

UNDERSTANDING ANAEROBIC OXIDATION OF METHANE IN
A CLIMATE-MANIPULATED NORTHERN PEATLAND

By KAITLIN BRUNIK

BIOLOGY & ENVIRONMENTAL SCIENCE HONORS THESIS

Presented to the Department of Biology and the Environmental Studies
Program, University of Oregon, in partial fulfillment of the requirements
for the degree of Bachelor of Science

06/01/17

THESIS DEFENSE COMMITTEE APPROVAL

Approved: Scott Bridgham Date: 6/13/17

Dr. Scott Bridgham, Primary Adviser

Approved: Cristin Hulslander Date: 6/13/17

Dr. Cristin Hulslander, Biology Honors Faculty Representative

ABSTRACT

Although peatlands cover < 3% of the Earth's surface, they are among the most important terrestrial ecosystems partially because they are responsible for roughly 10% of global methane (CH₄) flux. The consumption of CH₄ (methanotrophy) is an important control on wetland emissions of this greenhouse gas. Anaerobic oxidation of methane (AOM) was thought to be unimportant in peatlands; however, recent studies suggest that this process is ubiquitous in freshwater wetlands, but report a wide range of rates of AOM in peatlands. Due to the lack of understanding of the magnitude and controls over AOM, it is not currently included in Earth system models. The Spruce and Peatland Responses Under Climatic and Environmental Change (SPRUCE; <http://mnspruce.ornl.gov>) experiment is assessing how northern peatland ecosystems react to a changing climate with a regression-based, ecosystem-scale climate manipulation that incorporates surface and deep (up to 2 m depth) peat heating from 0 to +9 °C above ambient. Soil cores were collected throughout the 2016 growing season following 13 months of deep peat heating and 14 months of subsequent whole-ecosystem warming (surface and deep heating) at 30, 50, 75, 125, and 200 cm depths from each enclosure at the SPRUCE site. Samples were slurried with a 1:3 mixture of peat and porewater (collected from the same plot and depth) and anaerobically incubated within 1°C of in situ temperatures for approximately three weeks using a radioactive tracer method. AOM was measured by the accumulation of tritiated water over time and CH₄ production rates were determined with gas chromatography. We found that AOM was ubiquitous throughout the entire peat profile, with the highest rates occurring at the surface and then decreasing with depth. Additionally, rates of AOM were the greatest at the beginning of the incubation and decreased over time, indicating that organic or inorganic terminal electron acceptors may be driving this process in peatlands. Finally, there was suggestive evidence that

temperature is positively correlated with rates of AOM. Collectively, these results suggest that AOM may be important process in northern peatlands, warranting further study and consideration in Earth system models.

ACKNOWLEDGEMENTS

I would like to say thank you to everyone who has helped me through this long journey of completing an honors thesis. First off I would like to thank Scott Bridgham for accepting me into his lab and providing such a wonderful experience to learn and grow. Thank you to Anya Hopple for her guidance and patience in teaching me everything I know about lab protocol to mentoring me through my first thesis. Many thanks to the entire lab, including Laurel Pfiefer-Meister, for their constant encouragement and feedback. I would like to thank Jason Keller and Glenn Woerndle for guiding me at the SPRUCE field site. Thank you to Peter O'Day for supporting my research with the University of Oregon OURS program. Lastly, but most certainly not least, a HUGE thank you to my family who constantly took interest in my research and was always there to encourage me along the way and my friends for their continuous support. This research was supported by U.S. Department of Energy's Office of Science DE-SC0008092, NICHD Summer Research Program at the University of Oregon NIH-R25HD070817 and the University of Oregon Alden Award.

TABLE OF CONTENTS

INTRODUCTION	7
MATERIALS AND METHODS	13
SPRUCE PROJECT	13
SITE DESCRIPTION	14
FIELD SAMPLING METHOD	15
INCUBATION	15
RADIOACTIVE TRACER TECHNIQUE	16
QUENCH	17
DATA ANALYSIS	18
RESULTS	19
SEASON EFFECT	19
DEPTH EFFECT	19
TEMPERATURE TREND	20
AOM THROUGH TIME	20
DISCUSSION	20
SEASON EFFECT	20
DEPTH EFFECT	21
AOM THROUGH TIME	22
TEMPERATURE TREND	22
AOM COMPARED TO PRODUCTION	23
CONCLUSION	25
REFERENCES	27
FIGURES AND TABLES	30

INTRODUCTION

Wetland ecosystems are defined by three main characteristics: the presence of hydrologic indicators, hydric soils, and biota adapted to live in the wet/anaerobic conditions. Wetland soils are saturated with water, which limits oxygen availability and slows the rate at which microbes can break down organic matter (Meronigal et al., 2004). Thus, imbalances between net primary production and decomposition lead to the vast accumulation of soil organic matter in wetlands, particularly in peat-forming wetlands (peatlands) which store approximately 500 Pg of carbon (C) globally, representing roughly one third of terrestrial soil C stocks (Bridgman et al., 2006). Though peatlands can sequester large amounts of C, they are also disproportionately responsible for methane (CH₄) flux from wetland ecosystems.

Wetland ecosystems emit 15-40% of global CH₄ emissions, making them the single largest natural source of CH₄ (Denman et al., 2007). Despite covering < 3% of the Earth's surface, peatlands account for roughly 10% of global CH₄ flux (Abdalla et al., 2016). Methane (CH₄) is an extremely potent greenhouse gas with 45 times the sustained-flux global warming potential of CO₂ over a 100-year timeframe (Neubauer & Meronigal, 2015). This means that a certain mass of CH₄ will trap 45 times as much heat in the atmosphere as an equivalent mass of CO₂. Thus, minor changes in peatlands CH₄ emissions can have important implications for current and future global climate.

Most peatlands are found above 40°N latitude where the largest temperature changes are projected to occur over the next century (Kirtman et al., 2013). It is largely presumed that as global temperatures increase, peatlands will produce more greenhouse gases, including CH₄, generating a positive feedback loop that further enhances global temperature. The Spruce and Peatland Responses Under Climatic and Environmental Change (SPRUCE) experiment is an

interdisciplinary research project run by the Oak Ridge National Laboratory and the US Forest Service aimed at assessing how northern peatland ecosystems react to a changing climate. To enhance mechanistic understanding of C cycling, the SPRUCE experiment implements a regression-based, ecosystem-scale climate manipulation that incorporates surface and deep peat heating in northern peatlands in Minnesota. One objective of the SPRUCE project is to facilitate the development of Earth system models which aim to project the total CH₄ flux from an ecosystem.

CH₄ flux represents the difference between CH₄ production and consumption. CH₄ is produced in peatlands through a series of microbially-mediated steps (Figure 1). First, soil microorganisms receive a biopolymer input, such as lignin and/or cellulose, from plant litter, which is then transformed by exo-cellular enzymes into a less complex monomer, such as glucose and/or other simple sugars. The monomers then undergo microbial fermentation that produces low molecular weight fatty acids and alcohols. A secondary fermentation reaction or acetogenesis further breaks down the fatty acids and alcohols into CO₂ and H₂ or acetate. Methanogenic archaea are then able to utilize these substrates to produce CH₄ through two different respiratory pathways, hydrogenotrophic or acetoclastic methanogenesis. In hydrogenotrophic methanogenesis, H₂ is oxidized into CH₄ and CO₂ acts as the terminal electron acceptor whereas in acetoclastic methanogenesis, acetate is split into CH₄ and CO₂.

CH₄ that is produced in peatlands follows several different emission pathways (Figure 2), including diffusion, ebullition, or plant-mediated transport into the atmosphere. Plant-mediated transport is responsible for 30-100% of CH₄ flux (Bridgham et al., 2013), while a portion of the remaining CH₄ undergoes oxidation reactions that produce CO₂, which is subsequently released to the atmosphere. Despite wetlands being largely anaerobic, aerobic oxidation of CH₄ (Figure 3)

is an important process in CH₄ cycling and has been shown to consume between 40% and 70% of gross CH₄ production in wetlands (Megonigal et al., 2004).

In other systems, anaerobic oxidation of methane (AOM) is an important process, consuming as much as 90% of the CH₄ produced in marine systems (Bridgham et al., 2013). The mechanism for AOM in marine environments has been linked to aggregates of methanogenic bacteria and sulfate reducing bacteria (SRB), which have been found in a few different marine sediments (e.g. Boetius et al 2000; Orphan et al., 2001; Michaelis et al., 2002). In the sediments SRB use sulfate as an alternative terminal electron acceptor to complete anaerobic oxidation of CH₄ rather than oxygen, which is only found in aerobic environments. Since freshwater ecosystems tend to have low concentrations of sulfates and sulfate was regarded as the sole oxidant in anaerobic environments, AOM was thought to be negligible in these ecosystems. New research in freshwater ecosystems has found other terminal electron acceptors such as nitrate (Hu et al, 2014), iron (Crowe et al. 2011), manganese (Zehnder et al., 1980) capable of driving AOM in freshwater environments, leading to new paths of research regarding CH₄ cycling.

Historically, it was thought that AOM was largely unimportant in peatlands because these ecosystems typically lack high concentrations of inorganic terminal electron acceptors (Caldwell et al., 2008). Recent studies have observed AOM in peatlands; although, the mechanism is unknown. It could be either inorganic and/or organics TEAs TEAs (Smemo & Yavitt, 2011; Hu et al., 2014) or reverse methanogenesis (Blazewicz et al., 2012) TEAs act as substitutes for O₂ in anaerobic conditions and are used by microbes to oxidize CH₄. Reverse methanogenesis occurs when the enzymes that carry out methanogenesis act in reverse to consume CH₄ rather than produce it. However, while AOM could prove to be a significant mechanistic constraint on CH₄

emissions from these ecosystems, it is currently not included in Earth system models due to a lack of understanding of the magnitude and controls over this process.

Few studies have investigated AOM in freshwater wetlands (Table 1). Gupta et al. (2013) was one of the first studies to look at AOM across latitude and peatland type. The locations of their 15 peatland sites ranged subarctic Canada (James Bay Lowlands) to the cool-temperate United States (central Appalachians). They found the AOM to be quantitatively important across the 15 different sites, with the highest rates of AOM occurring in the 5A' Fen in James Bay Lowlands, Canada (day 20 = $0.61 \pm 0.45 \mu\text{mol CH}_4 \text{ g soil}^{-1} \text{ d}^{-1}$, day 40 = $0.41 \pm 0.042 \mu\text{mol CH}_4 \text{ g soil}^{-1} \text{ d}^{-1}$). While Gupta et al. (2013) did not find any significant relationship between climate factors or latitudinal position, they found that AOM rates varied significantly by peatland (site-averaged AOM rates range = $0.024 \pm 0.0026 - 0.61 \pm 0.45 \mu\text{mol CH}_4 \text{ g soil}^{-1} \text{ d}^{-1}$). The highest rates of AOM occurred in fens which they hypothesized to be because the groundwater supply brings in new TEAs to sustain AOM. To better understand the mechanisms controlling AOM in wetlands, Gupta et al. (2013) added TEAs to the incubation bottles and measured porewater ion concentrations. They found AOM was not predicted by the addition of TEAs (sulfate, nitrate or ferric iron) or correlated with porewater ion concentrations, although they found that James Bay Lowlands, which had the highest rates of AOM, also had the highest concentrations of sulfate. The researchers suggested that, under certain conditions, AOM is carried out with sulfate acting as the TEA although they acknowledged that it is perhaps more likely that organic substrates act as TEAs.

Gupta et al. (2013) also found that AOM rates were not predicted by CH_4 production rates, which was the opposite of Blazewicz et al. (2012) who found the rate of AOM to be strongly correlated with the amount of CH_4 produced. Blazewicz et al. (2012) studied AOM in

tropical peat (Puerto Rico) and boreal peat (Alaska) and found AOM to be quantitatively important at both sites. Higher rates of AOM occurred in the boreal peat ($0.021 \pm 0.002 \mu\text{mol CH}_4 \text{ g soil}^{-1} \text{ d}^{-1}$) than the tropical peat ($0.0029 \pm 0.0005 \mu\text{mol CH}_4 \text{ g soil}^{-1} \text{ d}^{-1}$). Blazewicz et al. (2012) added TEAs during their experiments and found both CH_4 production and AOM were inhibited by the addition of TEAs; however, after some time both AOM and methanogenesis began. Blazewicz et al. (2012) hypothesized that AOM was mediated by methanogens in the peat completing reverse methanogenesis, the enzymatic reverse of CH_4 production.

Smemo & Yavitt (2007) were also unable to determine the mechanism for AOM but they suggested that humic acids act as TEAs in carbon-rich systems, such as peatlands. Smemo & Yavitt's (2007) found averaged rates of AOM ($1.47 \pm 0.22 \mu\text{mol CH}_4 \text{ g soil}^{-1} \text{ d}^{-1}$) in a New York minerotrophic fen to be much higher than site-averaged rates reported by Gupta et al. (2013) and by Blazewicz et al. (2012). Along with the minerotrophic fen in New York, Smemo & Yavitt (2007) also looked at three fens in Minnesota, dominated by *Sphagnum* spp., and a mixed mire in northern Sweden. They found that AOM is spatially and temporally variable, with higher rates of AOM occurring in minerotrophic fens that are not *Sphagnum*-dominated.

AOM has long been known to be an important process in marine environments, but it was thought to be insignificant in freshwater systems due to lower availability of suitable TEAs. However, Segarra et al. (2015) found rates of AOM in freshwater wetlands ($0.2 \mu\text{mol CH}_4 \text{ g soil}^{-1} \text{ d}^{-1}$) that were comparable to those observed in marine systems. Additionally, they found AOM occurring up to 40 cm into the soil profile. They found the highest rates in tidal freshwater wetlands in Georgia ($>2.65 \pm 0.09 \mu\text{mol CH}_4 \text{ g soil}^{-1} \text{ d}^{-1}$), where it was suggested that sulfate was the primary mechanism for AOM, although a fraction of the AOM was likely carried out using alternative TEAs which were readily available in the Georgia soils. Segarra et al. (2015)

suggested these high rates of AOM in freshwater wetlands were a result of sulfate reduction potentially acting as an alternative TEA, but found that the addition of TEAs suppressed AOM.

In attempts to elucidate the mechanism by which AOM is carried out, Shi et al. (2017) compared rates of AOM in cultivated rice paddy-peatlands that received long term nitrogen fertilizer inputs to those of adjacent undisturbed peatlands in northeastern China. AOM occurred at both sites and the rice paddy-peatland, with nitrate additions, had higher rates of AOM than those without nitrate additions. Shi et al. (2017) suggest that denitrification-dependent AOM might be the mechanism, but rates of AOM also likely depend on the amount of nitrate applied to the wetland, along with the depth nitrate is able to penetrate in the anoxic layer. They also found that rates of oxidation decreased logarithmically with time over the course of the incubations, suggesting that TEAs are involved in AOM and are being depleted over the incubation period. Hu et al. (2014) looked at a natural wetland, an urban wetland, and a cultivated wetland (nitrogen fertilizer inputs) in southeastern China. They found that rates of AOM increased in all wetlands with the addition of nitrite, but not with the addition of sulfate. Other studies (Smemo & Yavitt, 2007) have acknowledged denitrification- and nitrate-dependent AOM as potential mechanisms by which AOM is carried out, but note that most northern peatlands are nitrogen limited and, thus, lack the necessary TEAs.

The few studies looking at AOM in wetlands have shown that it is occurring, but there are still many questions to be answered about the magnitude of this process and the controls that govern it. The goal of my thesis is to provide a more holistic view of peatland CH₄ cycling in northern peatlands in the face of climate change. AOM experiments across season, depth, and climate manipulation were carried out using peat samples collected from the Spruce and Peatland Responses Under Climatic and Environmental Change experimental site in northern Minnesota. I

hypothesized that 1) AOM will be an important process throughout the whole peat profile (down to 2 m depth), with higher rates at the surface, 2) increasing temperature will increase rates of AOM, and 3) rates of AOM will decrease through time, suggesting the utilization of TEAs in AOM.

MATERIALS AND METHODS

SPRUCE Project

The Spruce and Peatland Responses Under Climatic and Environmental Change (SPRUCE) experiment is an interdisciplinary research project run by the Oak Ridge National Laboratory and the US Forest Service (Hansen et al., 2017). The experiment is in a *Picea mariana* [black spruce] and *Sphagnum* spp. dominated bog in northern Minnesota, in the Marcell Experimental Forest (47°30.1710 N, 93°28.9700 W). The overall objective of the SPRUCE experiment is to assess the response of a northern peatland ecosystem to changes in climate with a regression-based, ecosystem-scale climate manipulation that incorporates surface and deep (> 2 m) peat heating.

Ecosystem warming is achieved via large open-top enclosures (12 m diameter by 8 m high) that combine deep peat heating and air warming. There are 10 enclosed plots, 2 plots receive no heat treatment (controls) and 8 plots receive elevated temperature treatments at +2.25, +4.5, +6.75 and +9 °C above the recorded readings in the ambient plots. Vertically installed below-ground heaters warm deep peat via electrical resistance heating. The air temperature is maintained above ambient via propane-fired heating and ventilation units. The open enclosures allow for natural inputs from the environment including precipitation (rain and snow), while still allowing for fine-scale climate manipulation (Krassovski et al., 2015).

Site Description

The SPRUCE experimental site (S1 Bog) is an 8.1-ha raised dome peat bog that was harvested in two successive strips cut that were 5 yards apart in 1969 and 1974 (Verry et al. 1981). The bog is ombrotrophic, meaning it receives water and nutrient inputs primarily from atmospheric sources. The bog has a perched water table and there is little regional groundwater influence (Hansen et al. 2016). The peat ranges in depth from 2 to 3 m throughout the experimental site, but can be as deep as 9 m in some small areas (Parsekian et al. 2012). The peatland has well-decomposed acidic peat (pH ~4) to varying depths and is overlain by 30–100 cm of less decomposed peat (Boelter and Verry 1977). S1 bog has a sub-humid continental climate that experiences extreme temperature fluctuations both diurnally and seasonally (Verry et al. 1988). Over the last 40 years, mean annual air temperature has increased by about 0.4°C per decade (<http://nrs.fs.fed.us/ef/marcell/data/>).

The experimental site has a hummock and hollow microtopography at the surface, with a typical relief ranging between 10 and 30 cm between the tops of the hummocks and the bottoms of the hollows. The bog is dominated by the tree species *Picea mariana* (commonly known as black spruce). The bryophyte layer is dominated by various species of *Sphagnum* moss. *Sphagnum magellanicum* is found on the drier hummocks and *S. angustifolium* in the hollows. The understory supports ericaceous shrubs, which prefer acidic soils, including evergreen shrubs as well as *Vaccinium angustifolium* (deciduous common blueberry). Sedges such as *Carex trisperma* and *Eriophorum spissum* (cotton grass), as well as forbs *Sarracenia purpurea* (northern pitcher plant) and *Smilacina trifolia* (three-leaved false Solomon's seal) are found dispersed throughout the bog (Tfaily et al. 2014).

Field sampling method

Soil cores were collected at 30, 50, 75, 125, and 200 cm below the soil surface from each enclosure at the SPRUCE site in June, July, August, and October 2016. Surface peat (0-30 cm) was collected using a modified hole saw and the deeper peat (30-200 cm) was collected using a Russian peat corer (Tfaily et al. 2014). Approximately 8 g of peat was taken from the core at each depth and placed in a 72 mL serum bottle, which was immediately sealed and flushed with N₂ for 15 minutes to remove any remaining oxygen.

Porewater samples were collected from 1.25 cm-diameter PVC piezometers installed at 25, 50, 75, 100, and 200 cm depths in each enclosure using a peristaltic pump. Stagnant porewater was pumped out of the PVC piezometers 24 hours prior to sample collection. Approximately 30 mL of anaerobic porewater were collected at each depth. The porewater along with the peat samples were stored on ice during overnight shipment to the University of Oregon.

Incubation

Anaerobic incubations were done within 1°C of in situ temperatures using samples slurried with a 1:3 mixture of peat and porewater collected from the same plot and depth. Samples were slurried in an anaerobic chamber with a N₂ atmosphere (<5% H₂ in the presence of a palladium catalyst, COY) approximately 24 hours after field collection from S1 bog. Next, they were flushed with N₂ for 15 minutes to begin the incubation. 0.8 µCi of ³H-CH₄ were added to each incubation bottle 2 days into the incubation period, after the first gas measurement was taken, which allowed CO₂ and CH₄ to build up in the headspace. One mL headspace and aqueous samples were then collected four times over the course of 2-3 weeks (4, 6, 10, 18 days from the beginning of the incubation), with headspace CH₄ and CO₂ analyzed simultaneously using an SRI gas chromatograph equipped with a methanizer and flame ionization detector. Aqueous

samples were placed in the freezer after collection and kept frozen until they were pre-treated (method described below).

Heat-killed peat samples served as controls and were autoclaved to stop any biological activity and incubated using the same procedure. They were incubated at the median temperature of the S1 samples.

Radioactive tracer technique

We adapted the radiotracer technique used in this experiment to measure anaerobic CH₄ oxidation from Valentine et al. (2001). In the incubation bottles the ³H-label of CH₄ is converted to ³H-H₂O via anaerobic oxidation of CH₄. 1mL aqueous samples were analyzed by liquid scintillation counting. Prior to analysis on the liquid scintillation analyzer, the samples were pretreated to remove dissolved tritiated CH₄.

Initially, attempts were made to drive off the dissolved tritiated CH₄ by bubbling the samples in the scintillation vials for 15 minutes with N₂. After bubbling, the heat killed controls still showed elevated levels of radioactivity. The next proposed solution to drive off the dissolved tritiated CH₄ from the samples was to alter the dissolution state of the dissolved CH₄ by adding 1mL of 4 M KCl solution to the scintillation vial. The samples were placed under a strong vacuum (> -9 psi) for 48 hours to remove any residual ³H-CH₄ that came out of the samples because of the salting. After 48 hours, the samples were removed from the vacuum and 3 mL of 50% Scintisafe cocktail was added and allowed to react with the sample overnight. The levels of radioactivity in the samples were measured on the liquid scintillation analyzer. Salting and placing the samples under vacuum significantly reduced the levels of radioactivity in the heat killed controls; the remaining radioactivity was due to background hydrogen isotopic exchange, where the ³H-label hydrogen in the CH₄ exchanges places with hydrogen on the H₂O

molecule. This process occurs randomly as molecules in the samples move around and bump into each other; thus, levels of hydrogen isotopic exchange were measured in heat killed controls and accounted for as control radioactivity and then subtracted out during calculations of sample rates of AOM.

Quench

Quench occurs in samples when the liquid scintillation analyzer is unable to detect the energy being emitted by the radioisotope because the signal is reduced via physical quench, chemical quench or color quench. As a result, inaccurate levels of radioactivity are measured in each sample. To account for the peat chemistry and colored, dissolved organic matter's effect on the radioactivity measured, 0.3 mL of 0.001 μCi of $^3\text{H}\text{-H}_2\text{O}$ stock solution was added to each vial after its initial run on the scintillation counter and allowed to react for an hour. The samples were then re-measured on the scintillation counter post-addition. Without quench we would expect to see a 0.0003 $\mu\text{Ci}/\text{mL}$ increase in radioactivity measured and with quench the actual radioactivity measured post-addition is lower. The percent variance of each sample was used when accounting for quench in the calculations.

$$\% \text{ Variance} = \frac{\text{Actual radioactivity}(\mu\text{Ci}/\text{mL}) - \text{Expected radioactivity}(\mu\text{Ci}/\text{mL})}{\text{Expected radioactivity}(\mu\text{Ci}/\text{mL})} \times 100$$

Actual radioactivity = radioactivity measured post-addition

Expected radioactivity = initial radioactivity measured + 0.003 $\mu\text{Ci}/\text{mL}$

Data analysis

The amount of CH₄ anaerobically oxidized was calculated using the equation below.

$$\text{CH}_{4\text{ox}} (\mu\text{mol}) = ({}^3\text{H}_2\text{O} (\mu\text{Ci}) / \text{C}^3\text{H}_{4\text{added}} (\mu\text{Ci})) * \text{CH}_{4\text{ave}} (\mu\text{mol})$$

CH_{4ox} (μmol) = the amount of CH₄ that was anaerobically oxidized

³H₂O (μCi) = the total amount of radioactivity/bottle, measured as ³H₂O

C³H_{4added} (μCi) = the amount of tritiated radioactivity added as CH₄ = 0.8 μCi

CH_{4ave} (μmol) = the amount of available CH₄, averaged between the amount at the time of addition (T1) and the amount at the time of measurement (Tx)

To calculate the total amount of radioactivity per sample bottle, ³H₂O (μCi), the initial radioactivity measured was corrected for sample quench, control radioactivity and tritium decay and then multiplied by the amount of porewater in the sample. Sample quench was calculated on a per sample basis as noted above. Control radioactivity was determined from median radioactivity of 8 control sample measured throughout the 2016 growing season. Tritium decay was corrected on a sample basis from when the tritium was assayed to when the sample was measured on the liquid scintillation analyzer.

$$\text{Rate of AOM} (\mu\text{mol g}^{-1} \text{d}^{-1}) = \text{CH}_{4\text{ox}} (\mu\text{mol}) / \text{dry peat weight (g)} / \text{time elapsed (days)}$$

I analyzed the data using the statistical package IMB SPSS Statistics version 23 (IMB Corp, 2015). I used a repeated measures ANOVA and, when appropriate, Tukey's HSD, to

determine the effects of season, depth, incubation time-point, and temperature on rates of CH₄ oxidation. For all analysis, except CH₄ oxidation over incubation time, measurements 48-hours after the tracer was added were used to examine the relationship between AOM and the independent variables. CH₄ oxidation over incubation time analyzed the first three measurements (2, 4, 8 days after the tracer was added). I used a general linear model along with regression analysis to determine the relationship between rates of CH₄ oxidation and incubation temperature.

RESULTS

Season effect

In June and July 2016, we observed AOM occurring across all experimental treatments and depths, except for three samples in June 2016 that were below our detection limit (detection limit $\approx 1.0 \times 10^{-6}$ $\mu\text{mol dry g peat}^{-1} \text{ day}^{-1}$; Table 2). AOM rates in June and July 2016 ranged from 1.84×10^{-6} to 0.43 and 6.80×10^{-6} to 0.0052 $\mu\text{mol dry g peat}^{-1} \text{ day}^{-1}$, respectively. In August and October 2016, AOM rates were below detection except for three samples collected from one of the warmest enclosures (+9°C) that consumed 0.00052 $\mu\text{mol CH}_4 \text{ g soil}^{-1} \text{ d}^{-1}$ (30 cm) and two that averaged 0.00042 ± 0.0003 $\mu\text{mol CH}_4 \text{ g soil}^{-1} \text{ d}^{-1}$ (50 cm) in October 2016. Accordingly, AOM rates for August and October were not included in the statistical analysis. We found no significant effect of month on rates of AOM in June and July (repeated measures ANOVA: $F_{1,23} = 0.372$, $p = 0.548$), and depths effects (see below) were also not dependent on month (repeated measures ANOVA: $F_{1,4} = 0.339$, $p = 0.849$).

Depth effect

In June and July 2016, AOM rates decreased by approximately two orders of magnitude from the surface peat (30 cm depth) to 75-200 cm depth (repeated measures ANOVA: $F_{4,23} =$

12.36, $p < 0.001$, Figure 4). Specifically, AOM rates at 30 cm were highest (Tukey's HSD, $p < 0.001$), followed by the 50 cm depth increment (Tukey's HSD, $p < 0.05$), with rates at the deeper depths (75-200 cm) uniformly low.

Temperature trend

In June and July 2016, we observed a marginally significant effect of temperature on AOM rates (repeated measures ANOVA: $F_{6,23} = 2.109$, $p = 0.091$). Since we were particularly interested in a potential temperature effect on rates of AOM, we used linear regression to further investigate this relationship within significantly different depth increments. At 30 cm depth, the temperature effect was not statistically significant ($p = 0.282$), but there was a positive trend of increased AOM at warmer temperatures (Figure 5). At 50 cm depth, there was a marginally significant increase of AOM with warmer temperatures ($p = 0.078$, Figure 6). However, this relationship was largely driven by one point at 4°C. When that point was removed from the analysis, there was still a positive, but insignificant trend ($p > 0.05$). In the combined deeper depths (75, 125 and 200cm), AOM increased with warmer temperatures ($p = 0.012$, Figure 7).

AOM through time

In a comparison of rates of AOM measured at 2, 4, and 8 days, AOM rates decreased through time (Figure 8; repeated measures ANOVA: $p < 0.01$). Specifically, for both June (Figure 8a) and July (Figure 8b) 2016, AOM rates were greatest on day 2 and similar on days 4 and 8.

DISCUSSION

Season effect

While we found no significant seasonal effects on rates of AOM between June and July 2016, we were unable to detect this process in August and October 2016, suggesting that rates of

AOM may be lower later in the growing season. This contrasts with previous studies that have not noted any differences in rates of AOM over seasonal sampling periods (Smemo & Yavitt, 2007; Hu et al., 2014). Segarra et al. (2015) measured AOM both during summer and winter and found it to be an important process even during the non-growing season. Many studies did not measure AOM over the course of the year or growing season, providing no context regarding the potential effects of season on the rates of AOM reported (Blazewicz et al., 2012; Gupta et al., 2013; Shi et al., 2017). Our results suggest a trend of lower rates of AOM later in the growing season, highlighting the need for further study of the seasonal controls over AOM.

Depth effect

We observed AOM throughout the entire peat profile (up to 2 m deep), which is the first time AOM has been documented this deep in the peat profile. Most of the previous studies limited the study of AOM to the top 40 cm or shallower (Blazewicz et al., 2012; Gupta et al., 2013; Seggara et al., 2015). Seggara et al. (2015) measured AOM at 10 cm increments down to 40 cm and noted that rates of AOM were highest in surface soil samples (0-10 cm) that were anoxic and permanently water-saturated. Surface peat is an important area of focus because it had the highest rates of AOM (Figure 4). The mechanism of AOM has been suggested in previous studies to be associated with either alternative terminal electron acceptors (TEAs) or reverse methanogenesis. TEAs act as substitutes for O_2 in anaerobic conditions and are used by microbes to oxidize CH_4 . Reverse methanogenesis occurs when the enzymes that carry out methanogenesis act in reverse to consume CH_4 rather than produce it. We hypothesize higher rates occur at the surface because as the water table fluctuates over time, it introduces oxic conditions to the surface peat and recharges reduced TEAs. These TEAs can then be used again by microorganisms to carry out AOM. Thus, deep peat (75, 125, and 200 cm) may have much

lower rates of AOM because it is limited by the availability of TEAs as these depths are not subject to water-table fluctuations.

AOM through time

Rates of AOM were greatest at the earliest timepoint measured (2 days) and decreased over time in both June and July 2016 (Figure 8a, 8b). This trend also suggests that TEAs are likely involved in carrying out AOM in this peatland because, as the TEA pool is depleted by CH₄ consuming microorganisms, the rate of AOM decreases. Other proposed AOM mechanisms, such as reverse methanogenesis (Blazewicz et al., 2012), do not require the presence of TEAs to occur and are not supported by our results. If AOM was carried out by reverse methanogenesis, rates of AOM would stay relatively consistent over time because the reaction is not limited by the availability of reactants. The SPRUCE site (S1 Bog) is an ombrotrophic bog and has very low nutrient inputs and low concentrations of inorganic TEAs, such as nitrate and sulfate (Lin et al., 2014), suggesting that humic substances could be acting as TEAs for microorganisms performing AOM. Previous studies have documented the use of organic TEAs during anaerobic incubations measuring peatland CH₄ and CO₂ production (Keller et al., 2009), providing support for our hypothesis that humic substances could be driving AOM at S1 Bog.

Temperature effect

Little is known about what controls rates of AOM in peatlands and understanding how climate change, specifically temperature increases, will affect rates of AOM is particularly interesting. Previous examination of climate factors in 15 different peatlands revealed no significant relationships between temperature, precipitation, and rates of AOM (Gupta et al., 2013). Segarra et. al (2015) also did not attribute changes in rates of AOM to temperature at their study sites. In our study, we found a weakly significant effect between temperature and rates of

AOM, providing one of the first indications that climate factors may affect rates of AOM. Further breaking down the relationship between temperature and rates of AOM by significantly different depth increments revealed that the significance of the relationship varied by depth. The relationship was statistically non-significant at 30 cm depth, marginally significant at 50 cm depth and significant at the deeper depths (75, 125 and 200 cm depth). Despite varying statistical significance, all depths showed positive trends between temperature and rates of AOM, suggesting that in the face of climate change AOM may help reduce CH₄ flux to the atmosphere (which is presumed to increase with temperature increases; Wilson et al. 2016). At deeper depths, temperature explained as much as 10% of the variation in rates of AOM, where higher rates of AOM were correlated with warmer temperatures (Figure 7). Hopefully, with further study of AOM, the relationship between rates of AOM and temperature will become clearer and will facilitate the development of better informed Earth system models.

AOM compared to production

Despite the ubiquitous occurrence of AOM throughout the entire peatland profile in June and July 2016, we found that only a very small percentage of the CH₄ produced was consumed through this process (Table 3). In June 2016, a greater percentage of the CH₄ produced was consumed via AOM compared to July 2016 which had higher production rates and lower consumption rates (Table 4). Overall, the amount of CH₄ consumed via AOM was less than 1%, except in June 2016 at the 30 cm depth increment where there was an outlier measurement that caused average AOM to be 11.2% of the CH₄ produced.

Rates of AOM and the amount of CH₄ consumed via AOM were much lower in this study compared to previous studies (Table 1). Average rates of AOM range from $0.0029 \pm 0.0005 \mu\text{mol C g soil}^{-1} \text{ d}^{-1}$ in tropical mineral soils in Puerto Rico (Gupta et al., 2013) to $>2.65 \pm$

0.09 $\mu\text{mol C g soil}^{-1} \text{ d}^{-1}$ in tidal freshwater wetlands in coastal Georgia (Segarra et al., 2015). In our study rates ranged from 1.84×10^{-6} to $0.43 \mu\text{mol dry g peat}^{-1} \text{ day}^{-1}$, which was lower than most of the rates reported in previous literature. It's likely that the tidal freshwater wetland had the highest rate of AOM because of the rapid rate of water flowing through the system, which replenishing the TEA pool, while the tropical mineral soils likely have the lowest rate from high levels of precipitation washing away potential TEAs for AOM. Our study may have had lower rates due to the low availability of traditional TEAs and low rates of water flowing through the S1 Bog, along with methodological limitations. In the literature, the percentage of CH_4 consumption via AOM varied from 0.267% (Gupta et al., 2013) to 357% (Segarra et al., 2015). Despite the wide range in percent consumption in the literature, most of the percentages calculated for these studies were orders of magnitude higher than the percentages reported in this study. This discrepancy could be due to methodological limitations affecting rates of CH_4 consumption and production during our incubations.

In this study, we adapted a radioisotope tracer method previously used by Valentine et al. (2001) to measure AOM by quantification of $^3\text{H-H}_2\text{O}$ produced, which is one of the by-products (the other by-product is CO_2) of AOM. Smemo & Yavitt (2007) and Segarra et al. (2015) reported the highest rates of AOM and used a $^{14}\text{CH}_4$ tracer and measured the amount of $^{14}\text{CO}_2$ produced rather than a $^3\text{H-CH}_4$ tracer and measuring the $^3\text{H-H}_2\text{O}$ produced. To directly measure AOM using a tritiated CH_4 tracer, we slurried peat samples with porewater collected from the same enclosure and depth; however, we also completed a parallel experiment that was done in the same way, except without porewater addition. Lack of porewater addition consistently reduced net CH_4 production across all depths and sampling events, often resulting in even net CH_4 consumption (Figure 9). Rates of net CH_4 consumption in surface peat were often as high as

one $\mu\text{mol C g soil}^{-1} \text{ d}^{-1}$ and orders of magnitude greater than our direct measurements of AOM using tritiated CH_4 . We hypothesize that decreasing porewater content often results in net CH_4 consumption because (1) methanogens (CH_4 -producing microorganisms) can be C limited, and the porewater results in the addition of dissolved organic matter, and/or (2) the additional porewater inhibits the diffusion of CH_4 to the microbial consortia responsible for CH_4 oxidation, resulting in low rates of AOM. These results highlight the potential importance of AOM in northern peatlands and the need for further study of the rates and controls over this process to put it into an in-situ context, as well as inform modelling predictions of ecosystem-scale CH_4 flux.

CONCLUSION

Due to the lack of understanding of the magnitude and controls over AOM in peatlands, this process is not currently included in Earth system models; the accuracy of which relies upon a complete understanding of peatland CH_4 cycling in response to climate change. In an effort to bridge this knowledge gap, we measured rates of AOM at multiple depths (30-200 cm) within a northern peatland that is the subject of an ecosystem-scale climate manipulation. In June and July 2016, we observed AOM occurring throughout the entire peatland profile (up to 2 m deep) and found that rates of CH_4 consumption decreased with depth and incubation time.

Additionally, we provided suggestive evidence that rates of AOM may increase with increasing temperature. Taken together, these results suggest that AOM may be important process in northern peatlands and that it could potentially act as a constraint on the presumed positive feedback loop of rising temperatures on CH_4 emissions from peatlands. Given these implications, future studies should continue to focus on the role of AOM in peatlands, including identifying the mechanistic controls over CH_4 consumption, to enhance our understanding of ecosystem- and global-scale anaerobic C cycling.

REFERENCES

- Abdalla, M., et al. "Emissions of methane from northern peatlands: a review of management impacts and implications for future management options." *Ecology and Evolution* (2016).
- Blazewicz, S. J., et al. "Anaerobic oxidation of methane in tropical and boreal soils: ecological significance in terrestrial methane cycling." *Journal of Geophysical Research: Biogeosciences* 117.G2 (2012).
- Boelter, D. H., and E. S. Verry. "Peatland and water in the northern Lake States." *General Technical Report NC-31. St. Paul, MN: US Dept. of Agriculture, Forest Service, North Central Forest Experiment Station* (2006).
- Boetius, A., et al. "A marine microbial consortium apparently mediating anaerobic oxidation of methane." *Nature* 407.6804 (2000): 623-626.
- Bridgham, S. D., et al. "The carbon balance of North American wetlands." *Wetlands* 26.4 (2006): 889-916.
- Bridgham, S. D., et al. "Methane emissions from wetlands: biogeochemical, microbial, and modeling perspectives from local to global scales." *Global Change Biology* 19.5 (2013): 1325-1346.
- Caldwell, S. L., et al. "Anaerobic oxidation of methane: mechanisms, bioenergetics, and the ecology of associated microorganisms." *Environmental science & technology* 42.18 (2008): 6791-6799.
- Crowe, S. A., et al. "The methane cycle in ferruginous Lake Matano." *Geobiology* 9.1 (2011): 61-78.
- Denman, K. L., et al. "Couplings between changes in the climate system and biogeochemistry." *Climate change 2007: The physical science basis*. Cambridge University Press, (2007).
- Gupta, V., et al. "Stable isotopes reveal widespread anaerobic methane oxidation across latitude and peatland type." *Environmental science & technology* 47.15 (2013): 8273-8279.
- Hanson, P. J., et al. "Intermediate-scale community-level flux of CO₂ and CH₄ in a Minnesota peatland: putting the SPRUCE project in a global context." *Biogeochemistry* 129.3 (2016): 255-272.
- Hu, B-l., et al. "Evidence for nitrite-dependent anaerobic methane oxidation as a previously overlooked microbial methane sink in wetlands." *Proceedings of the National Academy of Sciences* 111.12 (2014): 4495-4500.

IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp.

Keller, J. K., Pamela B. Weisenhorn, and J. Patrick Megonigal. "Humic acids as electron acceptors in wetland decomposition." *Soil Biology and Biochemistry* 41.7 (2009): 1518-1522.

Kirtman, B., et al. "Near-term climate change: projections and predictability." (2013).

Krassovski, M. B., et al. "A comprehensive data acquisition and management system for an ecosystem-scale peatland warming and elevated CO₂ experiment." *Geoscientific Instrumentation, Methods and Data Systems* 4.2 (2015): 203-213.

Lin, X., et al. "Microbial community stratification linked to utilization of carbohydrates and phosphorus limitation in a boreal peatland at Marcell Experimental Forest, Minnesota, USA." *Applied and environmental microbiology* 80.11 (2014): 3518-3530.

Megonigal, J. P., M. E. Hines, and P. T. Visscher. "Anaerobic metabolism: linkages to trace gases and aerobic processes, edited by: Schlesinger, WH." *Biogeochemistry* 8.

Michaelis, W., et al. "Microbial reefs in the Black Sea fueled by anaerobic oxidation of methane." *Science* 297.5583 (2002): 1013-1015.

Neubauer, S. C., and J. P. Megonigal. "Moving beyond global warming potentials to quantify the climatic role of ecosystems." *Ecosystems* 18.6 (2015): 1000-1013.

Orphan, V. J., et al. "Methane-consuming archaea revealed by directly coupled isotopic and phylogenetic analysis." *science* 293.5529 (2001): 484-487.

Parsekian, A. D., et al. "Uncertainty in peat volume and soil carbon estimated using ground-penetrating radar and probing." *Soil Science Society of America Journal* 76.5 (2012): 1911-1918.

Segarra, K. E. A., et al. "High rates of anaerobic methane oxidation in freshwater wetlands reduce potential atmospheric methane emissions." *Nature communications* 6 (2015).

Shi, Y., et al. "Using ¹³C isotopes to explore denitrification-dependent anaerobic methane oxidation in a paddy-peatland." *Scientific Reports* 7 (2017).

Smemo, K. A., and J. B. Yavitt. "Anaerobic oxidation of methane: an underappreciated aspect of methane cycling in peatland ecosystems?." *Biogeosciences* 8.3 (2011): 779-793.

Smemo, K. A., and J. B. Yavitt. "Evidence for anaerobic CH₄ oxidation in freshwater peatlands." *Geomicrobiology Journal* 24.7-8 (2007): 583-597.

Tfaily, M. M., et al. "Organic matter transformation in the peat column at Marcell Experimental Forest: humification and vertical stratification." *Journal of Geophysical Research: Biogeosciences* 119.4 (2014): 661-675.

Valentine, D. L., et al. "Water column methane oxidation adjacent to an area of active hydrate dissociation, Eel River Basin." *Geochimica et Cosmochimica Acta* 65.16 (2001): 2633-2640.

Verry, E. S., K. N. Brooks, and P. K. Barten. "Streamflow response from an ombrotrophic mire." (1988).

Wilson, R. M., et al. "Stability of peatland carbon to rising temperatures." *Nature communications* 7 (2016): 13723.

Zehnder, A. JB, and T. D. Brock. "Anaerobic methane oxidation: occurrence and ecology." *Applied and Environmental Microbiology* 39.1 (1980): 194-204.

FIGURES AND TABLES

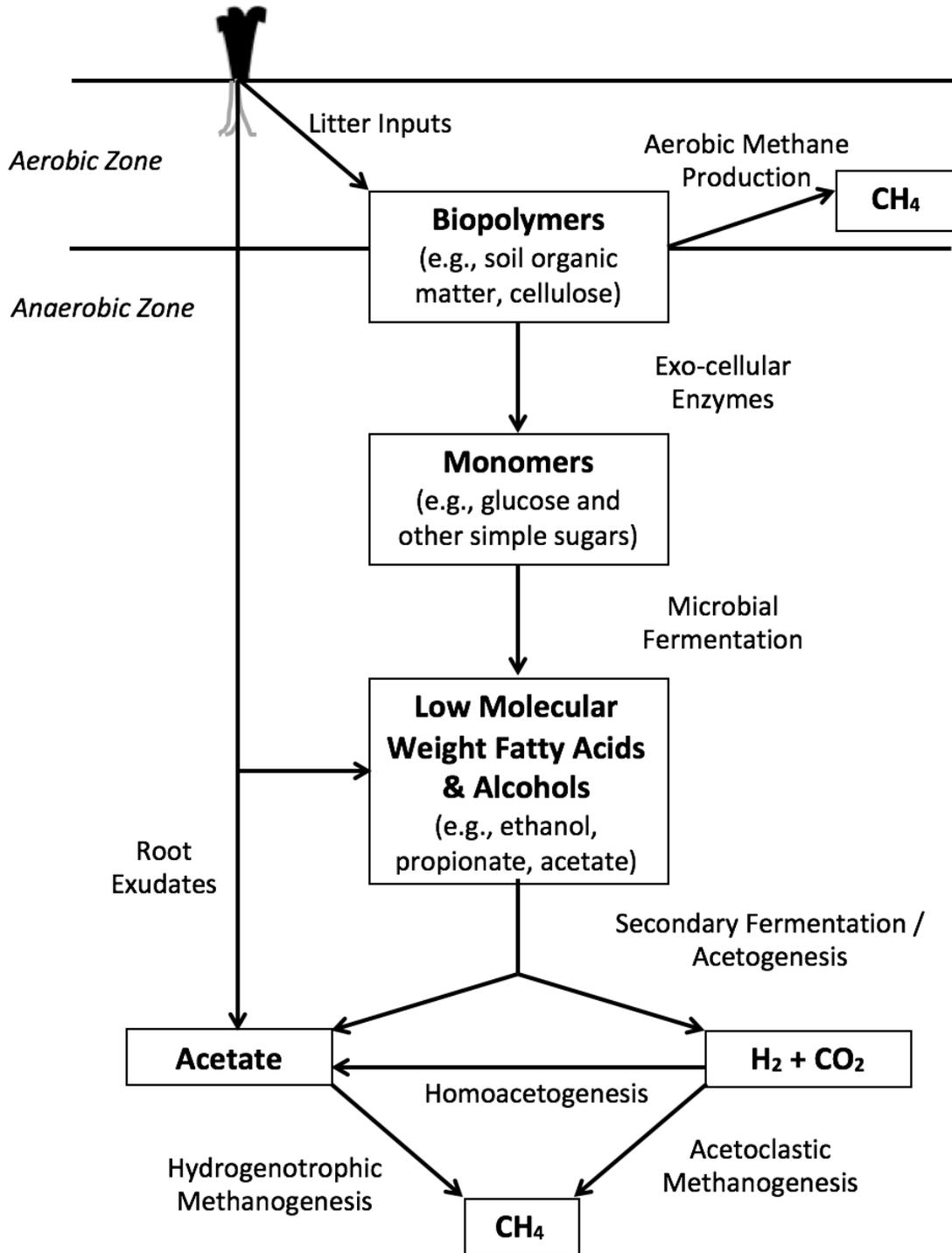


Figure 1: Microbially-mediated production of methane in peatlands. White boxes are pools of carbon and arrows show microbial processes. (modified from Bridgham et al., 2013).

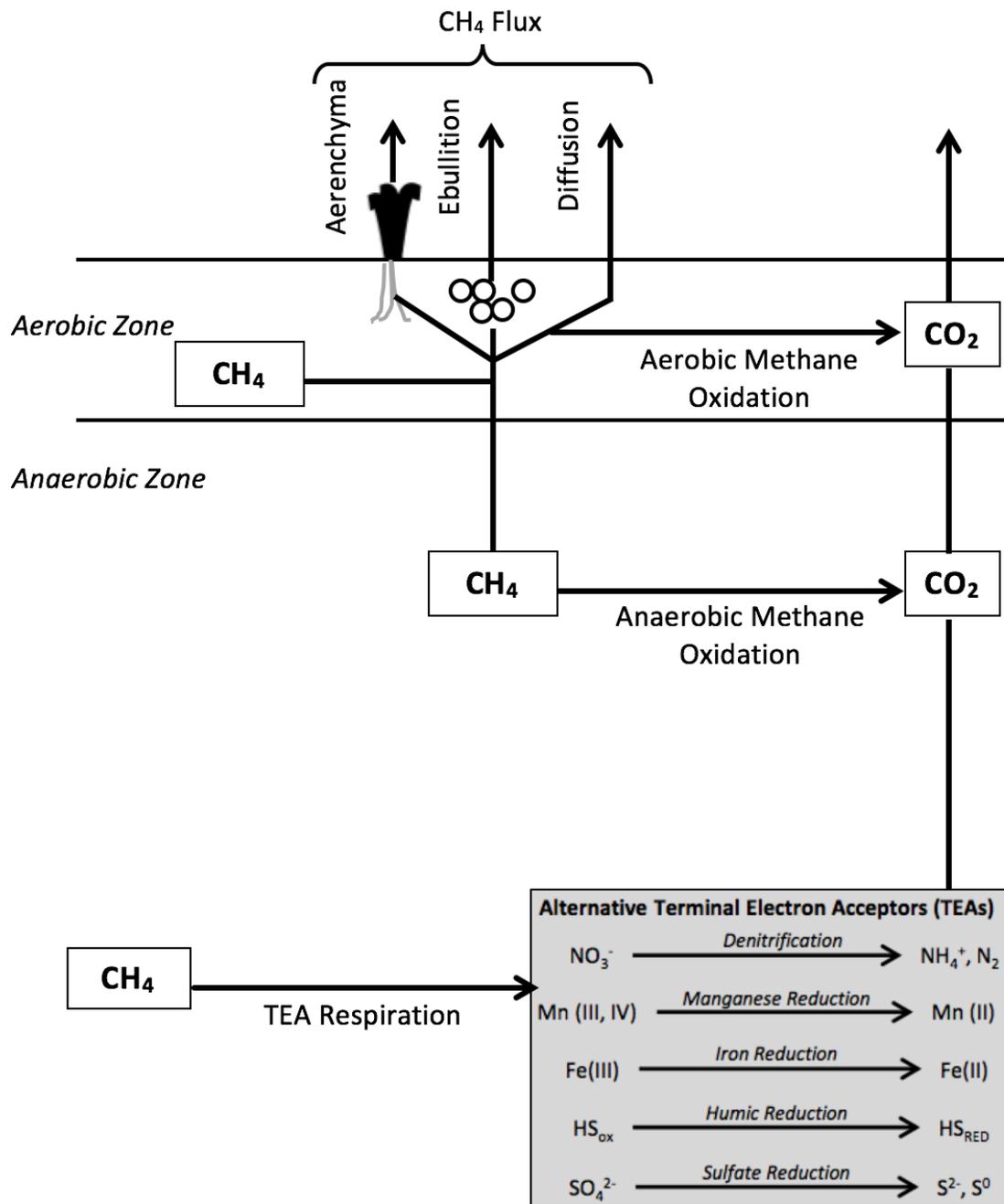


Figure 2: Transport and consumption of methane in peatlands. White boxes are pools of carbon and arrows show microbial processes. (modified from Bridgham et al., 2013).



Figure 3: Aerobic oxidation of methane, with oxygen acting as the terminal electron acceptor.

Table 1: AOM in freshwater wetlands.

Location	Rate of AOM ($\mu\text{mol g}^{-1} \text{ soil day}^{-1}$)	Percentage of AOM to total CH_4 Production	Method	Study
Michigan Hollow – NY	Average rate = 1.47 ± 0.22 Maximum rate = 15.2		N=350, used specific and non-specific methanogenic inhibitors, both stable isotopic tracer and ^{13}C fractionation techniques	Smemo & Yavitt 2007
Boreal peat soil Alaska, USA	0.021 ± 0.002	0.267%	15cm depth, $^{13}\text{CH}_4$ addition, rate calculated from day 10-15	Blazewicz et al. 2012
Tropical mineral soil Puerto Rico	0.0029 ± 0.0005	0.826%	15cm depth, $^{13}\text{CH}_4$ addition, rate calculated from day 45-60	Blazewicz et al. 2012
Michigan Hollow	0-20d = 0.075 ± 0.018 0-40d = 0.13 ± 0.0086	0-20d = 7.58% 0-40d = 11.50%	15-30cm depth, $^{13}\text{CH}_4$ addition, destructive sampling at day 3, 20, 40, headspace evacuated and bubbled	Gupta et al. 2013
White River Rich Fen	0-20d = 0.18 ± 0.006 0-40d = 0.14 ± 0.0078	0-20d = 5.17% 0-40d = 6.66%	15-30cm depth, $^{13}\text{CH}_4$ addition, destructive sampling at day 3, 20, 40, headspace evacuated and bubbled	Gupta et al. 2013
White River Int. Fen	0-20d = 0.14 ± 0.015 0-40d = 0.11 ± 0.0026	0-20d = 2.47% 0-40d = 2.49%	15-30cm depth, $^{13}\text{CH}_4$ addition, destructive sampling at day 3, 20, 40, headspace evacuated and bubbled	Gupta et al. 2013
Channel Fen	0-20d = 0.22 ± 0.14 0-40d = 0.15 ± 0.034	0-20d = 20.47% 0-40d = 13.95%	15-30cm depth, $^{13}\text{CH}_4$ addition, destructive sampling at day 3, 20, 40, headspace evacuated and bubbled	Gupta et al. 2013
Big Run Bog	0-20d = 0.12 ± 0.0086 0-40d = 0.12 ± 0.014	0-20d = 7.19% 0-40d = 6.58%	15-30cm depth, $^{13}\text{CH}_4$ addition, destructive sampling at day 3, 20, 40, headspace evacuated and bubbled	Gupta et al. 2013

Big Lake Fen	0-20d = 0.097 ± 0.032 0-40d = 0.22 ± 0.037	0-20d = 24.67% 0-40d = 9.34%	15-30cm depth, ¹³ CH ₄ addition, destructive sampling at day 3, 20, 40, headspace evacuated and bubbled	Gupta et al. 2013
SA460 Fen	0-20d = 0.11 ± 0.006 0-40d = 0.10 ± 0.0086	0-20d = 12.99% 0-40d = 8.99%	15-30cm depth, ¹³ CH ₄ addition, destructive sampling at day 3, 20, 40, headspace evacuated and bubbled	Gupta et al. 2013
5A' Fen	0-20d = 0.61 ± 0.45 0-40d = 0.41 ± 0.042	0-20d = 115.66% 0-40d = 38.51%	15-30cm depth, ¹³ CH ₄ addition, destructive sampling at day 3, 20, 40, headspace evacuated and bubbled	Gupta et al. 2013
White River Poor Fen	0-20d = 0.085 ± 0.020 0-40d = 0.099 ± 0.0017	0-20d = 7.37% 0-40d = 8.79%	15-30cm depth, ¹³ CH ₄ addition, destructive sampling at day 3, 20, 40, headspace evacuated and bubbled	Gupta et al. 2013
Buckles Bog	0-20d = 0.024 ± 0.0026 0-40d = 0.041 ± 0.0043	0-20d = 27.72% 0-40d = 35.07%	15-30cm depth, ¹³ CH ₄ addition, destructive sampling at day 3, 20, 40, headspace evacuated and bubbled	Gupta et al. 2013
Bog on Permafrost	0-20d = 0.010 ± 0.0035 0-40d = 0.022 ± 0.016	** 0-20d = N.A. 0-40d = 7.98%	15-30cm depth, ¹³ CH ₄ addition, destructive sampling at day 3, 20, 40, headspace evacuated and bubbled	Gupta et al. 2013
Mclean Bog	0-40d = 0.076 ± 0.0052	0-40d = 3.94%	15-30cm depth, ¹³ CH ₄ addition, destructive sampling at day 3, 20, 40, headspace evacuated and bubbled	Gupta et al. 2013
Dryden Bog	0-20d = 0.091 ± 0.014 0-40d = 0.072 ± 0.023	0-20d = 2.78% 0-40d = 2.65%	15-30cm depth, ¹³ CH ₄ addition, destructive sampling at day 3, 20, 40, headspace evacuated and bubbled	Gupta et al. 2013
S2 Bog	0-20d = 0.079 ± 0.0035 0-40d = 0.072 ± 0.0095	0-20d = 5.50% 0-40d = 9.93%	15-30cm depth, ¹³ CH ₄ addition, destructive sampling at day 3, 20, 40, headspace evacuated and bubbled	Gupta et al. 2013

S1 Bog	0-20d = 0.056 ± 0.0035 0-40d = 0.092 ± 0.0069	0-20d = 35.52% 0-40d = 7.24%	15-30cm depth, $^{13}\text{CH}_4$ addition, destructive sampling at day 3, 20, 40, headspace evacuated and bubbled	Gupta et al. 2013
*Freshwater peat soil of Florida Everglades	Maximum rate = 0.54 ± 0.03	92.68%	24hr incubation, $^{14}\text{CH}_4$ tracer, surface – 40cm	Segarra et al. 2015
*Coastal organic rich wetland in Arcadia National Park, Maine	Maximum rate = 0.16 ± 0.04	350%	24hr incubation, $^{14}\text{CH}_4$ tracer, surface – 40cm	Segarra et al. 2015
*Tidal freshwater wetland in coastal Georgia	Maximum rate = $>2.65 \pm 0.09$	357%	24hr incubation, $^{14}\text{CH}_4$ tracer, surface – 40cm	Segarra et al. 2015
Xiazhuhu wetland, Southeastern China	$3.1 \times 10^{-4} \pm 6 \times 10^{-5}$ – $5.43 \times 10^{-3} \pm 1.9 \times 10^{-4}$	No production rate reported	Depths = 20-30, 50-60, 90-100, NO_2^- and $^{13}\text{CH}_4$ addition, CO_2 measured with continuous flow isotopic ratio MS	Hu et al. 2014
Xixi wetland, Southeastern China	$6.8 \times 10^{-4} \pm 1.3 \times 10^{-4}$ – $4.92 \times 10^{-3} \pm 4 \times 10^{-5}$	No production rate reported	Depths = 20-30, 50-60, 90-100, NO_2^- and $^{13}\text{CH}_4$ addition, CO_2 measured with continuous flow isotopic ratio MS	Hu et al. 2014
Paddy field, Southeastern China	$1.68 \times 10^{-3} \pm 3 \times 10^{-5}$ – $2.04 \times 10^{-3} \pm 6 \times 10^{-5}$	No production rate reported	Depths = 20-30, 50-60, 90-100, NO_2^- and $^{13}\text{CH}_4$ addition, CO_2 measured with continuous flow isotopic ratio MS	Hu et al. 2014
Peatland in Jinchuan, Northeast China	0-1hr = 0.70 36hr = 0.096	No production rate reported	[reported rate after 1hr incubation] 36hr incubation (sampling at 1, 6, 12, 24, 30, 36hr), $^{13}\text{CH}_4$ addition, 50-60cm depth	Shi et al. 2017

Fertilized patty-peatland in Jinchuan, Northeast China	0-1hr = 0.53 36hr = 0.047	No production rate reported	36hr incubation (sampling at 1, 6, 12, 24, 30, 36hr), ¹³ CH ₄ addition, 50-60cm depth	Shi et al. 2017
--	------------------------------	-----------------------------	---	-----------------

*converted to $\mu\text{mol g}^{-1} \text{ soil day}^{-1}$, assuming a bulk density of 0.1 g/cm^3

**N.A., gross production rate reported was negative

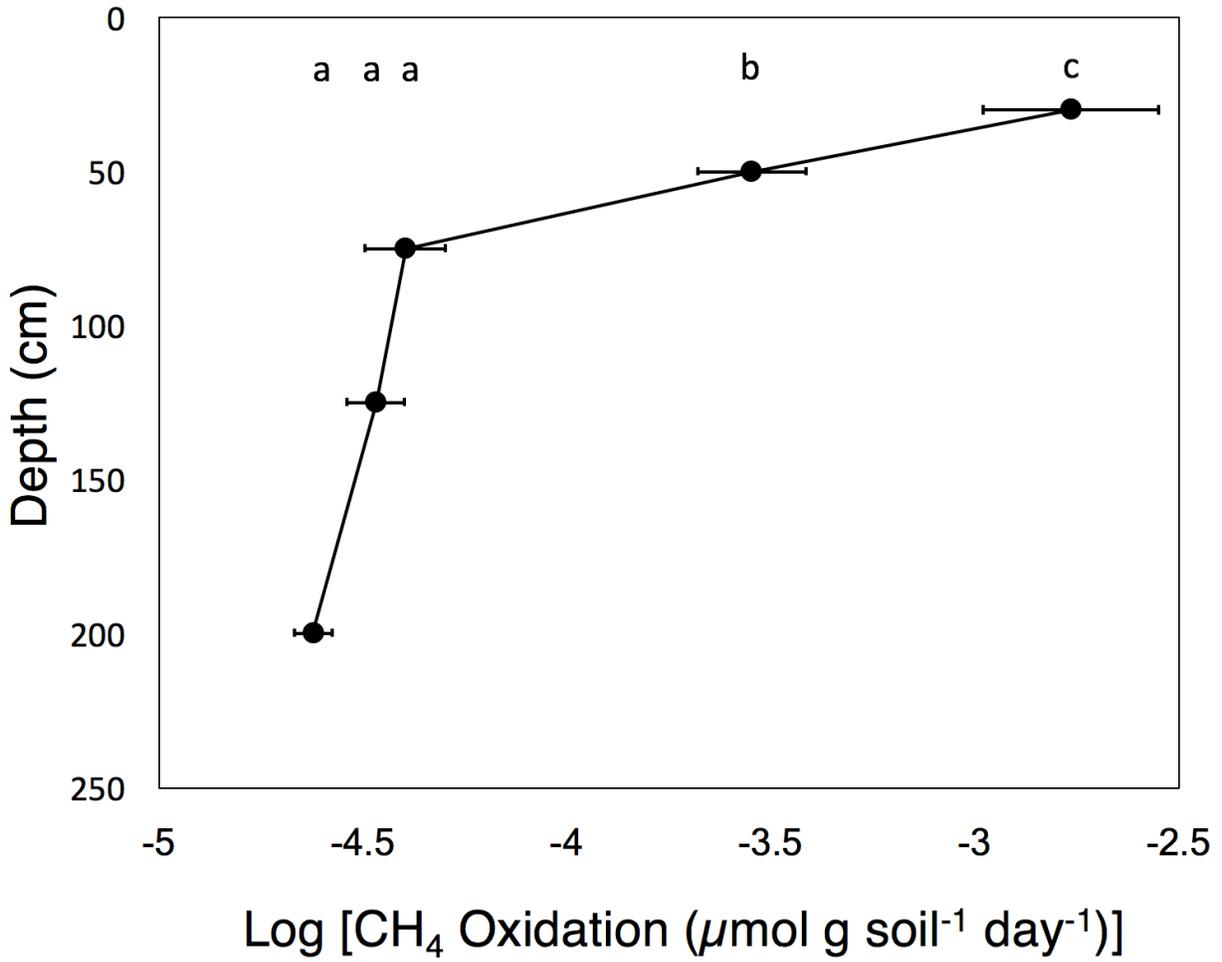


Figure 4: The average log of CH₄ anaerobically oxidized throughout the peat profile in June and July 2016. Significant differences at p < 0.001 are denoted with different lower case letters.

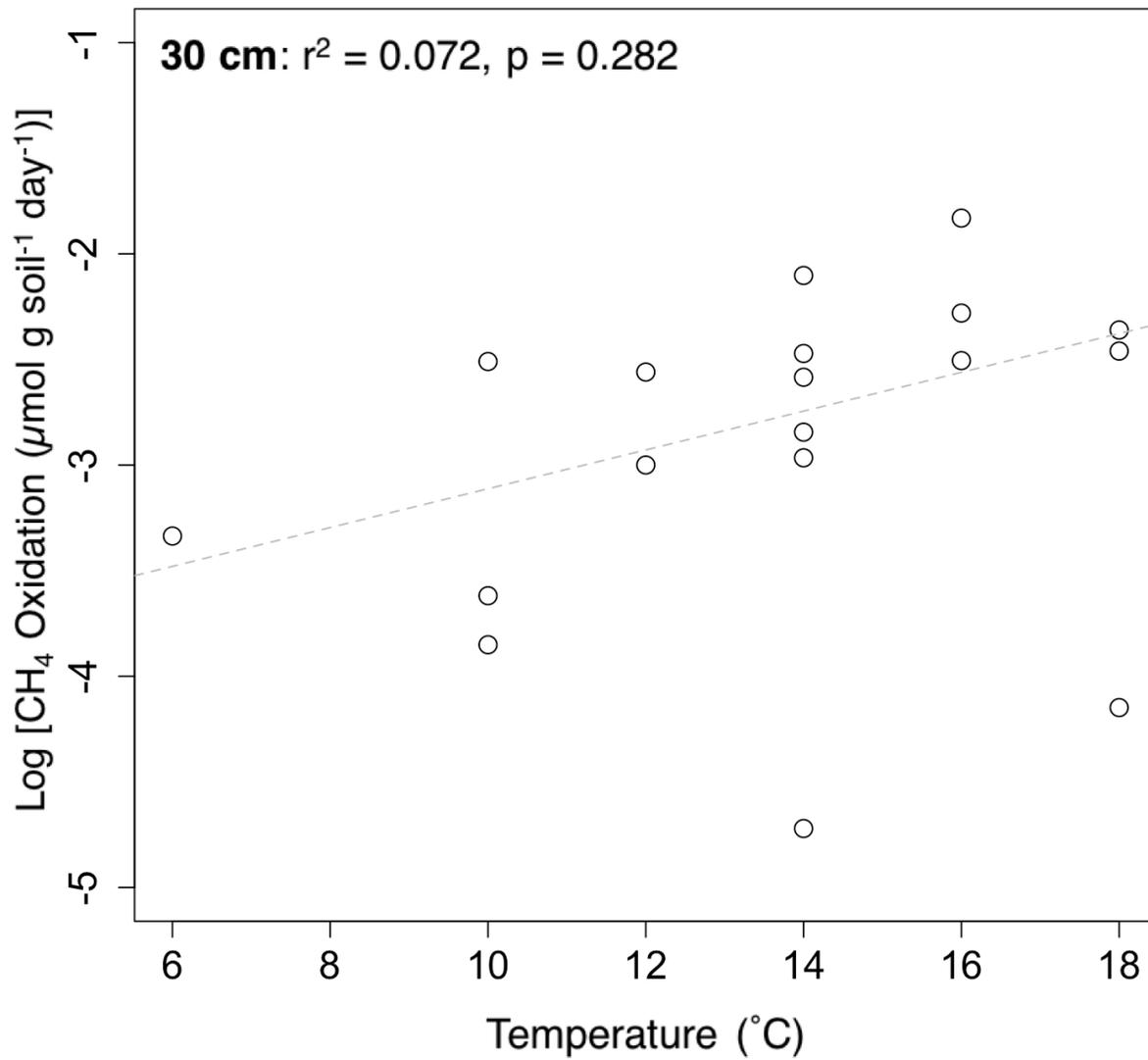


Figure 5: AOM temperature response from surface (30 cm) peat samples collected in June and July 2016. Note the non-significant relationship between temperature and CH₄ oxidized ($p = 0.282$).

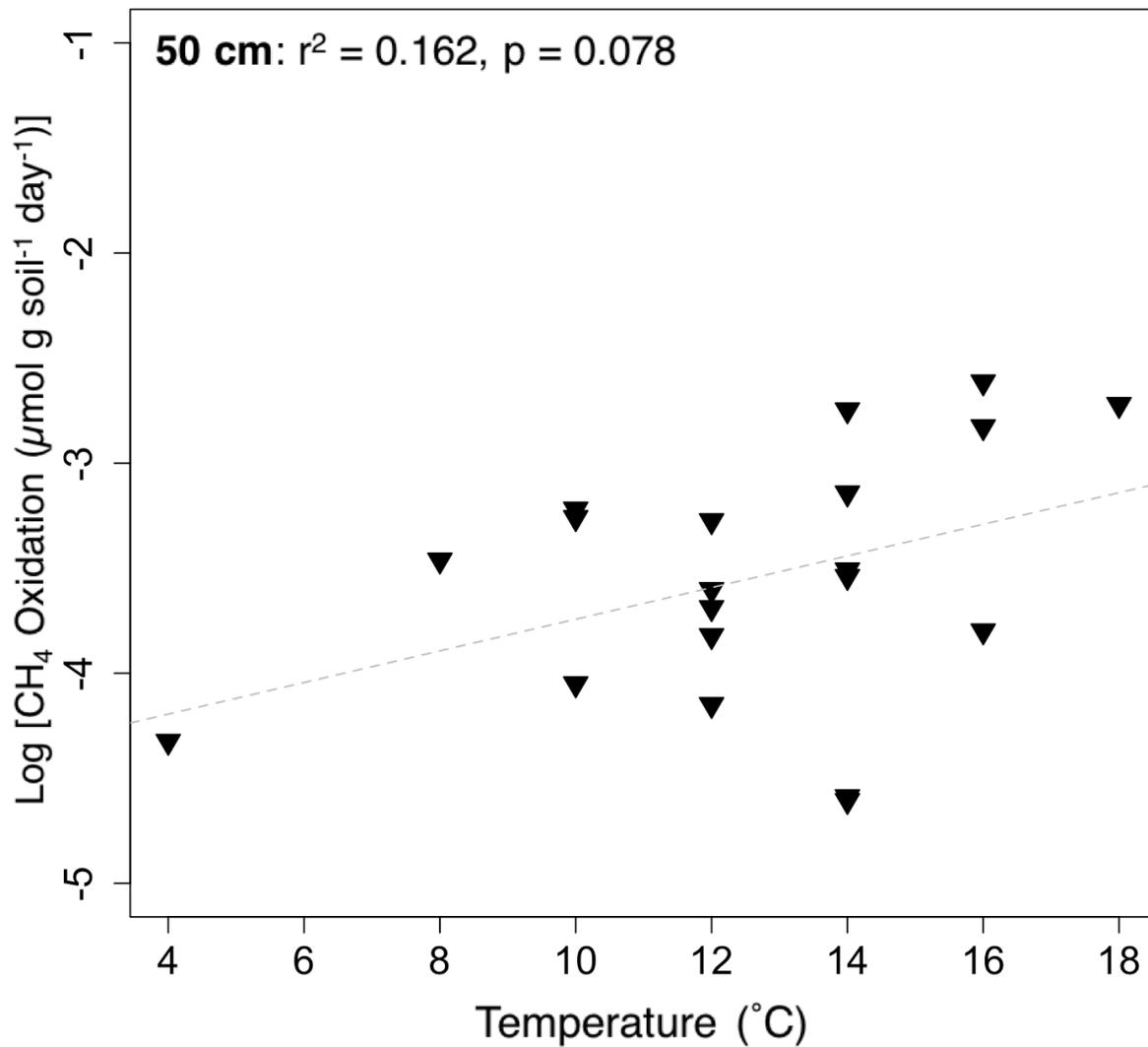


Figure 6: AOM temperature response from surface (50 cm) peat samples collected in June and July 2016. Marginally significant positive relationship between temperature and CH₄ oxidized ($p = 0.078$).

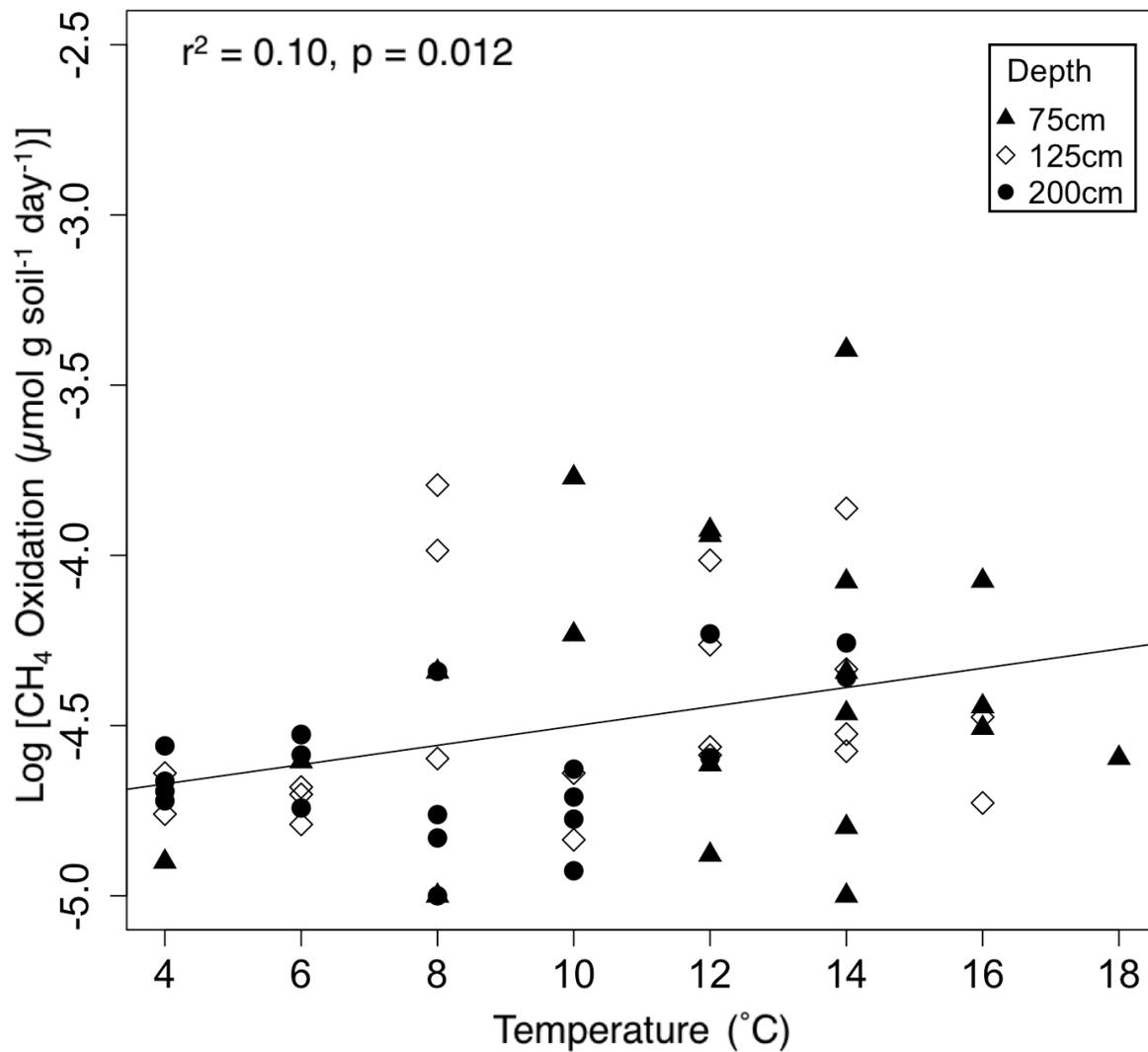


Figure 7: AOM temperature response from deep (75, 125 and 200 cm) peat samples collected in June and July 2016. Significant positive relationship between temperature and CH₄ oxidized at depth (p = 0.012).

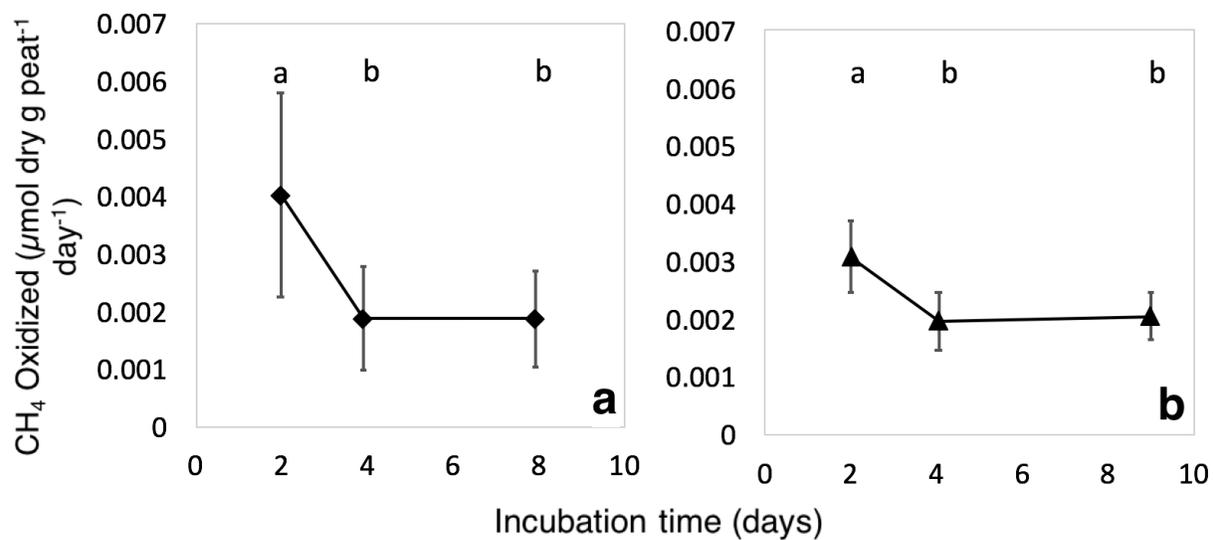


Figure 8: AOM rates over incubation time. Each point represents the average of all surface measurements (a) in June and (b) in July 2016. Significant differences are denoted with lower case letters.

Table 2: Average rates of AOM across all plots and depths for June and July 2016. No detectable rates were observed in August and October 2016.

	Average rate of AOM ($\mu\text{mol dry g peat}^{-1} \text{ day}^{-1}$)
June 2016	0.00081 ± 0.00037
July 2016	0.00065 ± 0.00018

* June AOM average excludes outlier from plot 17, 30 cm.

Table 3: Rates of AOM compared to production rates for June and July 2016 observed during the same anaerobic incubation. Average Production and AOM ($\mu\text{mol dry g peat}^{-1} \text{ day}^{-1}$) \pm St. Er.

Depth	June 2016		July 2016	
	Production ($\mu\text{mol dry g peat}^{-1} \text{ day}^{-1}$)	AOM ($\mu\text{mol dry g peat}^{-1} \text{ day}^{-1}$)	Production ($\mu\text{mol dry g peat}^{-1} \text{ day}^{-1}$)	AOM ($\mu\text{mol dry g peat}^{-1} \text{ day}^{-1}$)
30 cm	0.39 ± 0.090	0.046 ± 0.043	0.69 ± 0.13	0.0025 ± 0.00061
50 cm	0.040 ± 0.007	0.00037 ± 0.00017	0.16 ± 0.031	0.00081 ± 0.00026
75 cm	0.014 ± 0.0049	0.000063 ± 0.000038	0.024 ± 0.0029	0.000053 ± 0.000015
125 cm	0.0051 ± 0.0024	0.000026 ± 0.000012	0.014 ± 0.0037	0.000046 ± 0.000015
200 cm	0.0014 ± 0.00029	0.000013 ± 0.0000044	0.0037 ± 0.0012	0.000020 ± 0.0000043

* June AOM 30cm average includes outlier from plot 17.

Table 4: Percentage of CH₄ produced that was consumed via AOM in June and July 2016.

Depth	June: percent CH ₄ consumed via AOM	July: percent CH ₄ consumed via AOM
30 cm	11.8%	0.362%
50 cm	0.925%	0.506%
75 cm	0.45%	0.221%
125 cm	0.51%	0.329%
200 cm	0.929%	0.541%

* June percent CH₄ consumed via AOM includes outlier from plot 17 at 30 cm depth.

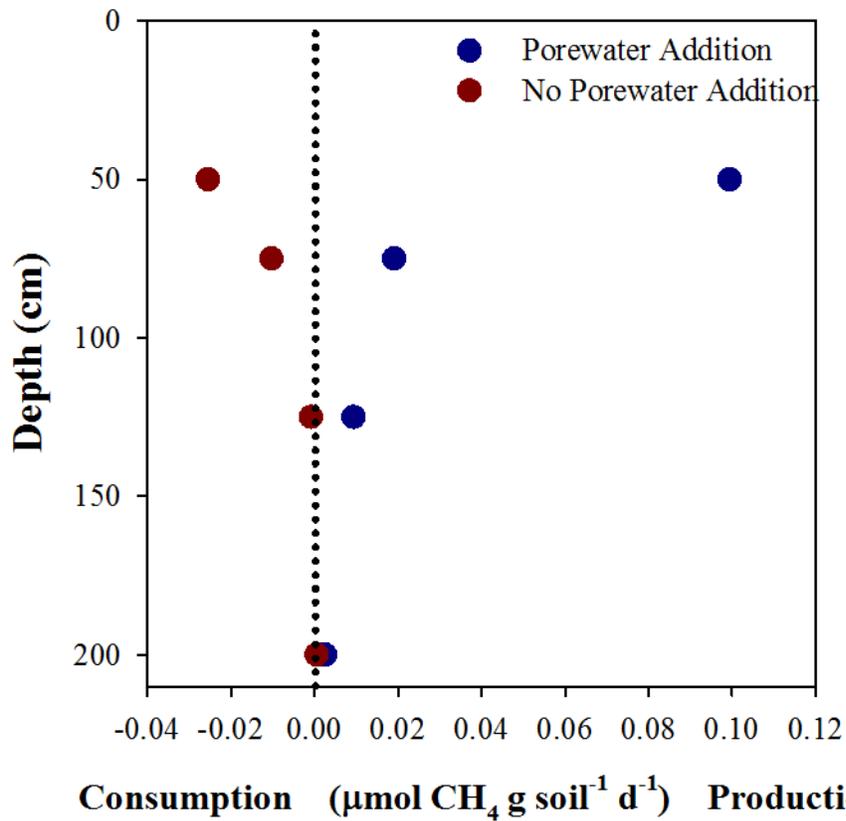


Figure 9: Each point represents the average methane production or consumption of all samples from a specific depth following 10 and 11 months (June and July 2016) of WEW. Figure provided by Anya Hopple.