LONG-TERM WARMING AND ELEVATED CARBON DIOXIDE EFFECTS ON THE ANAEROBIC OXIDATION OF METHANE IN A NORTHERN PEAT BOG

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THESIS ABSTRACT

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Freshwater wetlands are responsible for ~ 20% of global methane (CH_4) emissions. While previous studies have highlighted the importance of aerobic oxidation and marine anaerobic oxidation of CH_4 (AOM), limited research has been conducted on AOM in freshwater ecosystems. Here, a pilot experiment in riverine sediments verified the $^{13}CH_4$ tracer method used and indicated low rates of AOM with no effect of instantaneous temperature change. Results from a long-term warming and CO_2 enhancement experiment in a northern Minnesota, USA bog showed warming increases AOM rates more than CH_4 and CO_2 production. Thus, with increasing temperatures, AOM might consume a larger proportion of net CH_4 production. In a methodology component of the experiment, we found weak evidence that incubations with added porewater underrepresent AOM rates. This study highlights the importance of AOM in CH_4 cycling in freshwater ecosystems and the need for continued research.

This thesis includes unpublished co-authored material.

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1. INTRODUCTION

I wrote Chapter 1 with text edits provided by Scott Bridgham.

1.1 Importance of Wetlands on Climate Change

While wetlands provide numerous ecological goods and services, they are also the largest natural source of global methane (CH_4) emissions (Saunois et al. 2020). Although CH_4 has a lower atmospheric residency than CO_2 (~10 years vs 100 years, respectively), CH_4 has 45 times the sustained-flux global warming potential over 100 years (Neubauer and Megonigal 2015). After 100 years, most of the radiative forcing (97%) from the original CH_4 can be attributed to the oxidation of CH_4 into CO_2 (Neubauer and Megonigal 2015). Most CH_4 emissions (~ 60%) are a result of anthropogenic activity (waste treatment, coal and oil usage, livestock production, etc.) (Saunois et al. 2020). However, wetlands are responsible for roughly 20% of total CH_4 emissions with an average global flux of 149 Tg CH_4 yr^{-1} from 2008 to 2017 (Saunois et al. 2020). To fully understand future climate change scenarios, wetland CH_4 cycling and processes that might impact fluxes need to be understood under different warming regimes.

Peatlands are wetlands where decomposition is slow because of waterlogging and organic matter accumulates into peat soil. They differ from mineral-soil in that they have high organic matter content and a peat profile that usually extends several meters (Frolking et al. 2001, Yu 2012). While they cover less than 3% of the global surface, they contain half of the total soil carbon and are significant environments for carbon sequestration (Bridgham et al. 2013, Nichols and Peteet 2019). Generally, peatlands are divided into two major categories: fens and bogs. Fens receive water and nutrients from groundwater movement, are typically more alkaline, and have high rates of primary productivity. By contrast, bogs receive water and nutrients only from atmospheric precipitation, are usually highly acidic, and have lower rates of primary productivity (Wheeler and Proctor 2000). Due to the minerotrophic nature of fens, they tend to produce more

CH_4 than comparable bog systems (Medvedeff et al. 2015). As well as spatial classifications, peatlands can also be categorized vertically. The most surface level, the acrotelm, is a layer of peat that is usually aerobic and consists of vegetation, roots, and freshly decayed organic matter. It typically has high rates of decay, hydrological conductivity, and thus CH_4 production (Clymo 1984, Clymo and Bryant 2008). Deeper, the recently termed mesotelm is periodically aerobic with high rates of decomposition and carbon turnover, while the deepest layer, the catotelm, is permanently saturated with large stores of organic matter and carbon that are resistant to decay and can be thousands of years old (Clymo 1984, Clymo and Bryant 2008).

The majority of CH_4 is released from tropical ecosystems. (Bloom et al. 2010, Saunois et al. 2020). However at northern latitudes, emissions from wetlands are significantly higher than anthropogenic emissions (Saunois et al. 2020). Most increases in global CH_4 emissions post-2006 can be attributed to elevated production from microbial sources in the tropics, including wetlands (Lan et al. 2021). However, with rising global temperatures, it is possible that the substantial concentration of carbon in northern peatlands (40°– 70° N) will show heightened CH_4 production as well (Dean et al. 2018). This increase in CH_4 emissions could led to a positive feedback loop: Emissions promote the greenhouse gas effect which increases global temperature and, in turn, promotes the production of more CH_4 (Curry 2007, Schädel et al. 2016, Dean et al. 2018). Extensive research has been conducted on CH_4 production, but few studies have examined the response of CH_4 consumption to increased temperatures in northern peatlands.

1.2 Methane Production

Methane production (methanogenesis) can be classified into three types: thermogenic, pyrogenic, and biogenic (Monteil et al. 2011, Dean et al. 2018). Thermogenic methane is produced through deep sea vents, gas seeps, and the anthropogenic burning of fossil fuels like

coal, oil, and natural gas. These processes emit high rates of $^{12}CH_4$ relative to $^{13}CH_4$ (Cicerone and Oremland 1988). Pyrogenic production occurs when there is incomplete combustion of organic matter during forest fires or the burning of biomass. It heavily favors the production of $^{13}CH_4$ and is 10% more enriched with ^{13}C than production from other sources (Schwietzke et al. 2016). Finally, biogenic production occurs when microbes generate CH_4 under anaerobic conditions. Conditions can be natural (wetlands), anthropogenic (rice paddies and wastewater treatment), or involve living creatures (rumens of livestock) (Bridgham et al. 2013). Microbes involved in biogenic production can also be present in deep sea vents although thermogenic production remains more prevalent (Dean et al. 2018). Like thermogenic production, biogenic sources tend to heavily favor $^{12}CH_4$ production (99%) which dilutes the concentration of $^{13}CH_4$ in the atmosphere (Conrad 2005, Monteil et al. 2011). Since this thesis examines emissions from wetlands, we will be primarily focusing on biogenic production.

For biogenic methanogenesis to occur, complex organic matter must first be depolymerized into simple substrates by enzymes, and then degraded into H_2 and acetate by fermenting bacteria (Reeburgh 2007, Borrel et al. 2011). Fermentation occurs when organic matter acts as both an electron donor and acceptor in anaerobic respiration to form alcohols, acids, and H_2 that are utilized in other reactions (Costa and Leigh 2014). The final step in biogenic methanogenesis is performed by anaerobic microbes (methanogens, domain Archaea). There are three distinct pathways utilized by methanogens: 1) Hydrogenotrophic methanogenesis is the reduction of CO_2 using H_2, 2) acetoclastic methanogenesis is the cleavage of acetate into CH_4 and CO_2, and 3) methylotrophic methanogenesis is the disproportionated reaction of carbon in a methyl group that is first reduced to methyl-coenzyme M before producing CH_4 (Canfield et al. 2005). Acetoclastic methanogenesis is the most important production pathway in boreal and

temperate ecosystems; however, studies have also shown that hydrogenotrophic methanogenesis can produce between 0% - 100% of the CH_4 in freshwater ecosystems (Conrad 1999). Within peatlands, bogs typically have low rates of methanogenesis due to their low pH and highly recalcitrant organic matter derived from *Sphagnum* mosses and woody plant material. By contrast, fens have higher rates of methanogenesis as pH is higher and dissolved organic matter from sedges is more labile (Chanton et al. 2008, Ye et al. 2012).

In anaerobic environments, the biogenic mineralization of organic matter is not very energetically favorable since it has a relatively low energy yield (Megonigal et al. 2004). Methanogenesis can be limited as it competes for fermentation byproducts with inorganic terminal electron acceptors (TEAs): nitrate, ferric iron, manganese (III, IV), and sulfate (in order of thermodynamic favorability) (Megonigal et al. 2004). In addition, studies have shown that quinone moieties in humic substances act as TEAs (Lovley et al. 1996, Scott et al. 1998, Smemo and Yavitt 2007, Keller and Takagi 2013). As these reduction reactions are more energetically favorable and can be major inhibitors to methanogenesis, rates of methanogenesis remain low until favorable TEAs have been consumed (Canfield et al. 2005, Keller and Takagi 2013, Ye et al. 2014, Rush et al. 2021). In coastal wetlands, high levels of salinity have been shown to suppress methanogenesis (Poffenbarger et al. 2011), with salinity acting as a surrogate for sulfate concentrations. However, studies have shown peatlands tend to have low concentrations of inorganic TEAs (Keller and Bridgham 2007), although organic TEAs can be very important in these systems (Keller and Takagi 2013, Rush et al. 2021). Methylotrophic methanogenesis does not compete with TEAs for substrate uptake and is able to occur in ecosystems where TEAs are not yet depleted. Despite this, methylotrophic methanogenesis rates remain low in those

freshwater systems, and have only been shown to be important in saline systems (Lomans et al. 2001, Zalman et al. 2018).

Several other environmental factors can impact rates of methanogenesis. *Sphagnum* moss is known to secrete phenolic and aromatic compounds that are inhibitory to CH_4 production (Rasmussen et al. 1995, Ye et al. 2014). The leaching and decomposition of *Sphagnum* can also produce organic acids that lower peat pH where there are few basic cations to buffer the soil (Ye et al. 2012). This low pH can directly inhibit methanogenesis as well as decrease fermentative activity and thereby fermentation products like acetate (Ye et al. 2012). Recent studies have shown that *Sphagnum* cover might decrease under warmer and drier conditions. In the same warming experiment as this thesis (see below), *Sphagnum* decreased from 90% to 22% cover with a + 9 °C temperature increase (Norby et al. 2019). High rates of methanogenesis are also closely tied to high levels of rapid root turnover and root exudation. Root exudation can release labile organic compounds necessary for fermentation and thereby promote CH_4 production (Vann and Patrick Megonigal 2003). Long-term studies have shown that fine root production will increase under warming conditions perhaps promoting CH_4 production (Malhotra et al. 2020).

There are several ways that CH_4 can be emitted into the atmosphere: plant-mediated transport, ebullition, and diffusion. Plant-mediated transport refers to the movement of CH_4 through emergent plants' aerenchyma allowing it to bypass peat layers (Shannon et al. 1996). Ebullition (or bubble events) refers to the transport of dissolved CH_4 through bubbles from deep, saturated peat. Depending on the environment and season, ebullition can occur infrequently through large emission events or at a higher frequency through smaller emissions events (Glaser et al. 2004, Goodrich et al. 2011). Finally, CH_4 can also diffuse along a concentration gradient

which can be coupled with ebullition (Gogo et al. 2011). The frequency of all these events is increased in warmer months due to increased primary production allowing for higher rates of methanogenesis (Shannon et al. 1996, Gogo et al. 2011, Gill et al. 2017).

1.3 Methane Consumption

When CH_4 loses electrons and becomes oxidized, it is converted into the less potent greenhouse gas, CO_2. After emission from the environment, most CH_4 is oxidized by hydroxyl radicals in the stratosphere (Megonigal et al. 2004). However, there is also a smaller CH_4 sink through soil-residing microbes (methanotrophs) which oxidize CH_4 through chemoautotrophy. Methanotrophs can be found in a variety of ecosystems. In wetlands, they are found most often near surface soil and in symbiotic relationships with *Sphagnum* moss. (Bridgham et al. 2013). The best known CH_4 consumption pathway is aerobic oxidation. During diffusion from anaerobic soil to the atmosphere, CH_4 can be oxidized by methanotrophs in surface aerobic environments. Along this pathway 40 – 70% of the gross CH_4 produced might be consumed, regulating total CH_4 emissions (Megonigal et al. 2004). As these methanotrophs can be substrate limited (O_2 and CH_4), the highest consumption rates are found at the aerobic/anaerobic interface where both substrates are found in abundance (Megonigal et al. 2004). Globally, the highest CH_4 consumption occurs in tropical and subtropical regions where there are high levels of soil moisture and warm temperatures (Curry 2007).

There are several phyla of aerobic methanotrophs: Gammaproteobacteria (type I), Alphaproteobacterial (type II), and Verrucomicrobia (Guerrero-Cruz et al. 2021). Type I have high rates of survival and fecundity under optimal conditions but do not respond well to disturbance and stress. By contrast, Type II have low rates of growth in optimal conditions but tend to survive well under stress (Hanson and Hanson 1996). Type II is more oligotrophic and

tends to outcompetes type I in low CH_4 conditions, but type I is favored under low O_2/high CH_4 conditions. (Knief and Dunfield 2005). The more recently discovered Verrucomicrobia is believed to have significant impacts on aerobic consumption in volcanic and geothermal environments (Guerrero-Cruz et al. 2021). Aerobic pathways have been highly studied for decades, and until recently were thought to be the only terrestrial CH_4 consumption pathways.

In contrast to aerobic environments, CH_4 can also be oxidized in anaerobic environments through the anaerobic oxidation of methane (AOM). While marine environments represent only a fraction of the global CH_4 budget (~ 2%), they provide valuable insights into anaerobic CH_4 flux (Canfield et al. 2005, Saunois et al. 2020). Rates of methanogenesis are low in marine environments since methanogens are typically found deep in the water column where there are low concentrations of dissolved organic matter (Reeburgh 2007). However, studies have found that modest rates of methanogenesis can occur in deep marine sediments where substrates are more available (Treude et al. 2005). As in terrestrial environments, methanogenesis is an energetically unfavorable process, and methanogens have difficulty outcompeting other microbes for substrates (Reeburgh 2007). In marine systems, where the most common competitor is sulfate reducers, methanogenesis can typically only occurs when sulfate is exhausted from the system (Reeburgh 2007). Concentrations of sulfate decrease at deeper depths, but so do substrates, allowing for limited methanogenesis (Canfield et al. 2005). Hydrogenotrophic methanogenesis is the main production process since acetate is commonly depleted by sulfate reducers (Reeburgh 2007).

By contrast, AOM works in combination with sulfate reduction and is commonly found at the interface of CH_4 and sulfate concentrations in marine sediments (Canfield et al. 2005). In this process, CH_4 acts as an electron donor to reduce sulfate to hydrosulfide (Canfield et al. 2005).

Studies have found that up to 85% of the CH_4 produced in marine environments is consumed through AOM in the water column (Reeburgh 2007). These rates are in part dependent on sulfate, CH_4, and O_2 concentrations and can vary by season, depth, and salinity (Treude et al. 2005). Until recently, AOM was thought to only occur in marine environments as sulfate is quickly consumed in freshwater systems leading to low concentrations (Canfield et al. 2005).

While there have been extensive studies on aerobic oxidation and AOM in marine systems, limited research has been conducted on the impact of AOM in freshwater ecosystems. Recent studies have shown that AOM is not only coupled with the reduction of sulfate, but also with the reduction of nitrate, ferric iron, manganese, humic acid, and other TEAs (Smemo and Yavitt 2007, Beal et al. 2009, Valenzuela et al. 2017, Fan et al. 2020). Despite this new interest in freshwater AOM, there has been no consensus on the importance of AOM in freshwater CH_4 flux. AOM rates vary widely among sites, seasons, studies, and methodologies (Table 1). Previous freshwater studies have shown that AOM consumes between -2 – 284% of the CH_4 produced (Table 1). Thus, the importance of AOM in freshwater systems remains speculative and based on only a few studies.

The importance of AOM in CH_4 cycling merits further examination in the context of a globally changing climate. Previous studies have shown that as temperature rises, so do methanogenesis and overall CH_4 emissions as primary production and decomposition increase (Bridgham and Richardson 1992, Updegraff et al. 2001, Yvon-Durocher et al. 2014). Although increasing temperature has not been found to change the relative methanogen abundance, it might allow for more optimal methanogenic growth conditions (Zeikus and Winfrey 1976, Wilson et al. 2016, Hopple et al. 2020). Aerobic methanotrophs also show a temperature preference with models showing that a 5 °C global temperature increase would result in an 8%

increase in mean annual uptake because of changes in both temperature and soil moisture (Curry 2007). Methanotrophs in anaerobic hydrothermal vents and sediments were also found to be temperature sensitive (Kallmeyer and Boetius 2004). While this information cannot be directly transferred to mesophiles, it indicates that freshwater methanotrophs might also be temperature sensitive. Studies have shown increases in potential marine AOM up to 20 °C (Treude et al. 2005), but it has not yet been determined if temperature is a major driver of freshwater AOM relative to CH_4 production.

Table 1: Length of incubation, AOM rates, and percentage of gross CH_4 produced consumed by AOM in 5 recent studies Adapted from McCullough et al., 2019.

Study	**Length of Incubation**	**AOM Rate (umol/ g dry peat/ day**	**% of Gross CH_4 Production Consumed by AOM**
Gupta et al. 2013	40 days	Mean (overall): 0.13 Mean (fens): 0.17 Range (fens): 0.10 - 0.41 Mean (bogs): 0.067 Range (bogs): 0.022 - 0.099	Mean (overall): 37.5 Mean (fens): 28.4 Range (fens): 3 - 115.7 Mean (bogs): 47.1 Range (bogs): -2 - 284
Blazewicz et al. 2012	~ 80 days	Mean (overall): 0.012 Mean (Alaska): 0.021 Mean (Puerto Rico): 0.0029	Mean: 0.5 Range: 0.3 - 0.8
Miller et al. 2019	40 days	Mean: 2.32	Mean: 29.5 Range: 25 - 34
Martinez-Cruz et al. 2017	204 days	Mean: 0.11	Mean: 32
Segarra et al. 2015	1 day	Mean (overall): 0.13 Range: 0.10 - 1.71	Mean: 91.3 Range: 78.1 - 98.9
Smemo and Yavitt 2007	15 days	Mean: 1.47 Range: 0.086 - 15.2	Mean: 41.7 Range: 17.4 - 63.5

1.4 Previous AOM Methodology

As discussed above, AOM rates are highly varied in previous research on freshwater ecosystems, and this may be partially due to inconsistent experimental design among studies. Some previous analyses of AOM in freshwater environments used long incubation periods and indirect methods to estimate rates (Table 1). Long incubation times allow for the buildup of CH_4, which may partially explain rate discrepancies among experiments. Stable isotope tracers ($^{13}CH_4$ and $^{14}CH_4$) are commonly used to measure AOM (Blazewicz et al. 2012, Segarra et al. 2015). Prior experiments with $^{13}CH_4$ used a GC mass spectrometer (Smemo and Yavitt 2007, Gupta et al. 2013, Valenzuela et al. 2017), but cavity ring-down spectrometers are becoming more widely available. This is the first experiment to our knowledge to use a cavity ring-down spectroscopy to determine AOM rates.

Many previous experiments have studied AOM *in vitro* by creating a peat and porewater slurry (typically 1:1) and sometimes injecting CH_4 into the headspace (Smemo and Yavitt 2007, Gupta et al. 2013, Hopple 2018). Henry's Law indicates that CH_4 is relatively insoluble in water (0.0014 and 0.033, Henry's Law constants for CH_4 and CO_2 at 298 °K, respectively), so we have hypothesized that the addition of porewater creates a diffusional barrier that limits the methanotrophs' access to headspace CH_4 (Fig. 1). As a result, researchers may be underestimating AOM rates. *In situ*, CH_4 is unlikely to be a limiting resource as most wetlands have high concentrations of porewater CH_4. Concentrations can be so ubiquitous in porewater that it rises to the surface in ebullition bubbles (as discussed above) (Smemo and Yavitt 2007). Thus, the current *in vitro* experiments may not be good representation of what is occurring *in situ*.

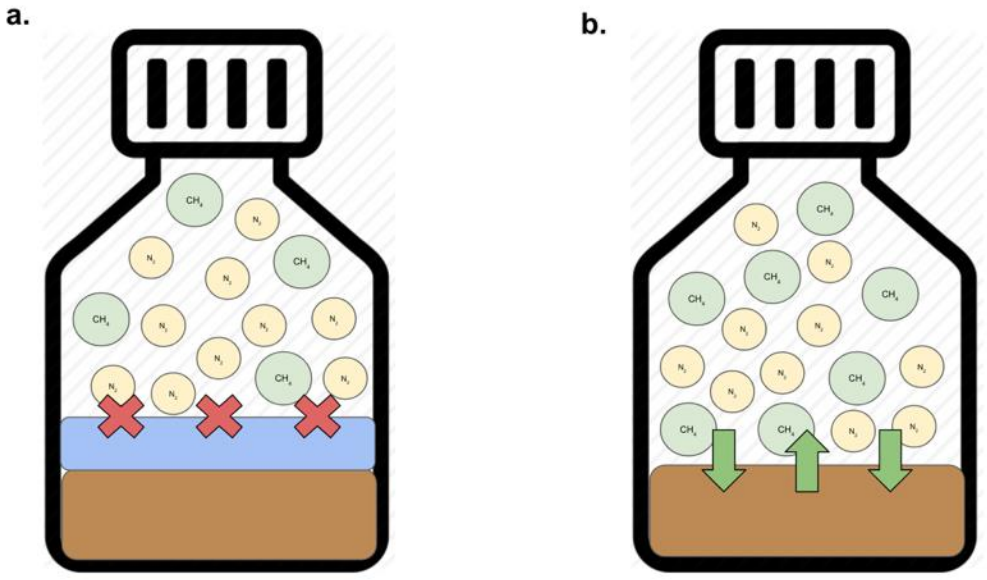

Figure 1. (a) Portrayal of potential porewater impact. Porewater limits diffusion of CH_4 from the headspace and availability to methanotrophs. (b) In the absence of porewater, CH_4 can be easily accessed by methanotrophs.

A previous study compared net CH_4 production and consumption in peat from our study site (Chapter 3) and found that surface samples with porewater addition produced CH_4 whereas without added porewater net CH_4 consumption occurred (Fig. 2, Hopple 2018). This was hypothesized to be due to the diffusional barrier of the added porewater. However, this study was limited by not directly measuring AOM, but we directly tested the impact of porewater on rates of AOM across temperatures and depths (Chapter 3). We have hypothesized that without the addition of porewater, more CH_4 is available for consumption by methanotrophs which more accurately reflects the conditions *in situ*.

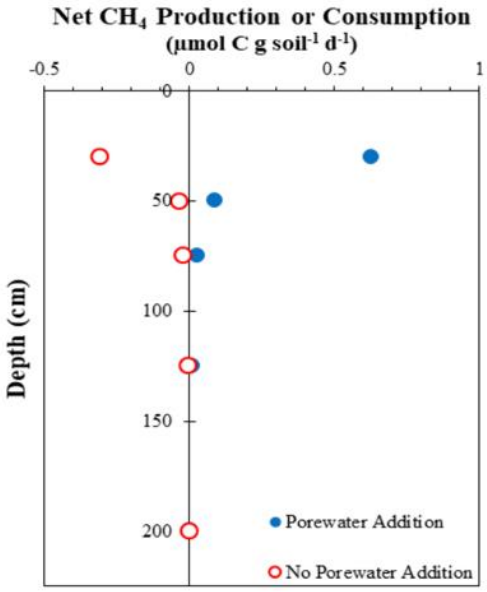

Figure 2. Net CH_4 production and consumption across depth with and without the addition of porewater (Hopple 2018).

1.5 Hypotheses

This thesis tests three main hypotheses: 1) in a northern peatland ecosystem, AOM will consume a significant percentage of CH_4 production and partially regulate total CH_4 emissions, 2) AOM and gross CH_4 production rates will be temperature sensitive and will increase proportionally across a temperature gradient, and 3) the addition of porewater creates a diffusional barrier and depresses methanotrophs' access to CH_4 in the headspace, thus underrepresenting rates of AOM *in situ*.

One of our main objectives in this manuscript was to examine AOM in response to temperature. Chapter 2, "Pilot experiment" verifies the $^{13}CH_4$ tracer method, determines the optimal time to measure AOM, and provides preliminary evidence for temperature-dependency for AOM microbes.

Chapter 3 is titled "Long-term warming increases AOM rates". This chapter built off research in the prior chapter by examining AOM in response to long-term warming. Chapter 3 was conducted in an ombrotrophic peat bog at SPRUCE where a whole ecosystem warming and

CO_2 enhancement experiment is occurring. Our objective was to determine the role of AOM in CH_4 cycling and the relative importance of temperature, depth, elevated CO_2, and water saturation on rates of production and anaerobic consumption in a northern peatland. All chapters of this thesis were made possible with contributions from co-author, Scott Bridgham. We intend to publish Chapter 3 with co-authors Laura McCullough, Anya Hopple, and Jason Keller.

2. PILOT EXPERIMENT

The laboratory and field work for this chapter was conducted by Scott Bridgham and myself based on methodology developed by Scott Bridgham and Laura McCullough. I preformed the calculations, data analysis, and visualizations for this chapter. Text edits were provided by Scott Bridgham. This experiment served as a test for our methodology and preliminary data for temperature impacts on AOM and CH_4 production rates.

2.1 Introduction

Understanding temperature effects on carbon cycling is of the utmost importance as global temperatures continue to increase. With a changing global environment, the most recent IPCC report predicts losses in biodiversity, nutrient overloading, loss of habitat for endangered species, and other devasting impacts on freshwater ecosystems (Caretta et al. 2022). Perhaps the most concerning aspect of climatic shifts is the impact on greenhouse gas flux and the potential ensuing positive feedback loop (Dean et al. 2018). The potent greenhouse gas, methane (CH_4), typically has high production rates in anaerobic wetland environments and has a sustained global warming potential 45 times that of CO_2 over 100 years despite having a lower atmospheric lifetime (Neubauer and Megonigal 2015). While studies have researched the impact of increased temperature on CH_4 emissions, little is understood about the underlying anaerobic microbial pathways that ultimately determine CH_4 production and consumption in freshwater ecosystems.

The production of CH_4 (methanogenesis) can occur in several different ways. These can be anthropogenic (through agriculture, the burning of fossil fuels, wastewater treatment, etc.) or natural (through forest fires, deep sea vents, biogenic production by microbes in wetlands) (Bridgham et al. 2013, Schwietzke et al. 2016). Methanogenesis by microbes (methanogens) occurs through the degradation of organic matter in anaerobic environments and provides the microbes with products necessary for their metabolism (Canfield et al. 2005). Since this

fermentation process has a relatively low energy yield, it is typically outcompeted by microbes for terminal electron acceptors (TEAs): (nitrite, ferric iron, manganese III and IV, and sulfate, in order of thermodynamic favorability) (Megonigal et al. 2004, Reeburgh 2007). More recent studies have also shown quinone moieties in humic compounds also act as TEAs (Keller and Takagi 2013). Since methanogens are usually outcompeted in these systems, rates of methanogenesis usually remain low until concentrations of favorable TEAs have been reduced (Ye et al. 2014, Rush et al. 2021). Methanogenesis can also be sensitive to environmental pH, root exudation, salinity, and soil carbon lability (Vann and Patrick Megonigal 2003, Ye et al. 2012).

When CH_4 is oxidized and loses electrons, it is converted into CO_2, a less potent greenhouse gas. While most CH_4 is oxidized in the stratosphere by hydroxyl radials, there are also consumption pathways by soil-residing microbes (methanotrophs) (Megonigal et al. 2004). Previous studies have shown that aerobic oxidation is an important regulator of CH_4 flux and can consume 40 – 70% of CH_4 along the diffusion pathway (Megonigal et al. 2004). Yet, limited research has been conducted on the importance of the anaerobic oxidation of methane (AOM). AOM methanotrophs form symbiotic relationships with other microbes to utilize TEAs and oxidize CH_4; thus high levels of TEAs typically result in high levels of consumption (Canfield et al. 2005). Until recently, AOM was thought to only occur with sulfate reducers in marine environments (Canfield et al. 2005), but studies have shown that AOM can occur in freshwater systems with many TEAs (see above) (Smemo and Yavitt 2007, Beal et al. 2009). Knowledge remains limited about AOM processes in freshwater ecosystems, yet this information is crucial in predicting CH_4 consumption under global warming regimes.

As well, in working to understand CH_4 flux, all pertinent environments must be considered. A recent study found that freshwater fluvial sources account for ~ 17% of inland water fluxes (Saunois et al. 2020). Yet, limited research has been conducted on CH_4 emissions from these systems. Estimates have placed global fluvial emissions around 1.5 Tg CH_4 yr^{-1} (Bastviken et al. 2011), but this study suffers from a low sample size and measured area. A more recent study found that fluvial sources emit approximately 15% of what wetlands emit with an average global emission rate of 26.8 Tg CH_4 yr^{-1} (Stanley et al. 2016). Given that temperature does not work in isolation, there has not been conclusive evidence that increases in fluvial temperature impact CH_4 emissions (Stanley et al. 2016). However, given studies from other environments show increases in emissions with temperature (Updegraff et al. 2001, Yvon-Durocher et al. 2014), it seems likely that fluvial emissions will also increase under warmed conditions. Fluvial systems are a potentially underrepresented CH_4 source that might be impacted by changes in global temperature. Here, we seek to address how temperature might affect rates of CH_4 production and consumption under anaerobic conditions in a temperate fluvial system.

This pilot experiment tested the optimal time to measure AOM and was used to reverify the stable isotope tracer method of injecting $^{13}CH_4$ to measure CH_4 production and consumption. Determining the optimal time to measure AOM allows us to maximize sensitivity to AOM rates while minimizing microbial biomass turnover. The stable isotope tracer methods has been utilized in other studies (Blazewicz et al. 2012), but not with a cavity ring-down spectrometer, and we used this experiment to ensure its success before starting the experiment at SPRUCE described in Chapter 3.

2.2 Methods

2.2.1 Site Description

This study was conducted at Millrace Creek, Eugene, Oregon, USA. It is a fluvial system on the University of Oregon campus with a loose, silty sediment riverbed. This 1.33 mile stream was diverted from the Willamette River and historically used as a waterpower source for a local sawmill (See et al. 2019). As the flow is controlled by the Willamette River, the water level can vary dramatically, but the creek is on average 40 ft wide with a depth of less than 2 ft (See et al. 2019). While there is a mix of native and invasive herbaceous plants and trees, the most dominant species are *Fraxinus latifolia*, *Acer macrophylum*, *Psuedotsuga menziesii*, *Populus balsamifera*, and *Thuja plicata* (See et al. 2019). Due to the creek's close proximity to human developments, it has excess nutrient concentrations, low dissolved oxygen levels, high concentrations of heavy metals, and a fluctuating pH (See et al. 2019). It serves as stormwater runoff during the wet season and is not known to provide a habitat for large aquatic fauna (See et al. 2019).

2.2.2 Sample Collection

Since the primary motivation for this experiment was to verify the AOM stable isotope tracer method, there was no temporal or spatial replication. Sediment samples (20 g) were collected in October 2020 from the Millrace riverbed ~ 3 feet from the bank and under ~ 1 foot of water. Samples were collected at one timepoint during midmorning. Centrifuge tubes (50 mL) were filled to the top to limit O_2 exposure and quickly transferred to 120 mL serum bottles with blue butyl rubber stoppers. All bottles were N_2-flushed for 15 minutes to remove any O_2 contamination and injected with 19.4 mL N_2 to over-pressurize samples. *In situ* water temperature was 11 °C.

2.2.3 Laboratory Methods

Samples were divided into two equal groups and injected with 0.60 mL $^{13}CH_4$ or $^{12}CH_4$ (timepoint 0) for a target headspace concentration of 1000 ppm. Samples (3 replicates per temperature and isotope treatment) were incubated at one of five temperatures (12, 16, 18, 20, 24 °C). A malfunction changed an incubation temperature form 8 °C to 18 °C, leaving increments unevenly spaced. After 7 hours (timepoint 1), 1 mL was pulled from each sample and run on an SRI gas chromatograph model 8610C with a flame ionization detector and methanizer. An additional 1 mL was pulled from the sample and injected into a 120 mL N_2-flushed serum bottle over-pressurized with 19 mL of N_2 for later analysis on a Picarro G2201-i analyzer for isotopic CO_2/CH_4 with the small stable isotope module (SSIM) attached. Samples (10 mL) were injected into the SSIM for isotopic analysis. Gas analysis was run on all samples again after 24 hours (timepoint 2) and 48 hours (timepoint 3) to determine how timing might impact measured rates of CH_4 production and consumption. Dead controls were used to verify the recovery of injected headspace CH_4. Controls were produced by adding autoclaved deionized water (20 mL) to a 120 mL serum bottle and injecting $^{13}CH_4$ (to a final headspace concentration of 1000 ppm). Controls were only run on the gas chromatograph after 48 hours.

Moisture content was determined by drying 20 g subsamples in a drying oven at 60 °C for several days. Average pH (7.22) was determined upon collection by using a pH probe on 3 sediment samples.

2.2.4 Calculations

This experiment used the stable isotope tracer method where $^{12}CH_4$ samples controlled for background rates of $^{13}CO_2$ production (Blazewicz et al. 2012). While biogenic methanogenesis strongly favors the production of $^{12}CH_4$ (~ 99%) versus $^{13}CH_4$ (~ 1%) (Conrad 2005), AOM only marginally prefers to utilize $^{12}CH_4$ (Wilson et al. 2019). We injected samples

with $^{13}CH_4$ to measure $^{13}CO_2$ production over time, which allowed us to measure anaerobic oxidation of $^{13}CH_4$. Background controls (samples with $^{12}CH_4$ injected) were created so we could correct for differences in background production of $^{13}CO_2$. Net CH_4 was found by calculating the change in CH_4 concentration over the course of the experiment.

Equation 1: AOM rate for pilot experiment

$$AOM = \frac{(^{13}CO_{2,T1,L} - {}^{13}CO_{2,T1,B}) \times (CH_{4,ave})}{(^{13}CH_{4,ave}) \times g\ dry\ weight \times (T1 - T0)}$$

Equation 2: Net CH_4 production rate for pilot experiment

$$Net\ CH_4\ Production = \frac{(CH_{4,T1})}{g\ dry\ weight \times (T1 - T0)}$$

Where $^{13}CO_{2,\ T1,\ L}$ is the $^{13}CO_2$ production in the $^{13}CH_4$-labeled samples from T0 to T1;

$^{13}CO_{2,\ T1,\ B}$ is the background $^{13}CO_2$ production in $^{12}CH_4$-injected samples from T0 to T1;

$CH_{4,\ ave}$ is the average total CH_4 concentration from T0 to T1;

$^{13}CH_{4,\ ave}$ is the average $^{13}CH_4$ concentration between T0 and T1;

T0 and T are timepoints 0 and 1, respectively.

Equations were repeated for T2, T3, T0 – T3, and T1 – T3.

2.2.5 Data Analysis

Statistical analyses were completed using mixed linear effects model in R (package lme4, version 1.3.1073) to determine the significance of temperature (fixed) and time (random) on

rates of AOM, CH_4 production, and CO_2 production. Distributions of rates were examined, and CO_2 production was logged to improve its normality. Timepoints were treated as a categorical variable and significant differences ($p < 0.05$) among timepoints were found using a Tukey's test. Timepoints represent the rates that occurred since the previous timepoint (e.g., T1 indicates the rate from T0 to T1). Negative AOM values were assumed to be random variation around our detection limit.

2.3 Results

Anaerobic oxidation of CH_4 varied little across temperature ($p = 0.56$) with many values below detection (Fig. 3). However, the interaction between time and temperature marginally impacted AOM ($p = 0.067$). AOM consumed only a small percentage of CH_4 produced (-2.84% to 5.21%). The negative values were likely due to random noise in the procedure. Two $^{12}CH_4$-labeled samples had net negative CH_4 production and positive CO_2 production suggesting the differences were real and AOM occurred.

In contrast to AOM, net CH_4 production increased with temperature across all the timepoints ($p = 0.042$, Fig. 4), although temperature explained little of the variation in production ($R^2 = 0.09$). We found no difference in CO_2 production across temperatures (Fig. 5, $p = 0.21$), and CO_2 production was roughly double the production of CH_4.

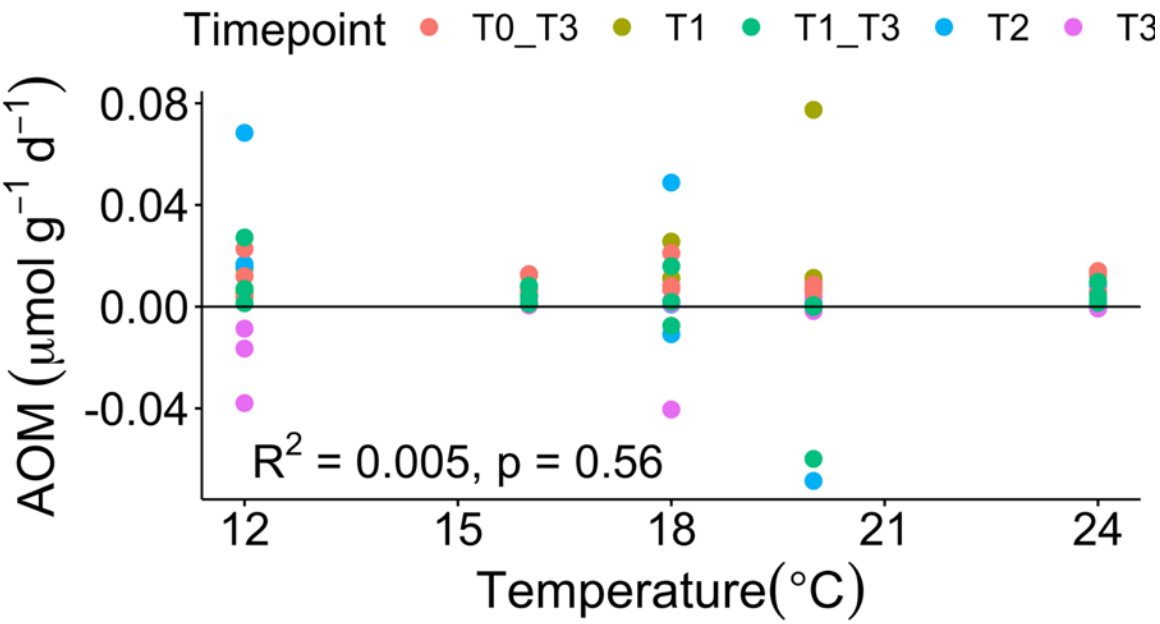

Figure 3. AOM rates plotted against temperatures (12 – 24 °C). Color indicates experimental timepoint. Ex. T0_T3 indicates the time from T0 to T3.

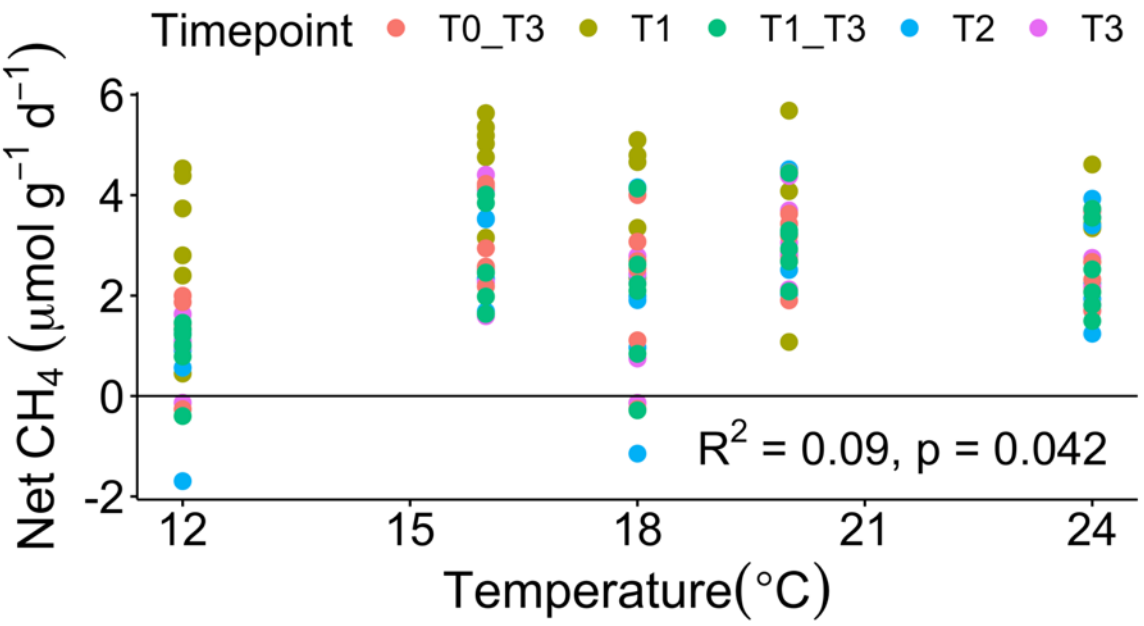

Figure 4. Net CH_4 production rates plotted against temperatures (12 –24 °C). Color indicates experimental timepoint. Ex. T0_T3 indicates the time from T0 to T3.

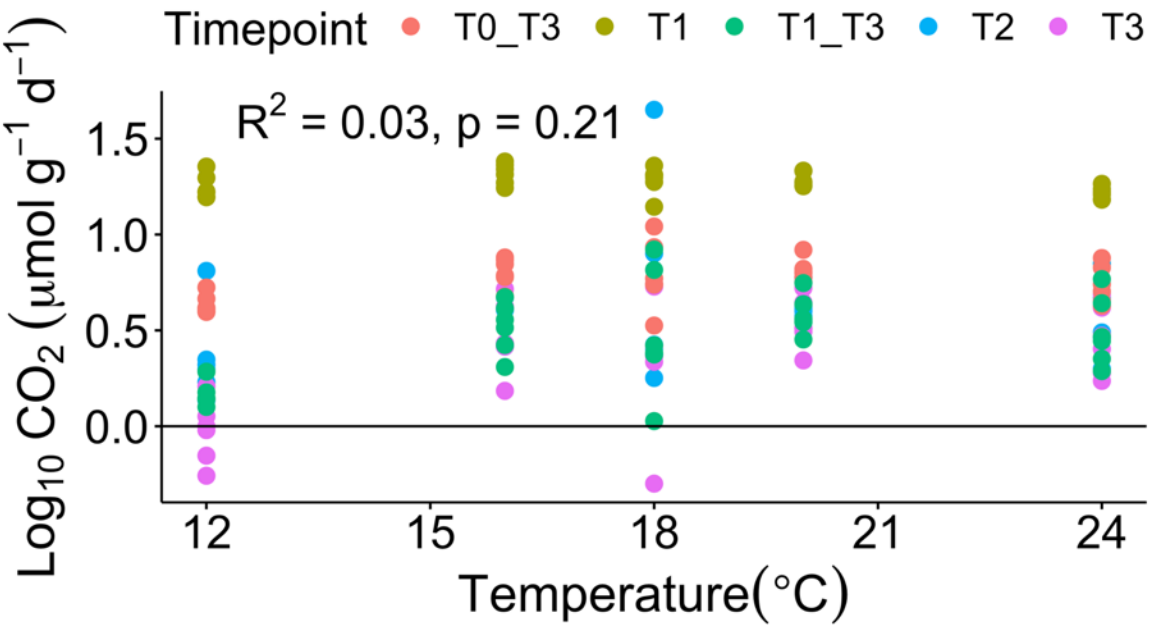

Figure 5. CO_2 production rates plotted against temperatures (12 – 24 °C). Color indicates experimental timepoint. Ex. T0_T3 indicates the time from T0 to T3.

One of the primary goals of this methods experiment was to determine the optimal time to measure AOM. Anaerobic oxidation of CH_4 decreased over time ($p = 0.048$) with most rates below detection at T3 (Fig. 6). CH_4 was highest at T1 and constant across all other timepoints (Fig. 7, $p < 0.001$). CO_2 production showed the most variability with the highest production at T1 and lowest rates at T3 and T1-T3 (Fig. 8, $p < 0.001$).

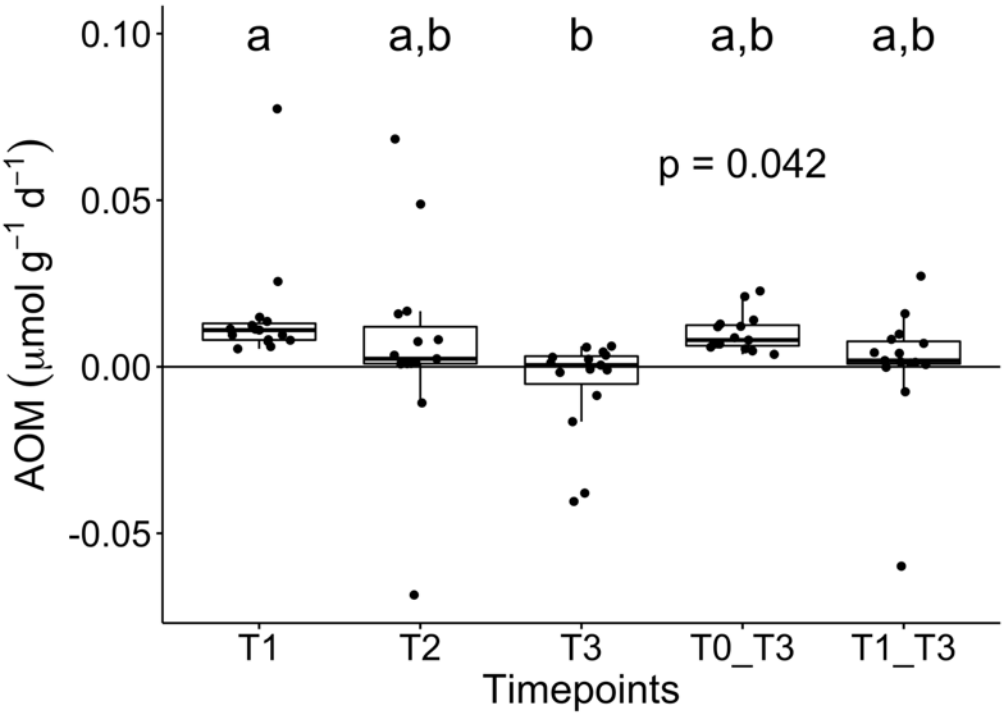

Figure 6. AOM rates across all experimental timepoints. Ex. T0_T3 indicates the time from T0 to T3.

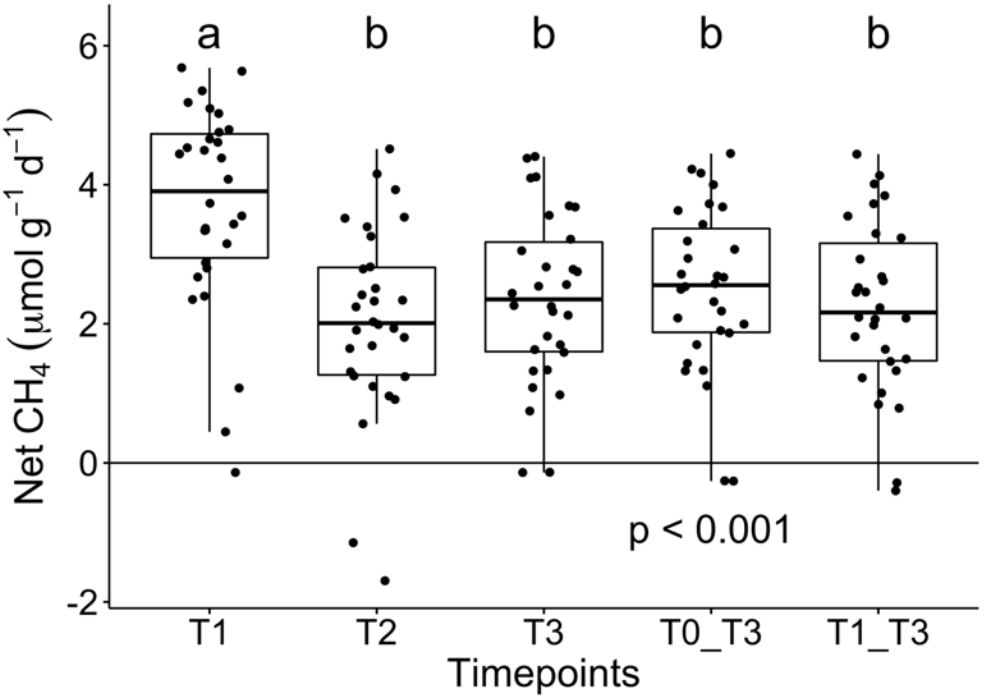

Figure 7. CH_4 production rates across all experimental timepoints. Ex. T0_T3 indicates the time from T0 to T3.

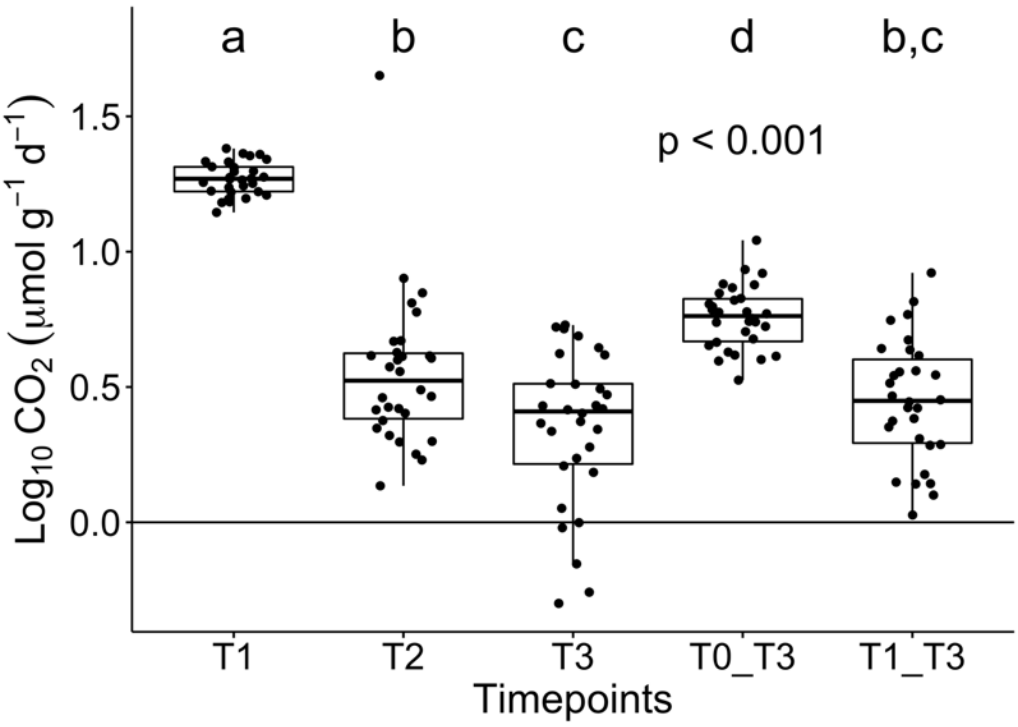

Figure 8. CO_2 production rates across all experimental timepoints. Ex. T0_T3 indicates the time from T0 to T3.

2.4 Discussion

Rates were not as responsive to a relatively instantaneous change in temperature as we anticipated. Although CH_4 production significantly increased with rising temperature, it explained little variation, and temperature had no effect on AOM and CO_2 production. Longer-term warming studies have shown that rising temperatures increases methanogenesis due to the increased degradation of organic matter, greater availability of fermentation products, and increased microbial growth (Curry 2007, Hopple et al. 2020). As well, previous studies have shown that CH_4 and CO_2 production in wetlands generally increase with temperature to an optimum, but production can be detectable at much lower temperatures (Metje and Frenzel 2005, Kolton et al. 2019). However, given the relatively short time span for the study, it is unlikely that increasing temperature would allow for optimum microbial growth (Schulz and Conrad 1996, Gill et al. 2017). The high rates of methanogenesis might indicate that there are low

concentrations of TEAs at Mill Race (Megonigal et al. 2004). If that is the case, AOM might be limited as there is a lack of TEAs to complete the redox reaction.

The limited temperature impact could be in part explained by the abrupt increase in temperature post-collection. Microbial communities sometimes take time to adapt to warmer conditions and might have already been adjusted to the *in situ* temperature. Furthermore, sample collection took place in late fall and *in situ* sediment temperature was lower than what some studies regard as optimal for microbial activity in freshwater ecosystems (Zeikus and Winfrey 1976, Kolton et al. 2019). Since temperature changes were relatively instantaneous, there were no cascading ecosystem effects on vegetation, water table, and nutrient availability which could have strong impact on microbial processes (Wilson et al. 2016, Hopple et al. 2020).

Rates of AOM, net CH_4 production, and CO_2 production were all significantly impacted by the length of incubation. The highest rates of production and consumption occurred after 7 hours and leveled off at 24 and 48 hours. As we did not inject CO_2 into our samples, we can feel reasonably confident that the CO_2 production rates are real. Greater CH_4 production at 7 hours could be driven by decreased substrate availability at latter timepoints or a larger injection of CH_4 into the headspace at the beginning of the experiment than we planned. The results from our deionized water controls support this as they were all consistently higher than expected. It is also possible that the decrease in production over time was real and, like CO_2 production, driven by decreasing substrate availability. AOM was less significantly impacted by timing and differences in rates could be in part explained by the decrease in CH_4 production over time, as CH_4 concentration is part of the equation used to measure AOM. Based on the results of this experiment, we measured rates after 48 hours in the subsequent experiment in Chapter 3. This was deemed an optimal time to maximize our sensitivity to measure AOM while minimizing the

likelihood of microbial biomass turnover contributing to recovered $^{13}CO_2$ after labeling. We also found that the large sample-to-sample variation in total CO_2 production reduced our sensitivity to AOM. In the following study, we use samples prior to $^{13}CH_4$ injection to measure background rates of $^{13}CO_2$ production instead of separate $^{12}CH_4$ controls which showed great variability.

It is of note that the sediments had higher rates of methanogenesis than what is typically measured at the SPRUCE location used during the subsequent experiment (Gill et al. 2017, Kolton et al. 2019). However, unlike the Chapter 3 site location, the temperature in this experiment was not changed *in situ* over many years and only adjusted for the samples after they had been collected and placed in incubators. The SPRUCE location allowed us to investigate system-wide, long-term temperature changes that have permeated through the ecosystem to get a better idea of sustained temperature effects on anaerobic carbon cycling.

2.5 Conclusion

Few studies have examined rates of CH_4 production and AOM in fluvial ecosystems. Here we showed that temperature had a minor impact on CH_4 production and no effect on CO_2 production and AOM. It is possible that the microbes were already adjusted to their *in situ* temperature and sudden warming had a minimal impact on overall production rates. The timing of sampling impacted CH_4 production and consumption, but rates stabilized after 7 hours. Production rates might have been higher due to differences in planned headspace CH_4 injections at the beginning of the incubation or decreased concentrations of labile substrate over time. This study helped us determine that 48 hours was the optimal time to measure AOM in Chapter 3, because it allows us to maximize sensitivity to AOM while minimizing the possibility of microbial biomass turnover. Importantly, this approach also allowed us to reverify that the $^{13}CH_4$ tracer method can measure even very low rates of AOM before starting Chapter 3 of this thesis.

3. LONG-TERM WARMING INCREASES AOM RATES

The laboratory and field work for this chapter was conducted by Scott Bridgham and myself based on methodology developed by Laura McCullough and research conducted by Anya Hopple and Jason Keller. I preformed the calculations, data analysis, and visualizations for this chapter. Text edits were provided by Scott Bridgham.

3.1 Introduction

Wetlands are the largest natural emitter of methane (CH_4), a highly potent greenhouse gas with the potential to exacerbate climate change. Despite having a lower atmospheric residency than CO_2 (~10 years vs 100 years, respectively), CH_4 has 45 times the sustained flux global warming potential over a 100 year time frame (Neubauer and Megonigal 2015). While most CH_4 emissions come from anthropogenic sources (land usage, burning of fossil fuels, wastewater treatment, etc.), wetlands are responsible for ~ 20% of global CH_4 emissions (Bridgham et al. 2013, Saunois et al. 2020). There has been particular concern for how CH_4 emissions from northern peatlands will respond to changing climate. While peatlands cover just 3% of the global surface, they are responsible for storing massive amounts of soil carbon in deep organic peat (Bridgham et al. 2013). Bloom et al. (2010)suggested that most increases in global CH_4 emissions from 2003 to 2007 can be attributed to increases in northern peatlands, but the mechanics behind CH_4 flux in these northern peatlands are not completely understood.

Biogenic CH_4 production (methanogenesis) occurs when complex organic matter is degraded into simple substrates by enzymes and fermenting bacteria in an anaerobic environment (Borrel et al. 2011). As methanogenesis is not an energetically favorable process, it is typically outcompeted by other microbes utilizing terminal electron acceptors (TEAs) to consume fermentative substrates (CO_2, H_2, and acetate) (Canfield et al. 2005, Costa and Leigh 2014).

Studies have found that sulfate, nitrite, manganese (III and IV), ferric iron, and humic compounds can act as TEAs and suppress methanogenesis (Megonigal et al. 2004, Keller and Takagi 2013). Methanogenesis can also be limited by recalcitrant soil organic matter, acidic soil, and low root turnover (Vann and Patrick Megonigal 2003, Chanton et al. 2008, Ye et al. 2012, 2014). In soil, CH_4 can be emitted through plant-mediated transport, ebullition, and diffusion (Shannon et al. 1996, Gogo et al. 2011).

Soil-residing methanotrophs can consume CH_4 and oxidize it into the less potent greenhouse gas, CO_2 (Megonigal et al. 2004). While methanotrophs can exist in many environments, in peatlands they are found most often in surface peat and in symbiotic relationships with *Sphagnum* (Bridgham et al. 2013). Aerobic methanotrophy is regarded as major regulator of CH_4 emissions and can consume 40 – 70% of the CH_4 produced in soils (Megonigal et al. 2004). However, there is another oxidation pathway known as the anaerobic oxidation of methane (AOM), first discovered in marine environments where it is coupled with the reduction of sulfate into hydrosulfide (Canfield et al. 2005). AOM in marine environments can also control CH_4 emissions with a recent study finding that AOM sometimes consumes up to 85% of the CH_4 produced in marine water columns (Reeburgh 2007). It was widely believed that AOM only occurred in marine systems, because freshwater environments typically have low concentrations of sulfate (Canfield et al. 2005). However, recent studies have shown that AOM frequently occurs in freshwater systems with many different TEAs, both inorganic and organic (Smemo and Yavitt 2007, Beal et al. 2009, Valenzuela et al. 2017). Despite extensive knowledge on aerobic oxidation and marine AOM, data about AOM in freshwater systems remains inconclusive and highly varied. Here, we seek to understand the importance of AOM in freshwater CH_4 cycling.

The most recent IPCC report found that CH_4 emissions have the potential to increase with climate warming from carbon-rich wetland soils (Caretta et al. 2022). Yet, studies examining AOM consumption of CH_4 production remain inconclusive as to its relative importance (see Chapter 1) with AOM varying widely among sites, methodologies, and even within studies. Given that AOM in aerobic and marine environments are major regulators of CH_4 emissions, we seek to understand the role of AOM in freshwater ecosystems. Moreover, there is evidence that increasing temperature will increase methanotrophy in aerobic sediments, marine sediments, and hydrothermal vents (Kallmeyer and Boetius 2004, Treude et al. 2005, Curry 2007), but to our knowledge, no study has examined the response of AOM in freshwater systems to increasing temperatures. While this evidence on methanotrophy in other environments is not directly transferable, it indicates that AOM in freshwater ecosystems might also be temperature sensitive. In order to more fully understand how wetlands will respond to changing global temperatures, here we have examined AOM and its relationship to CH_4 production under warmed conditions in a northern bog.

Prior understanding of how greenhouse gas emissions will change under warming conditions have mostly relied on short-term experiments that do not consider cascading ecological effects. To remedy this, the SPRUCE (Spruce and Peatland Responses Under Changing Environments) experiment seeks to understand how warming and elevated CO_2 conditions will impact northern peatland ecosystems over the course of ten years. This whole ecosystem warming experiment heats vegetation and peat aboveground and belowground across a temperature gradient. A study conducted at SPRUCE after three years of warming showed increased CH_4 production and emissions under warmed conditions, especially in more surface-

level peat (Hopple et al. 2020). However, it is not yet understood how AOM will be impacted under long-term warming conditions.

Finally, many laboratory studies examining anaerobic carbon dynamics, including most AOM experiments, create a slurry of peat and porewater in a serum bottle. A prior study observed net CH_4 production vs. consumption in samples with and without added porewater, respectively, in samples from SPRUCE (Hopple 2018). Since CH_4 is relatively insoluble, we have hypothesized that the porewater creates a diffusional barrier that slows transfer of CH_4 in the headspace to AOM microbes. If that is the case, experiments with added porewater might be underrepresenting AOM and conversely overestimating net CH_4 production. As well, in our laboratory design, we use cavity ring-down spectroscopy to determine AOM rates. This experiment is the first to our knowledge to use this methodology.

In this study, we have hypothesized that 1) in a northern peatland ecosystem, AOM will consume a significant percentage of CH_4 production and partially regulate total CH_4 emissions, 2) AOM and gross CH_4 production rates will be temperature sensitive and will increase proportionally across a temperature gradient, and 3) the addition of porewater creates a diffusional barrier and depresses methanotrophs' access to CH_4 in the headspace, thus underrepresenting rates of AOM *in situ*.

3.2 Methods

3.2.1 Site Description

Our study site is S-1 Bog in Marcell Experimental Forest in northern Minnesota, USA at the border of temperate deciduous and boreal ecosystems (N 47° 30.476'; W 93° 27.162'). This site is classified as an ombrotrophic bog, and the vegetation is predominantly black spruce (*Picea mariana)*, *Sphagnum* spp. mosses, and ericaceous shrubs (Hanson et al. 2017). The peat is

on average 2 to 3 m deep with 90% older than 3,000 years (Hanson et al. 2017). S-1 Bog was strip-cut in 1969 and 1974 to examine tree regrowth following logging.

The S-1 Bog is characterized by an acrotelm (0 – 30 cm) of living moss, roots, and fresh organic litter. This relatively young layer has high rates of decomposition, high hydraulic activity, and a fluctuating water table. Due to the high proportion of soil organic matter derived from *Sphagnum*, which has lower N content than vascular plants, the acrotelm has a high C:N ratio (Tfaily et al. 2014). This surface is highly acidic with a pH of ~ 4 vs ~ 6 at 3 m depth (Griffiths and Sebestyen 2016). The mesotelm (30 – 75 cm) has less frequent water table fluctuations and intense decomposition. The low C:N ratio is due in part to N immobilization in biomass and the formation of stable fulvic and humic compounds with high N levels (Tfaily et al. 2014). The highest rates of humification are seen in this mesotelm transition zone. The catotelm (> 75 cm) is peat that is permanently water-saturated and acts as long-term storage for organic matter and carbon (Tfaily et al. 2014). In S-1 Bog, the catotelm has low decomposition rates and C:N ratios consistent with the mesotelm (Tfaily et al. 2014). There are high levels of vertical hydrology in S-1 Bog so the labile dissolved organic carbon found in more surface peat can move into deeper layers with relative ease (Tfaily et al. 2018). Microbes preferentially decompose this labile peat over the recalcitrant, older, deeper peat (Tfaily et al. 2014).

There is great variation in carbon flux among plots in S-1 Bog, so it has yet to be confirmed if the site acts as a net carbon source or sink (Griffiths et al. 2017). The carbon stock of the upper 2 m of S-1 Bog is 158 kg C m^{-2} which falls within the average rage for peatlands (Griffiths et al. 2017). With regards to CH_4 production, a recent study found high abundances of hydrogenotrophic and methylotrophic methanogens across plots (Kolton et al. 2019).

3.2.2 The SPRUCE Experiment

The SPRUCE (Spruce and Peatland Responses Under Changing Environments) experiment is a ten-year experiment, led by Oak Ridge National Laboratory and designed to determine the impact of increased temperatures and elevated CO_2 on peatland function both above and belowground. Deep peat warming began in summer 2014, ecosystem warming began in summer 2015, and elevated CO_2 levels began in summer 2016 (Hanson et al. 2017).

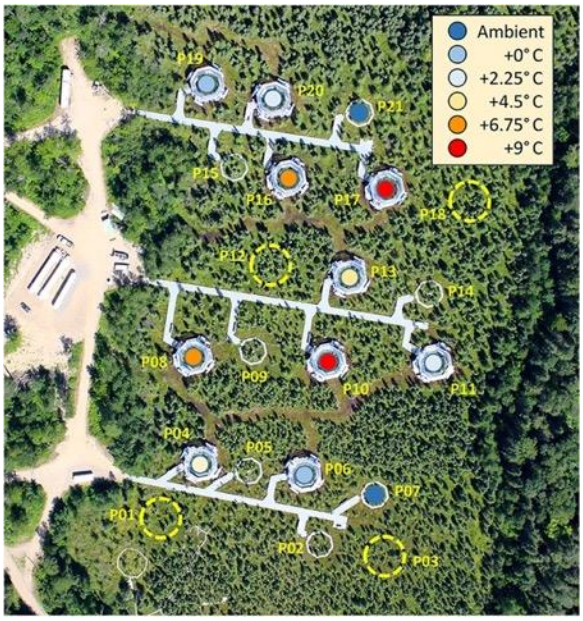

Figure 9. Aerial view of SPRUCE S-1 Bog. Chamber temperatures are shown in colored legend. (Hanson et al., 2017)

Chambers (7 m tall x 12.8 m diameter) have temperatures set to + 0, + 2.25, + 4.5, + 6.75, and + 9 °C above ambient and CO_2 at ambient or approximately twice ambient at each of the 5 temperature manipulations (Fig. 9). Chambers are open-top and heated aboveground using forced air and belowground (to 3 m depth) using electric resistance cables (Hanson et al. 2017).

3.2.3 Sample Collection

Samples were collected in July 2021 from the SPRUCE chambers. A periodic drought occurred prior to sampling so water tables were depressed, especially in heated plots. Peat samples (20 g) were collected at five depths (20 - 30, 30 - 50, 50 - 75, 100 - 125, and 175 - 200 cm) from each plot. (From here on out, depths will be referred to by the deepest depth in each increment.) A Russian corer was used for deeper depths and a serrated knife was used for the 30 cm increment. An additional peat sample (20 g) was collected for the porewater addition treatment (see below). Upon collection, samples were placed in 120 mL serum bottle capped with blue butyl rubber stopper and N_2 flushed within five minutes of collection to maintain anaerobic conditions. Extra peat samples were collected from each depth and placed in watertight plastic bags for moisture determination.

PVC piezometers with 1.25 cm diameters (at 25, 50, 75, 100, 150, and 200 cm depths) were flushed the day before porewater collection. Porewater samples (60 mL) were collected using a peristaltic pump from the first two piezometers that contained water in each plot. We intended to collect from the 25 and 50 cm depths, but a majority of the 25 cm piezometers were dry. Most porewater samples (70%) were collected from 50 and 75 cm depths with 15% from deeper depths and 15% from the 25 cm piezometer. Porewater was later added to peat samples of the same depth (see 3.2.4 Laboratory Methods) while holding all else constant to determine the impact of porewater on CH_4 production and AOM. Samples were placed in 70 mL serum bottles, capped, and N_2-flushed. All samples were then put on ice for shipment back to Eugene, Oregon, USA for processing. Water table and soil temperature data were collected from the SPRUCE monitoring network.

3.2.4 Laboratory Methods

Samples were incubated at 7 temperatures corresponding to the closest average *in situ* measurements from the week prior to collection, July 2 – 8 (within 1.5 °C). Porewater (40 mL) was added to necessary samples using a syringe to prevent oxygenation, and all bottles were N_2-flushed for 15 minutes and over-pressurized by 11 mL N_2. Deionized water (DI) controls were created by adding 20 mL autoclaved DI water to each of five serum bottles, N_2 flushing for 15 minutes, and injecting $^{13}CH_4$ (to a final headspace concentration of 1000 ppm). Control samples were run on the gas chromatograph and stable isotope analyzer to ensure the isotope stock solution was accurate.

After samples were incubated for 24 hours (timepoint 1), the headspace was homogenized using a syringe. From each serum bottle, a 1 mL sample was run on an SRI gas chromatograph model 8610C with a flame ionization detector and methanizer with appropriate standards. A 10 mL sample was pulled and injected into a 120 mL N_2-flushed serum bottle for later analysis on a Picarro G2201-i analyzer for isotopic CO_2/CH_4 with the small stable isotope module (SSIM) attached. A 10 mL sample (1 mL for DI controls) was pulled from each bottle and injected into the Picarro for analysis. Samples were diluted in serum bottles because the Picarro can only accurately assess samples with less than 20 ppm $^{13}CH_4$. Timepoint 1 was used to determine background $^{13}CO_2$ production prior to $^{13}CH_4$ isotope addition. Before being placed back in the incubator, $^{13}CH_4$ (to a final headspace concentration of 1000 ppm) was injected into each bottle.

After 48 hours (timepoint 2), the serum bottles were analyzed using the same method except a 1 mL sample from each bottle was stored in a 120 mL N_2-flushed serum bottle for use on the Picarro. Samples for the stable isotope analyzer were smaller than at timepoint 1 (1 mL vs

10 mL), because the addition of $^{13}CH_4$ greatly increased $^{13}CH_4$ gas concentrations in the serum bottles.

pH was measured in each sample using a pH probe after gas analysis. Using the extra peat collected in the field, 20 g of each sample was placed in a drying oven for several days at 60 °C to determine moisture content.

3.2.5 Calculations

Total CH_4 and CO_2 concentrations were found by using the Ideal Gas Law, Henry's Law and pH (Bridgham and Ye 2013). Several samples were rerun on the gas chromatograph and stable isotope analyzer to verify results. To account for this, samples were repressurized and concentrations were adjusted for dilution differences.

We based our methodology on the stable isotope tracer method. Biogenic methanogenesis heavily favors the production of $^{12}CH_4$ (~ 99%) as opposed to $^{13}CH_4$ (~ 1%) (Conrad 2005), but AOM only marginally discriminates against $^{13}CH_4$ (Wilson et al. 2019). By injecting samples with $^{13}CH_4$, we can measure the rates of $^{13}CO_2$ production and thus, how much $^{13}CH_4$ is being oxidized through AOM. We used the CO_2 isotopic discrimination in samples prior to isotope injection (timepoint 1) and total CO_2 produced during timepoint 2 to account for background $^{13}CO_2$ production. Previous studies relied on samples spiked with $^{12}CH_4$ to determine background $^{13}CO_2$ production (as seen in Chapter 2) (Blazewicz et al. 2012). However, we found in the pilot experiment that the large sample-to-sample variation in total CO_2 production reduced our sensitivity to measure AOM. Net CH_4 was measured by calculating the difference in CH_4 concentration over the course of the experiment, and gross CH_4 production was calculated by summing net CH_4 production and AOM. Negative AOM values were set to zero as we assumed that these values were random variation around our detection limit of approximately 0.012 µmol

g^{-1} day^{-1}. Detection limit was calculated by doubling a one-sided 95% confidence interval of the negative samples.

Equation 3 : AOM rate for SPRUCE experiment

$$AOM = \frac{(^{13}CO_{2,T2} - {}^{13}CO_{2,B}) \times (CH_{4,ave})}{(^{13}CH_{4,ave}) \times g\ dry\ weight \times (T2 - T1)}$$

Equation 4: Net CH_4 production rate for SPRUCE experiment

$$Net\ CH_4\ Production = \frac{(CH_{4,T2})}{g\ dry\ weight \times (T2 - T1)}$$

Equation 5: Gross CH_4 production rate for SPRUCE experiment

$$\text{Gross } CH_4 \text{ Production} = \text{Net } CH_4 \text{ Production} + \text{AOM}$$

Where $^{13}CO_{2,\,T2}$ is the $^{13}CO_2$ production in the $^{13}CH_4$-labeled samples from T1 to T2;

$^{13}CO_{2,\,B}$ is the background $^{13}CO_2$ production from T1 to T2, found by multiplying the CO_2 production from T1 to T2 by CO_2 atom % of samples at T1. This assumes that the background isotopic fractionation rate for CO_2 in a sample does not change over the incubation, but accounts for sample-to-sample variation in CO_2 production;

$CH_{4,\,ave}$ is the average total CH_4 concentration from T1 to T2;

$^{13}CH_{4,\,ave}$ is the average $^{13}CH_4$ concentration between T1 and T2;

T1 and T2 are timepoints 1 and 2, respectively.

3.2.6 Data Analysis

All statistics were done in R (version 1.3.1073). Data transformations were determined using univariate Box-Cox approach with powerTransform (package car). Values for gross CH_4 production and net CH_4 production were logged to better fit a normal distribution. Data for CO_2 production were square root transformed, and AOM rates were cube root transformed to improve fit. The percent AOM of net CH_4 production was arcsine transformed. Non-transformed data are shown in figures unless otherwise specified to make it easier to visualize results. The effects of temperature, depth, and elevated CO_2 on the response variables were determined using a linear regression model (package stats). Depth was treated as a categorical variable and significant differences ($p < 0.05$) among depths were found using a Tukey's test. We also examined the effect of porewater addition in samples with and without porewater in the first two depths in which we could collect porewater samples using a one-way ANOVA (package car). The samples with added porewater were only used to determine porewater effects and not for other analyses. Q_{10} coefficients for AOM and gross CH_4 production were found by using rates at the highest temperature and lowest temperature with a nonnegative AOM rate. Rates were determined using regression equations across the temperature gradient.

Equation 6: Q_{10} equation for SPRUCE experiment

$$Q_{10} = \left(\frac{r_1}{r_0}\right)^{\frac{10}{\triangle T}}$$

Where r_1 is the rate of production or consumption at T1 found by using a regression equation;

r_0 is the rate of production or consumption at T0 found by using a regression equation;

$\triangle T$ is the change in temperature between T1 and T0;

T1 is the highest temperature and T0 is the lowest temperature with a nonnegative AOM rate.

Even though the SPRUCE plots were warmed throughout the peat profile, depth and temperature were somewhat confounded in our experimental design. Deeper depths were cooler (Fig 10, $p = 0.002$), and there was limited temperature overlap between 30 cm and 200 cm (30 cm was never less than 10 °C and 200 cm was never greater than 15 °C). The result of this cofounding of temperature and depth was that adding an interaction term into the regression model reduced the significance of the main factors. Consequently, we mainly examine main effects and qualitatively refer to interactions among terms when appropriate.

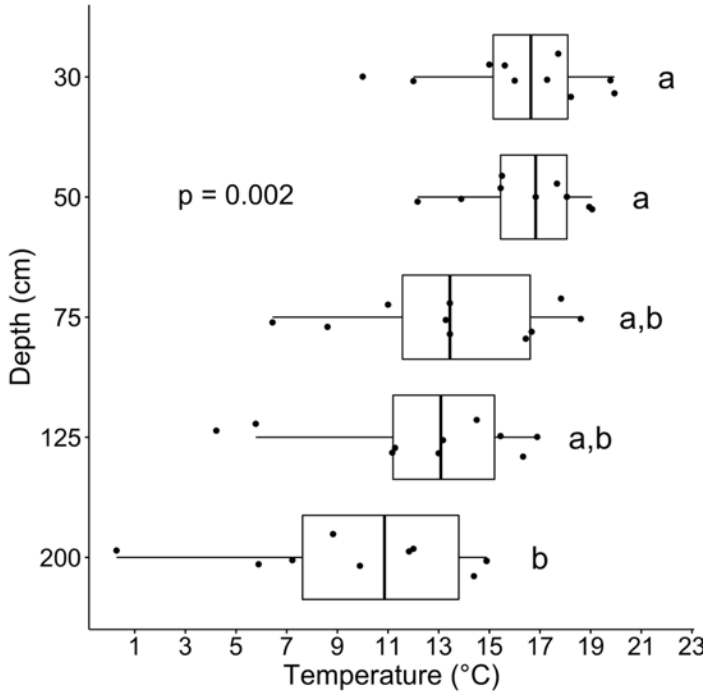

Figure 10. Temperature (°C) across depth in all plots. Letters indicate significant differences among depths.

Since some peat samples were collected above the water table, we also wanted to examine if samples collected above and below the water table had different rates when incubated anaerobically in the laboratory. All but one nonsaturated sample was in the 30 cm depth interval, so we limited our analysis of the saturation effect to this depth.

3.3 Results

We found no impact of water saturation on rates of CH_4 production, CO_2 production, or AOM. Elevated CO_2 only affected CO_2 production (see 3.3.3 Carbon Dioxide Production), so it is only mentioned in that section.

3.3.1 Methane Production

We did not observe net CH_4 consumption in this experiment. Net CH_4 production was not affected by temperature (Fig. 11a, $p = 0.25$), and the greatest production occurred in the surface 30 cm depth (Fig. 11b, $p < 0.001$). The impact of whole-ecosystem warming on net CH_4 production has decreased over time in the most surface level depth (Fig. 12).

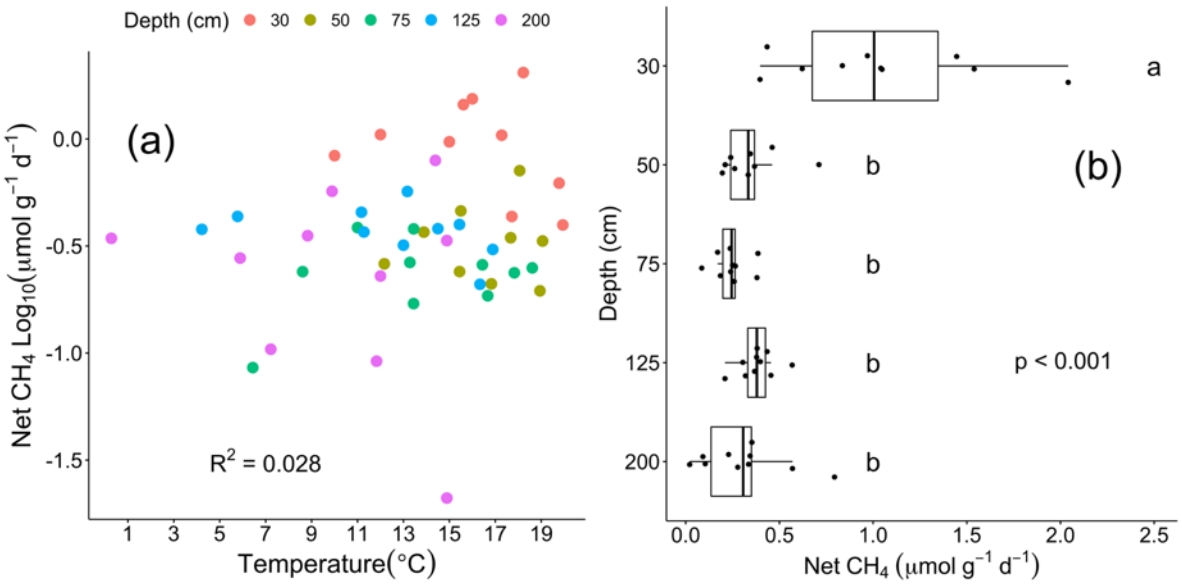

Figure 11. (a) Net CH_4 production (Log_{10}) across temperature (°C). Color indicates depth. (b) Boxplot of net CH_4 production by depth. Letters indicate significant differences among depths.

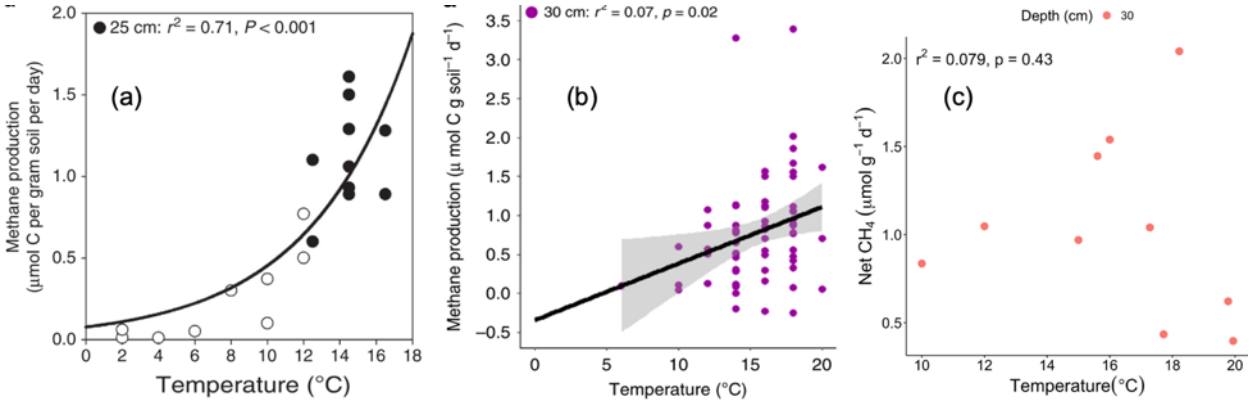

Figure 12. Net CH_4 production across temperature in the most surface-level depth measured after (a) one year of whole-ecosystem warming (Wilson et al. 2016), (b) three years of whole-ecosystem warming (Hopple et al. 2020), and six years of whole-ecosystem warming.

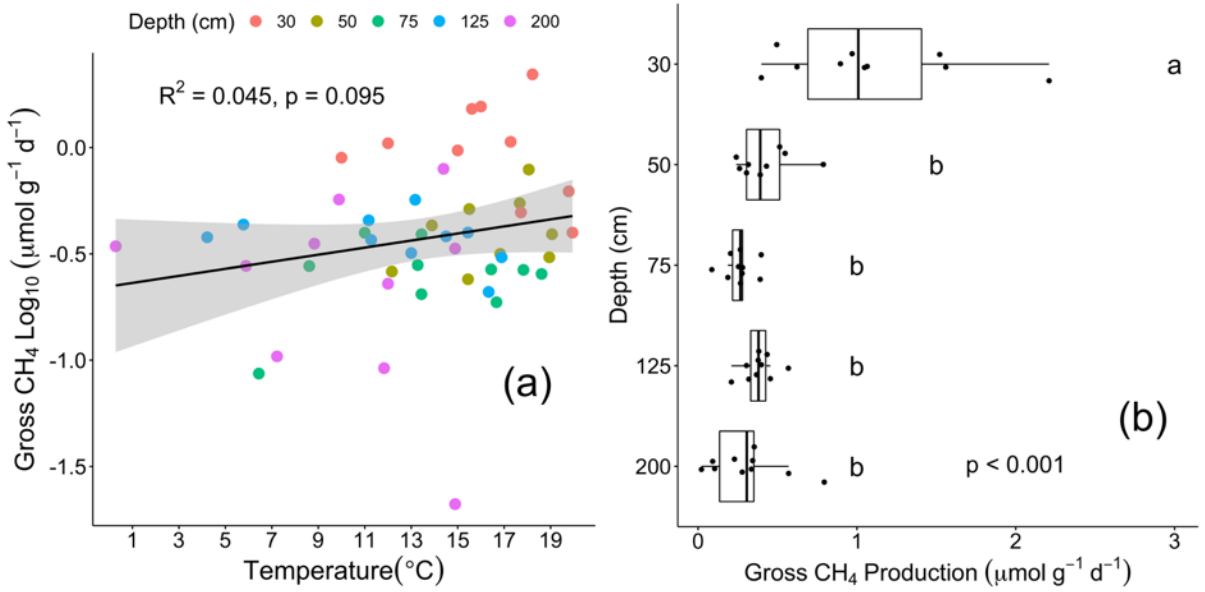

Figure 13. (a) Gross CH_4 production (log_{10}) across temperature (°C). Color indicates depth. y = 0.0166x – 0.6534 (b) Boxplot of gross CH_4 production rates by depth. Letters indicate significant differences among depths.

Temperature had a marginally significant impact on gross CH_4 production (Fig. 13a, p = 0.095) with a Q_{10} of 3.04. As with net CH_4 production, the greatest production occurred at the surface 30 cm depth (Fig. 13b, p < 0.001). When we examine CH_4 production visually, we see that higher temperatures and more surface level depths tended to have higher rates, although the depth effect appears to be stronger.

3.3.2 AOM

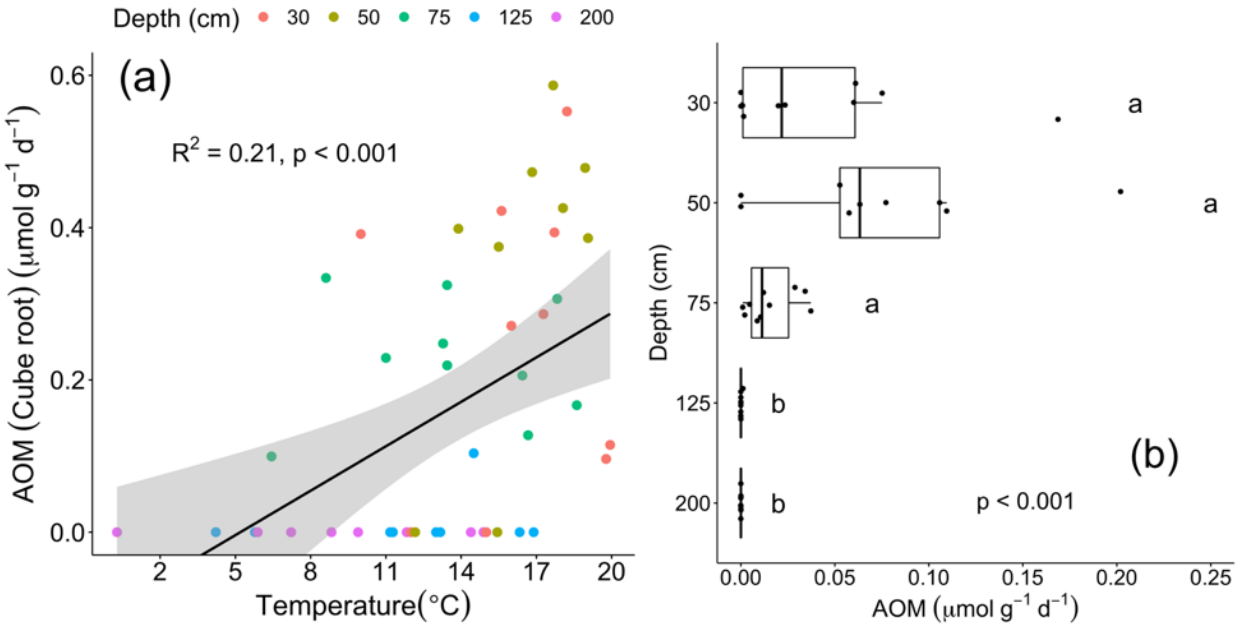

Figure 14. (a) AOM (cube root) across temperature (°C). Color indicates depth. y = 0.0195x – 0.1011 (b) Boxplot of AOM by depth. Letters indicate significant differences among depths.

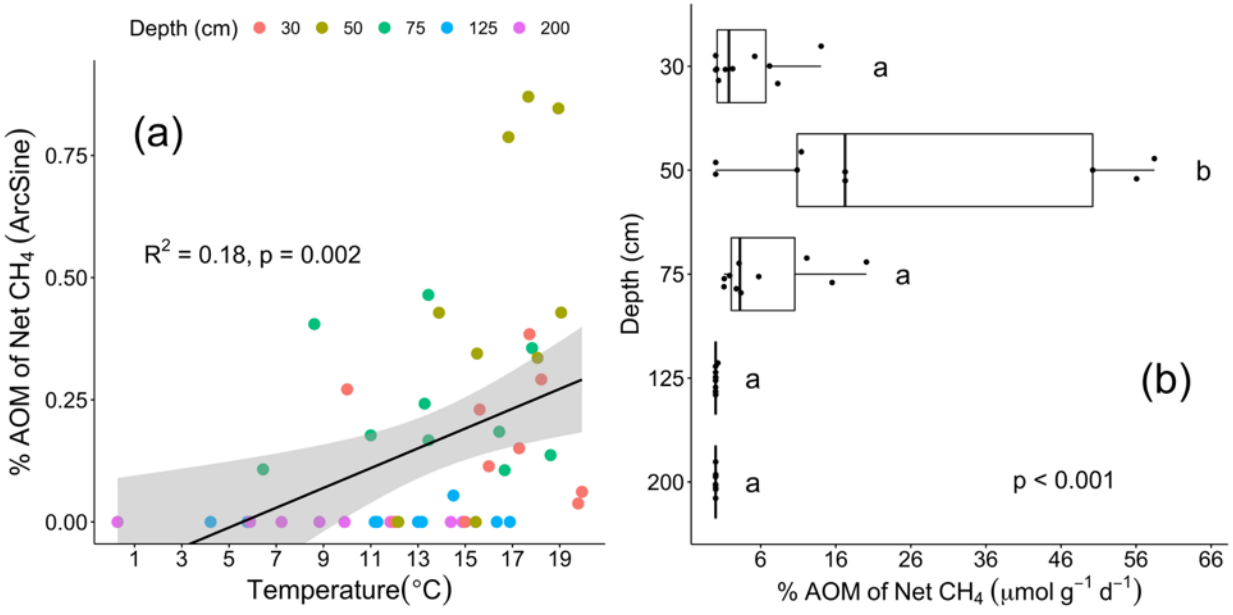

Figure 15. (a) Percent AOM of net CH_4 production (Arcsine) across temperature (°C). Color indicates depth. y = 0.0203x – 0.1128 (b) Boxplot of the percent AOM of net CH_4 production by depth. Letters indicate significant differences among depths.

Higher AOM rates were observed at warmer temperatures (Fig. 14a, $p < 0.001$). The Q_{10} coefficient was 4.47, and measurable rates were seen more often at temperatures above 8 °C. AOM was higher in surface peat ($p = 0.002$) with a visual peak at 50 cm (Fig. 14b). AOM was generally below detection at deeper depths (125 – 200 cm).

A higher percentage of net CH_4 production was consumed by AOM with increased temperature (Fig. 15a, $p = 0.002$). The highest percentage of AOM consumption occurred at 50 cm (Fig. 15b, $p < 0.001$) with most deeper depths (125 – 200 cm) consuming 0% of net CH_4 production within our limits of detection for AOM.

3.3.3 Carbon Dioxide Production

Temperature did not have an impact on CO_2 production (Fig. 16a, $p = 0.73$), but production was marginally greater in the 30 cm depth (Fig. 16b, $p = 0.08$). Unlike CH_4 production and AOM, elevated CO_2 in plots marginally increased CO_2 production (Fig. 17, $p = 0.09$).

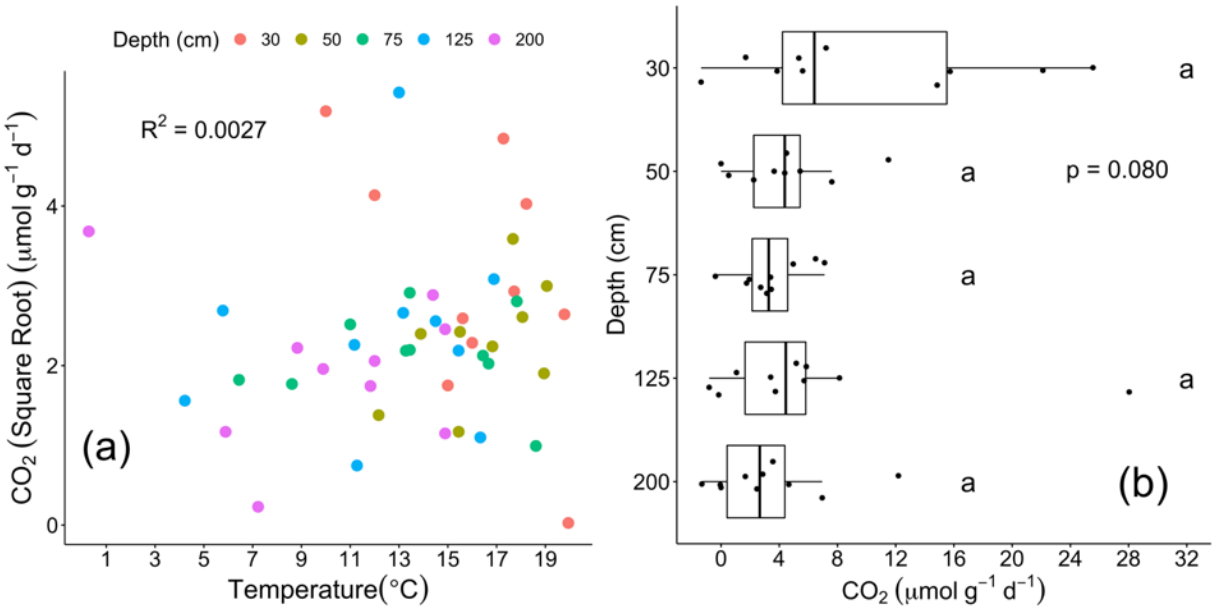

Figure 16. (a) CO_2 production (square root) across temperature (°C). Color indicates depth. (b) Boxplot of CO_2 production rates by depth. Letters indicate significant differences among depths.

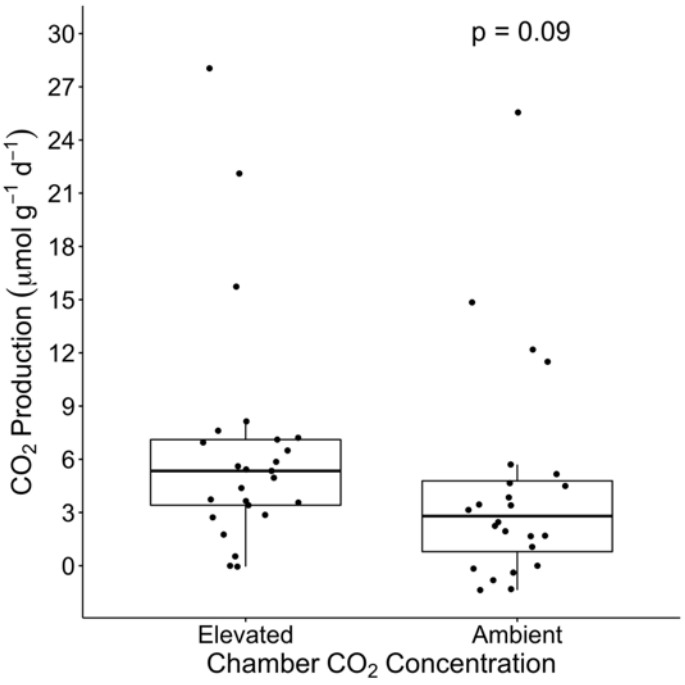

Figure 17. CO_2 production in elevated and ambient CO_2 treatments in plots.

3.3.4 Effect of Porewater

Since SPRUCE manipulated temperature with its predictable positive effect on evapotranspiration, water tables varied widely by plot. We collected porewater from the 2 most surface level depths in each plot which gave us an uneven distribution of sample depths. We collected most samples from 50 and 75 cm with limited measurements from deeper depths.

We did not observe a direct effect of porewater on AOM ($p = 0.68$) when it was examined alone as a main effect. However, temperature had a positive effect in samples with and without porewater addition as expected (Fig. 18a, interaction $p = 0.79$). When we added depth to the model, AOM marginally increased in samples without porewater (Fig. 18b, $p = 0.079$), with this effect apparent only in surface depths (Fig. 18b).

There was an increase in gross (Fig. 19a, $p = 0.007$) and net (Fig. 19b, $p = 0.005$) CH_4 production with the addition of porewater. However, there was no observable impact of

porewater addition on CO_2 production (Fig. 20, p = 0.31). Since AOM was frequently below detection, the percent AOM of net CH_4 production did not respond to porewater addition (Fig. 21, p = 0.67).

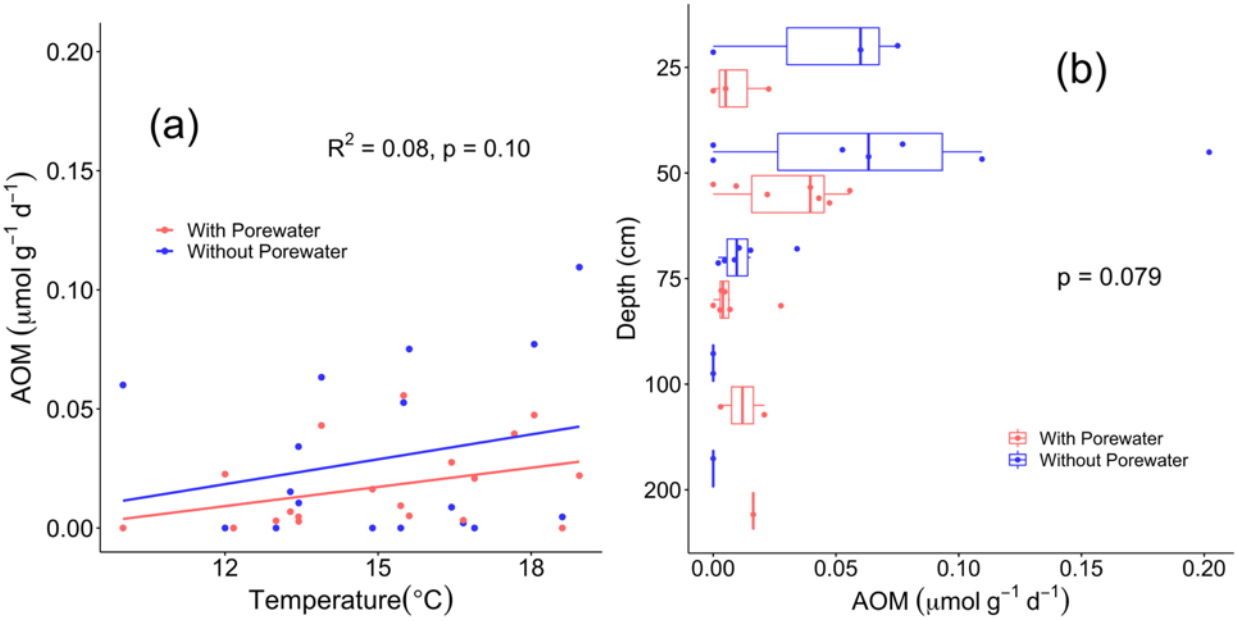

Figure 18. (a) AOM across temperature (°C). Color indicates porewater addition or control. (b) Boxplot of AOM with and without porewater by depth. Color indicates porewater addition or control.

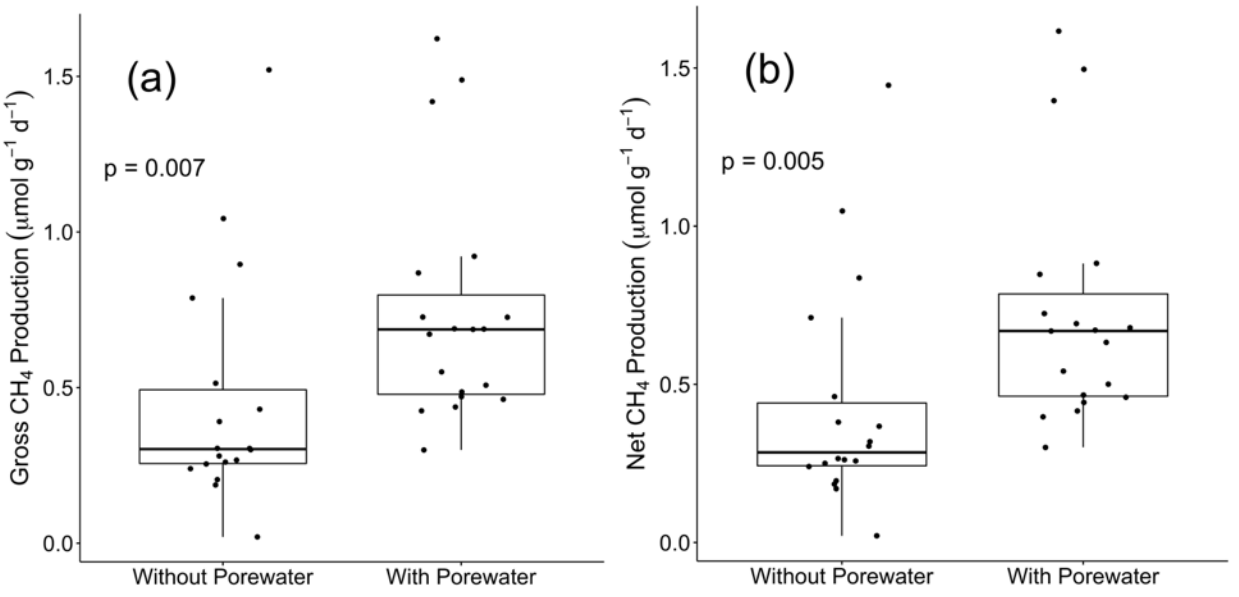

Figure 19. (a) Boxplot of gross CH_4 production with and without porewater (b) Boxplot of net CH_4 production with and without porewater

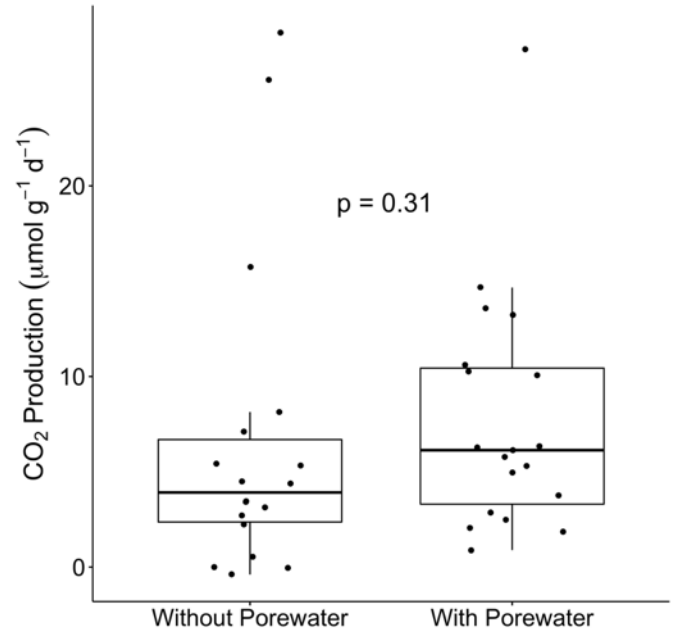

Figure 20. Boxplot of CO_2 production with and without porewater

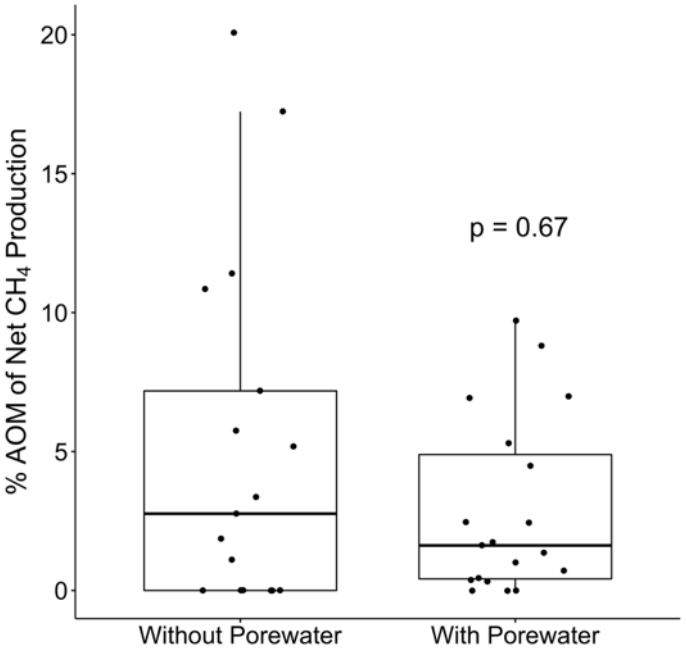

Figure 21. Boxplot of percent AOM of net CH4 production without and without porewater

3.4 Discussion

It is important to acknowledge with a one-time sampling that depth was closely tied to water saturation and temperature, although whole-ecosystem warming reduced the typical depth-

temperature relationship seen in unmanipulated systems. The surface-level increments exhibited the warmest temperatures and 60% of our 30 cm depths were aerobic. However, there was no evidence that treating both aerobic and anaerobic peat under anaerobic conditions yielded different results.

3.4.1 Depth Effects

We confirmed our first hypothesis that AOM is an important regulator of CH_4 production in at least this northern peat bog. In the mesotelm, AOM on average consumed a quarter of net CH_4 production with some samples consuming over half. This high percentage is attributed to high levels of AOM and modest levels of CH_4 production. The highest AOM rates occurred in the mesotelm (50 cm) with visually somewhat lower rates in the 75 and 30 cm depths. Negligible AOM occurred at deeper depths. Segarra et al. (2015) also found increased rates of AOM in peat close to the surface where there are high concentrations of TEAs. At S-1 bog, humic compounds are a major TEA and are found in high concentrations in the mesotelm (Tfaily et al. 2014, Rush et al. 2021). In warmer conditions with seasonal reductions in the water table, mesotelm peat can become aerated and TEAs, like humic compounds, can be oxidized (Rush et al. 2021). After rewetting, large stores of TEAs become available for reduction with AOM (Rush et al. 2021). The interface of high levels of humic compounds and CH_4 (see below) allow for increased AOM rates.

As seen in previous studies at SPRUCE, net CH_4 production was highest in the most surface-level increment (Wilson et al. 2016, Hopple et al. 2020). The high concentration of labile, recent decayed organic matter near the surface provides substrates for methanogens (Schulz and Conrad 1996). As seen in our experiment and previous studies, even in warmed conditions, deep peat produces limited CH_4, and much of the CH_4 that is produced is derived from surface dissolved organic matter (Hopple et al. 2019, 2020, Rush et al. 2021). Similarly, we

found the highest CO_2 production in the acrotelm. The high production in the surface could be driven by high decomposition rates as recent studies using radiocarbon analysis have found that CO_2 production in SPRUCE is driven by surface dissolved organic carbon (Wilson et al. 2016).

Elevated CO_2 only had a positive effect on anaerobic CO_2 production, possibly driven by increased vascular plant growth. Previous studies at SPRUCE have shown that elevated CO_2 increased CH_4 production at surface depths which was ascribed to increased available acetate and more labile root exudates (Hopple et al. 2020), but we did not observe this after six years of warming in a single mid-summer sampling.

3.4.2 Temperature Sensitivity

This is the first study to our knowledge that examines long-term temperature changes on AOM in freshwater wetlands. Our second hypothesis, that in a long-term warming experiment, CH_4 production, CO_2 production, and AOM would be similarly temperature sensitive, was only partially supported. We observed a modest positive temperature effect on AOM at more surface level depths (30 – 75 cm) and a marginal positive effect on gross CH_4 production, but not on net CH_4 or CO_2 production. In contrast, Gill et al. (2017) found at SPRUCE that methanogenesis is more sensitive to temperature increases than aerobic methanotrophy. Increases in temperature might allow for more optimal AOM microbial growth (Kolton et al. 2019) and increased reduction of organic matter (see above) (Rush et al. 2021). Intriguingly, the result of these differential temperature effects is that the percentage of net CH_4 consumed by AOM increased with increasing temperature. If this trend is observed in other northern peatlands under long-term warming conditions, it is possible that AOM will regulate greater percentages of CH_4 production with rising global temperatures. Further research should expand on this study to determine how universal these trends are across peatlands.

The increase in gross CH_4 in our study can partially be explained by warmer temperatures increasing the rate at which organic matter decays and breaks down into simple substrates, thus increasing methanogenesis (Schulz and Conrad 1996, Shannon et al. 1996). Studies at SPRUCE have also shown decreases in substrate concentrations under warming conditions are likely due to increased use by methanogens (Hopple et al. 2020). It is unlikely that methanogens in our study were beyond their temperature optimum, which was found to be 30 °C at SPRUCE (Kolton et al. 2019). Studies conducted relatively early in the warming manipulation at SPRUCE have found that warming led to higher rates of net CH_4 production and total emissions (Wilson et al. 2016, Gill et al. 2017, Kolton et al. 2019, Hopple et al. 2020). However, the strength of the warming effect has decreased through time (Hopple et al. 2020), and our study suggests this decrease is continuing.

We ascribe this result to years of warming having allowed for cascading ecological effects to manifest themselves at SPRUCE, like increases in nutrient availability, prevalence of ericaceous shrubs and ectomycorrhizal fungal rhizomorphs as well as the desiccation of *Sphagnum* (McPartland et al. 2019, Norby et al. 2019, Iversen et al. 2022). For example, warmer temperatures might allow microbes utilizing other TEAs to become metabolically active and substrate limit methanogens. Acetate has been shown to pool at lower temperatures before sulfate reducers are metabolically active, but with temperature increases, sulfate reducers can deplete acetate from the system (Shannon and White 1996). A study in S-1 Bog showed that warmed conditions increased the rate at which organic matter is used as a TEA and created more competition for acetate (Rush et al. 2021). As well, drawdowns in the water table at higher temperatures have the potential to oxidize TEAs, including organic matter, and periodically decrease the anaerobic zone, both of which can decrease CH_4 production (Hopple et al. 2020,

Rush et al. 2021). Recent studies have shown increases in available ammonium and phosphate in SPRUCE which might impact organic matter decomposition, CH_4 production, and AOM (Keller et al. 2005, 2006, Iversen et al. 2022). Other indirect effects include the increases in vascular plant growth, especially ericaceous shrubs, and the loss of *Sphagnum* (McPartland et al. 2019, Norby et al. 2019). While we do not know relative importance of each of these factors, we can anticipate that they will have direct and indirect impacts on CH_4 production and AOM.

CO_2 production in our study was minimally impacted by the temperature gradient. In SPRUCE, CO_2 production is mediated by two anaerobic communities that have local maximal growth at 4 °C and 20 °C (Kolton et al. 2019). Since most of our temperatures (4 – 19 °C) are located between these two maxima, there might be less consistency in how CO_2 production reacts to these temperature changes. Previous studies at SPRUCE have shown temperature has a widely variable impact on CO_2 production. Increases in CO_2 production at higher temperatures were seen only at the most surface level depth (Wilson et al. 2016, McCullough 2019), at the deepest depth (Hopple et al. 2020), or not at all (Hopple 2018). Since all these studies at SPRUCE were conducted at different times since the warming initiation, there might be differences in how CO_2 production is affected by temperature over time. Given the wide variety of data surrounding CO_2 production at SPRUCE, we cannot conclusively say how CO_2 production might be impacted by temperature, but at best, there is a weak positive effect.

3.4.3 Porewater Impacts

There was weak support for our third hypothesis that the addition of porewater would suppress rates of AOM through a diffusional barrier. We found that the addition of porewater marginally decreased AOM rates in depths above 75 cm, and, as seen in a previous study at SPRUCE, increased CH_4 production (Hopple 2018). Both our study and Hopple (2018) provide evidence for increased AOM in surface samples without porewater. The previous study observed

net CH_4 consumption in surface level depths when samples were not slurried (Hopple 2018). However, our study did not observe net consumption at any depth. For net CH_4 consumption to occur, AOM must outpace gross CH_4 production. There are several possible reasons for differences between these studies. Our study only measured rates once, whereas Hopple (2018) measured rates at multiple timepoints and did not see net consumption for every measurement. There also might be discrepancies because of time differences in the collection period. Hopple (2018) collected samples (August 2015 – October 2016) shortly after the initiation of SPRUCE, while our collection occurred after six years of whole ecosystem warming and might have been impacted by cascading ecological effects as described above.

There are several possible explanations for the increase in CH_4 production and decrease in AOM with the addition of porewater. As hypothesized, samples with porewater may have lower rates of AOM since CH_4 is relatively insoluble and has difficulty diffusing through the porewater barrier. This would suppress AOM and potentially underrepresent *in situ* rates where CH_4 is ubiquitous. Porewater also contains labile dissolved organic matter that can be fermented into simple substrates (CO_2, H_2, and acetate) used to fuel methanogenesis (Medvedeff et al. 2015, Hopple et al. 2019). Many laboratory studies of CH_4 production add porewater to soil samples to create a slurry, yet our results here and those of Hopple (2018) suggest adding porewater might add labile dissolved organic matter, potentially enhancing methanogenesis, and suppressing AOM. Overall, evidence suggests that adding porewater to make slurries might be a poor experimental design if the objective of a study is to estimate *in situ* rates of anaerobic carbon cycling.

3.5 Conclusion

Evidence from this study supports the hypothesis that AOM plays an important role in CH_4 cycling in freshwater wetlands. We observed instances where over half of CH_4 production

was consumed by AOM, potentially driven by the high concentrations of humic compounds acting as TEAs restricting methanogenesis and promoting AOM. This study found that AOM can regulate CH_4 flux in northern peatlands and should be considered an important part of CH_4 cycling.

Increased temperature not only led to higher gross CH_4 production, it also led to higher AOM consumption rates. The effect of temperature on net CH_4 production and emission in the SPRUCE has decreased through time. Increased temperatures might have allowed for more optimal microbial growth and faster decomposition rates but cascading ecological effects could have limited total emissions. Changes in water table levels, nutrients concentrations, microbial communities and vegetation all have the potential to impact CH_4 flux and should be studied to fully appreciate how rising global temperatures will impact CH_4 emissions. Our study provides evidence for marginal increases CH_4 production and higher increases in AOM consumption after six years of a whole ecosystem warming experiment.

Finally, the addition of porewater marginally decreased AOM rates and substantially increased CH_4 production. This might be attributed to a diffusional barrier blocking AOM microbial access to headspace CH_4 and to porewater dissolved organic matter breaking down into simple substrates for higher rates of methanogenesis. Adding porewater and peat to make slurries in experimental design might overestimate CH_4 production and underestimate AOM in surface level depths and should be used with caution.

This study was limited by a singular collection in the field that did not allow for nuance between timepoints and environmental conditions, as evidenced by not seeing net CH_4 consumption in any sample, and the partial confounding of soil temperature and depth. Further experiments should examine the full suite of processes controlling CH_4 dynamics across both

time and temperature conditions. At a single timepoint, our results indicate that assessments of CH_4 flux in wetlands would be more accurate if they incorporate AOM and that AOM may become a more important regulator of CH_4 emissions with increases in global temperature.

4. CONCLUSION

I wrote Chapter 4 with text edits provided by Scott Bridgham.

As the earth continues to warm at unprecedented rates, it is important to understand the mechanisms behind global CH_4 flux. This study expanded upon the current understanding of AOM's importance in freshwater wetland CH_4 cycling. AOM consumed the highest percentage of net CH_4 production in the mesotelm in part due to high concentrations of humic compounds both suppressing CH_4 production and promoting AOM. As supported in previous studies, CH_4 production and CO_2 production were highest in the surface increment possibly due to enhanced decomposition and increased microbial activity. As the evidence from this study supports the importance of AOM in freshwater CH_4 cycling, it merits further study in a globally changing context.

This study is the first to our knowledge to examine the impact of long-term temperature changes on AOM in freshwater systems. Our study suggests that long-term temperature increases will alter rates of CH_4 production and AOM. After six years of whole ecosystem warming in an ombrotrophic peat bog, both gross CH_4 production and AOM rates increased in response to temperature. However, since AOM rates increased more significantly, it obscured any temperature impact on net CH_4 production. Our study suggests that under warmed conditions, AOM will consume a greater percentage of CH_4 consumption. To create the most accurate assessment of freshwater CH_4 flux, these impacts should be examined in context with other cascading ecological effects under warming conditions.

To our knowledge, our study is also the first to examine the addition of porewater to experimental methodology of AOM. There is a suggestion that the addition of porewater to *in vitro* AOM experiments is not representative of rates seen *in situ* especially at more surface level depths. The addition of porewater to samples might both hinder AOM microbes from accessing

CH_4 in the headspace because of the diffusional barrier and promote methanogenesis through increased dissolved organic matter. Further research should be conducted to verify the role of porewater in this methodology.

AOM remains an understudied process in CH_4 cycling in freshwater environments. Results from our study indicate that AOM acts as an important regulator in CH_4 emissions in temperate peatlands. Changes in climate might lead to increases in CH_4 production and AOM across peatland environments, although our study indicates that AOM will consume a larger percentage of net CH_4 with increasing temperatures. Peatlands contain massive stores of soil carbon, and more comprehensive studies are necessary to determine the full impacts of climate change on these stores. Future studies should work to include the impacts of warming temperature on full ecosystem effects and their interactions to get the best representation of climate change scenarios.

APPENDIX

SUPPLEMENTAL DATA

Supp. Table 1. All raw data isotope values for the pilot experiment.

			Timepoint 1 (7 hours)				Timepoint 2 (24 hours)				Timepoint 3 (48 hours)			
Isotope	Temperature (°C)	Replicate	$^{13}CH_4$ (μmol)	$^{12}CH_4$ (μmol)	$^{13}CO_2$ (μmol)	$^{12}CO_2$ (μmol)	$^{13}CH_4$ (μmol)	$^{12}CH_4$ (μmol)	$^{13}CO_2$ (μmol)	$^{12}CO_2$ (μmol)	$^{13}CH_4$ (μmol)	$^{12}CH_4$ (μmol)	$^{13}CO_2$ (μmol)	$^{12}CO_2$ (μmol)
$^{13}CH_4$	8	1	2.570	7.494	0.384	33.724	3.297	23.709	0.772	65.842	3.572	43.818	1.044	92.115
$^{13}CH_4$	8	2	4.837	7.464	0.438	38.525	4.203	13.414	0.548	48.358	4.491	19.719	0.679	59.965
$^{13}CH_4$	8	3	4.599	9.148	0.460	39.163	3.954	19.284	0.566	49.775	4.423	31.626	0.693	61.183
$^{13}CH_4$	12	1	4.460	7.841	0.342	30.378	3.827	13.790	0.518	39.228	3.689	20.521	0.522	42.686
$^{13}CH_4$	12	2	4.316	8.629	0.358	31.748	4.004	14.043	0.437	37.236	4.147	22.076	0.510	45.271
$^{13}CH_4$	12	3	4.048	7.514	0.455	39.617	2.954	10.965	0.571	48.274	2.982	15.760	0.593	50.972
$^{13}CH_4$	16	1	4.303	9.444	0.491	43.354	3.821	19.417	0.708	62.256	4.040	32.009	0.859	75.608
$^{13}CH_4$	16	2	4.076	9.322	0.480	42.674	3.769	16.525	0.603	53.463	4.055	27.515	0.752	66.735
$^{13}CH_4$	16	3	3.710	9.563	0.382	33.792	3.589	16.763	0.504	44.727	3.769	24.436	0.588	52.199
$^{13}CH_4$	20	1	4.172	9.787	0.422	37.351	4.121	23.274	0.603	53.579	4.380	39.191	0.774	69.062
$^{13}CH_4$	20	2	4.627	7.041	0.449	33.176	3.783	19.755	0.576	50.909	4.285	37.993	0.824	73.053
$^{13}CH_4$	20	3	4.273	8.138	0.386	33.780	3.877	20.351	0.561	49.507	4.180	35.102	0.736	65.392
$^{13}CH_4$	24	1	3.222	6.749	0.327	28.677	3.820	22.839	0.661	58.254	4.122	40.901	0.941	83.144
$^{13}CH_4$	24	2	4.214	8.157	0.379	33.061	3.883	22.579	0.592	52.194	4.208	40.478	0.821	72.545
$^{13}CH_4$	24	3	4.157	6.519	0.342	30.106	3.805	15.072	0.488	43.043	3.940	25.709	0.625	55.427
$^{12}CH_4$	8	1			0.296	26.457	0.129	12.111	0.369	33.331	0.168	15.799	0.398	35.808
$^{12}CH_4$	8	2			0.501	45.173	0.024	2.215	1.131	102.238	0.013	1.179	1.311	118.524
$^{12}CH_4$	8	3			0.472	42.526	0.263	24.722	0.595	53.714	0.412	38.888	0.743	67.076
$^{12}CH_4$	12	1			0.331	29.788	0.136	12.778	0.415	37.456	0.204	19.273	0.472	42.139
$^{12}CH_4$	12	2			0.438	39.455	0.023	2.147	0.547	49.300	0.012	1.144	0.628	56.733
$^{12}CH_4$	12	3			0.366	33.023	0.141	13.219	0.442	39.905	0.197	18.550	0.504	45.452
$^{12}CH_4$	16	1			0.389	35.132	0.200	18.821	0.564	50.833	0.332	31.318	0.705	63.645
$^{12}CH_4$	16	2			0.452	40.760	0.300	28.211	0.633	57.172	0.531	50.109	0.918	82.959
$^{12}CH_4$	16	3			0.490	44.274	0.317	29.865	0.675	60.903	0.534	50.368	0.910	81.915
$^{12}CH_4$	20	1			0.445	40.091	0.321	30.178	0.705	63.697	0.554	52.204	1.000	90.161

$^{12}CH_4$	20	2			0.400	36.095	0.150	14.130	0.559	50.522	0.261	24.566	0.681	61.356
$^{12}CH_4$	20	3			0.400	36.101	0.212	19.897	0.589	52.978	0.359	33.895	0.766	69.059
$^{12}CH_4$	24	1			0.313	28.075	0.182	17.124	0.436	39.355	0.277	26.107	0.531	47.852
$^{12}CH_4$	24	2			0.322	29.010	0.149	14.041	0.410	37.011	0.239	22.533	0.515	46.488
$^{12}CH_4$	24	3			0.377	34.050	0.209	19.689	0.507	45.817	0.353	33.309	0.674	60.475

Supp. Table 2. All raw data isotope values for SPRUCE experiment.

				Timepoint 1 (Background)				Timepoint 2			
Plot	Depth (cm)	Pore water	Temperature (°C)	$^{13}CH_4$ (μmol)	$^{12}CH_4$ (μmol)	$^{13}CO_2$ (μmol)	$^{12}CO_2$ (μmol)	$^{13}CH_4$ (μmol)	$^{12}CH_4$ (μmol)	$^{13}CO_2$ (μmol)	$^{12}CO_2$ (μmol)
4	25	-	17.28	0.044	4.284	0.320	28.824	4.659	8.554	1.722	150.690
4	50	-	16.83	0.023	2.237	0.186	16.424	4.245	3.138	1.171	50.789
4	75	-	13.44	0.014	1.310	0.071	6.225	3.929	1.871	0.917	63.633
4	75	+	13.44	0.019	1.817	0.576	51.107	6.722	2.242	1.086	93.692
4	100	-	13.00	0.027	2.588	0.122	10.726	3.811	3.293	1.465	132.988
4	100	+	13.00	0.024	2.348	0.584	51.767	5.761	2.910	0.931	81.634
4	200	-	12.00	0.023	2.218	0.102	9.001	4.453	2.836	0.362	33.323
6	25	-	10.00	0.012	1.185	0.145	13.029	4.721	2.095	1.123	89.557
6	25	+	10.00	0.008	0.793	0.375	33.677	6.536	2.231	0.832	76.419
6	50	-	10.83	0.040	3.875	0.180	15.909	3.324	4.871	0.337	29.202
6	50	+	10.83	0.033	3.148	0.364	32.352	5.738	3.707	0.605	54.678
6	75	-	6.44	0.024	2.328	0.105	9.315	3.647	2.348	0.252	21.884
6	100	-	4.22	0.021	2.045	0.075	6.554	4.375	2.563	0.126	11.513
6	200	-	0.28	0.063	6.075	0.272	23.992	4.181	6.764	0.929	84.524
8	25	-	18.22	0.081	6.652	0.264	23.845	4.308	13.969	1.124	84.534
8	50	-	17.67	0.013	1.253	0.097	8.706	3.719	2.556	1.523	71.366
8	50	+	17.67	0.012	1.187	0.366	32.478	6.218	2.373	0.793	57.917
8	75	-	16.44	0.032	3.030	0.169	14.968	3.763	4.055	0.424	35.084
8	75	+	16.44	0.020	1.894	0.557	49.323	5.220	2.164	1.355	110.186
8	100	-	14.50	0.013	1.265	0.062	5.439	4.371	1.721	0.329	28.640
8	200	-	11.83	0.052	4.972	0.250	22.122	4.456	5.695	0.658	58.879
10	25	-	17.72	0.002	0.236	0.344	31.420	4.456	0.930	0.965	65.095
10	50	-	19.06	0.008	0.674	0.072	6.453	5.062	1.616	1.193	71.781
10	75	-	17.83	0.013	1.234	0.093	8.226	3.911	1.648	0.573	41.432
10	100	-	16.89	0.019	1.817	0.096	8.482	3.930	2.387	0.513	45.416
10	100	+	16.89	0.021	2.022	0.587	52.073	6.341	2.373	1.146	96.392
10	200	-	14.89	0.042	4.038	0.226	19.818	3.130	4.122	0.221	19.602

10	200	+	14.89	0.037	3.528	0.371	32.850	4.894	4.106	1.463	126.992
11	25	-	15.61	0.083	7.929	0.411	36.485	4.436	11.464	0.679	53.979
11	25	+	15.61	0.077	7.372	0.641	57.085	4.934	10.177	1.073	95.101
11	50	-	13.89	0.019	1.848	0.117	10.410	4.739	2.533	0.680	37.541
11	50	+	13.89	0.024	2.296	0.526	46.743	5.671	2.668	1.160	86.778
11	75	-	11.00	0.014	1.363	0.098	8.735	4.569	1.966	0.440	35.087
11	100	-	11.17	0.026	2.509	0.121	10.736	4.704	3.019	0.312	27.831
11	200	-	8.83	0.041	3.969	0.208	18.488	4.053	5.133	0.438	39.352
13	25	-	16.00	0.051	4.865	0.184	16.506	3.144	11.441	0.388	32.767
13	50	-	15.50	0.015	1.471	0.104	9.386	4.143	3.209	0.595	36.021
13	50	+	15.50	0.017	1.598	0.376	33.460	5.677	2.647	1.231	90.266
13	75	-	13.28	0.012	1.175	0.077	6.808	4.766	1.527	0.452	32.433
13	75	+	13.28	0.015	1.423	0.307	27.294	6.166	1.819	0.831	70.671
13	100	-	13.17	0.019	1.863	0.117	10.235	4.976	2.270	0.364	32.901
13	200	-	9.89	0.016	1.574	0.095	8.328	4.988	2.257	0.208	19.318
16	25	-	19.78	0.008	0.787	1.342	123.005	4.503	1.733	1.574	144.043
16	50	-	18.06	0.023	2.222	0.192	17.270	4.260	4.910	0.725	46.080
16	50	+	18.06	0.027	2.570	0.190	17.036	5.095	4.049	0.428	26.249
16	75	-	16.67	0.011	1.089	0.059	5.244	4.092	1.428	0.289	24.710
16	75	+	16.67	0.012	1.098	0.465	41.253	6.379	1.314	0.804	69.581
16	100	-	15.44	0.014	1.287	0.066	5.819	4.498	1.599	0.222	20.331
16	200	-	14.39	0.044	4.174	0.172	15.213	3.856	5.221	0.342	30.588
17	25	-	19.94	0.036	3.480	0.171	15.314	3.395	5.954	0.070	5.900
17	50	-	18.94	0.082	0.751	0.075	6.789	4.318	0.960	0.866	21.647
17	50	+	18.94	0.009	0.822	0.340	30.157	5.057	1.103	0.584	43.288
17	75	-	18.61	0.010	0.969	0.117	10.429	4.522	1.152	0.113	7.924
17	75	+	18.61	0.018	1.744	0.729	64.596	6.021	2.506	1.061	95.077
17	100	-	16.33	0.023	2.223	0.176	15.533	3.359	2.920	0.161	14.800
17	200	-	14.89	0.046	4.366	0.217	19.164	3.137	6.201	0.489	44.507
19	25	-	12.00	0.010	0.978	0.310	28.409	4.403	3.572	0.932	86.442
19	25	+	12.00	0.012	1.171	0.471	42.575	6.683	2.722	1.120	95.905
19	50	-	12.17	0.060	5.825	0.301	26.740	3.421	7.451	0.342	30.537
19	50	+	12.17	0.052	4.974	0.684	61.068	5.061	5.460	0.758	68.151
19	75	-	8.61	0.014	1.392	0.077	6.904	4.228	1.742	0.358	17.457
19	100	-	5.78	0.013	1.230	0.051	4.527	4.589	2.107	0.378	36.161
19	200	-	5.89	0.032	3.124	0.141	12.462	3.938	3.775	0.133	12.443
20	25	-	15.00	0.054	5.223	0.293	26.249	4.078	7.777	0.353	32.288
20	50	-	15.44	0.018	1.706	0.086	7.647	3.808	2.442	0.083	7.629
20	50	+	15.44	0.020	1.913	0.452	40.254	6.506	2.205	0.712	59.421

20	75	-	13.44	0.016	1.515	0.081	7.205	4.318	2.105	0.298	23.400
20	75	+	13.44	0.027	2.193	0.528	46.919	5.864	2.651	0.638	55.956
20	100	-	11.28	0.016	1.524	0.064	5.707	4.079	1.958	0.028	2.602
20	200	-	7.22	0.081	7.804	0.306	27.068	3.106	8.408	0.223	20.230

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