EXAMINING ANAEROBIC OXIDATION OF METHANE IN A NORTHERN PEAT BOG

by

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THESIS ABSTRACT

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Globally, about one-third of annual methane (CH_4) emissions from natural sources come from freshwater wetlands. Scientists need a strong understanding of CH_4 cycling to predict how climatic shifts will affect future CH_4 emissions. Anaerobic oxidation of CH_4 (AOM) is an important factor in CH_4 cycle models in marine systems, but it has so far been excluded from freshwater CH_4 cycle models which balance production and aerobic consumption. However, evidence for AOM as an influential part of CH_4 cycling in freshwater ecosystems is mounting, revealing that traditional methods for measuring CH_4 production and modeling CH_4 cycling may need updating. Here, we present a new method for measuring AOM and gross CH_4 production simultaneously during incubation using a $^{13}CH_4$ tracer. This study supports existing evidence that AOM is an influential part of CH_4 cycling in peatlands and presents evidence that the process can occur to a depth of at least 2 meters.

This thesis includes unpublished co-authored material.

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TABLE OF CONTENTS

LIST OF FIGURES

Figure Page

Figure Page

LIST OF TABLES

Table Page

LIST OF SCHEMES

Scheme Page

I. INTRODUCTION

I wrote the introduction chapter with editing provided by Scott Bridgham.

Anaerobic Carbon and Methane Cycling

In ecosystems of every kind, scientists are pushing to better understand how environmental processes will be affected by future environmental change. Projecting shifts in greenhouse gas production in carbon-rich ecosystems is of particular interest because of its potential to create feedback loops with climate change (Dean et al., 2018).

Methane (CH_4) is a potent greenhouse gas that is a product of anaerobic decomposition, with a global warming potential that is about 30 times greater, and a sustained-flux global warming potential that is about 45 times greater, over a 100-year period than carbon dioxide (CO_2; Neubauer & Megonigal, 2015, Armstrong et al., 2015, Myhre et al., 2013). Future climate is likely to be greatly impacted by slight changes in atmospheric CH_4 concentrations because of the high global warming potential.

Total annual CH_4 emissions were about 548 Tg CH_4 yr^{-1} between 1980 and 2010 (Kirschke et al., 2013). Wetlands were the largest natural source of CH_4, contributing about one third of the total global emissions (Bridgham et al., 2013, Saunois et al., 2016, Kirschke et al., 2013). Bottom-up models use mechanistic modules of varying sophistication for CH_4 production, transport to the atmosphere, and consumption to estimate global CH_4 emissions (Saunois et al., 2016). This technique yields estimated emissions of 185 (153-227) Tg CH_4 yr^{-1} from natural wetlands (Saunois et al., 2016, Dean et al., 2018). Top-down models, on the other hand, take measured gradients of atmospheric CH_4 and sometimes its isotopic $\delta^{13}C$ signature to estimate what portion of emissions are coming from different sources across the Earth, like natural wetlands, wildfires, or agriculture (Saunois et al., 2016). These models estimate slightly lower emissions of 167 (127-202) Tg CH_4 yr^{-1} (Saunois et al., 2016, Dean et al., 2018). Unfortunately, uncertainty around global wetland CH_4 emissions is around 50%, highlighting the need for a better understanding of CH_4 cycling in these ecosystems (Saunois et al., 2016).

In wetlands, CH_4 production has a positive relationship with both temperature and water-table level (Walter & Heimann, 2000, Dean et al., 2018). The bulk of wetland CH_4

is emitted from tropical wetlands (Dean et al., 2018). However, northern peatlands are very carbon-dense and emit substantial amounts of CH_4 despite having much lower temperatures (Loisel et al., 2014). Wetlands may increase CH_4 production under a warmer climate, creating a positive feedback loop between CH_4 production and temperature increase due to the greenhouse effect (van Winden et al., 2012, Dean et al., 2018). However, net CH_4 emissions depend on both CH_4 production and CH_4 oxidation, and there are both aerobic and anaerobic pathways of CH_4 oxidation (Whalen & Reeburgh, 2000, Zhuang et al., 2004, Segarra et al., 2015).

Importance of Northern Peatlands

Peatlands are wetlands that are formed due to low hydraulic conductivity of the thick peat layer that leads to waterlogged conditions, further promoting the accumulation of partially decomposed organic matter, which has a high water-holding capacity (Clymo, 1984, Foster et al., 1988). Cool temperatures in northern latitudes further slow the turnover of soil organic carbon (Yu, 2012). Northern peatlands cover about 3% of global terrestrial area, or about $4x10^6$ km^2, and hold about one third of the global soil C (about 500 Gt), a disproportionately high stock of C per unit area (Yu, 2012, Loisel et al., 2014).

Peatlands emit globally significant amounts of CH_4 because of their very high organic content and anaerobic conditions (Frolking et al., 2006, Mikaloff Fletcher et al., 2004). Because of the short atmospheric life time of CH_4 versus CO_2 (Joos et al., 2013, Myhre et al., 2013), when the greenhouse gas balance is examined over a long time scale (500 + years), the cooling effect of carbon sequestration is dominant over CH_4 emissions (Whiting & Chanton, 2001, Frolking et al., 2006, Loisel et al., 2014). Northern peatlands are estimated to sequester between 15-46 g C m^{-2} yr^{-1} by Turunen et al. (2002) and to hold about 1,497 Mg C ha^{-1} (Bridgham, 2014). Loisel et al. (2014) created a database of 268 bogs, fens, and permafrost peatlands in North America and Eurasia and presented peat properties and C and nitrogen (N) accumulation rates during the Holocene. The average estimated C sequestration rates across northern peatlands was 22.9 ± 2 g C m^{-2} yr^{-1} (Loisel et al., 2014).

It is likely that CH_4 production will increase with warmer temperatures, unless significant water table draw-down occurs, which would increase decomposition of soil

organic carbon (SOC) to CO_2 (Ise & Moorecroft, 2006, Clark et al., 2009, van Winden et al., 2012). Because of the role that northern peatlands play in the global CH_4 emissions, it is important to have a strong understanding of the mechanisms behind their CH_4 cycling to model how their emissions will be affected by environmental change. Models for CH_4 cycling in wetlands balance produced CH_4 with aerobically consumed CH_4 to estimate net emissions (Cao et al., 1996, Arah & Stephen, 1998, Matthews, et al., 2000, Walter & Heimann, 2000). These models may be oversimplified, though, because it assumes that anaerobic oxidation of CH_4, a globally significant factor in marine CH_4 cycling, is not occurring in these freshwater systems.

Anaerobic Oxidation of Methane (AOM)

Anaerobic Oxidation of CH_4 (AOM) was first observed in the 1970's (Barnes & Goldberg, 1976, Martens & Berner, 1974) in marine systems, where methanotrophs have since been found to consume about 90% of CH_4 that is produced (Hinrichs & Boetius, 2002, Reeburgh, 2007). Studies have linked AOM in marine systems to archaebacteria in groups ANME-1 to -3 that exist in association with sulfate-reducing bacteria (Hinrichs et al., 1999, Michaelis et al., 2002, Nauhaus et al., 2005, Treude et al., 2007, Bhattarai et al., 2017, Yanagawa et al., 2018) and a fourth group of archaea that couple AOM with denitrification (Raghoebarsing et al., 2006).

Associations between methanotrophs and sulfate-reducing bacteria are common in marine environments where both substrates exist, and sulfate has been identified as the main terminal electron acceptor (TEA) driving AOM in these environments (Barnes & Goldberg, 1976, Treude et al., 2005). More recently, manganese, and iron have also been identified as TEAs associated with marine AOM and it is suggested that manganese and iron-dependent AOM are also an important part of the marine CH_4 cycle despite having slower rates than sulfate-dependent AOM (Beal et al., 2009, Ettwig et al., 2016, Valenzuela et al., 2019). Availability of sulfate and CH_4 have been seen to limit AOM in marine systems, but otherwise there is little understanding of environmental controls (Nauhaus et al., 2005).

Until recently, AOM was assumed to be an unimportant part of the CH_4 cycle in freshwater systems. Much of the skepticism surrounding AOM in freshwater ecosystems has been based on the limited availability of sulfate and other suitable TEAs.

Additionally, AOM is difficult to detect when using incubation experiments because of the overwhelming signal of CH_4 production (Smemo & Yavitt, 2011). A study done in 1980 by Zehnder and Brock presented evidence of AOM occurring simultaneously to CH_4 in freshwater lake sediment and digested sewage sludge production. Another study (Smith et al., 1991) examined CH_4 oxidation in a sand and gravel aquifer and observed both aerobic and anaerobic CH_4 oxidation in the freshwater system. Since then, AOM has been observed in several other types of freshwater ecosystems, including arctic and sub-arctic lakes, boreal peatlands, and tropical wetlands (Smemo & Yavitt, 2007, Blazewicz et al., 2012, Gupta et al., 2013, Nordi et al., 2013, Martinez-Cruz et al., 2017 Valenzuela et al., 2017). The research on AOM in freshwater systems is only beginning to identify the importance of this factor in wetland CH_4 cycling, and a much larger body of evidence is needed to understand the role that AOM plays and what controls it.

Scientists are particularly interested in the occurrence of AOM in peatlands because of the significant role they play in the global CH_4 cycle. A study done by Smemo and Yavitt (2007) provided evidence that AOM is an important factor in CH_4 cycling in northern peatlands, consuming a significant amount of the CH_4 produced, and nearly as much CH_4 as was oxidized aerobically. The study reported an average AOM rate of 1.47 ± 0.22 μmol g dry peat^{-1} day^{-1}, or 47.1% of produced CH_4, and suggested that AOM is limited by CH_4 porewater concentrations (Smemo & Yavitt, 2007). In contrast, Blazewicz et al. (2012) observed an AOM rate in Alaskan peat of only 0.021 ± 0.002 μmol g dry peat^{-1} day^{-1}. A study of AOM across 15 peatlands in North America that span a 1500 km latitudinal gradient and vary in hydrology, vegetation, and soil chemistry was done by Gupta et al. (2013). AOM was observed in all peatlands included in the study, with minerotrophic fens having higher rates than ombrotrophic bogs, on average (Gupta et al. 2013). Rates of AOM were reported at a minimum of 0.017 μmol g dry peat^{-1} day^{-1} in a permafrost bog and a maximum of 0.511 μmol g dry peat^{-1} day^{-1} in a minerotrophic fen, with an overall average rate of 0.251 μmol g dry peat^{-1} day^{-1} for the 15 study sites (Gupta et al., 2013).

Studies have now identified TEAs being used in AOM in multiple freshwater ecosystems. A coupling between denitrification and AOM was identified in agricultural

runoff by Raghoebarsing et al. (2006). Iron-dependent AOM was demonstrated in Danish freshwater lake sediment (Nordi et al., 2103). Other studies have since added observations of AOM coupled to Fe reduction in freshwater systems, including peatlands (Ettwig et al., 2016, Miller et al., 2019). One study has even presented evidence suggesting that AOM could be occurring with the humic-fraction of organic matter acting directly as a TEA in a tropical, coastal, organic wetland (Valenzuela et al., 2017). Despite the building evidence for TEAs used in different ecosystems, the TEA at play in nutrient-poor peatlands remains elusive (Gupta et al., 2013).

Many of the studies on AOM in freshwater environments are not conducted in a manner to infer '*in situ*', and especially ecosystem or global scale, rates. One reason scaling up may not be appropriate is because there has been some evidence that AOM may be limited by CH_4 availability (Smemo & Yavitt, 2007), but most incubation experiments have not attempted to achieve *'in situ'* porewater CH_4 concentrations (Blazewicz et al., 2012, Gupta et al., 2013, Miller et al., 2019). It can also be a problem to scale up if only shallow depths are studied (Smemo & Yavitt, 2007, Blazewicz et al., 2012, Gupta et al., 2013, Segarra et al., 2015, Miller et al., 2019), or if only a small range of environmental conditions are captured, for example if measurements are only made in the summer (Blazewicz et al., 2012, Miller et al., 2019). Most AOM studies use long-term incubations and measure CO_2 as the product of AOM using a tracer (Blazewicz et al., 2012, Gupta et al., 2013, Miller et al., 2019). It is important to consider incubation length in these studies because they are long enough to be concerned with the tracer getting assimilated into biomass and being respired again as CO_2 (Gupta et al., 2013).

Based upon two seasonal AOM measurements in each of three wetlands along the East Coast of the USA, Segarra et al. (2015) scaled up to estimate that globally AOM in freshwater wetlands consumes about 200 Tg CH_4/year, reducing their possible CH_4 emissions by about 50% (Segarra et al., 2015). This estimate for AOM accounted for a soil depth of 40 cm (Segarra et al., 2015). Some freshwater systems have much deeper soil profiles, like northern peatlands which average depths of 1.3-2.3 m and have maximum depths of 15-20 m (Clymo et al., 1998, Turunen et al., 2002). If this greater depth were accounted for, the global estimate for AOM in freshwater wetlands could be much higher. Additionally, for the AOM incubation, samples headspaces were purged

with pure CH_4, to achieve high porewater concentrations (Segarra et al., 2013). This method probably gets close to *'in situ'* concentrations for some freshwater wetlands but is probably much too high for many of them. This study also only examined three sites, and scaling up makes a bold assumption that all freshwater wetlands will have similar rates to these three wetlands.

Altogether, evidence suggests that AOM is an important factor in CH_4 cycling across many freshwater ecosystems, including northern peatlands. This means that conventional methods of measuring CH_4 production may be underestimating gross CH_4 production if they assume no oxidation is occurring due to anaerobic conditions. It also suggests that measured fluxes and models based on the simplified CH_4 cycle are attributing all CH_4 consumption to aerobic oxidation, while anaerobic oxidation may be just as important. A much better understanding of AOM and the environmental factors that control its rates is needed to estimate global rates more accurately and to project how they might shift in relationship to CH_4 production with environmental change. The mounting evidence for AOM in freshwater systems also suggests the need for an updated model of CH_4 cycling in freshwater wetlands that incorporates AOM.

As part of a long-term, ecosystem scale, climate manipulation experiment, this study focused on CH_4 cycling, and specifically AOM, in a northern ombrotrophic bog (S1) undergoing a long-term whole-ecosystem manipulation of temperature and elevated atmospheric CO_2. We also incubated peat from depths down to 2 m, allowing us to study whether these processes are depth dependent. The main objectives of this study were to develop a method to simultaneously measure AOM and gross CH_4 production using a $^{13}CH_4$ tracer, measure AOM and gross CH_4 production to a depth of 2 m in a Minnesota bog, determine the relative importance of AOM within the site's CH_4 cycle, and identify whether depth, and temperature have a strong influence on AOM at S1 bog. All three chapters of this thesis were made possible with the contributions of co-author Scott Bridgham, and we plan on publishing chapter II along with co-authors Cory LeeWays, Anya Hopple, Jason Keller, and Paul Hanson.

II. DATA

The method for measuring AOM and gross CH4 production described in this chapter was primarily developed by myself and Scott Bridgham. Additional help in the lab and in the field was provided by Cory LeeWays and Anya Hopple. Jason Keller and Paul Hanson also helped with field work. This work was made possible by funding attained by Scott Bridgham and Jason Keller. Besides being the primary contributor to developing the method, I did all the calculations, analyzed and visualized the data, wrote the data chapter with editing from Scott Bridgham, and either conducted lab work myself or lead others in conducting lab work.

Introduction

Understanding global carbon (C) cycling in natural ecosystems has become increasingly important in the light of a changing climate. The methane (CH_4) cycle is a particularly influential part of the C cycle due to its potency as a greenhouse gas and, therefore, its ability to cause a positive feedback loop with climate (Dean et al., 2018). Wetlands are responsible for the largest portion of natural CH_4 emissions, contributing about a third of the total (Bridgham et al., 2013, Kirschke et al., 2013, Saunois et al., 2016). Northern peatlands are a globally significant source of CH_4 and store a disproportionately high amount of organic C for their areal extent making them of exceptional interest in terms of wetland C cycling (Frolking et al., 2006, Yu et al., 2012, Loisel et al., 2014).

Models of CH_4 cycling wetlands, and specifically peatlands, are important for predicting how CH_4 emissions could shift in the future (Cao et al., 1996, Arah & Stephen, 1998, Walter & Heimann, 2000, Zhuang et al., 2004). The major processes involved in these models are CH_4 production and aerobic consumption, which should equal net emissions to the atmosphere but do not always match up well with measured emissions (Potter, 1997, Arah & Stephen, 1998, Smemo & Yavitt, 2006). One of the possible reasons for this discrepancy is that these models assume that CH_4 consumption is not occurring in the anaerobic zone even though anaerobic CH_4 consumption has been shown

to occur in many marine systems (Hoehler et al., 1994, Michaelis et al., 2002, Treude et al., 2005, Moran et al., 2008, Knab et al., 2009, Beal et al., 2009).

Studies have been reporting observations of anaerobic oxidation of CH_4 (AOM) in marine systems since the 1970s and it has been identified as a key process in marine CH_4 cycling (Martens & Berner, 1974, Barnes & Goldberg, 1976). As much as 90% of the CH_4 produced in marine systems has been shown to be consumed through AOM, primarily using sulfate as a terminal electron acceptor (TEA; Barnes & Goldberg, 1976, Hinrichs & Boetius, 2002, Nauhaus et al., 2005, Beal et al., 2009). More recently studies have started to report evidence of AOM in freshwater systems like peatlands and lakes (Smith et al., 1991, Smemo & Yavitt, 2007, Nordi et al., 2013, Gupta et al., 2013, Martinez-Cruz et al., 2017). Specifically, evidence is building that AOM may act as an important constraint on CH_4 emissions in peatlands (Smemo & Yavitt, 2007, Blazewicz et al., 2012, Gupta et al., 2013, Segarra et al., 2015, Miller et al., 2019).

Many studies of AOM in freshwater systems implicitly underestimate AOM rates by conducting experiments at low CH_4 concentrations, not representative of *in situ* conditions, despite evidence that AOM is dependent on substrate availability (Smemo & Yavitt, 2007). Although some studies consider that AOM might be CH_4 limited, they do not aim for *in situ* concentrations during incubations and instead add enough CH_4 so the process should not be limited by its availability (Blazewicz et al., 2012). Additionally, these studies often have incubation periods that are lengthy enough to cause concern about microbial assimilation of the tracer into biomass later getting respired as CO_2 (Blazewicz et al., 2012, Gupta et al., 2013, Miller et al., 2019). It is also common for AOM to only be measured within the surface peat profile while peat deposits are often several meters deep (Turunen et al., 2002, Blazewicz et al., 2012, Segarra et al., 2015, Miller et al., 2019). Some of the studies that have examined AOM in northern peatlands take rates that were measured during incubation experiments and scale up to estimate for all northern peatland area (Gupta et al., 2013, Segarra et al., 2015). With the limitations of AOM incubation experiments mentioned above, the appropriateness of scaling up these incubation rates is questionable.

Accurate models of global CH_4 cycling are imperative for scientists to predict how climate change will alter CH_4 emissions. Because it is such a potent greenhouse gas, small shifts in atmospheric CH_4 concentrations could impact global climate and potentially create a positive feedback loop (Dean et al., 2018). It is becoming clear through mounting evidence that AOM is a key factor in CH_4 cycling and that it needs to be integrated into models to fully understand how it constrains CH_4 emissions (Knittel & Boetius, 2009, Smemo & Yavitt, 2011, Segarra et al., 2015). To do this, however, scientists need to gain a much stronger understanding of the environmental controls behind AOM and how rates will be impacted by climate change.

As part of a novel ecosystem-scale climate manipulation experiment in a northern ombrotrophic bog, we examined CH_4 cycling, and specifically AOM and gross CH_4 production, in the context of climate change using ^{13}C-tracer techniques. Our major objectives were to: (1) to develop a novel method to simultaneously measure AOM and gross CH_4 production under as in situ conditions as possible with a $^{13}CH_4$ tracer, (2) measure net and gross CH_4 production and AOM at depths down to 2 m in the peat profile across a wide range of *in situ* temperatures in a northern peatland, and (3) identify whether temperature or depth influenced rates of AOM.

Methods

Site Description

The Spruce and Peatland Response Under Changing Environments (SPRUCE) project is a novel ecosystem-scale climate manipulation experiment located in the USDA Forest Service Marcell Experimental Forest in S1 bog (8.1 ha) in north-central Minnesota (47°30.476’ N, 93°27.162’ W). S1 Bog is an ombrotrophic black spruce - *Sphagnum* bog with on average 2-3 m deep peat (Parsekian et al., 2012). The site was strip cut in 1969 and 1974, and the SPRUCE experimental chambers are in these strips with their smaller-statured and lower density trees.

The SPRUCE project was designed to be a long-term experiment that will continue for a decade. It was set up in a regression-based design with the aim of understanding how the bog will respond to different levels of warming and elevated CO_2. There are five warming treatments (0, 2.25, 4.5, 6.75, 9° C above to ambient) each with

two open-top enclosures, and one of these enclosures is treated with elevated CO_2 levels (+500 ppm). Deep peat heating was implemented in June 2014, heating the peat profile down to 3 m (Krassovski et al., 2015, Hanson et al., 2017). Whole ecosystem warming started in 2015 with warming of the air, and elevated CO_2 was initiated in 2016 (Hanson et al., 2017). Enclosures are 12 m diameter by 8 m tall and contain natural bog vegetation, including trees. Sub-surface corrals installed to the mineral soil hydrologically isolate each plot to allow for experimental feedbacks on the water-table level. The SPRUCE experimental protocol is discussed in-depth in Hanson et al. (2017) and the belowground geochemistry at S1 Bog is discussed in Tfaily et al. (2014).

Sample Collection

Peat and porewater samples were collected from S1 bog in August and October of 2018 at 5 depth increments (20-30, 40-50, 50-75, 100-125, and 175-200 cm) throughout the peat profile. Peat cores were collected using a 5 cm diameter peat auger. Three 25 mL glass serum vials were filled with about 7 g peat from each depth. Samples were then capped with thick blue butyl septa and aluminum crimp tops, flushed with N_2 for 10 minutes in the field, and then placed on ice.

Porewater was collected from 1.25 cm diameter PVC piezometers from depths corresponding to peat samples (30, 50, 75, 100, and 200 cm) in each plot (Wilson et al., 2016). Samples were collected using a peristaltic pump without exposure to the atmosphere during collection. Porewater was injected into N_2-flushed glass serum that were sealed with septa and crimp tops, and stored on ice.

Incubations

Samples were shipped overnight to the University of Oregon, where they were put into dark incubators set within 1° C of their *in situ* soil temperature from the previous week at S1 bog. Samples were randomly divided into two batches that were processed one day apart within 3 days of collection.

Samples were brought into an anaerobic glovebox with an atmosphere of N_2/H_2 (98%/2%), and porewater was added to corresponding peat until there was a headspace of about 5.5 cm^3. Before sealing the vials, we carefully stirred the slurry to make sure there were no bubbles in the peat. Three replicate samples were prepared for each depth in each plot. After the samples were taken out of the glovebox, they were bubbled with ultra-high

purity (UHP) N_2 for 10 minutes to ensure there was no remaining CH_4, and only a minimal amount of CO_2 left in the form of dissolved inorganic carbon. Samples were then placed back in their respective incubators. Killed controls were prepared by autoclaving about 7 g peat in serum vials and deionized water. These controls were treated exactly like live 75 cm samples throughout the incubation (see below).

After 48 hours of incubation (i.e., T1), we sampled the headspaces of one set of replicates for CH_4 and CO_2 concentrations using an SRI 8610c gas chromatograph with a flame ionization detector and a methanizer. These concentrations were used to calculate rates of net CH_4 and CO_2 production at *in situ* temperatures at low CH_4 concentrations. After samples were analyzed at T1, gas production, CH_4 was added at high concentrations that reflect *in situ* porewater concentrations (Zalman et al., 2018, Table 1) using the ideal gas law and Henry's law calculations. ^{13}C-labeled CH_4 (99% ^{13}C; Sigma-Aldrich) was added as a stable isotope tracer at a ratio of 1:2 $^{13:12}CH_4$. Two of the three replicates for each sample were injected with this isotopic ratio, while the third replicate was injected with the same volume of only $^{12}CH_4$ as a live control.

Depth (cm)	**CH_4 Concentration (mM)**
30	0.25
40	0.35
75	0.45
125 & 200	0.70

Table 1. Typical S1 bog porewater CH_4 concentrations at the depths sampled for the AOM incubation (Zalman et al., 2018).

To confirm the initial concentration of CH_4 in the samples for the AOM incubation, we sampled headspaces of the vials again on the gas chromatograph 30 minutes to an hour after CH_4 addition (i.e., T2). At this stage we also took 20 random headspace samples to measure the initial isotopic signature of headspace CH_4. Using a

N_2-flushed syringe with a stopcock, we removed 0.3 cm^3 from headspaces and injected them into UHP N_2-flushed, 120 mL glass serum vials sealed with blue butyl rubber septa for storage and appropriate dilution for analysis (see below). Then, samples were placed back into incubators for another 48 hours for the AOM incubation.

At the end of this incubation period, the headspaces of all vials were sampled again for CH_4 and CO_2 concentrations (i.e., T3). We also took a sample from each headspace and injected them into UHP N_2-flushed vials to measure final $^{13}CH_4$ isotopic signature. All isotopic gas samples were analyzed using a Picarro Small Sample Isotope Module II (SSIM II), employing injection through a syringe. The analytical error of this injection method was tested with four groups of five replicate samples, each group with a different atom percent, and we found the instrument be accurate within an average of 0.024 ± 0.0044 atom percent for the range measured in our experiment. Lastly, we measured pH of the samples.

Calculations

Total concentrations of CH_4 and CO_2 in the samples were calculated using Henry's Law and pH. Before measuring CH_4 and CO_2 on the gas chromatograph, we measured the pressure inside each vial to correct for the volume of headspace lost inside the pressure gauge, we took a set of 5 vials, added 1cm^3 gas to the headspace and calculated what the pressure should be. We repeated this adding 2, 3, 4 and 5 cm^3 gas to the headspace, and plotted the results to form a standard curve with measured pressure versus percent of total gas in headspace lost. Using this equation, we were able to use the pressure that we measured before measuring gas concentration, and back-calculate what the total concentration was before the pressure was taken.

AOM consumes $^{12}CH_4$ and $^{13}CH_4$ in proportion to their availability except for a small discrimination factor (1.012‰, Martens et al., 1999). Consequently, we calculated the AOM rate based on the disappearance of $^{13}CH_4$ using equation 1 for all live samples (i.e., labeled and unlabeled) and the labeled dead controls (Fig. 1). The production of $^{13}CH_4$ in the live controls was very small relative to change in $^{13}CH_4$ in the labeled samples, so this correction made little difference in the final rates. In contrast to AOM, methanogenesis primarily produces $^{12}CH_4$, again except for a small discrimination factor (-60‰ or 1.05 atom %, Quay et al., 1988, Whiticar, 1993). Consequently, gross CH_4

production was also calculated for all samples and controls using equation 2 based upon the dilution of the $^{13}CH_4$ by the predominant production of $^{12}CH_4$ during the incubation (Fig. 1). Preliminary calculations with "dummy data" with widely varying initial CH_4 concentrations and rates of both methanogenesis and AOM showed that using the final CH_4 concentration for the gross CH_4 calculation provided rates within a few percent of the actual rate in all scenarios. This approach has the advantage that gross CH_4 calculations were not dependent on small changes in net CH_4 concentrations at the very high CH_4 concentrations that we used, and we observed larger changes in atom percent.

Equation 1:

$$AOM = \frac{\left(\left(\frac{A\%\ ^{13}CH_{4,L,T2}}{100} * \mu mol\ CH_{4,L,T2}\right) - \left(\frac{A\%\ ^{13}CH_{4,L,T3}}{100} * \mu mol\ CH_{4,L,T3}\right) - \left(\left(\frac{A\%\ ^{13}CH_{4,C,T2}}{100} * \mu mol\ CH_{4,C,T2}\right) - \left(\frac{A\%\ ^{13}CH_{4,C,T3}}{100} * \mu mol\ CH_{4,C,T3}\right)\right)\right) * \mu mol\ CH_{4,L,T2}}{(\mu mol\ ^{13}CH_{4,L,T2} - umol\ ^{13}CH_{4,C,T2}) * g\ dry\ peat * day}$$

Where: A% = atom %, L = ^{13}C labeled samples, C = control ^{12}C samples, T2 = initial AOM time point, T3 = final AOM time point.

Equation 2:

$$Gross\ CH_4\ Production = \frac{\left(\left(\frac{A\%\ ^{13}CH_{4,L,T2}}{100} - \frac{A\%\ ^{13}CH_{4,L,T3}}{100}\right) * \mu mol\ CH_{4,L,T3}\right)}{\frac{A\%\ ^{13}CH_{4,L,T2}}{100}}$$

Dead controls had rates of AOM and gross CH_4 production that fell within the range of the live samples within the batch they were run with. We examined the equations extensively and checked our assumptions experimentally in the lab by quantifying recovery of $^{13}CH_4$ and $^{12}CH_4$ in autoclaved deionized water samples run the

same way as the live samples (see next section). Total CH_4 concentration in dead controls changed by an average of 3.4 and 10.8% in August and October, respectively, between initial and final measurements. Unfortunately, we were not able to explain what caused these rates in dead controls. Consequently, we subtracted the average rate of dead controls from the batch of vials they were run with for both rates.

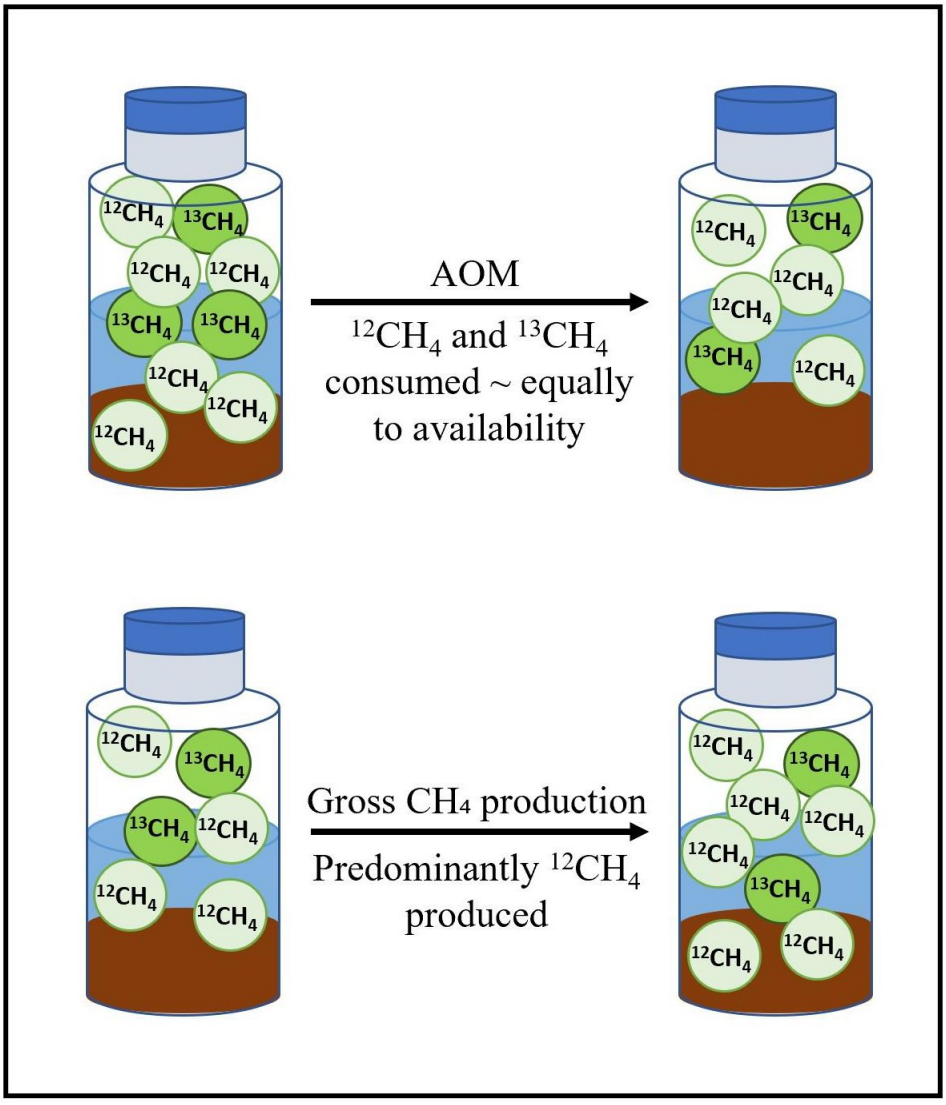

Figure 1. Graphic of AOM and gross CH_4 production illustrating how atom% in samples is altered by each of these processes.

Determining Limit of Detection

Because the net change in CH_4 concentration is a parameter in the AOM calculation and uncertainty about our ability to detect small changes in CH_4 at very high concentrations, we quantified our analytical sensitivity to measure net changes in CH_4. Using the same size vial and headspace as the AOM experiments but filled with only deionized water, vials were injected with one of four concentrations of CH_4 spanning the range used in our experiments. Headspace CH_4 concentrations were measured on the gas chromatograph after sitting for 24 hours at room temperature. We compared the measured CH_4 concentrations with the amount of CH_4 that we added to the vial and used the difference to calculate standard deviation (5.15%) for recovery of CH_4. Then, we performed a 1-tailed t-test for 80, 90, and 95% confidence intervals which were used to determine our limit of detection (LOD) for changes in CH_4 concentration in the incubations. The final LOD was a change of 8.9%, 6.8%, and 4.4% in total CH_4 concentration for 95%, 90% and 80% confidence that a real change in concentration occurred.

Data analysis

Data for net CH_4 and CO_2 production, and gross CH_4 production were log-transformed to improve their distribution. Rates of AOM and net change in CH_4 at *in situ* concentrations were already normally distributed. All rates were analyzed using mixed effects linear models in R (package nlme, version 3.4.1). Models were run for net CH_4 and CO_2 production, AOM rates (for 95, 90, and 80% confidence intervals), net change in CH_4 at high concentrations, and gross CH_4 production using temperature as a continuous variable, depth as a categorical variable, and plot as a random variable. Tukey's tests were used to examine significant differences ($p < 0.05$) between depths when appropriate.

Results

CH_4 & CO_2 production at low concentrations

Net production of CH_4 at low CH_4 concentrations (i.e., T1) decreased with depth (Fig. 2, $p < 0.0001$) with or without a 30 cm outlier (value 2.85 μmol g dry peat^{-1} day^{-1}), and increased with temperature ($p < 0.0001$), but the temperature effect depended upon depth ($p = 0.021$). Methane production was greatest in the 30 cm depth ($p < 0.001$) but

did not differ among the other depths. Methane production increased with temperature in both surface and deep samples (Fig. 3, p = 0.029 and Fig. 4, p = 0.0001), but with a steeper slope in the surface peat. In contrast, neither depth (Fig. 5, p = 0.500) nor temperature (p = 0.113) affected the production of CO_2.

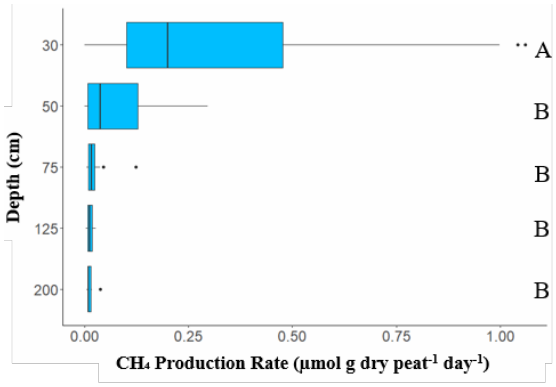

Figure 2. Net CH_4 production by depth at low initial CH_4 concentrations (excluding one outlier from the 30 cm depth).

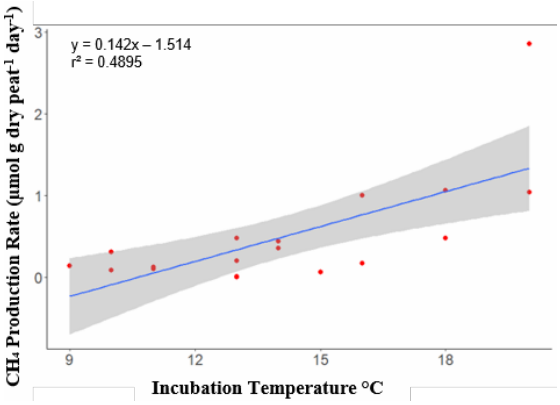

Figure 3. Net CH_4 production relative to temperature for the 30 cm samples at low initial CH_4 concentrations.

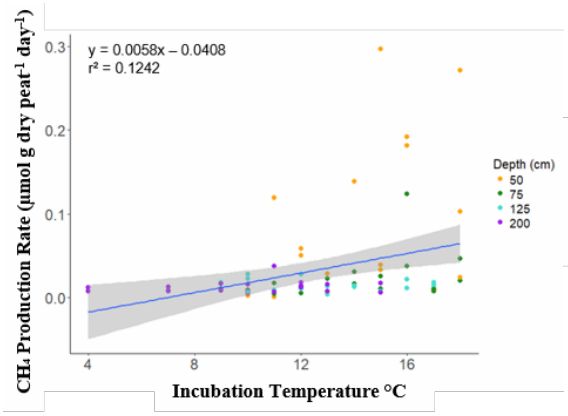

Figure 4. Net CH_4 production relative to temperature for samples from 50, 75, 125, and 200 cm at low initial CH_4 concentrations.

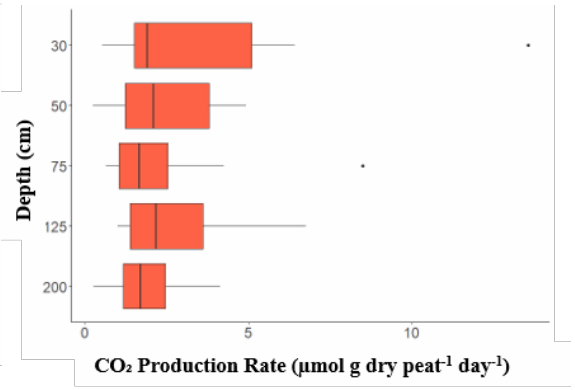

Figure 5. CO_2 production plotted by depth for all samples at low initial CH_4 concentrations.

Net change in CH_4 at high concentrations

The net change in CH_4 at high CH_4 concentrations (i.e., T3) generally decreased with depth (Fig. 6, p = 0.005) but was not affected by temperature (p = 0.63). Rates from

the top 75 cm of the profile were higher than those from 125 cm (p = 0.015, 0.03, 0.02) and samples from 200 cm were not different from any other depth (Fig. 6). Generally, CH_4 concentrations increased over the incubation (62.5% positive), but 37.5% of the rates had decreasing CH_4 concentrations, generally because of the subtraction of the high rates in the dead controls. Rates of net change in CH_4 at high concentrations had no notable relationship with CH_4 production rates at low CH_4 concentrations.

The net change in CH_4 was qualitatively similar when a conservative 95 % analytical confidence interval was applied to the data. The net change in CH_4 decreased with depth (Fig. 7, p = 0.012) but was not influenced by temperature (p = 0.220). Rates of net change in CH_4 at 125 cm were lower than those at 50 and 75 cm (p = 0.05, 0.02) and neither 30 nor 200 cm were different from other depths (Fig. 7). For rates of net change in CH_4, 60.4% were positive, 33.3% were negative, and six (6.3%) had no net change in CH_4. Generally similar results were obtained using 90% and 80% analytical confidences (Fig. S1 and S2).

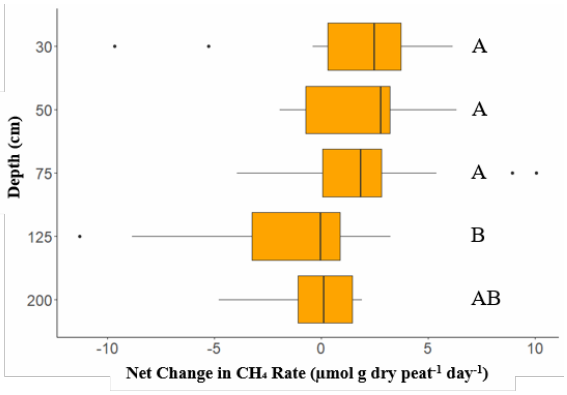

Figure 6. Net change in CH_4 concentration (no confidence interval applied) by depth.

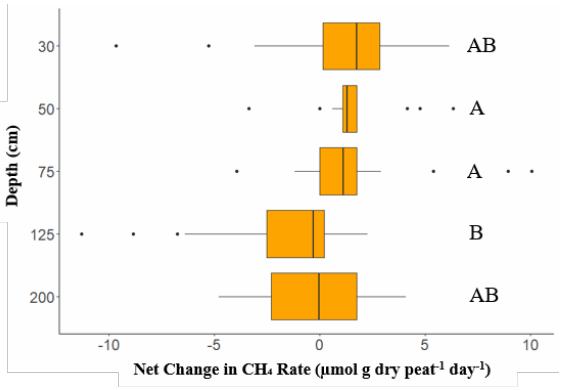

Figure 7. Net change in CH_4 concentration with 95% analytical confidence interval by depth.

Change in Atom %

The greatest decrease in atom percent $^{13}CH_4$ occurred in the surface 30 cm increment (Fig. 8, p < 0.0001), whereas there was no difference among the other depths with or without one outlier. Methane production should decrease the atom percent because primarily because about 99.95% of what is produced is $^{12}CH_4$, but there was only

a decrease in the 30 cm depth (p =0.005), no significant change at the 50 cm depth (p = 0.35), and an increase at 75, 125, and 200 cm depth (p = 0.086, 0.001, 0.0005). Temperature did not influence change in atom percent (p = 0.34).

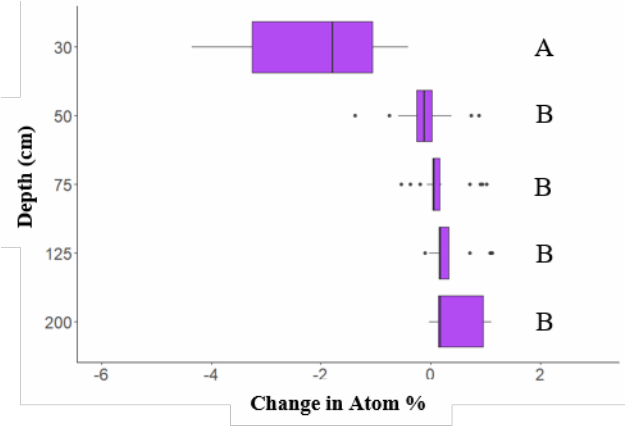

Figure 8. Net change in atom % by depth, excluding one outlier (value = -16.9%).

AOM

Rates of AOM did not vary by depth (Fig. 9, p = 0.138) or temperature (p = 0.143) when no analytical confidence interval for the net change in CH_4 concentration was applied. When a 95% confidence interval was used, depth (Fig. 10, p = 0.011) but not temperature (p = 0.902 affected AOM rates. However, only depths of 50 and 125 cm were different from each other (p = 0.105). Rates were positive at 30 cm using no analytical confidence interval (p = 0.05), negative at 50 cm depth using a 95% confidence interval (p = 0.039), and positive at 125 cm under no analytical confidence interval or a 95% confidence interval (p = 0.08, 0.06), while rates at 75 and 200 cm were never different from zero (p = 0.36, 0.47). Using either no analytical confidence interval for net change in CH_4 or a 95 % confidence interval, 60.4% of the rates were negative. Using 80% and 90% confidence intervals gave similar results (Fig. S2). Generally, as net change in CH_4 increased AOM decreased (Figs. 11,12).

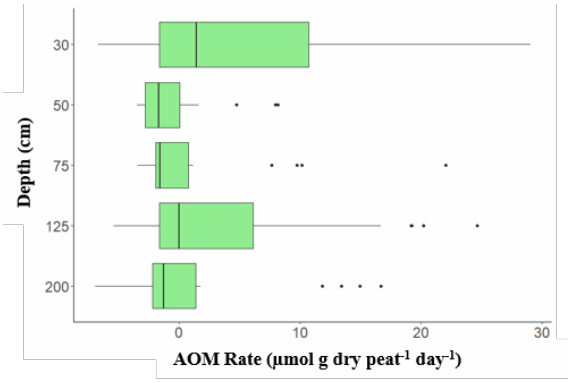

Figure 9. AOM rates (no analytical confidence interval applied) by depth.

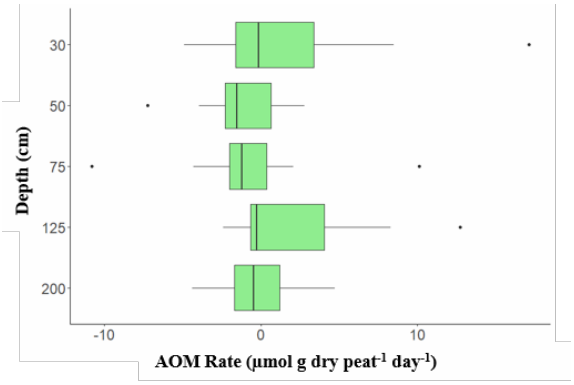

Figure 10. AOM rates using 95% analytical confidence interval by depth.

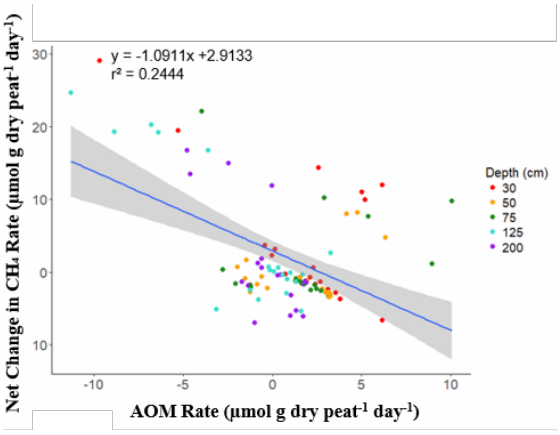

Figure 11. AOM rates relative to net change in CH_4 (no analytical confidence interval applied) by depth.

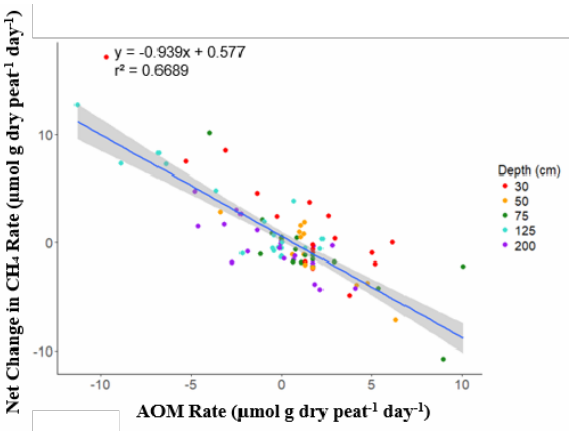

Figure 12. AOM rates relative net change in CH_4 (95% analytical confidence interval applied) by depth.

Gross CH_4 Production

Gross CH_4 production decreased with depth (Fig. 13, $p < 0.0001$) and increased with temperature ($p < 0.0001$) but the temperature effect was depth-dependent ($p < 0.0001$). Surface (30 cm) samples had higher rates of gross CH_4 production than all other depths ($p < 0.01$) and depths below the surface did not differ from each other. Depths of 30 and 50 cm had positive rates of gross CH_4 production ($p = 0.0005, 0.053$) while rates for depths of 75, 125, and 200 cm were not different from zero ($p = 0.14, 0.48, 0.24$). Mean and median gross CH_4 production at the surface (2.641 and 1.025 µmol g dry peat^{-1}

day^{-1}) were about 77 and 8 times as great as those in deep (50, 75, 125, 200) samples, respectively (0.034 and 0.132 µmol g dry peat^{-1} day^{-1}). Gross production of CH_4 increased with temperature for both surface samples (Fig. 14, $p < 0.0001$) and deep samples (Fig. 15, $p = 0.035$). Rates of AOM had a positive relationship with gross CH_4 production (Figs. 16, 17), but there was an odd dispersion of outliers in the middle of these regressions that were identified as samples from one of the August batches. There was no notable relationship between gross CH_4 production and net change in CH_4 at high concentrations. Methane production under low concentrations, however, had a positive relationship with gross CH_4 production at high CH_4 concentrations (Fig. 18).

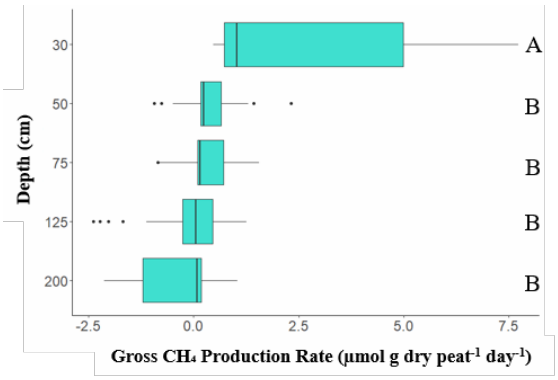

Figure 13. Gross CH_4 production by depth.

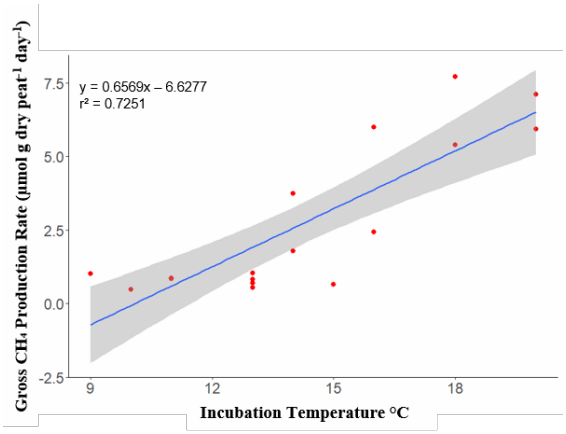

Figure 14. Gross CH_4 production relative to temperature for samples from 30 cm.

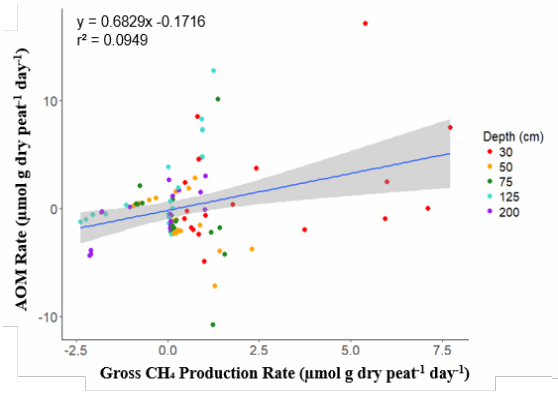

Figure 15. Gross CH_4 production relative to temperature for samples from 50, 75, 125, and 200 cm.

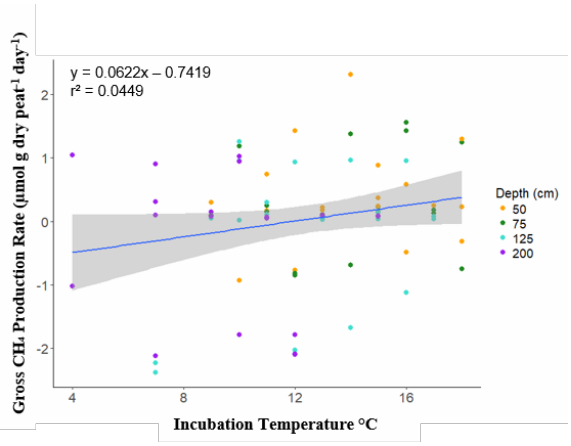

Figure 16. Gross CH_4 production relative to AOM (no analytical confidence interval applied) for all depths.

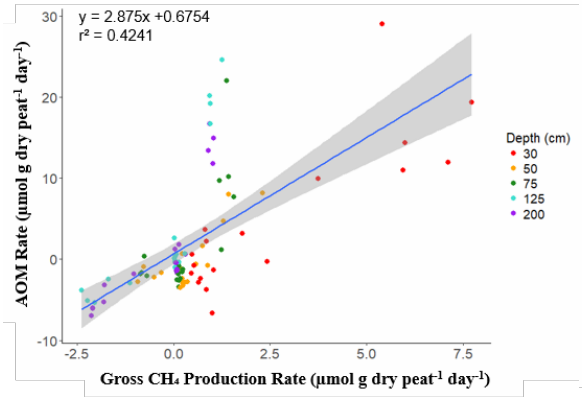

Figure 17. Gross CH_4 production relative to AOM (for 95% analytical confidence interval) for all depths.

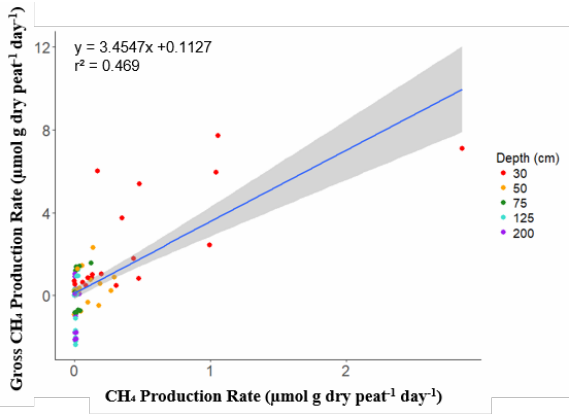

Figure 18. Gross CH_4 production at high CH_4 concentrations relative to CH_4 production under low concentration for all depths.

Discussion

Comparison of AOM methods

The traditional method for measuring CH_4 production starting with a N_2-flushed headspace is often considered a gross rate, although some AOM could potentially be occurring (Smemo & Yavitt, 2006, van Winden et al., 2012). If AOM is dependent on CH_4 availability like some studies suggest, it is probably very limited during incubations that start with a N_2 headspace meaning it likely is close to a gross process (Smith et al., 1991, Smemo & Yavitt, 2007, van Winden et al., 2012). However, some studies have noticed that potential CH_4 production and aerobic consumption do not match well with measured emissions, and AOM could be one explanation for this (Smemo & Yavitt, 2006). Negative net CH_4 production has also occasionally been observed in incubations of peat from S1 bog when starting with a N_2-flushed headspace (Hopple, 2018).

Most studies that examine AOM in freshwater systems spike samples with $^{13}CH_4$ and measure the increase of $^{13}CO_2$ as the product of AOM over time (Blazewicz et al., 2012, Gupta et al., 2013, Miller et al., 2019). Although using the increase of $^{13}CO_2$ may be a more straightforward way of measuring AOM than measuring the disappearance of $^{13}CH_4$, this method is not without limitations. Longer-term incubations are used to measure the production of $^{13}CO_2$ over time amidst the much higher $^{12}CO_2$ production that is happening in the background (Table 2; Blazewicz et al., 2012, Gupta et al., 2013). These long-term incubations are valuable, but also introduce the noise of potential recycling of ^{13}C that may have accumulated as microbial biomass. However, one study observed no ^{13}C enrichment of peat due to microbial assimilation after 3 days of incubation, suggesting that our samples should have had no $^{13}CH_4$ produced due to turnover of biomass during the 48-hour incubation period (Gupta et al., 2013).

Study	Length of Incubation	CH_4 in Porewater (mM)	AOM Rate (μmol g dry peat^{-1} day^{-1})	% of Gross CH_4 Production Consumed by AOM
Gupta et al. 2013	40 days	0.039	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	(Alaska): 0.047 (Puerto Rico): 0.11	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	0.10	Mean: 2.32	Mean: 29.5 Range: 25 - 34
Martinez-Cruz et al. 2017	204 days	0.10	Mean: 0.11	Mean: 32
Segarra et al. 2015	1 day	NA	Mean (overall): 0.13 Range: 0.10 – 1.71	Mean: 91.3 Range: 78.1 – 98.9
Smemo & Yavitt 2007	15 days	Varying concentrations; NA	Mean: 1.47 Range: 0.086 - 15.2	Mean: 41.7 Range: 17.4 – 63.5

Table 2. Compares different studies in freshwater systems of AOM and their methods, including incubation length, porewater CH_4 concentration (when enough information was available to calculate; NA if not available). We used the average bulk density from S1 top 0.5 m (0.155 g cm^{-3}) to convert rates expressed in cm^3 to g.

Another possible limitation of many AOM studies in peatlands is that only surface peat is examined (Smemo & Yavitt, 2007, Blazewicz et al., 2012, Gupta et al., 2013, Segarra et al., 2015, Miller et al., 2019). Peatlands are known to have depths much deeper than the 0.5 m that most studies look at, with average depths of 1.3 – 2.3 m (Turunen et al., 2002). Both CH_4 production and pooling of CH_4 are known to occur in some peatlands to at least a depth of 2 m (Smemo & Yavitt, 2006, Clymo & Bryant, 2008, Zalman et al., 2018), so it is possible that AOM plays an important role in CH_4 cycling at depths beyond the surface 0.5 m.

One limitation of our study was the use of analytical confidence intervals for detecting changes in CH_4 concentrations as a conservative measure for AOM rates because of the reduced ability to measure small changes in CH_4 at very high concentrations. We also had to subtract the AOM rates of dead controls from live samples which may have reduced the accuracy of calculated rates. However, even after applying the most conservative confidence interval (95%) and subtracting rates from dead controls, the average rates we calculated exceed many of the rates that have been measured in other studies (Table 2).

In future experiments we would measure both consumption of $^{13}CH_4$ and the production of $^{13}CO_2$, as the latter may prove to be the more sensitive method for measuring AOM. We also realized that although AOM may be limited by the availability of CH_4, the method for measuring AOM may also be limited by too high of CH_4 concentrations which can lead to reduced sensitivity in detecting changes in CH_4 concentrations. We are also interested in looking more closely into whether AOM is concentration dependent in future experiments.

CH_4 production and AOM rates

Most studies that look at AOM and gross CH_4 production calculate gross production rates by adding AOM rates to measured net CH_4 production values (Gupta et al., 2013, Miller et al., 2019). Our method for measuring gross production using the dilution of $^{13}CH_4$ was more direct, and we were interested in comparing the net rates of change in CH_4 that we measured at *in situ* concentrations to a net rate calculated as the difference between AOM and gross CH_4 production. Rates calculated by difference and those that were measured were closely related, especially when a 95% analytical confidence interval for change in CH_4 concentration was applied, and the slope was reasonably close to 1 (Figs. 19, 20). Like measured rates of net change in CH_4, rates calculated by difference showed no relationship to CH_4 production rates that were measured starting with a N_2 headspace.

Gross CH_4 production under *in situ* concentrations was reasonably well correlated with net CH_4 production starting with a headspace of N_2 ($r^2 = 0.47$, Fig. 18), which provides some level of confidence in the gross CH_4 production technique. However, gross CH_4 production was 5.4 times higher at the surface and 5.2 times higher accounting

for the whole 2 m peat profile than CH_4 production starting with a headspace of N_2. Thus, it is possible that relatively high rates of AOM occurred even in incubations starting with a N_2-flushed headspace. Giving further credence to this supposition, we have previously observed occasional net consumption of CH_4 in S1 Bog when starting incubation with a N_2 headspace (Hopple, 2018).

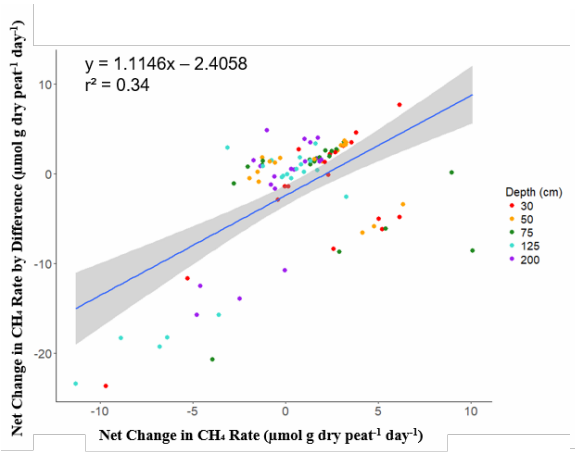

Figure 19. Measured net change in CH_4 plotted against net change in CH_4 calculated by the difference between AOM and gross CH_4 production.

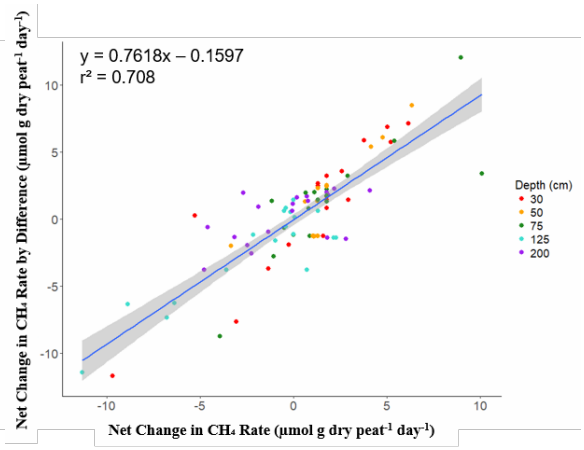

Figure 20. Measured net change in CH_4 (for 95% analytical confidence interval) plotted against net change in CH_4 calculated by the difference between AOM and gross CH_4 production.

In 2013 another study observed AOM in S1 bog in peat samples down to 45 cm, estimating a rate between 0.056 ± 0.003 and 0.092 ± 0.007 µmol g dry peat^{-1} day^{-1} (Table 2; Gupta et al., 2013). In comparison, our study estimates a higher average rate of 4.76 ± 2.21 (using no analytical confidence interval) and 1.701 ± 1.223 µmol g dry peat^{-1} day^{-1} (using the analytical 95% confidence interval) when accounting for samples to a depth of 30 cm, and between 2.18 ± 1.65 (using no confidence interval) and 0.24 ± 0.69 µmol g dry peat^{-1} day^{-1} (using the 95% confidence interval) when accounting for a depth of 50 cm. Average surface AOM rates in our study span a range that includes the average AOM rate measured by Smemo and Yavitt (2007) in a variety of northern peatlands (Table 2). One possibility for why some of our average rates are higher than those measured by

Gupta et al (2013) could be because our study had higher concentration of CH_4 during incubation (Table 2); the incubation was also substantially shorter (2 vs. 40 days) in our attempt to more closely approximate *in situ* rates. With some studies indicating the AOM is limited by the availability of CH_4 (Smith et al., 1999, Smemo & Yavitt, 2007), lower CH_4 porewater concentrations could lead to lower rates of AOM.

This same study calculated an average gross CH_4 production rate of 0.158 ± 0.32 μmol g^{-1} d^{-1} during the first twenty days of their incubation (Gupta et al., 2013). In comparison, our study measured a much higher average gross production rate of 2.641 ± 0.611 μmol g^{-1} d^{-1} for peat when accounting for the top 30 cm and 1.50 ± 0.36 μmol g^{-1} d^{-1} from the top 0.5 m of the peat profile in S1 bog. Gross production of CH_4 was also measured in an Alaskan peatland and an average rate of 0.144 μmol cm^3 d^{-1} was estimated, which is also comparatively low to our study (Miller et al., 2019). Gross CH_4 production rates in both the aforementioned studies are closer to our studies average net CH_4 production rates of 0.494 ± 0.035 and 0.288 ± 0.085 μmol g^{-1} d^{-1} that were measured starting with a N_2 headspace for the surface 30 cm and 50 cm, respectively.

Methane production was highest at the surface for net CH_4 production starting with a N_2 headspace and for gross CH_4 production. Two previous studies at S1 Bog show higher CH_4 production rates at the surface at low concentrations, supporting our results (Wilson et al., 2016, Hopple et al., in prep). We also found that CH_4 production became negative in deep peat when porewater CH_4 was increased from very low to *in situ* concentrations. The only way for gross CH_4 production to be negative is for the atom percent of $^{13}CH_4$ to increase over the incubation, which we saw at 125 and 200 cm (Equation 2, Fig. 8, $p < 0.0001$). Other than measurement error, if CH_4 production and AOM are the only processes affecting the isotopic composition of CH_4, the only way for this to occur is for rates of AOM to be much larger than CH_4 production given that about 99.95% of CH_4 production is as $^{12}CH_4$ and the small isotopic discrimination of AOM against $^{13}CH_4$ (1.012‰, Martens et al., 1999). In support of this idea, the net change in CH_4 at higher CH_4 concentrations were negative and in directional agreement with gross CH_4 production below 1 m depth. In contrast, rates of CH_4 production under low CH_4 concentrations were positive but very small below 50 cm depth. Other studies have

observed similarly low rates of CH_4 production in the deep peat of northern peatlands, including S1 bog (Putkinen et al., 2009, Tfaily et al., 2014, Wilson et al., 2016, Hopple et al., in prep). High CH_4 concentrations at depth under *in situ* conditions may well enhance AOM and inhibit methanogenesis.

Rates of gross CH_4 production that exceed AOM rates are how pools of CH_4 form within the peat profile. At S1 bog, porewater CH_4 concentrations are highest at 125 and 200 cm (Table 1), but in our study both gross and rates of net change in CH_4 agree that more CH_4 is being consumed than produced at these depths. Studies have found modern dissolved organic carbon in deep peat and have attributed this to downward advection (Corbett et al., 2013). Similarly, vertical diffusion could be occurring with CH_4 throughout the peat profile and could explain how CH_4 pools as deep as 2 m even when net consumption is occurring (Clymo & Bryant, 2008).

Gross CH_4 production rates were generally higher in our study than AOM rates (that used analytical confidence intervals) down to 1 m depth (Fig. S3). Below 1 m depth AOM increased and gross production became negative, indicating net consumption of CH_4. AOM consumed between 39.5 – 64.4% of the gross CH_4 production in the top 30 cm of the peat profile, and between 35.6 - 77.1% of the gross CH_4 production down to 2 m depth (Figs. 21, 22). Another study reported AOM to consume as much as 64% of gross CH_4 production during incubations with Minnesota peat (Smemo & Yavitt, 2007, Table 2). Globally, in freshwater wetlands, it is estimated that AOM could be consuming over 50%, or 200 Tg CH_4 annually, of the potential annual CH_4 emission rate of 127-227 Tg CH_4 estimated from these ecosystems (Segarra et al., 2015, Table 2). Northern peatlands alone have been estimated to have annual AOM rates of 1.6-49 Tg CH_4 (Gupta et al., 2013). These results strongly suggest AOM is an important constraint on global CH_4 emissions.

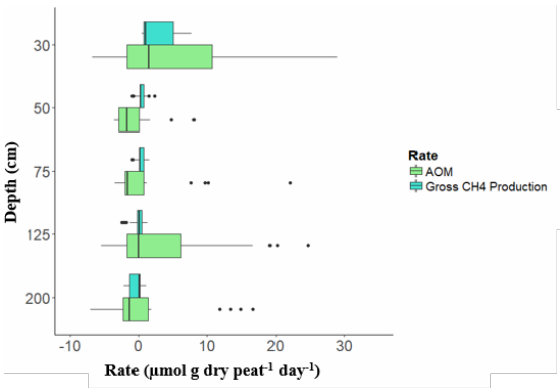

Figure 21. Boxplots comparing AOM rates using no confidence interval to rates of gross CH_4 production by depth.

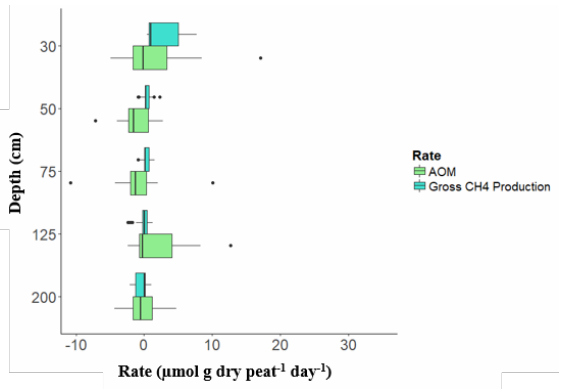

Figure 22. Boxplots comparing AOM rates using the 95% confidence interval to rates of gross CH_4 production by depth.

Temperature effects

Considering this study was conducted within the context of climate change at the SPRUCE project, we were particularly interested in how the processes we measured varied over a range of temperatures (4 - 20° C). Rates of CH_4 production starting with a N_2 headspace and gross CH_4 production starting with *in situ* CH_4 concentration were both positively affected by increased temperature (Figs. 3, 4, 14, 15). This observation is upheld by research from many studies that have closely linked temperature to CH_4 production (Dunfield et al., 1993, Walter & Heimann, 2000, Saunois et al., 2016, Wilson et al., 2016, Dean et al., 2018, Hopple et al., in prep). However, CO_2 production, AOM, and net change in CH_4 starting with *in situ* CH_4 concentrations did not vary with temperature. Another study done at the SPRUCE site in S1 bog found temperature to have no effect on CO_2 flux or CO_2 production rates below 75 cm (Wilson et al., 2016). However, this study did see CO_2 production rate increase with temperature in the surface 25 cm (Wilson et al., 2016). Additionally, a more recent study at the SPRUCE site found temperature to increase CO_2 production in peat below 75 cm (Hopple et al., in prep). The effect of temperature on AOM rates may have been offset by the increase in CH_4 concentration with depth. The complicated interplay between AOM and gross CH_4 production described above may also have caused the lack of a temperature response in

the net change in CH_4 at *in situ* concentrations. Another potential explanation for why AOM and net change in CH_4 were not related to temperature could be our use of analytical confidence intervals for calculating these two processes. This is because some values were identified by confidence intervals as having no net change in CH_4 when they may have changed but not enough to meet our cut-off criteria.

Conclusion

We present novel methods for measuring AOM and gross CH_4 production at high *in situ* concentrations of porewater CH_4 with a short incubation period. While the high concentrations of porewater CH_4 presented challenges in determining meaningful net changes in CH_4 over time, multiple lines of evidence suggest that the methods gave meaningful results. While there is no comparable method for directly measuring gross CH_4 production, it would be useful in the future to compare the consumption of $^{13}CH_4$ to the production of $^{13}CO_2$ in determining AOM rates at high porewater CH_4 concentrations. We were perplexed by the high rates of AOM in the dead controls despite multiple experiments showing good recovery of added $^{13}CH_4$ in deionized water. It would also be useful to further explore if some abiotic mechanism is consuming $^{13}CH_4$ in our experimental protocol.

Anaerobic oxidation of CH_4 has been shown to be globally important as a part of CH_4 cycling in freshwater wetlands, even in nutrient-poor systems like northern peat bogs where availability of inorganic terminal electron acceptors is scarce (Smemo & Yavitt, 2007, Blazewicz et al., 2012, Gupta et al., 2013, Miller et al., 2019). Our study lends to the body of evidence that AOM is occurring in northern peat bogs, and that it is occurring at significant rates compared to measured gross production. Even as evidence of AOM as a global constraint of CH_4 emissions mounts, there is a gap in the knowledge concerning how AOM will be affected by future climate change. It is likely that CH_4 production will increase in some ecosystems as temperature increases, but it is important to understand how AOM will shift to accurately predict future CH_4 emissions.

III. CONCLUSION

I wrote the conclusion chapter with editing provided by Scott Bridgham.

The evidence presented in this study supports the idea that AOM is an influential part of the CH_4 cycle in freshwater wetlands. We presented a new method for measuring both AOM and gross CH_4 production under more *in situ* incubation conditions. Moreover, gross CH_4 production using the new method agreed reasonably well with calculated gross production by difference of AOM and net change in CH_4, providing some confidence in the method. Moreover, gross CH_4 production rates were higher at the surface and lower at depth (125 & 200 cm) than CH_4 production starting with a N_2 headspace, suggesting that traditional CH_4 production techniques may be underestimating CH_4 production at the surface and CH_4 consumption in deep peat.

Models of CH_4 cycling in freshwater wetlands would be more accurate if they incorporated AOM, considering the evidence that it could be consuming over half of potential CH_4 production, which is supported by our study (Smemo & Yavitt, 2007, Segarra et al., 2015). This would be especially true if improved incubation techniques lead to AOM and gross CH_4 production rates that are closer to those occurring *in situ*. Considering the potency of CH_4, having a strong understanding of how the ecosystems that account for the largest portion of natural emissions cycle CH_4 is of great importance (Bridgham et al., 2013, Kirschke et al., 2013, Saunois et al., 2016). Greater accuracy in modeling of CH_4 cycling allows scientists to better predict how emissions will shift with changing climate and whether they will play into a positive feedback loop with climate (Dean et al., 2018). More research needs to be done on AOM in freshwater wetlands, especially peatlands, examining environmental controls over the process so it can be appropriately included in CH_4 models.

Peatland ecosystems, their CH_4 emissions, and their vast stores of old organic carbon rely on cold temperatures and waterlogged conditions for stability (Clymo, 1984, Clymo et al., 1998, Yu et al., 2012). Changes in climate and the CH_4 cycle in peatlands could lead to an increase in CH_4 emissions (van Winden et al., 2012, Wilson et al., 2016, Hopple et al., in prep) with the potential to affect global climate (Dean et al., 2018). These facts highlight the need for more accurate models of CH_4 cycling in peatlands that

include AOM so scientists can better predict how these methanogenic ecosystems will respond to future climate change.

APPENDIX

SUPPLEMENTAL FIGURES

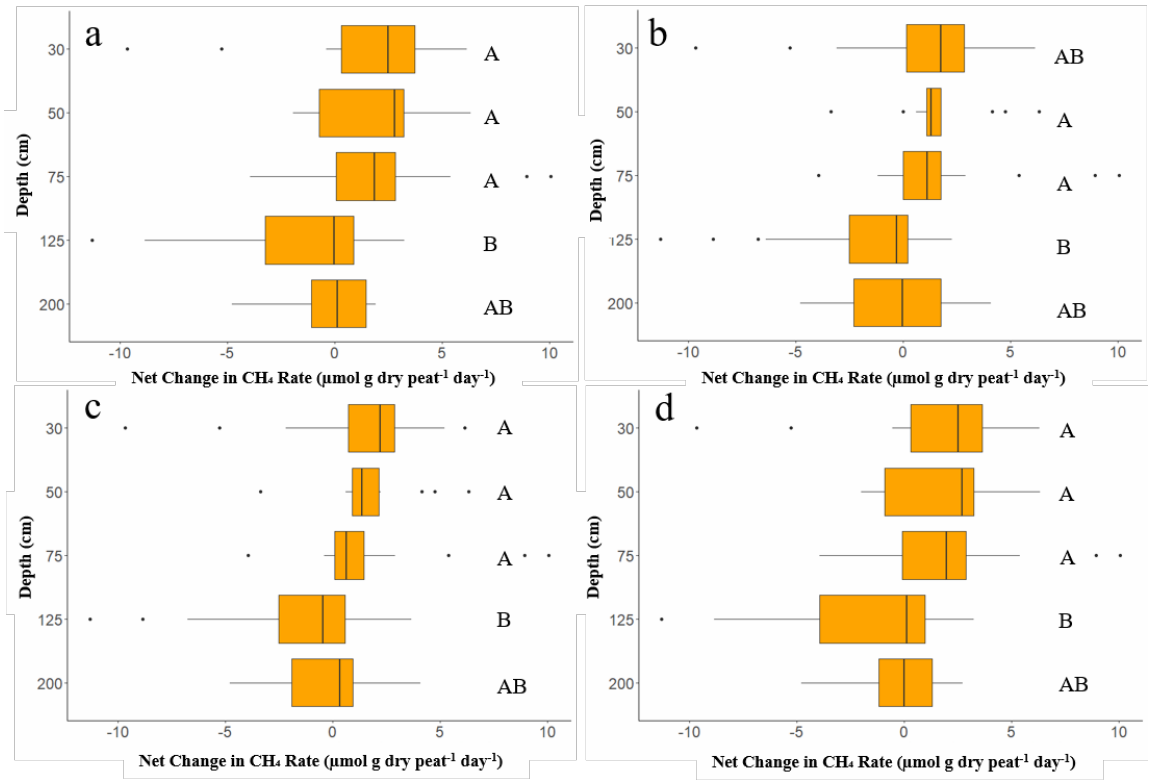

Figure S1. Net change in CH_4 for a) no analytical confidence interval, b) 95% confidence interval, c) 90% confidence interval, and d) 80% confidence interval for all depths. Capital letters indicate significant differences between depths.

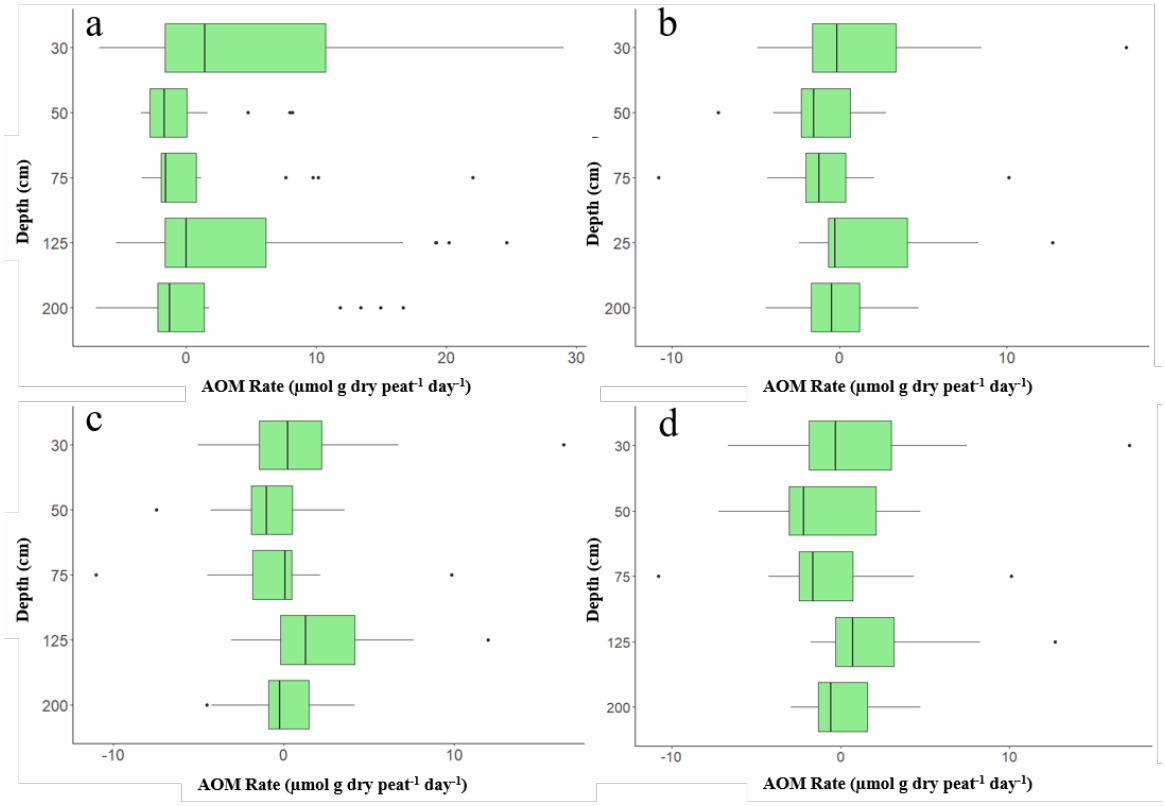

Figure S2. AOM for a) no analytical confidence interval, b) 95% confidence interval, c) 90% confidence interval, and d) 80% confidence interval for all depths.

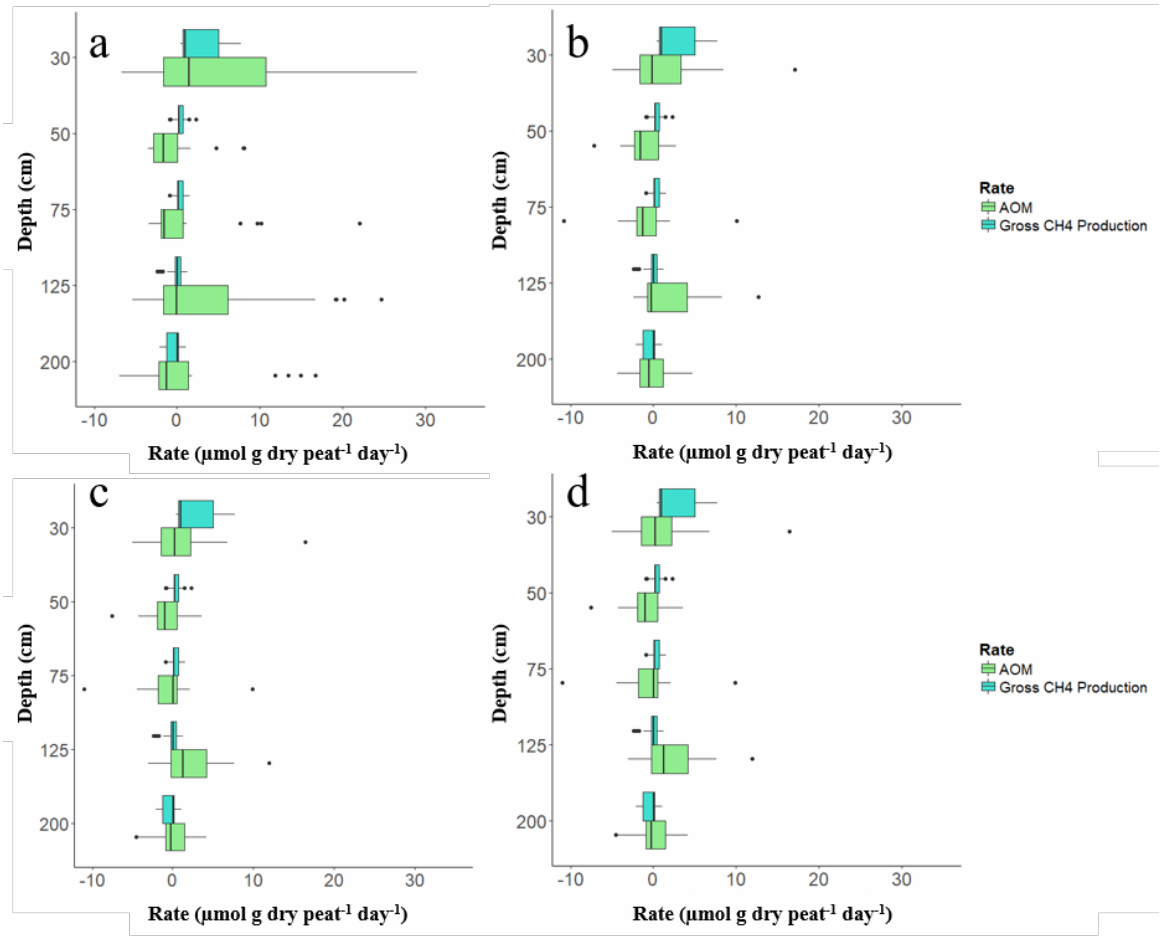

Figure S3. Rates of AOM for a) no confidence interval, b) 95% confidence interval, c) 90% confidence interval, and d) 80% confidence interval plotted with Gross CH_4 production for all depths.

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