenesis, however hysteresis like behaviour in different chambers in **papers III** and **IV** indicated alternative pathways may be underway. This result indicates that spatial and temporal development of CH_4 emissions may not be dominated by one functional group, but rather a combination of different functional groups taking advantage of substrate availability during various times of the year. The presence of all metabolic pathways and in **papers I, II, II** and **IV** suggests a dynamic community with a high resilience (**paper II**) to different environmental change.

Similar results were observed in **paper III**, where the sub-arctic peatland under permafrost degradation returned a $\delta^{13}C\text{-}CH_4$ signal indicating acetoclastic methanogenesis, but the taxonomic community and functional genes were dominated by the hydrogenotrophic pathway. We believe that although the acetoclastic and methylotrophic functional groups remain in low abundances compared to hydrogenotrophic, the entire community is dynamic and responds to favourable condition like a surplus of usable substrates, but then becomes dormant when their ecological niche is not favourable.

High spatial and temporal variability in CH_4 fluxes were consistent in **papers I, II, III** and **IV** and is in line with previous research (Rinne et al., 2022, Sun et al., 2013). **Paper I** observed high spatial variability between low, medium and high emitting mesocosms but small differences in the composition of both taxa and functional genes. The same spatial variability was observed in **paper III,** where the variability of CH_4 fluxes was related to the rate of which permafrost degrades, in addition to the dominant vegetation and microbial community. After upscaling the CH_4 fluxes in **paper III**, it became apparent that the mire at Katterjåkk (most permafrost

degraded site) has the highest area-weighted emission of CH_4 compared to the mires at Kursflaket (rapid permafrost degradation) and Storflaket (slow permafrost degradation). Suggesting that total area of wet surfaces and rate of permafrost degradation becomes an important factor when up-scaling CH_4 fluxes from peatland scale to regional.

Paper II and **IV** highlighted both temporal and spatial variability in temperate peatlands. **Paper IV** concluded that the spatial variability in $\delta^{13}C\text{-}CH_4$ is larger than temporal variability and is driven by differences in substrate ability rather than methanotrophy as previously believed (Chowdhury and Dick, 2013). While **paper II**, observed large temporal variability in response to drought. Consistent across all papers was the high spatial and temporal variability of peatland CH_4 emissions. Traditional drivers have helped explain the variation in CH_4 fluxes, but here we see that inclusion of microbial data can help explain spatial and temporal variation, unless you are looking at one dominant vegetation type (**paper I**).

These results become important when designing future studies. If we focus primarily on peak growing season, we may begin to make assumptions that may not be correct. By simply sampling in the peak growing season, we may miss important information, we need to expand our sampling regime to include higher frequency on a temporal scale and more spatially over different peatlands (especially in arctic regions undergoing permafrost thaw). This combined with a metatranscriptomics approach will allow the research community to understand better the roles other functional groups play when not dominant, and under what conditions these change. Thus, allowing a better understanding of the dynamics governing CH_4 production and consumption allowing for me robust models.

Conclusions and future outlook

This thesis discusses the functional potential of methane producing and consuming microorganisms from temperate to sub-arctic mires. We address the spatial and temporal variability observed in CH_4 fluxes and attempt to explain the links between these two factors. In addition, we address the impact of drought on the methanogen and methanotroph communities and conclude that the communities are resilient and adaptive to environmental change.

We found the captured metagenomics method particularly useful when researching such a small component of the whole metagenomic community. Captured metagenomics provides an alternative to studying the entire DNA pool of metagenomic communities. This method makes it possible to target thousands of key genes related to methanogen and methanotroph metabolism, while avoiding lengthy lab hours and massive sequencing efforts required of large-scale metagenomic study.

To conclude this thesis, we will return to the thesis aims and address them individually. In **Thesis aim I** we wanted to determine if analysing the whole metagenome of CH_4 producing and consuming community acts as a good proxy for predicting CH_4 fluxes. We found that the overall structure and function of the CH_4 producing and consuming microbial community does not explain the variation in CH_4 fluxes under typical peatland conditions. However, we identified that the presence of acetoclastic and methylotrophic methanogens plus type I, II and *Verrucomicrobia* methanotrophs indicates that the microbial community holds the ability to produce and consume CH_4 via alternate metabolic pathways. This is important in terms of peatlands under future climate pressure, where we can see methanogenic and methanotrophic communities hold the functional potential to respond to altered nutrient status, hydrology, or peat chemistry.

Thesis aim II Identified whether the CH_4 producing or consuming community are resilient to drought. We found that drought is a strong disturbance to CH_4 fluxes, and significantly affects the functional potential of the microbial community in favour of more methanotrophy. The relative abundance of methanotrophs increased by 8% and the shift in dissimilarity was driven by type II *Methylocella*, *Methylosinus* and type I: *Methylococcus*. Interestingly, the relative abundance of methanogens also increased, primarily from members of genera *Methanoregula*, *Methanosarcina* and *Methanosphaerula* that possess expanded genomic features

that enable better adaptation to oxidative environments. Therefore, our results suggest that specific functional groups respond differently to drought events due to advantageous genomic traits, giving a competitive edge when under oxidative stress.

Thesis aim III addressed how sub-arctic peatland microbial communities respond to permafrost thaw at different degradation rates (slow, medium, fast) and whether this change is reflected in GHG emissions. We found that sub-arctic peatlands under different rates of permafrost thaw yield different magnitudes of GHG flux. Peatlands with fast permafrost degradation yielded the largest variation between thaw categories, indicating that the microbial community may be responding to newly available substrate previously inaccessible in permafrost to microbial degradation.

Finally, **thesis aim IV** investigated whether temporal variation in CH_4 emission rates and their associated $\delta^{13}C\text{-}CH_4$ values reflect the composition of the CH_4 producing and consuming microbial community. The chamber measurements that showed large and systematic spatial variations in $\delta^{13}C\text{-}CH_4$ of up to 15‰, but smaller and less systematic temporal variation. The temporal variation in methanogenesis is likely due to the differences in substrate availability rather than methanotrophy. The analysis of the microbial community revealed that the functional potential to produce CH_4 via the hydrogenotrophic and acetoclastic pathways is present, thus enabling shifts in $\delta^{13}C\text{-}CH_4$ to reflect both spatial and seasonal changes in the availability of substrate for methanogenesis.

Future applications of the methods used in this thesis can be applied to any environment where CH_4 is produced biologically. Particular interest should be paid to peatland restoration projects where understanding the spatial and temporal responses in carbon fluxes becomes important in deciding whether restoration projects act as sinks or sources of carbon long term.

The work conducted in this thesis was performed in natural environments. However, CH_4 sources are both natural and anthropogenic as described in the introduction of this thesis. Therefore, the application of methods used in this thesis can be applied to any biological production of CH_4. Applications include anaerobic decomposition in restored wetlands; paddy rice fields; livestock production systems (including intrinsic fermentation and animal waste and anaerobic decomposition of organic waste in landfills).

As with all research, no one method can answer all questions but rather a combination. We suggest the combined use of metagenomics and transcriptomics in the future. The addition of a metatranscriptomics approach will enable us to analyse the activity of the whole meta-community, opening possibilities to investigate further processes important in peatland carbon cycling (i.e., CO_2 and N_2O cycles). Rather than seeing the functional potential of the community (metagenomics), metatranscriptomics approach will allow us to specific activity

levels of the microorganisms present in these environments. Thus, a combined approach to see the potential and current activity of the community will enable further conclusions in peatland research.

Researching temporal variability of CH_4 emissions is of particular importance due to variability of CH_4 emission and the relative contribution from acetoclastic and hydrogenotrophic pathways during the season. **Paper IV** highlighted that the magnitude of CH_4 fluxes is substrate dependant, therefore a combine substrate and metatranscriptomics approach could confirm this hypothesis.

Due to the rapid change observed in the sub-arctic, special attention should be paid to these environments. A distinct lack of all year measurements exists due to the difficulty of sampling during the cold season. This represents a key research gap in our understanding of peatland functioning. The addition of metatranscriptomic approach combined with concentrations of CH_4 substrates with year-round measurements will allow for easier integration of genomic data in models.

After applying these techniques to natural peatlands, we encourage the application of these methods on peatland restorations projects. The European Union has set a target to restore 650 000 km^2 of terrestrial environments by 2030. The restoration targets should include ecosystems that play an important role as nature-based solutions for climate change mitigation and adaptation. Ecosystems with high carbon storage or absorption potential have been highlighted as an important target.

With this European Union restoration goal in mind, the application of methods used in this thesis are of particular importance. Kitson and Bell (2020) highlighted that drained peatlands have dysfunctional microbial communities, which can lead to net carbon emissions. Rewetting of drained peatlands is therefore an environmental priority, yet our understanding of the effects of peatland drainage and rewetting on microbial communities is still incomplete. Therefore, with increased attention being paid to restoration work, understanding the microbial dynamics is important in steering restorative work to obtain the best possible outcome for carbon storage.

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"I wish I could do better by you
Cause that's what you deserve
You sacrifice so much of your life
In order for this to work
While I'm off chasing my own dreams
Sailing around the world
Please know that I'm yours to keep
My beautiful girl"

References

ÅKERMAN, H. J. & JOHANSSON, M. 2008. Thawing permafrost and thicker active layers in sub-arctic Sweden. *Permafrost and Periglacial Processes,* 19**,** 279-292.

ANDERSEN, R., CHAPMAN, S. J. & ARTZ, R. R. E. 2013. Microbial communities in natural and disturbed peatlands: A review. *Soil Biology and Biochemistry,* 57**,** 979-994.

BAIRD, A. J., BECKWITH, C. W., WALDRON, S. & WADDINGTON, J. M. 2004. Ebullition of methane-containing gas bubbles from near-surface Sphagnum peat. *Geophysical Research Letters,* 31.

BELYEA, L. R. & MALMER, N. 2004. Carbon sequestration in peatland: patterns and mechanisms of response to climate change. *Global Change Biology,* 10**,** 1043-1052.

BRANDT, F. B., MARTINSON, G. O., POMMERENKE, B., PUMP, J. & CONRAD, R. 2015. Drying effects on archaeal community composition and methanogenesis in bromeliad tanks. *FEMS Microbiology Ecology,* 91.

BRÄUER, S. L., BASILIKO, N., M. P. SILJANEN, H. & H. ZINDER, S. 2020. Methanogenic archaea in peatlands. *FEMS Microbiology Letters,* 367.

BRIDGHAM, S. D., CADILLO-QUIROZ, H., KELLER, J. K. & ZHUANG, Q. 2013. Methane emissions from wetlands: biogeochemical, microbial, and modeling perspectives from local to global scales. *Global Change Biology,* 19**,** 1325-1346.

BRIDGHAM, S. D., PASTOR, J., DEWEY, B., WELTZIN, J. F. & UPDEGRAFF, K. 2008. RAPID CARBON RESPONSE OF PEATLANDS TO CLIMATE CHANGE. *Ecology,* 89**,** 3041-3048.

BRUMFIELD, K. D., HUQ, A., COLWELL, R. R., OLDS, J. L. & LEDDY, M. B. 2020. Microbial resolution of whole genome shotgun and 16S amplicon metagenomic sequencing using publicly available NEON data. *PLOS ONE,* 15**,** e0228899.

CADILLO-QUIROZ, H., BRÄUER, S., YASHIRO, E., SUN, C., YAVITT, J. & ZINDER, S. 2006. Vertical profiles of methanogenesis and methanogens in two contrasting acidic peatlands in central New York State, USA. *Environ Microbiol,* 8**,** 1428-40.

CHADBURN, S. E., AALTO, T., AURELA, M., BALDOCCHI, D., BIASI, C., BOIKE, J., BURKE, E. J., COMYN-PLATT, E., DOLMAN, A. J., DURAN-ROJAS, C., FAN, Y., FRIBORG, T., GAO, Y., GEDNEY, N., GÖCKEDE, M., HAYMAN, G. D., HOLL, D., HUGELIUS, G., KUTZBACH, L., LEE, H., LOHILA, A., PARMENTIER, F.-J. W., SACHS, T., SHURPALI, N. J. & WESTERMANN, S. 2020. Modeled Microbial Dynamics Explain the Apparent Temperature Sensitivity of Wetland Methane Emissions. *Global Biogeochemical Cycles,* 34**,** e2020GB006678.

CHOWDHURY, T. R. & DICK, R. P. 2013. Ecology of aerobic methanotrophs in controlling methane fluxes from wetlands. *Applied Soil Ecology,* 65**,** 8-22.

CHRISTENSEN, T. R. 2010. Methane and Climate Change. *Earthscan***,** 27-42.

CHRISTENSEN, T. R., PANIKOV, N., MASTEPANOV, M., JOABSSON, A., STEWART, A., ÖQUIST, M., SOMMERKORN, M., REYNAUD, S. & SVENSSON, B. 2003. Biotic controls on CO2 and CH4 exchange in wetlands – a closed environment study. *Biogeochemistry,* 64**,** 337-354.

CHROŇÁKOVÁ, A., BÁRTA, J., KAŠTOVSKÁ, E., URBANOVÁ, Z. & PICEK, T. 2019. Spatial heterogeneity of belowground microbial communities linked to peatland microhabitats with different plant dominants. *FEMS Microbiology Ecology,* 95.

CONRAD, R. 2020. Importance of hydrogenotrophic, aceticlastic and methylotrophic methanogenesis for methane production in terrestrial, aquatic and other anoxic environments: A mini review. *Pedosphere,* 30**,** 25-39.

COOPER, M. D. A., ESTOP-ARAGONÉS, C., FISHER, J. P., THIERRY, A., GARNETT, M. H., CHARMAN, D. J., MURTON, J. B., PHOENIX, G. K., TREHARNE, R., KOKELJ, S. V., WOLFE, S. A., LEWKOWICZ, A. G., WILLIAMS, M. & HARTLEY, I. P. 2017. Limited contribution of permafrost carbon to methane release from thawing peatlands. *Nature Climate Change,* 7**,** 507-511.

CRILL, P. M., BARTLETT, K. B., HARRISS, R. C., GORHAM, E., VERRY, E. S., SEBACHER, D. I., MADZAR, L. & SANNER, W. 1988. Methane flux from Minnesota Peatlands. *Global Biogeochemical Cycles,* 2**,** 371-384.

CUI, M., MA, A., QI, H., ZHUANG, X. & ZHUANG, G. 2015. Anaerobic oxidation of methane: an "active" microbial process. *Microbiologyopen,* 4**,** 1-11.

DEAN, J. F., MIDDELBURG, J. J., RÖCKMANN, T., AERTS, R., BLAUW, L. G., EGGER, M., JETTEN, M. S. M., JONG, A. E. E., MEISEL, O. H., RASIGRAF, O., SLOMP, C. P., ZANDT, M. H. & DOLMAN, A. J. 2018. Methane Feedbacks to the Global Climate System in a Warmer World. *Reviews of Geophysics,* 56**,** 207-250.

DEDYSH, S. N. & KNIEF, C. 2018. Diversity and Phylogeny of Described Aerobic Methanotrophs. *In:* KALYUZHNAYA, M. G. & XING, X.-H. (eds.) *Methane Biocatalysis: Paving the Way to Sustainability.* Cham: Springer International Publishing.

DINSDALE, E. A., EDWARDS, R. A., HALL, D., ANGLY, F., BREITBART, M., BRULC, J. M., FURLAN, M., DESNUES, C., HAYNES, M., LI, L., MCDANIEL, L., MORAN, M. A., NELSON, K. E., NILSSON, C., OLSON, R., PAUL, J., BRITO, B. R., RUAN, Y., SWAN, B. K., STEVENS, R., VALENTINE, D. L., THURBER, R. V., WEGLEY, L., WHITE, B. A. & ROHWER, F. 2008. Functional metagenomic profiling of nine biomes. *Nature,* 452**,** 629-632.

DLUGOKENCKY, E. J., BRUHWILER, L., WHITE, J. W. C., EMMONS, L. K., NOVELLI, P. C., MONTZKA, S. A., MASARIE, K. A., LANG, P. M., CROTWELL, A. M., MILLER, J. B. & GATTI, L. V. 2009. Observational constraints on recent increases in the atmospheric CH4 burden. *Geophysical Research Letters,* 36.

DLUGOKENCKY, E. J., HOUWELING, S., BRUHWILER, L., MASARIE, K. A., LANG, P. M., MILLER, J. B. & TANS, P. P. 2003. Atmospheric methane levels off: Temporary pause or a new steady-state? *Geophysical Research Letters,* 30.

ESCOBAR-ZEPEDA, A., VERA-PONCE DE LEÓN, A. & SANCHEZ-FLORES, A. 2015. The Road to Metagenomics: From Microbiology to DNA Sequencing Technologies and Bioinformatics. *Frontiers in Genetics,* 6.

ETMINAN, M., MYHRE, G., HIGHWOOD, E. J. & SHINE, K. P. 2016. Radiative forcing of carbon dioxide, methane, and nitrous oxide: A significant revision of the methane radiative forcing. *Geophysical Research Letters,* 43**,** 12,614-12,623.

FENNER, N. & FREEMAN, C. 2011. Drought-induced carbon loss in peatlands. *Nature Geoscience,* 4**,** 895-900.

FERRY, J. G. 2011. Fundamentals of methanogenic pathways that are key to the biomethanation of complex biomass. *Current opinion in biotechnology,* 22**,** 351-357.

FIERER, N., BARBERÁN, A. & LAUGHLIN, D. C. 2014. Seeing the forest for the genes: using metagenomics to infer the aggregated traits of microbial communities. *Frontiers in Microbiology,* 5.

FINLAYSON, C. M. & MILTON, G. R. 2018. Peatlands. *In:* FINLAYSON, C. M., MILTON, G. R., PRENTICE, R. C. & DAVIDSON, N. C. (eds.) *The Wetland Book: II: Distribution, Description, and Conservation.* Dordrecht: Springer Netherlands.

FOFANA, A., ANDERSON, D., MCCALLEY, C. K., HODGKINS, S., WILSON, R. M., CRONIN, D., RAAB, N., TORABI, M., VARNER, R. K., CRILL, P., SALESKA, S. R., CHANTON, J. P., TFAILY, M. M. & RICH, V. I. 2022. Mapping substrate use across a permafrost thaw gradient. *Soil Biology and Biochemistry,* 175**,** 108809.

FREITAG, T. E., TOET, S., INESON, P. & PROSSER, J. I. 2010. Links between methane flux and transcriptional activities of methanogens and methane oxidizers in a blanket peat bog. *FEMS Microbiology Ecology,* 73**,** 157-165.

GALAND, P. E., FRITZE, H., CONRAD, R. & YRJÄLÄ, K. 2005. Pathways for Methanogenesis and Diversity of Methanogenic Archaea in Three Boreal Peatland Ecosystems. *Applied and Environmental Microbiology,* 71**,** 2195-2198.

GALAND, P. E., SAARNIO, S., FRITZE, H. & YRJÄLÄ, K. 2002. Depth related diversity of methanogen Archaea in Finnish oligotrophic fen. *FEMS Microbiol Ecol,* 42**,** 441-9.

GALAND, P. E., YRJÄLÄ, K. & FRITZE, H. 2003. Microsite-dependent changes in methanogenic populations in a boreal oligotrophic fen. *Environmental Microbiology,* 5**,** 1133-1143.

GRAVEL, D., BELL, T., BARBERA, C., COMBE, M., POMMIER, T. & MOUQUET, N. 2012. Phylogenetic constraints on ecosystem functioning. *Nature Communications,* 3**,** 1117.

GREEN, S. M. & BAIRD, A. J. 2013. The importance of episodic ebullition methane losses from three peatland microhabitats: a controlled-environment study. *European Journal of Soil Science,* 64**,** 27-36.

GREENUP, A. L., BRADFORD, M. A., MCNAMARA, N. P., INESON, P. & LEE, J. A. 2000. The role of Eriophorum vaginatum in CH4 flux from an ombrotrophic peatland. *Plant and Soil,* 227**,** 265-272.

GUERRERO-CRUZ, S., VAKSMAA, A., HORN, M. A., NIEMANN, H., PIJUAN, M. & HO, A. 2021. Methanotrophs: Discoveries, Environmental Relevance, and a Perspective on Current and Future Applications. *Frontiers in Microbiology,* 12.

GUPTA, V., SMEMO, K. A., YAVITT, J. B., FOWLE, D., BRANFIREUN, B. & BASILIKO, N. 2013. Stable Isotopes Reveal Widespread Anaerobic Methane Oxidation Across Latitude and Peatland Type. *Environmental Science & Technology,* 47**,** 8273-8279.

HUGELIUS, G., LOISEL, J., CHADBURN, S., JACKSON, R. B., JONES, M., MACDONALD, G., MARUSHCHAK, M., OLEFELDT, D., PACKALEN, M., SIEWERT, M. B., TREAT, C., TURETSKY, M., VOIGT, C. & YU, Z. 2020. Large stocks of peatland carbon and nitrogen are vulnerable to permafrost thaw. *Proceedings of the National Academy of Sciences,* 117**,** 20438-20446.

IPCC 2021. Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change Masson-Delmotte, V., P. Zhai, A. Pirani, S.L. Connors, C. Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M.I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J.B.R. Matthews, T.K. Maycock, T. Waterfield, O. Yelekçi, R. Yu, and B. Zhou (eds.). *Cambridge University Press*.

JOABSSON, A., CHRISTENSEN, T. R. & WALLÉN, B. 1999. Vascular plant controls on methane emissions from northern peatforming wetlands. *Trends in Ecology & Evolution,* 14**,** 385-388.

JUOTTONEN, H., TUITTILA, E.-S., JUUTINEN, S., FRITZE, H. & YRJÄLÄ, K. 2008. Seasonality of rDNA- and rRNA-derived archaeal communities and methanogenic potential in a boreal mire. *The ISME Journal,* 2**,** 1157-1168.

KASTER, A.-K., MOLL, J., PAREY, K. & THAUER, R. K. 2011. Coupling of ferredoxin and heterodisulfide reduction via electron bifurcation in hydrogenotrophic methanogenic archaea. *Proceedings of the National Academy of Sciences,* 108**,** 2981-2986.

KEANE, B. J., INESON, P., VALLACK, H. W., BLEI, E., BENTLEY, M., HOWARTH, S., MCNAMARA, N. P., ROWE, R. L., WILLIAMS, M. & TOET, S. 2018. Greenhouse gas emissions from the energy crop oilseed rape (Brassica napus); the role of photosynthetically active radiation in diurnal N2O flux variation. *GCB Bioenergy,* 10**,** 306-319.

KEANE, J. B., TOET, S., INESON, P., WESLIEN, P., STOCKDALE, J. E. & KLEMEDTSSON, L. 2021. Carbon Dioxide and Methane Flux Response and Recovery From Drought in a Hemiboreal Ombrotrophic Fen. *Frontiers in Earth Science,* 8.

KEELING, C. D. 1958. The concentration and isotopic abundances of atmospheric carbon dioxide in rural areas. *Geochimica et Cosmochimica Acta,* 13**,** 322-334.

KIRSCHKE, S., BOUSQUET, P., CIAIS, P., SAUNOIS, M., CANADELL, J. G., DLUGOKENCKY, E. J., BERGAMASCHI, P., BERGMANN, D., BLAKE, D. R., BRUHWILER, L., CAMERON-SMITH, P., CASTALDI, S., CHEVALLIER, F., FENG, L., FRASER, A., HEIMANN, M., HODSON, E. L., HOUWELING, S., JOSSE, B., FRASER, P. J., KRUMMEL, P. B., LAMARQUE, J.-F., LANGENFELDS, R. L., LE QUÉRÉ, C., NAIK, V., O'DOHERTY, S., PALMER, P. I., PISON, I., PLUMMER, D., POULTER, B., PRINN, R. G., RIGBY, M., RINGEVAL, B., SANTINI, M., SCHMIDT, M., SHINDELL, D. T., SIMPSON, I. J., SPAHNI, R., STEELE, L. P., STRODE, S. A., SUDO, K., SZOPA, S., VAN DER WERF, G. R., VOULGARAKIS, A., VAN WEELE, M., WEISS, R. F., WILLIAMS, J. E. & ZENG, G. 2013. Three decades of global methane sources and sinks. *Nature Geoscience,* 6**,** 813-823.

KITSON, E. & BELL, N. G. A. 2020. The Response of Microbial Communities to Peatland Drainage and Rewetting. A Review. *Frontiers in Microbiology,* 11.

KORRENSALO, A., MÄNNISTÖ, E., ALEKSEYCHIK, P., MAMMARELLA, I., RINNE, J., VESALA, T. & TUITTILA, E. S. 2018. Small spatial variability in methane emission measured from a wet patterned boreal bog. *Biogeosciences,* 15**,** 1749-1761.

KUSHWAHA, S. K., MANOHARAN, L., MEERUPATI, T., HEDLUND, K. & AHREN, D. 2015. MetCap: a bioinformatics probe design pipeline for large-scale targeted metagenomics. *BMC Bioinformatics,* 16**,** 65.

LAI, D. Y. F. 2009. Methane Dynamics in Northern Peatlands: A Review. *Pedosphere,* 19**,** 409-421.

LAMBIE, S. C., KELLY, W. J., LEAHY, S. C., LI, D., REILLY, K., MCALLISTER, T. A., VALLE, E. R., ATTWOOD, G. T. & ALTERMANN, E. 2015. The complete genome sequence of the rumen methanogen Methanosarcina barkeri CM1. *Standards in Genomic Sciences,* 10**,** 57.

LIEBNER, S., SCHWARZENBACH, S. P. & ZEYER, J. 2012. Methane emissions from an alpine fen in central Switzerland. *Biogeochemistry,* 109**,** 287-299.

LIU, Y. & WHITMAN, W. B. 2008. Metabolic, phylogenetic, and ecological diversity of the methanogenic archaea. *Ann N Y Acad Sci,* 1125**,** 171-89.

LIVINGSTON, G. & HUTCHINSON, G. 1995. Enclosure-based measurement of trace gas exchange: applications and sources of error. *Biogenic trace gases: measuring emissions from soil and water,* 51**,** 14-51.

LONNSTAD, J. & LÖFROTH, M. 1994. Myskyddsplan för Sverige. Stockholm, Sweden: Naturvårdsverket.

LUND, M., LINDROTH, A., CHRISTENSEN, T. R. & STRÖM, L. 2007. Annual CO2 balance of a temperate bog. *Tellus B,* 59**,** 804-811.

MANOHARAN, L., KUSHWAHA, S. K., AHRÉN, D. & HEDLUND, K. 2017. Agricultural land use determines functional genetic diversity of soil microbial communities. *Soil Biology and Biochemistry,* 115**,** 423-432.

MANOHARAN, L., KUSHWAHA, S. K., HEDLUND, K. & AHREN, D. 2015. Captured metagenomics: large-scale targeting of genes based on 'sequence capture' reveals functional diversity in soils. *DNA Res,* 22**,** 451-60.

MCCALLEY, C. K., WOODCROFT, B. J., HODGKINS, S. B., WEHR, R. A., KIM, E.-H., MONDAV, R., CRILL, P. M., CHANTON, J. P., RICH, V. I., TYSON, G. W. & SALESKA, S. R. 2014. Methane dynamics regulated by microbial community response to permafrost thaw. *Nature,* 514**,** 478-481.

MERILÄ, P., LAINE, J., GALAND, P. E., YRJÄLÄ, K., FRITZE, H., TUITTILA, E. S. & KUKKO-OJA, K. 2006. Methanogen communities along a primary succession transect of mire ecosystems. *FEMS Microbiology Ecology,* 55**,** 221-229.

METCALFE, D. B., HERMANS, T. D. G., AHLSTRAND, J., BECKER, M., BERGGREN, M., BJÖRK, R. G., BJÖRKMAN, M. P., BLOK, D., CHAUDHARY, N., CHISHOLM, C., CLASSEN, A. T., HASSELQUIST, N. J., JONSSON, M., KRISTENSEN, J. A., KUMORDZI, B. B., LEE, H., MAYOR, J. R., PREVÉY, J., PANTAZATOU, K., ROUSK, J., SPONSELLER, R. A., SUNDQVIST, M. K., TANG, J., UDDLING, J., WALLIN, G., ZHANG, W., AHLSTRÖM, A., TENENBAUM, D. E. & ABDI, A. M. 2018. Patchy field sampling biases understanding of climate change impacts across the Arctic. *Nature Ecology & Evolution,* 2**,** 1443-1448.

MILLER, K. E., LAI, C.-T., DAHLGREN, R. A. & LIPSON, D. A. 2019. Anaerobic Methane Oxidation in High-Arctic Alaskan Peatlands as a Significant Control on Net CH4 Fluxes. *Soil Systems,* 3**,** 7.

MINASNY, B., BERGLUND, Ö., CONNOLLY, J., HEDLEY, C., DE VRIES, F., GIMONA, A., KEMPEN, B., KIDD, D., LILJA, H., MALONE, B., MCBRATNEY, A., ROUDIER, P., O'ROURKE, S., RUDIYANTO, PADARIAN, J., POGGIO, L., TEN CATEN, A., THOMPSON, D., TUVE, C. & WIDYATMANTI, W. 2019. Digital mapping of peatlands – A critical review. *Earth-Science Reviews,* 196**,** 102870.

MONDAV, R., MCCALLEY, C. K., HODGKINS, S. B., FROLKING, S., SALESKA, S. R., RICH, V. I., CHANTON, J. P. & CRILL, P. M. 2017. Microbial network, phylogenetic diversity and community membership in the active layer across a permafrost thaw gradient. *Environmental Microbiology,* 19**,** 3201-3218.

MOORE, T. R., ROULET, N. T. & WADDINGTON, J. M. 1998. Uncertainty in Predicting the Effect of Climatic Change on the Carbon Cycling of Canadian Peatlands. *Climatic Change,* 40**,** 229-245.

PEREIRA-MARQUES, J., HOUT, A., FERREIRA, R. M., WEBER, M., PINTO-RIBEIRO, I., VAN DOORN, L.-J., KNETSCH, C. W. & FIGUEIREDO, C. 2019. Impact of Host DNA and Sequencing Depth on the Taxonomic Resolution of Whole Metagenome Sequencing for Microbiome Analysis. *Frontiers in Microbiology,* 10.

POINDEXTER, C. M., BALDOCCHI, D. D., MATTHES, J. H., KNOX, S. H. & VARIANO, E. A. 2016. The contribution of an overlooked transport process to a wetland's methane emissions. *Geophysical Research Letters,* 43**,** 6276-6284.

POTTER, C., FREEMAN, C., GOLYSHIN, P. N., ACKERMANN, G., FENNER, N., MCDONALD, J. E., EHBAIR, A., JONES, T. G., MURPHY, L. M. & CREER, S. 2017. Subtle shifts in microbial communities occur alongside the release of carbon induced by drought and rewetting in contrasting peatland ecosystems. *Sci Rep,* 7**,** 11314.

RANTANEN, M., KARPECHKO, A. Y., LIPPONEN, A., NORDLING, K., HYVÄRINEN, O., RUOSTEENOJA, K., VIHMA, T. & LAAKSONEN, A. 2022. The Arctic has warmed nearly four times faster than the globe since 1979. *Communications Earth & Environment,* 3**,** 168.

RINNE, J., ŁAKOMIEC, P., VESTIN, P., WHITE, J. D., WESLIEN, P., KELLY, J., KLJUN, N., STRÖM, L. & KLEMEDTSSON, L. 2022. Spatial and temporal variation in δ13C values of methane emitted from a hemiboreal mire: methanogenesis, methanotrophy, and hysteresis. *Biogeosciences,* 19**,** 4331-4349.

RINNE, J., TUOVINEN, J.-P., KLEMEDTSSON, L., AURELA, M., HOLST, J., LOHILA, A., WESLIEN, P., VESTIN, P., ŁAKOMIEC, P., PEICHL, M., TUITTILA, E.-S., HEISKANEN, L., LAURILA, T., LI, X., ALEKSEYCHIK, P., MAMMARELLA, I., STRÖM, L., CRILL, P. & NILSSON, M. B. 2020. Effect of the 2018 European drought on methane and carbon dioxide exchange of northern mire ecosystems. *Philosophical Transactions of the Royal Society B: Biological Sciences,* 375**,** 20190517.

SALMON, E., JÉGOU, F., GUENET, B., JOURDAIN, L., QIU, C., BASTRIKOV, V., GUIMBAUD, C., ZHU, D., CIAIS, P., PEYLIN, P., GOGO, S., LAGGOUN-DÉFARGE, F., AURELA, M., BRET-HARTE, M. S., CHEN, J., CHOJNICKI, B. H., CHU, H., EDGAR, C. W., EUSKIRCHEN, E. S., FLANAGAN, L. B., FORTUNIAK, K., HOLL, D., KLATT, J., KOLLE, O., KOWALSKA, N., KUTZBACH, L., LOHILA, A., MERBOLD, L., PAWLAK, W., SACHS, T. & ZIEMBLIŃSKA, K. 2022. Assessing methane emissions for northern peatlands in ORCHIDEE-PEAT revision 7020. *Geosci. Model Dev.,* 15**,** 2813-2838.

SAUNOIS, M., STAVERT, A. R., POULTER, B., BOUSQUET, P., CANADELL, J. G., JACKSON, R. B., RAYMOND, P. A., DLUGOKENCKY, E. J., HOUWELING, S., PATRA, P. K., CIAIS, P., ARORA, V. K., BASTVIKEN, D., BERGAMASCHI, P., BLAKE, D. R., BRAILSFORD, G., BRUHWILER, L., CARLSON, K. M., CARROL, M., CASTALDI, S., CHANDRA, N., CREVOISIER, C., CRILL, P. M., COVEY, K., CURRY, C. L., ETIOPE, G., FRANKENBERG, C., GEDNEY, N., HEGGLIN, M. I., HÖGLUND-ISAKSSON, L., HUGELIUS, G., ISHIZAWA, M., ITO, A., JANSSENS-MAENHOUT, G., JENSEN, K. M., JOOS, F., KLEINEN, T., KRUMMEL, P. B., LANGENFELDS, R. L., LARUELLE, G. G., LIU, L., MACHIDA, T., MAKSYUTOV, S., MCDONALD, K. C., MCNORTON, J., MILLER, P. A., MELTON, J. R., MORINO, I., MÜLLER, J., MURGUIA-FLORES, F., NAIK, V., NIWA, Y., NOCE, S., O'DOHERTY, S., PARKER, R. J., PENG, C., PENG, S., PETERS, G. P., PRIGENT, C., PRINN, R., RAMONET, M., REGNIER, P., RILEY, W. J., ROSENTRETER, J. A., SEGERS, A., SIMPSON, I. J., SHI, H., SMITH, S. J., STEELE, L. P., THORNTON, B. F., TIAN, H., TOHJIMA, Y., TUBIELLO, F. N., TSURUTA, A., VIOVY, N., VOULGARAKIS, A., WEBER, T. S., VAN WEELE, M., VAN DER WERF, G. R., WEISS, R. F., WORTHY, D., WUNCH, D., YIN, Y., YOSHIDA, Y., ZHANG, W., ZHANG, Z., ZHAO, Y., ZHENG, B., ZHU, Q., ZHU, Q. & ZHUANG, Q. 2020. The Global Methane Budget 2000–2017. *Earth Syst. Sci. Data,* 12**,** 1561-1623.

SCHLESINGER, W. H. & BERNHARDT, E. S. 2013. Chapter 7 - Wetland Ecosystems. *In:* SCHLESINGER, W. H. & BERNHARDT, E. S. (eds.) *Biogeochemistry (Third Edition).* Boston: Academic Press.

SMEMO, K. A. & YAVITT, J. B. 2011. Anaerobic oxidation of methane: an underappreciated aspect of methane cycling in peatland ecosystems? *Biogeosciences,* 8**,** 779-793.

SMHI 2006. Klimat i förändring. En jämförelse av temperatur och nederbörd 1991-2005 med 1961-1990. . Norrköping, Sweden: Swedish Meteorological anf Hydrological Institute.

SMITH, C. J. & OSBORN, A. M. 2009. Advantages and limitations of quantitative PCR (Q-PCR)-based approaches in microbial ecology. *FEMS Microbiology Ecology,* 67**,** 6-20.

STRÖM, L., EKBERG, A., MASTEPANOV, M. & RØJLE CHRISTENSEN, T. 2003. The effect of vascular plants on carbon turnover and methane emissions from a tundra wetland. *Global Change Biology,* 9**,** 1185-1192.

STRÖM, L., MASTEPANOV, M. & CHRISTENSEN, T. R. 2005. Species-specific Effects of Vascular Plants on Carbon Turnover and Methane Emissions from Wetlands. *Biogeochemistry,* 75**,** 65-82.

STRÖM, L., TAGESSON, T., MASTEPANOV, M. & CHRISTENSEN, T. R. 2012. Presence of Eriophorum scheuchzeri enhances substrate availability and methane emission in an Arctic wetland. *Soil Biology and Biochemistry,* 45**,** 61-70.

SUN, L., SONG, C., MIAO, Y., QIAO, T. & GONG, C. 2013. Temporal and spatial variability of methane emissions in a northern temperate marsh. *Atmospheric Environment,* 81**,** 356-363.

THAUER, R. K., KASTER, A.-K., SEEDORF, H., BUCKEL, W. & HEDDERICH, R. 2008. Methanogenic archaea: ecologically relevant differences in energy conservation. *Nature Reviews Microbiology,* 6**,** 579-591.

THOM, M., BÖSINGER, R., SCHMIDT, M. & LEVIN, I. 1993. The regional budget of atmospheric methane of a highly populated area. *Chemosphere,* 26**,** 143-160.

TURUNEN, J., TOMPPO, E., TOLONEN, K. & REINIKAINEN, A. 2002. Estimating carbon accumulation rates of undrained mires in Finland–application to boreal and subarctic regions. *The Holocene,* 12**,** 69-80.

TVEIT, A. T., KISS, A., WINKEL, M., HORN, F., HÁJEK, T., SVENNING, M. M., WAGNER, D. & LIEBNER, S. 2020. Environmental patterns of brown moss- and Sphagnum-associated microbial communities. *Scientific Reports,* 10**,** 22412.

VAN WINDEN, J. F., REICHART, G.-J., MCNAMARA, N. P., BENTHIEN, A. & DAMSTÉ, J. S. S. 2012. Temperature-Induced Increase in Methane Release from Peat Bogs: A Mesocosm Experiment. *PLOS ONE,* 7**,** e39614.

VITT, D. H., HALSEY, L. A., BAUER, I. E. & CAMPBELL, C. 2000. Spatial and temporal trends in carbon storage of peatlands of continental western Canada through the Holocene. *Canadian Journal of Earth Sciences,* 37**,** 683-693.

WADDINGTON, J. M., ROULET, N. T. & SWANSON, R. V. 1996. Water table control of CH4 emission enhancement by vascular plants in boreal peatlands. *Journal of Geophysical Research: Atmospheres,* 101**,** 22775-22785.

WELTE, C. & DEPPENMEIER, U. 2014. Bioenergetics and anaerobic respiratory chains of aceticlastic methanogens. *Biochim Biophys Acta,* 1837**,** 1130-47.

WENDLANDT, K.-D., STOTTMEISTER, U., HELM, J., SOLTMANN, B., JECHOREK, M. & BECK, M. 2010. The potential of methane-oxidizing bacteria for applications in environmental biotechnology. *Engineering in Life Sciences,* 10**,** 87-102.

WILMOTH, J. L., SCHAEFER, J. K., SCHLESINGER, D. R., ROTH, S. W., HATCHER, P. G., SHOEMAKER, J. K. & ZHANG, X. 2021. The role of oxygen in stimulating methane production in wetlands. *Global Change Biology,* 27**,** 5831-5847.

WOODCROFT, B. J., SINGLETON, C. M., BOYD, J. A., EVANS, P. N., EMERSON, J. B., ZAYED, A. A. F., HOELZLE, R. D., LAMBERTON, T. O., MCCALLEY, C. K., HODGKINS, S. B., WILSON, R. M., PURVINE, S. O., NICORA, C. D., LI, C., FROLKING, S., CHANTON, J. P., CRILL, P. M., SALESKA, S. R., RICH, V. I. & TYSON, G. W. 2018. Genome-centric view of carbon processing in thawing permafrost. *Nature,* 560**,** 49-54.

WU, D., ZHAO, C., BAI, H., FENG, F., SUI, X. & SUN, G. 2021. Characteristics and metabolic patterns of soil methanogenic archaea communities in the high-latitude natural forested wetlands of China. *Ecology and Evolution,* 11**,** 10396-10408.

YU, Z., BEILMAN, D. W. & JONES, M. C. 2009. Sensitivity of northern peatland carbon dynamics to Holocene climate change. *Carbon cycling in northern peatlands,* 184**,** 55-69.

YVON-DUROCHER, G., ALLEN, A. P., BASTVIKEN, D., CONRAD, R., GUDASZ, C., ST-PIERRE, A., THANH-DUC, N. & DEL GIORGIO, P. A. 2014. Methane fluxes show consistent temperature dependence across microbial to ecosystem scales. *Nature,* 507**,** 488-491.

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