Impacts of anaerobic methane oxidation, electron acceptors, and physical controls on net methane emissions from northern peatlands in Alaska and Finland

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Kimberley Elizabeth Miller
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Approved:

David A. Lipson, Co-Chair

Chun-Ta Lai, Co-Chair

Randy A. Dahlgren

Committee in Charge

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ABSTRACT

In this dissertation I focused on some of the least-understood aspects of the carbon cycle in northern peatlands – the biological controls on production, the presence and importance of the anaerobic CH₄ consumption pathway, and the physical controls on emission. A more clear understanding of the controls on CH₄ emissions from critical northern peatland systems will help constraint predictive models of carbon-climate feedbacks.

In chapter 1, I evaluated the linkages between porewater CH₄, CO₂, and iron concentrations within the upper active layer of a chronosequence of wetland basins in Barrow, Alaska. Iron concentrations varied amongst basin ages, with younger basins containing more iron in the upper soil profiles. Basin age also correlated with the thickness of the organic layer. Basin-specific seasonal mean porewater CH₄ concentrations had a negative relationship with total Fe and Fe(III) concentrations; CH₄ concentrations were positively related to organic layer thickness. Thus, the highest seasonal mean concentrations of CH₄ were found in older basins with thick organic layers and low Fe loads. A manipulated experiment confirmed a direct suppression effect on net CH₄ fluxes following Fe(III) and humic acids soil amendments, thus connecting in situ CH₄ production and release with soil electron acceptor availability.

Chapters 2 and 3 present the findings of a pair of anoxic soil incubations that use stable isotope tracers to simultaneously determine methanogenesis and anaerobic oxidation of methane (AOM) rates. In both experiments, I used treatments to determine the effect of different electron acceptors on CH₄ cycling rates. The in vitro incubations of Alaskan soil showed a significant positive correlation between methanogenesis and AOM rates, and an increase in methanogenesis rates with increasing depth within the active layer. There was also an interaction between soil depth and the kinetic rate constant for AOM, suggesting that AOM increased with Fe(III)
presence in shallow soil depths. Genetic surveys of Barrow soils show 16S rRNA and mcrA gene evidence for microbes closely related to known methanotrophs in the ANME groups 2 and 3. In Barrow soil incubations, AOM rates were greater than methanogenesis rates, causing negative net CH$_4$ fluxes; net fluxes were lowest in shallow, Fe(III)-treated soils. Using soils from Finland, in vitro incubations revealed no relationship between rates of methanogenesis and AOM. Nitrate-treated soils showed a significant suppression of methanogenesis, and a significant delay in the onset of AOM. While methanogenesis was greater than AOM, leading to a net positive soil CH$_4$ flux, AOM consumed a considerable percentage of CH$_4$ produced (6-39%), constituting a formidable constraint on CH$_4$ emissions.

Chapter 4 presents the results of a year-round field campaign in Finland. The annual flux data show strong seasonality in the CH$_4$ fluxes. Interseasonal variations in carbon fluxes were not significantly related to either air or soil temperatures, although summer fluxes were positively related to air temperatures. There is also evidence for a substantial autumnal CH$_4$ burst, and a lesser but still distinguishable burst during spring soil thaw, which combined accounted for a significant portion of the annual landscape CH$_4$ flux. Summer CH$_4$ fluxes were measured in situ, which allowed for the collection of data on the frequency and magnitude of CH$_4$ ebullition events. Growing season CH$_4$ ebullition events contributed an additional 50% of the diffusive CH$_4$ atmospheric flux, and showed strong fine-scale heterogeneity within the wetland landscape.
INTRODUCTION

This dissertation examines the ecological controls on net ecosystem methane flux from peatlands in Arctic Alaska and northern Lapland Finland. This includes landscape-scale studies of carbon fluxes, and the discovery and quantification of the anaerobic methane oxidation sink.

Methane is potent greenhouse gas, with an increasing influence and capacity to alter global climate-carbon feedbacks (Grosse et al. 2011, Schuur et al. 2013, Vaughan et al. 2013). The future role of methane is particularly salient in northern peatland ecosystems, which store vast quantities of soil carbon, much of which is currently sequestered and protected in permafrost and seasonally-frozen soils (Schuur et al. 2008, Tarnocai et al. 2009). Arctic permafrost-affected soils are a critical aspect of the global biosphere-climate feedbacks, as atmospheric fluxes of carbon dioxide and methane may outpace soil carbon storage as climate warming continues. Despite the importance of methane in the global climate system, the ecological controls and drivers of methane flux from natural wetland systems are still mired in uncertainties, creating disparities between modelled flux projections and measured rates under field conditions. A better characterization of the controls on methane is necessary for understanding how Arctic wetland ecosystems will interact with and contribute to future carbon-climate feedbacks.

The North Slope of Alaska landscape is equally divided between shallow thermokarst lakes, and drained and re-vegetated lake footprints, hereafter called ‘basins’. After draining, thermokarst lake basins re-vegetate, organic matter accretes over time, and organic soil layers thicken. Basins create a natural chronosequence of soil development, as the landscape naturally shifts between lake and basin form. Basins are classified by age since draining into four age classes: young (0-50 years), medium (50-300 years), old (300-2000 years), and ancient (2000-5500 years) (Hinkel et al. 2003, Bockheim et al. 2004). The organic matter accretion
characteristic of basin succession creates an increasing thickness of the organic layer between the surface and underlying mineral and permafrost soil layers. Despite the changes that occur over the stages of succession, soil genetic surveys across basin age classes confirm the presence of comparable populations of methanogenic, iron and humic substance reducing microbes (Lipson et al. 2013a, Lipson et al. 2013b). Like most wetland systems, there is high heterogeneity on fine scales – in the basins, the greatest differences in ecosystem functioning have often been found along hydrological and microtopographical gradients within basins rather than between basins (Zona et al. 2011, Lipson et al. 2012). Since the basin chronosequence is such a dominant feature of the Alaskan North Slope, and has been linked with differential amounts of net carbon fluxes to the atmosphere (Zona et al. 2010, Sturtevant and Oechel 2013), the basin system provides a framework for evaluating the range of changes possible in ecosystem functioning.

The first chapter addresses some of the ecosystem controls on methane flux from a set of 16 basins near Barrow, AK. Using soil porewater surveys, we measured extractable iron and dissolved CH$_4$ and CO$_2$ concentrations and related them with basin-scale soil characteristics. This work was a natural extension of previous work in Barrow soils that hinted at a strong connection between methane and the soil Fe and humic substance components. Using the porewater surveys, I found evidence of an inverse relationship between seasonal averages of dissolved iron and methane concentrations present within the soils. There was also a strong relationship between basin organic layer thickness and the ratio of CO$_2$:CH$_4$ in soil pore waters, which was attributable to the interplay between Fe-reduction and methane production within the soil depth profiles. Having found an interesting negative relationship between soil methane and Fe concentrations, I conducted a manipulative experiment within a single medium-aged basin, wherein I measured the net fluxes of both CO$_2$ and methane from a set of 48 permanent soil
collars before and after additions of iron and humic acid treatment solutions. Net methane fluxes were significantly suppressed following the experimental addition of both iron and humic acids. The data summarized in the first chapter suggest that *in-situ* methane production and net fluxes are tied to alternative electron acceptor presence and availability, which is in turn related to soil development. With melting permafrost leading to an increasing amount of organic matter and mineral soil introduced into the active layer (Hinkel et al. 2003, Bockheim and Hinkel 2007), the mineral-carbon relationship is an important consideration in future modeling and projections.

The landscape surveys explored in the first chapter clarified two important aspects of the methane cycle in the Barrow basin system – the presence of iron and humic substance electron acceptors result in the decrease of methane present in the soil, and that these electron acceptors can actively decrease net methane fluxes. Since there was a link between acceptor presence and methane concentrations, it begged the question of the mechanism resulting in the methane decline. There are four possible mechanisms – 1) the direct suppression of methane production via microbial competitive exclusion (Reiche et al. 2008), 2) the indirect suppression of methane production by toxic intermediates of electron acceptors (Klüber and Conrad 1998, Thauer and Shima 2008, Laanbroek 2010), 3) the promotion of methanotrophy (Smemo and Yavitt 2011, Callaghan 2013, Segarra et al. 2013), or 4) some combination of the above.

The second chapter was an extension of the Barrow methane story, looking into one of the least understood aspects of net methane fluxes – the anaerobic oxidation of methane. The anaerobic oxidation of methane (or AOM) has been recognized for decades as a crucial methane sink in marine systems, where AOM consumes approximately 60-100% of methane produced and released from marine sediments, with a mechanism of methane oxidation linked with sulfate-reduction, from individual or consortia of microbes (Valentine 2002, Knittel and Boetius 2009).
While AOM is important in marine systems, it has only recently been considered potentially influential in terrestrial systems. After establishing the strong connection between Fe-cycling and methane dynamics in the basin landscape, Barrow became an ideal candidate for AOM testing. Not only is AOM more energetically favored when linked with an acceptor reduction pathway like sulfate or Fe-reduction (Larowe et al. 2008), but there are only a handful of studies of Arctic terrestrial AOM (Smemo and Yavitt 2011, Blazewicz et al. 2012, Gupta et al. 2013).

In order to determine the presence and relative importance of the AOM pathway in the peatlands near Barrow, we performed anoxic in vitro incubations of intact peat using stable isotope tracers. Chapter 2 summarizes the incubation results. Somewhat surprisingly, gross AOM rates surpassed gross CH$_4$ production rates in almost all the incubations, resulting in negative net fluxes from the soil. AOM and CH$_4$ production rates were positively correlated, bolstering the literature evidence for a substrate limitation on AOM rate potentials. CH$_4$ production rates decreased with increasing soil depth, which is somewhat surprising given genomic evidence that methanogenic presence peaks at 20-30 cm depths (Lipson et al. 2012, Lipson et al. 2013a), and there was no relationship between average rates of AOM and depth. AOM and CH$_4$ production rates were mostly insensitive to additions of Fe(III) and sulfate electron acceptors. However, there was a negative effect of soil depth on gross production rates within S-treated soils. There was also a negative interaction between Fe treatment and soil depth on the AOm rate constant, relating an increase in potential AOM with Fe additions in shallow soils. Soil genetic surveys show phylogenetic links between Barrow soil organisms and known anaerobic methanotrophs in ANME groups 2 and 3, and mcrA gene copies present that are associated with the leading theory for the AOM mechanism. The results suggest the prevalence and potential importance of the
AOM pathway to the maintenance of the overall carbon sink in Arctic peatland ecosystems, and some evidence that AOM is linked with Fe-cycling in this system.

At this point I diverge from the high Arctic in Barrow, and move to northern Lapland Finland. I spent a full year in Finland in an attempt to create a pan-Arctic approach to understanding the controls on northern wetland methane fluxes. Much like the Barrow wetlands, the Finland field site is a minerotrophic wet sedge fen, but average overall growing season methane flux was about 3-fold higher in the Finnish system. Soil mineral content was also much lower in this system than in Barrow. This created an interesting situation in which to ask the same set of questions as in Alaska, to determine the impact of baseline conditions in a pan-Arctic comparison of methane process patterns. The third chapter is a mirror of the Alaskan AOM experiments, in which I did an almost identical set of in vitro incubations, with three minor exceptions: the initial amount of methane spike in the headspace was only 1% (as opposed to 5% in the Alaskan AOM incubation), the mass and isotopic signature of both CH₄ and CO₂ were measured at each time point, and the electron acceptor treatments included Fe, nitrate and sulfate. Measured AOM rates in the Finnish soils showed potential consumption of between 6-39% of the methane produced, contributing approximately 1% of total carbon dioxide flux. One of the strengths of the Finnish AOM study was the quantification of AOM, using two different datasets to both confirm the presence and relative importance of this pathway to overall methane cycling. Of the nitrate, sulfate and ferric iron treatments, only the nitrate treatment affected process rates; the nitrate treatment suppressed methanogenesis. Other acceptor treatments were entirely disassociated from AOM and methane production rates. Also interesting was the lack of relationship between AOM and methanogenesis rates, as if the two pathways were more disconnected than in the Alaskan soils.
The coup de grace of the year in Finland was the field measurement of methane and CO$_2$ net field fluxes collected over the course of an entire year. Just as in the Alaskan landscape surveys, this study was meant to help clarify the larger-scale ecological controls on net methane fluxes. The fourth chapter relates the average annual flux data for methane and CO$_2$ in this system. I saw substantial variation between different basin depressions, confirming the strength of the heterogeneity within wetland systems. Perhaps the most influential aspect of this dataset to the scientific literature is the evidence for both autumnal and spring thaw methane bursts, collectively accounting for 26% of the annual methane flux. These ‘shoulder seasons’ are critical periods under climate change conditions, which disproportionately affect the edges of the thaw season. The autumnal burst was more than 5-fold larger than the spring burst. Despite the dogma of a close relationship between methane fluxes and temperature, the annual net methane rates were not related to either the 10 cm or 30 cm soil temperatures, but were slightly related to air temperatures. There was a strong effect of season on both CO$_2$ and methane flux rates, relating to changes in temperatures and snow depth. Methane ebullition, or bubbles, measured throughout the growing season augmented the summer methane source by an average of 50% additional methane, and was linked with fine-scale spatial heterogeneity within the wetland. Another mirror of the Alaskan data, I conducted an identical field manipulation experiment, adding soil amendments of ferric iron and humic acids mid-way through the growing season. Surprisingly, methane flux rates were insensitive to Fe(III) and humic acid soil amendments, although both treatments amplified CO$_2$ fluxes.

This dissertation contributes to our understanding of the controls on methane fluxes from northern peatland ecosystems, with a focus on the interaction between carbon cycling and Fe-cycling, and the annual seasonal fluctuations of carbon cycling. Importantly, we also found that
AOM is a significant potential sink of methane in both Alaskan and Finnish peatland systems, which must be considered in global modeling of this critical ecosystem and future climate projections.

REFERENCES


Chapter 1: Methane Suppression by Iron and Humic Acids in Soils of the Arctic Coastal Plain

ABSTRACT

Methane is a greenhouse gas estimated to provide an increasingly important role in climate forcing, yet large uncertainty persists in quantifying methane-climate interactions because the biological controls of methane cycling are poorly understood. Within anoxic soils, alternative electron acceptors such as iron and humic substances govern microbial metabolic function, and thus affect whether carbon is lost as methane. Emissions of carbon dioxide and methane may outpace storage as Arctic warming continues, making the vast carbon stores held in Arctic permafrost-affected soils critical in global biosphere-climate feedbacks. Here, we present data from wet sedge tundra landscapes near Barrow, Alaska that show an inverse relationship between dissolved iron and methane concentrations. Net methane fluxes were significantly suppressed following the experimental addition of iron and humic acids. These results suggest that in-situ methane production is tied to alternative electron acceptor availability. With melting permafrost leading to an increasing amount of organic matter and mineral soil introduced into the active layer, the mineral-carbon relationship must be taken into consideration when predicting how these vulnerable systems will behave and contribute to carbon-climate feedbacks now and into the future.

INTRODUCTION

Northern permafrost soils contain as much as half of the world’s belowground organic carbon (Tarnocai et al. 2009), a large proportion of which is held in cold, highly anoxic wetland
conditions. As the Arctic warms, melting permafrost creates an ever-expanding pool of soil carbon vulnerable to microbial decomposition and loss as carbon dioxide (CO₂) and methane (CH₄)(Schuur et al. 2008). As thaw depths increase, underlying mineral layers will contribute additional carbon, nutrients and minerals to the active soil layer, potentially altering microbial decomposition rates. One under-evaluated aspect of these permafrost-affected soils is the interaction between soil organic and mineral components (Heitmann et al. 2007, Keller and Bridgham 2007, Keller et al. 2009, Lipson et al. 2010, Blodau and Deppe 2012, Lipson et al. 2012, Bridgham et al. 2013, Friedman et al. 2013, Keller and Takagi 2013, Lipson et al. 2013a, Lipson et al. 2013b). The thermodynamic ladder model for anaerobic microbial respiration posits a series of redox zones relating to the potential energy yield of the dominant terminal electron acceptors (henceforth, we use ‘acceptors’) present. Within anoxic subsurface systems, if resources are limiting or there are microbial inhibition mechanisms at play, the differential energy yields of alternative acceptor reactions should determine relative rates of respiratory processes.

After decades of ecological work focused on soil respiration, there is increasing evidence that outside of a few simple systems, the boundaries of anoxic redox zones are blurry at best (Bethke et al. 2011). Mechanistically, overlapping redox zones indicate a lack of competition for energy (Allen et al. 2010), no direct inhibition (van Bodegom et al. 2004), and/or temporal or spatial heterogeneity in soil conditions (Khalil and Baggs 2005, Sommerkorn 2008). There is some evidence to suggest that microbial populations do not compete for carbon substrates within carbon-rich environments (Lovley and Phillips 1987, Holmer and Kristensen 1994, Sturtevant and Oechel 2013). In fact, thermodynamically-competitive microbial populations are often found coexisting in relatively small areas, as evidenced by both genomic and incubation studies of
homogenized wetland soils (Roden and Wetzel 1996, Segers 1998, Blodau et al. 2002, Lueders and Friedrich 2002, Gauci and Chapman 2006, Keller and Bridgham 2007, Knorr and Blodau 2009, Friedman et al. 2012, Inglett et al. 2012, Bridgham et al. 2013). The effects of the development of redox zonation in soil systems is muddled further, as methanogenesis, for example, can be both directly inhibited (van Bodegom et al. 2004) and/or stimulated by alternative acceptor-reduction (Reiche et al. 2008). Fine-scale variability in respiratory processes could also be linked to micro-scale variation in soil conditions, which are strongly influenced by the presence and species of vegetation (Roden and Wetzel 1996, von Fischer et al. 2010).

Substrate limitation and microsites do not fully explain carbon cycling dynamics within anoxic, carbon-rich soils, but the more undervalued dissimilatory pathways may hold some clue (Keller and Bridgham 2007, Keller et al. 2009, Lipson et al. 2010, Keller and Takagi 2013). In addition to the classic inorganic acceptors, organic acceptors (i.e. humic substances) are gaining recognition as redox-reactive species within soils, especially in northern wetlands where they are in high abundance (Heitmann et al. 2007, Blodau and Deppe 2012, Bridgham et al. 2013). The reduction of humic substances is thought to be thermodynamically favorable to CH₄ production (Cervantes et al. 2000, Blodau and Deppe 2012, Friedman et al. 2013, Keller and Takagi 2013), and may also function as an electron shuttle with other redox pathways, such as iron(Fe)-reduction (Lipson et al. 2012, Lipson et al. 2013b). CH₄-producing microbes are at the very bottom of the thermodynamic ladder, and should therefore be suppressed in the presence of alternative acceptors. Given the importance of understanding CH₄ production for predicting climate feedbacks (Schuur et al. 2013), the interaction between organic and inorganic acceptors and CH₄ deserves more attention within carbon- and acceptor-rich Arctic soils.
A model system for investigating the links between CH$_4$ and organic and inorganic acceptors is Barrow, Alaska, which boasts carbon- and mineral-rich permafrost-affected soils representative of the Alaskan Arctic Coastal Plain. This landscape is dominated by a time series, or chronosequence, of drained thaw lake basins (hereafter referred to simply as ‘basins’), which allows comparison of ecosystem function across stages of soil development (Hinkel et al. 2003, Bockheim et al. 2004, Lipson et al. 2013b, Sturtevant and Oechel 2013). This system is also relatively simplified thermodynamically, as the more commonly-studied acceptors such as nitrate, sulfate and manganese are relatively unavailable (Lipson et al. 2010), and the water table remains near the soil surface throughout most growing seasons (Liljedahl 2011). Barrow has carbon-, humic substances- and Fe-rich soils that vary along the basin chronosequence, making it an ideal site to study the interactions between methanogenic and alternative respiration pathways. Previous work from Barrow has shown net gas fluxes to have high CO$_2$:CH$_4$ ratios (>100) despite prevailing anoxic conditions, suggesting CH$_4$ suppression by alternative respiratory activities (Lipson et al. 2012, Friedman et al. 2013). This study examined the relationships between Fe and net CH$_4$ production in the context of landscape features using two years of observational data representing both spatial and temporal variability. We then utilized an *in-situ* manipulative experiment to test the direct relationship between net CH$_4$ fluxes and the dissimilatory reduction of Fe and humic substances.

**METHODS**

**SITE DESCRIPTION**

Observational data were collected from a total of 14 drained thaw lake basins in the area surrounding Barrow, Alaska over two growing seasons in 2010 and 2011. All basins were
located within the Barrow Environmental Observatory research reserve (71.2963 N, 156.5891 W), an area characteristic of the Alaskan North Slope acidic wet sedge tundra (Zona et al. 2010). The landscape is underlain by deep continuous permafrost, a mean seasonal active layer thickness of 36 cm (range: 30-90 cm), and an average summer air temperature of 3.3°C (June to August, 1960-present). Basins sampled were categorized by years since draining: young (0-50 years), medium (50-300 years), old (300-2000 years), and ancient (2000-5500 years) (Hinkel et al. 2003), and included low-centered, and flat polygons. Hydrologically, basins are inundated most of the growing season with periodic cycles of evaporative surface drying and rewetting by precipitation (Hinkel et al. 2001), yet the water table remains within 5 cm of the soil surface during all but extreme droughts (Olivas et al. 2010a, Liljedahl 2011, Lipson et al. 2012).

SOIL POREWATER

Transects of approximately 70 m of tundra matting were established within each basin. Ten soil porewater samplers (Rhizon, type MOM for metal studies, Sunvalley Solutions, Florida) were installed at a depth of 0-10 cm randomly along each transect. In 2010, we sampled soil porewater every 1-2 weeks from June to September from 8 basins, with 2 representatives from each of the four basin age classes. In 2011, we increased spatial replication to include 6 additional basins, for a total of 14 basins. Soil porewater samples were collected in additive-free vacutainers (Becton Dickinson) and stored in dark refrigeration.

Dissolved gas samples were prepared by equilibrating 1 mL of porewater with an N₂ headspace within an airtight 25-mL glass serum vial fitted with gas-impermeable butyl rubber stoppers (Geo-Microbial Technologies, Inc.). Equilibration units were gently shaken for 12 hours, then left undisturbed in a dark cabinet for 12 hours. Headspace samples were analyzed for
CO₂ and CH₄ concentrations on a gas chromatograph equipped with TCD and FID detectors. Samples for Fe analysis were collected into air-free vials, and acidified to pH < 2.0 with 1 M HCl without filtering, wrapped in wax and stored in Bitran airtight zip-top bags in dark refrigeration until analysis within 2 weeks of collection. Total Fe and Fe(II) were quantified from acidified samples using a 1,10-phenanthroline colorimetric method (Committee 1978, Lipson et al. 2010); Fe(III) content was calculated by difference.

SOIL SAMPLING

Frozen 30 cm-length soil cores were extracted in early June 2010 and 2011, 5 cm sections of which were immediately immersed in pre-weighed bottles containing 2 M HCl. Fe analysis was completed on all samples using the 1,10-phenanthroline method, as described above, with content calculations adjusted to include soil water. Multiple deep cores (40-80 cm) were collected from all basins in both 2010 and 2011 with a SIPRE core drill bit and small gas-powered engine. Soil water content, organic matter content (determined by loss upon combustion at 550°C), bulk density (estimated from core dimensions and water content analysis), and organic and active layer thicknesses were measured from each core. Basin data were computed by first averaging each core individually, then averaging all cores within a basin to produce a single, growing season average value for the various parameters of interest, independent of soil depth or within-season changes.

MANIPULATED EXPERIMENT

The manipulative field experiment was conducted at the Biocomplexity Experiment (BE) site, situated within and around a medium aged basin (Hinkel et al. 2003) at 71.284056 N,
156.596 W. The BE medium basin vegetation consists of *Sphagnum* mosses, and *Carex Aquitalis, Eriophorum scheuchzeri, Dupontia fisherii, Arctophila fulva* vascular plants (Olivas et al. 2010b). The BE basin has an average organic layer thickness of 12-15 cm (Lipson et al. 2010), average active layer thickness of 34 cm (Sturtevant et al. 2012), and moderate ice wedge polygonization. Three, 20 m transects were established within two adjacent depressed-center ice wedge polygons. Soil collars made of 25 cm tall sections of 10 cm-diameter thin-walled, polyvinyl-chloride (PVC) pipe were buried to a 21 cm depth. Twenty-four pairs of collars (less than 5 cm between paired collars) were installed for a total of 48 collars, equally distributed amongst transects. Twice-weekly measurements of soil pH, soil temperature and electrical conductivity were taken at 10 cm soil depth. Reduction-oxidation (redox) potential measurements were collected twice weekly from probes permanently installed outside of the soil collars at depths of 7 and 14 cm. Redox measurements were collected with an internal Ag/AgCl electrode, and converted to redox potential, Eh (relative to standard hydrogen electrode) by applying a correction factor of +212 mV.

TREATMENTS

Treatment solutions of Fe(III)-nitrilotriacetic acid and Sigma Aldrich Humic Acids were designed to achieve approximate final concentrations of 5 mM Fe(III) and 1g humic acids/L within the saturated soil within the collar. Background levels of Fe were measured for all collars, and a background range of 4-40 mg/g dry humic yield by mass dry soil for medium basins (Jaime Zlamal, personal communication), were used to determine the magnitude of possible treatment effects. Previous work in this location has shown that the commercial Sigma Aldrich humic acids product displays the same redox properties as those of local soil-derived humic acids
Given the similarity in redox properties between commercial and soil-extracted humic acids, we used the commercial product to aid in reproducibility for future experimentation.

Gas flux measurements began seven days after collar installation. Treatments of equal volume solutions of Fe(III) NTA, humic acids, or deionized water were injected evenly throughout the top 10 cm of soil within each collar after gas flux measurements were completed on July 26, 2012.

GAS FLUX MEASUREMENTS

A PVC end cap was modified with brass barb fittings and 90 m of 3.2 mm internal diameter UV-resistant Tygon plastic tubing (U.S. Plastics, Lima, OH, USA) to create a closed circulation system with a field gas analyzer. Airtight seals were obtained with foam tape and a rubber gasket placed around the outer edge of the soil collar which the PVC cap was seated onto. Following cap placement, there was a 45 second delay before measurement recording began, allowing the tubing volume to flush completely. Chambers were pressure vented with a 21G needle during measurements, and ambient air was flushed through the system for at least 3 minutes between measurements.

CO₂ and CH₄ fluxes were measured every Tuesday, Thursday and Saturday using a Fast Greenhouse Gas Analyzer (Los Gatos Research, Mountain View, CA, USA) for the period of July 17 to August 14, 2012. CO₂ and CH₄ concentrations (ppmv) were recorded every second for 300 seconds starting from the point when both gases had linear increases in concentration. R² values for all flux curves were greater than 0.8, with all but one having R² values greater than 0.9.
STATISTICS

Observational data were averaged by season for each basin individually. CO$_2$:CH$_4$ ratios were aggregated into basin-specific seasonal geometric averages, by first calculating CO$_2$:CH$_4$ values for each spatial point at every collection time, averaging each spatial point through time, and then taking a geometric mean of the spatially- and temporally-averaged CO$_2$:CH$_4$ ratios for each basin for a single growing season. Basins were compared with ANOVA and Tukey’s honest significant difference tests. Treatment effects in the manipulated experiment were determined using repeated measures ANOVA, with post-hoc ANOVAs and Tukey’s tests. Data were transformed when appropriate to conform to the assumptions of statistical tests used. All statistical tests were performed in R.

RESULTS

LANDSCAPE PATTERNS

We sought first to determine the relationship between soil parameters and mineral Fe concentrations within the chronosequence of basins near Barrow, AK. Previous work has shown that these soils are naturally Fe-rich, with Fe-reduction contributing significantly to anaerobic soil respiration in all basin ages (Lipson et al. 2010, Lipson et al. 2013b). As basins age, organic matter accretes over time, increasing the thickness of the organic layer, and therefore the burial depth of the underlying mineral layer (Hinkel et al. 2003). Average minimum organic layer thickness here represents a basin-specific seasonal mean minimum thickness (with ‘minimum’ referring to samples where organic-to-mineral transition exceeded our sample depth). We found an inverse relationship between bulk density and organic layer thickness (Figure 1.1a, p< 0.001),
and a positive relationship between organic matter content and organic layer thickness (Figure 1.1c, p<0.001). The expansion of the organic layer effectively provides separation from the underlying, more-dense mineral soil. The repercussions of this separation are seen in the negative relationship between total acid-extractable Fe content and organic matter content in the active layer (Figure 1.1d, p<0.001), and the positive relationship between Fe content and bulk density (p<0.001).

![Figure 1.1: Relationships between basin physical soil characteristics. Data represent soil cores taken in June and August 2011 from thirteen basins, with representations from young (n=3), medium (n= 3), old (n = 4), and ancient (n = 3) age classes.](image-url)
Despite the variability among similarly-aged basins, the basin age classifications maintain an overall increase in organic layer thickness over time (Table 1.1, p=0.018). However, this relationship is the least clear within old basins, possibly due to organic matter compaction and/or cryoturbation during this stage of development. Independent of the cause, the thinner organic layers found in old basins puts the active layer back into closer contact with the mineral layer (Lipson et al. 2013b), reflected in the bump in total acid-extractable soil Fe content in old basins (Table 1.1). Using organic layer thickness as a continuous proxy for soil development, we found a significant negative linear relationship between seasonal mean acid-extractable total Fe ($R^2=0.50$, $p=0.003$), Fe(II) content ($R^2=0.22$, $p=0.045$), and Fe(III) content in the soil (Figure 1.2a, $p=0.001$).

### Table 1.1. Soil properties by basin age class†

<table>
<thead>
<tr>
<th></th>
<th>Bulk Density (g/cm³)</th>
<th>Organic Matter (g/g)*</th>
<th>Total Fe (uM/cm³)</th>
<th>Minimum OLT (cm)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young</td>
<td>0.48 (0.1)</td>
<td>0.28 (0.1)a</td>
<td>97.9 (16)a</td>
<td>12.4 (3.4)a</td>
</tr>
<tr>
<td>Medium</td>
<td>0.44 (0.2)</td>
<td>0.30 (0.1)a</td>
<td>89.2 (22)ab</td>
<td>18.3 (1.7)ab</td>
</tr>
<tr>
<td>Old</td>
<td>0.45 (0.1)</td>
<td>0.37 (0.1)ab</td>
<td>94.4 (8)a</td>
<td>13.8 (1.1)a</td>
</tr>
<tr>
<td>Ancient</td>
<td>0.31 (0.0)</td>
<td>0.49 (0.0)b</td>
<td>68.0 (11)b</td>
<td>22.8 (1.2)b</td>
</tr>
</tbody>
</table>

† Basins sampled June/August 2011: young (n=3), medium (n=3), old (n=4), ancient (n=3)

*aValues are means and standard errors. Down each column, values with the same letter (or no letter) are not significantly different by Tukey’s test

*Significant ANOVA
Figure 1.2: Relationships between minimum organic layer thickness averaged by season for each basin and (a) acid-extractable Fe(III) content in the soils, (b) log of dissolved porewater concentrations of CH$_4$ and CO$_2$, and (c) basin-specific seasonal geometric mean CO$_2$:CH$_4$ ratios.
We did not find a strong relationship between CO\textsubscript{2} and CH\textsubscript{4} concentrations in our surveys. Another study from this area attributed a similar lack of correlation between net CO\textsubscript{2} and CH\textsubscript{4} fluxes to a lack of competition for carbon substrate (Sturtevant and Oechel 2013). There is a significant decline in dissolved CO\textsubscript{2} in the porewater with both increasing basin age class and organic layer thickness (p=0.036 and 0.009, respectively), although the relationship is only slightly negative. There is, however, a strong, positive correlation between dissolved porewater CH\textsubscript{4} and organic layer thickness (Figure 1.2b, p=0.012). The diverging concentration trends combine to create a most interesting pattern: the basin-specific seasonal geometric mean CO\textsubscript{2}:CH\textsubscript{4} ratios have a strong decline as organic layer thickness increases (Figure 1.2c, p<0.001).

METHANE, HAVE YOU MET IRON?

Porewater surveys captured the strong negative correlation between average seasonal dissolved Fe(III) and CH\textsubscript{4} concentrations (Figure 1.3a, p<0.001). Figure 1.3b shows the significant positive relationship between porewater Fe(III) content and basin seasonal geometric mean CO\textsubscript{2}:CH\textsubscript{4} (p=0.009). Thus, thicker organic layers corresponded to increasing overall dissolved CH\textsubscript{4} concentrations and decreasing Fe(III) concentrations. There was also a negative (but non-significant) relationship between porewater CH\textsubscript{4} concentrations and the ratio of Fe(III)/total Fe (p=0.18), which can be interpreted as indicating a constraint on methanogenesis by Fe(III)- and humic substance-reducing bacteria (Lipson et al. 2012).
Figure 1.3: Acid-extractable Fe(III) soil content as it relates to (a) the log of dissolved CH$_4$ in soil pore waters, and (b) seasonal geometric mean CO$_2$:CH$_4$ ratios. All data points represent single seasonal averages for each basin tested. One outlier (>3 s.d. above mean) from the dissolved CH$_4$ concentration data was removed.
MANIPULATIVE EXPERIMENT: THE METHANE-IRON LINK

To investigate the negative correlations observed between Fe(III) availability and CH$_4$ concentrations, we designed a manipulative field study to resolve a possible mechanistic relationship among Fe and humic substances concentrations, and net CH$_4$ flux. The field experiment measured net CO$_2$ and CH$_4$ fluxes from soils treated with solutions of biologically-available Fe(III) NTA (Thamdrup 2000) or Sigma Aldrich humic acids. Porewater concentrations confirmed that both total Fe and Fe(III) were significantly raised in Fe(III) NTA-treated collars after treatment introduction (both p<0.001). The significant suppression of CH$_4$ flux after both Fe(III) NTA and humic acid treatments, and the resulting difference in mean CO$_2$:CH$_4$ between Fe and the other treatments are shown in Table 1.2. The redox data presented in Table 1.2 convey the maintenance of reducing conditions, and reflect the slightly wetter soil profile (due to precipitation) that characterized the last half of the experimental period.

| Table 1.2. Manipulated Experiment Flux Rates and Soil Reduction Potential$^\dagger$ |
|--------------------------------------------------|--------------------------------------------------|------------------|------------------|------------------|
|                                      | CH$_4$ Flux (mg/m$^2$/h) | CO$_2$ Flux (mg/m$^2$/h) | CO$_2$:CH$_4$ Ratio | Redox Potential 7cm (mV) | Redox Potential 14cm (mV) |
| Pre-Treatment                      |                                      |                              |                              |                              |                              |
| Control                           | 1.40 (0.08)*                         | 2548.6 (120)*                 | 3310 (364)                   | 21.9 (29)                    | -97.5 (15)                   |
| Humic Acids                       | 1.24 (0.10)                         | 2480.1 (153)                  | 3638 (594)                   | 16.5 (39)                    | -101.9 (21)                  |
| Fe(III)-NTA                       | 1.22 (0.10)                         | 2632.7 (157)                  | 4883 (1493)                  | -0.3 (40)                    | -108.2 (21)                  |
| Post-Treatment                    |                                      |                              |                              |                              |                              |
| Control                           | 1.88 (0.10)$^a$                      | 3023.0 (150)                  | 3307 (417)$^a$               | -160.1 (13)                  | -104.2 (12)                  |
| Humic Acids                       | 1.41 (0.08)$^b$                      | 2941.8 (202)                  | 4436 (796)$^b$               | -161.6 (18)                  | -105.7 (17)                  |
| Fe(III)-NTA                       | 1.39 (0.09)$^b$                      | 3208.1 (231)                  | 5623 (1199)$^b$              | -165.6 (18)                  | -108.4 (17)                  |

$^\dagger$Values are means and standard errors. Down each column within each timing period (pre- or post-treatment), values with the same letter (or no letter) are not significantly different by Tukey’s test.

$^a$Significant ANOVA between pre- and post-treatment averages (not including treatment effects), p-value < 0.05.

Due to high spatial variability within this system, we paired control and treatment collars across the landscape. Landscape variability is accounted for by subtracting paired control collars from treatment collars. Figure 1.4 shows the difference effects of the Fe(III) NTA and humic
acid treatment additions on CH$_4$ flux. Using a repeated-measure ANOVA, we found control treatment CH$_4$ flux values were significantly higher than both the Fe and humic acid addition treatments (p<0.001). Most notably, the two time points immediately following treatment introduction (denoted by the vertical dotted line on July 26) had the farthest separation between the control and treatment flux means. It should be noted that while the two adjacent depressed-center ice wedge polygons expressed significantly different flux magnitudes leading to increased overall variance (i.e. one depression had consistently higher emission rates), the treatment effect was common. All treatments experienced peak CO$_2$ fluxes four days after treatment introduction on 31 July, leading to a significantly higher post-treatment average CO$_2$ flux, independent of treatment type (Table 1.2). However, there were no significant differences in treatment-averaged CO$_2$ fluxes for any time point during the experiment.

![Graph](image)

**Figure 1.4:** Time series of treatment-averaged differences (from control treatment mean) in CH$_4$ flux during the manipulative experiment in Barrow, AK. One outlier (>3 s.d. from mean) on 8/14
was removed from the control treatment mean calculation. Dotted vertical line on 7/26 represents when treatment solutions (Fe(III)NTA, humic acids, or DI water (control)) were injected into collars following flux measurements on that day. Dashed horizontal line represents no difference from average control treatment flux. Error bars represent the combined standard errors using error propagation.

DISCUSSION

ORGANIC LAYER THICKNESS IS A USEFUL METRIC

Average organic layer thickness is a robust measure for characterizing season-level physical soil qualities in this system. Basin age class alone does not capture the variability between basins of equal age, and more importantly, the variation within any given basin. Importantly, we simplified the data presented here, sacrificing small-scale vertical and horizontal distinction by averaging over the vertical profile, within basins, and then for entire growing seasons. These simplifications served to make the results more translatable across space and time, and provided statistical power that is otherwise lost to the surfeit of blocking variables required to account for spatial variability in these highly heterogeneous wetlands. It also ensured that we presented somewhat conservative relationships, more probable than correlations seen with less inclusive datasets. In our observational data, we found basin age categories do not clarify the relationships between average active layer organic matter and bulk density with the total acid-extractable Fe content, nor the overall decreasing pattern in total-acid extractable Fe content as seen in Table 1 and Figures 1b and 1d. All of these soil variables can be more reliably explained by the organic layer thickness (Figures 1a, 1c and 2a). Other work in this area showed
a similar decrease in extractable humic substances with basin age (Bockheim et al. 2004), and thus as organic layer thickness increases.

Biologically, landscape surveys in this area have shown significant decreasing net ecosystem CO₂ flux with basin age, attributed mainly to vegetation-related successional changes in the basin ecosystems (Zona et al. 2010, Sturtevant and Oechel 2013). Our results corroborate this relationship, both directly in the CO₂ signal decrease with increasing basin age, and indirectly, with negative correlation between CO₂ and organic layer thickness. In this instance, organic layer thickness stands as a proxy for vegetation accretion and succession.

THE METHANE STORY

In acidic Sphagnum and sedge wetlands, there is a theoretical dominance of the acetoclastic compared to the hydrogenotrophic pathway of methanogenesis, but the universality of this assumption is unclear (Rooney-Varga et al. 2007, Hines et al. 2008). Regardless of the particular combination of pathways, methanogenesis produces a CO₂:CH₄ ratio around 1 (Yavitt and Seidmann-Zager 2006); thus, higher CO₂:CH₄ ratios are evidence of the relative dominance of alternative acceptor reduction pathways (Keller et al. 2009). Among basins, we found a strong relationship between growing season geometric mean porewater CO₂:CH₄ ratios and organic layer thickness (Figure 2c). The CO₂:CH₄ ratio, although unable to demonstrate if it is CO₂ or CH₄ that is changing between different (or similar) ratio values, does provide context about the balance between methanogenic and alternate acceptor-respiring activity. Interestingly, changes to these CO₂:CH₄ ratios were driven primarily by the CH₄ component (Figures 2b and 2c). As organic layer thickness increases, we see an increase in CH₄ concentration, and a decrease in
both Fe(III) concentrations and CO₂:CH₄. These data support a strong connection between Fe and the proportion of carbon lost as CH₄.

There are a number of proposed mechanisms for CH₄ suppression in the presence of Fe-reduction, including direct inhibition by Fe(III) (van Bodegom et al. 2004). Alternatively, the ability to reduce Fe(III) has been observed in some methanogens (Bond and Lovley 2002, van Bodegom et al. 2004), therefore it is possible that certain methanogens reduce Fe(III) when it is available, and switch to a purely methanogenic metabolism when electron acceptors are depleted or not favored. This possibility is feasible given the observed mid-season shift from humic substance-reduction to Fe(III)-reduction, as peak CH₄ fluxes to the atmosphere are observed just prior to mid-season, when humics-reduction is peaking (Lipson et al. 2013b, Sturtevant and Oechel 2013). The coexistence of methanogenesis and Fe-reduction could also result in part from high electron donor availability in organic layers (Lovley and Phillips 1987, Holmer and Kristensen 1994), or microsite conditions (Olivas et al. 2011). Despite prevalent thermodynamic constraints on the methanogenic communities by Fe- and humic substance-reducing populations, CH₄ production was not (entirely) repressed in this system.

Our landscape surveys substantiate a mechanistic link between net CH₄ production and Fe(III)-reduction, both of which are well characterized by basin organic layer thickness. The link between CH₄ and organic layer thickness is potentially explained in three ways: 1) an increase in carbon available for CH₄ production with increasing thickness of the organic layer, 2) methanotrophy associated with the changes in soil aeration and/or the availability of alternative acceptors coupled with soil development, or 3) an active suppression of CH₄ production by a dominant Fe-reducing pathway. Within the Barrow system, the availability of carbon has not been found to limit carbon fluxes (Allen et al. 2010, von Fischer et al. 2010, Sturtevant and
Oechel 2013), casting doubt on the first possibility. We also did not find a strong relationship between CO₂ and CH₄ concentrations in our surveys, which can be attributed to a lack of competition for carbon substrate (Sturtevant and Oechel 2013). Aerobic methanotrophy has a predictably positive relationship with soil aeration, be it through evaporative drying of the upper soil layers or the oxygenation of the rhizosphere, which is related with vegetation (Whalen 2005, Bhullar et al. 2013). The biological controls on anaerobic methanotrophy are currently underexplored, with current examples of both inhibition and stimulation with acceptor abundance, and only a handful of in situ rate estimations that can be linked with living vegetation (Gupta et al. 2013). This pathway is perhaps best mentally grouped with methanogenesis itself, as they share many thermodynamic and physical limitations (Smemo and Yavitt 2011). Regardless of methanotrophic pathway, this mechanism is not exclusive of option 3. The third possibility, that CH₄ production is suppressed by Fe- and humic substance-reduction pathways, is addressed by our manipulative experiment.

MANIPULATED EXPERIMENT

We chose the site of the manipulative experiment based on the large apparent effect of Fe on CH₄ seen in the observational study. We found a basin with relatively low available Fe concentrations and high CH₄ fluxes. In this way, the experimental additions of Fe and humic substances would push conditions in the manipulated basin more towards those found in all other basins. There were no areas in our system where Fe and humic substances are absent.

The Fe(III) and humic acid treatments had indistinguishable suppression effects on CH₄ flux (see Figure 4), which is not surprising given the flexibility within certain microbial populations to reduce both Fe(III) and humic substances (Coates et al. 1998). In addition, there
were no significant differences in treatment-averaged CO$_2$ fluxes for any time point during the experiment, again consistent with the pathway-switching potential of Fe(III)- and humic substance-reducing microbes, and the high native load of acceptors in the soil system. The addition of both Fe and humic acids also might not have elicited an overwhelming difference in net CO$_2$ flux because the low natural CH$_4$ fluxes in the Barrow basin system (Zona et al. 2009, von Fischer et al. 2010, Sturtevant et al. 2012, Friedman et al. 2013, Sturtevant and Oechel 2013) mean that any experimental CH$_4$ suppression would have a small impact on the much larger CO$_2$ fluxes. Or that the addition of labile acceptor treatments promoted the re-oxidation of exhausted acceptors rather than direct reduction.

We have previously found evidence for a mid-season pathway shift in these soils, with humic substance-reduction dominating the early season, and then a swing to Fe(III)-reduction in the mid- and late-season (Lipson et al. 2013b). This work was done mid-season, when the electron accepting capacity of humic substances is therefore thought to be nearly exhausted, and Fe(III)-reduction has started shifting to become the dominant anaerobic respiratory pathway. Others have also found evidence that fermentative Fe-reduction (which does not compete with methanogenesis for electron donors) can stimulate carbon cycling, thus increasing the amounts of carbon substrate available within the anoxic layers (Reiche et al. 2008), creating a delayed treatment effect on CH$_4$ flux, which we did not see during the post-treatment period measured.

**IMPLICATIONS FOR THE FUTURE**

A warmer Arctic climate is likely to complicate soil chemistry patterns, as increased cryoturbation and permafrost thaw alter the soil profile. Cryoturbation causes deeper layers to mix into the upper soil horizons, effectively redistributing the mineral-rich upper permafrost
layer into the organic layer (Bockheim 2007). Additionally, the melting of shallow permafrost may supply an increased amount of Fe and other acceptors simply due to the increasing depth of the active layer. Permafrost thaw can also promote new basin development, in which case the landscape may become disproportionately populated with young basins (Hinkel et al. 2003). The high-Fe characteristic of the active layers of young or cryoturbated basins could have implications for carbon cycling in the future on the Alaskan Coastal Plain. However, the expansion of the mineral supply in the active layer of these soils will not occur in isolation from any melt-associated changes in the projected-to-be wetter hydrology, shifts in vegetation type and productivity, and rising temperatures, making this a highly relevant area for future research.

CONCLUSIONS

This work provides direct field evidence for a mechanistic link between Fe and CH₄ in Barrow wetland soils. We observed strong and consistent correlations between Fe and CH₄ at the landscape level, and validated these patterns with a manipulative field experiment. We also found that organic layer thickness is a useful landscape-level proxy for the relationship between Fe and CH₄ within the mineral-rich drained thaw lake basin landscapes of the Alaskan North Slope. This is in direct contrast to meta-analysis work by Olefeldt et al. (2013), who found organic layer thickness to be unhelpful in characterizing CH₄ fluxes on the biome level (Olefeldt et al. 2013). We posit that a strong relationship between organic layer thickness and CH₄ is reasonable only under conditions similar to those in our mineral-rich, shallow permafrost basin system. The basin succession fosters strong connections between landscape features and soil chemistry, and the high concentrations of soil Fe were important to establishing the landscape
and CH₄ link. This basic relationship between CH₄ and Fe is useful for understanding the complex CH₄ dynamics in many highly heterogeneous wetland systems.

The higher CO₂:CH₄ ratios correlated with thinner organic layers pose an interesting question for the future. Given the potential for new basin development within this system under warming conditions, we would expect a higher proportion of soil carbon lost as CO₂ rather than CH₄ in the Fe-rich young basins (Lipson et al. 2010, Lee et al. 2012, Lipson et al. 2012, Lipson et al. 2013a, Lipson et al. 2013b). This may be especially true as increasing thaw depths penetrate farther into the mineral layer releasing new Fe into the active layer, and if the hydrology alters to increase soil aeration and promote CH₄ oxidation. Warming temperatures in shallow permafrost soils may also increase the amount of cryoturbation (Bockheim 2007), leading to a greater amount of mineral components mixed into the active layer. By contrast, an increased amount of alternative acceptors present in the soil could have a stimulatory effect on net CH₄ release by enhancing overall carbon cycling (Reiche et al. 2008), particularly if melting permafrost exposes additional carbon stores and alters the hydrology of the system. Future work should focus on the vulnerability of methanogenic and methanotrophic pathways to alternative electron acceptors under warming scenarios.

ACKNOWLEDGEMENTS
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REFERENCES


Chapter 2: Anaerobic methane oxidation in high-Arctic Alaskan peatlands as a significant control on net CH$_4$ fluxes

ABSTRACT

The terrestrial consumption of the potent greenhouse gas methane (CH$_4$) is a critical aspect of future climate, as CH$_4$ concentrations in the atmosphere are projected to play an increasingly important role in global climate forcing. The anaerobic oxidation of methane (AOM) is an important consumptive pathway in marine systems, but has only recently been considered influential in terrestrial systems. In order to determine the presence and relative importance of the AOM pathway in peatlands near Barrow, Alaska, we performed *in vitro* anoxic incubations of intact peat using stable isotope tracers. Our results show average AOM rates of 14.95 nmol cm$^{-3}$ hr$^{-1}$ that surpasses the average rate of gross CH$_4$ production: 6.02 nmol cm$^{-3}$ hr$^{-1}$. We found AOM and CH$_4$ production rates were positively correlated, and CH$_4$ production rates decreased with increasing soil depth. CH$_4$ production was insensitive to additions of Fe(III), but there was a negative effect of soil depth on gross production rates within sulfate-treated soils. AOM rates were unaffected by sulfate additions, but there was a depth:Fe(III) interaction in the kinetic reaction constant, suggestive of AOM stimulation by Fe(III) in shallow soils (<10 cm). Soil genetic surveys show phylogenetic links between soil organisms and known anaerobic methanotrophs in ANME groups 2 and 3. These results suggest the prevalence and potential importance of the AOM pathway to the maintenance of the overall carbon sink in Arctic peatland ecosystems, and a possible link with Fe(III)-reduction.
INTRODUCTION

The anaerobic oxidation of methane (AOM) is an important aspect of global climate, as it constrains the atmospheric release of the powerful greenhouse gas methane (CH$_4$) from marine and terrestrial systems. In marine systems, AOM linked to sulfate-reduction consumes approximately 90% of methane before it can be released into the atmosphere (Valentine 2002, Knittel and Boetius 2009). In terrestrial systems, aerobic methane oxidation is regarded as a significant CH$_4$ sink, but there is increasing evidence that AOM can also be an important control on net CH$_4$ fluxes from soils and freshwater systems (Whalen 2005, Gupta et al. 2013). This is particularly salient in northern peatlands, which store vast quantities of soil carbon (Tarnocai et al. 2009), and traditionally act as sinks of carbon dioxide (CO$_2$) and sources of CH$_4$ to the atmosphere (Gorham 1991, Limpens et al. 2008, Oechel et al. 2014). Although northern peatlands are major sources of atmospheric CH$_4$ and have the potential to influence the global climate system, the ecological and physical constraints on both CH$_4$ production and consumption are still poorly understood. (Smemo and Yavitt 2011). The influence of the AOM pathway to net ecosystem CH$_4$ balance is especially enigmatic, as estimations of this pathway within northern soils are limited (Smemo and Yavitt 2007, Smemo and Yavitt 2011, Blazewicz et al. 2012, Zhu et al. 2012, Gupta et al. 2013), leaving open the possibility that AOM is an important aspect of CH$_4$ cycling in northern peatland systems.

A number of mechanisms have been identified for the AOM process. Marine AOM has been most commonly linked with sulfate-reduction, conducted by either a single sulfate-reducing bacterium or via the consortium of a sulfate-reducing bacterium with a methanotrophic archaeal partner (Knittel and Boetius 2009). Recent evidence has been found for alternative marine consortia between archaea and iron and manganese reducing bacterial partners (Beal et al. 2009,
Segarra et al. 2013, Riedinger et al. 2014). The impact of alternative electron acceptor presence is less clear in terrestrial AOM, where there has been much less success definitively linking AOM activity with a specific reductive pathway. The major exception to this is the identification of freshwater nitrate-dependent AOM, or ‘n-damo’, conducted by denitrifying bacteria with (Raghoebarsing et al. 2006, Hu et al. 2009, Kip et al. 2010) or without an archael consortium (Ettwig et al. 2010, Zhu et al. 2012, Shen et al. 2014). Although often not as conclusive as the n-damo pathway, there have been a number of studies supporting sulfate-independent terrestrial AOM, unsurprising since electron acceptors other than sulfate are more energetically favorable (Caldwell et al. 2008). To date, the interplay between electron acceptors and AOM in northern peatlands has been highly site-specific, and often unresolved even within sites (Smemo and Yavitt 2011), with a few exceptions where a link to iron-reduction was the most likely mechanism (Crowe et al. 2011, Sivan et al. 2011, Amos et al. 2012).

While the interaction between AOM and electron acceptors is still somewhat muddled, the impact of alternative electron acceptors is known to inhibit methanogenesis, through direct and indirect means (Schlesinger 1997, Reiche et al. 2008). Electron acceptors directly inhibit methanogenesis via microbial competition, as methanogenesis is less energetically-favored than other forms of anaerobic reduction (van Bodegom et al. 2004, Whalen 2005), or indirectly through toxic or thermodynamically-favorable intermediate products like NO$_2^-$ or H$_2$ (Klüber and Conrad 1998, Sorensen et al. 2001, Mohanty et al. 2006, Thauer and Shima 2008, Conrad 2009). This phenomenon presents an interesting juxtaposition, as the leading hypothesis for the process of AOM is a form of ‘reverse methanogenesis’ (Zehnder and Brock 1980, Caldwell et al. 2008, Moran et al. 2008, Scheller et al. 2010a, Scheller et al. 2010b, Callaghan 2013). Thus, the
suppression of methanogenesis by alternative electron acceptors could also theoretically impact the reverse process, AOM.

Despite the difficulty in identifying the syntrophic partnerships (or lack thereof) in freshwater systems, advances in the genetic characterization of the AOM pathway have given us methods for identifying the presence and activity of AOM-associated organisms, including 16S rRNA and functional genes such as pmoA, pMMO, and mmoX which code for methanotrophic-specific enzymes (Jugnia et al. 2006, Tuomivirta et al. 2009, Gupta et al. 2013). The n-damo pathway has also been linked with the Phylum NC10 (Hu et al. 2009, Ettwig et al. 2010, Zhu et al. 2012, Shen et al. 2014), allowing for the easy identification of organisms potentially linked with this form of AOM. In marine systems, archeal methanotrophs, also known as ‘ANME’ for ANaerobic MEthanotroph, have been differentiated into phylogenetically-distinct groups ANME-1 (with subgroups a and b), ANME-2 (with subgroups a, b, and c), and ANME-3 (Orphan et al. 2001, Knittel and Boetius 2009). The ANME groups do not cover all known members of the AOM community, particularly in terrestrial and some extreme environments (Dettling et al. 2007, Cadillo-Quiroz et al. 2008), but have been shown to display some interesting functional characteristics. For example, Mohanty et al. (2006) found type I methanotrophs were stimulated by nitrogenous fertilizers, while type II were inhibited , and Knoblauch et al. (2008) saw a shift from type I to type II aerobic methanotrophs in Siberian peat under warming conditions (Knoblauch et al. 2008).

Despite the advances in our understanding of the mechanisms and organisms associated with terrestrial AOM, there are still relatively few studies with measured rates, especially in Arctic systems. The drained thaw lake basin landscapes on the North Slope of Alaska offer a unique location for the measurement of terrestrial AOM. The landscape is populated with
vegetated drained thermokarst lake basins (hereafter referred to as ‘basins’), underlain by deep continuous permafrost, and creating a natural chronosequence of soil development (Hinkel et al. 2003, Bockheim et al. 2004). The chronosequence of basins is correlated with variable amounts of organic matter and iron (Fe)-rich mineral soils incorporated into the active layer and available to biological processes (Bockheim 2007, Lipson et al. 2010, Lipson et al. 2013b). Measurements of ecosystem respiration within the basins show high Fe-reduction rates, and a high native load of iron in the soil system, while having non-measurable amounts of nitrate and sulfate electron acceptors during the growing season (Lipson et al. 2010). The basin wetlands are currently net sources of CH₄ to the atmosphere and are projected to become wetter and warmer with climate change (Vaughan et al. 2013), creating ideal conditions for enhanced CH₄ production. As AOM has been shown to be substrate limited, and thus increases with increasing amounts of CH₄ present, the projections favoring CH₄ may in turn also optimize conditions for AOM (Jugnia et al. 2006, Prater et al. 2007, Smemo and Yavitt 2007). Warming Arctic temperatures can result in thermokarst melting and new basin development, meaning that the future North Slope may become disproportionally populated with younger basins (Bockheim et al. 2003, Hinkel et al. 2003). If the landscape develops more young basins, the dynamics in these basins are critical to understanding the climate forcing potential of the extensive carbon stocks held within this ecosystem.

To our understanding, there are no previously-published AOM rates within a high Arctic peatland system. Our research was motivated by the desire to determine if AOM affects net CH₄ fluxes in the high-Arctic wetland system near Barrow, Alaska, and if so, could the unique native biogeochemistry help elucidate the electron acceptor aspect of the AOM mechanism. In order to determine the importance of AOM and the influence of inorganic electron acceptors on AOM
rates within Barrow, AK peatland soils, we performed *in vitro* incubations of soils representing six different basins across the landscape, and the vertical profile of the active layer within each basin. We amended soils with sulfate (SO$_4^{2-}$) and ferric iron (Fe(III)-nitrilotriacetic acid) as electron acceptor treatments. Given the importance of Fe-reduction to the anaerobic carbon cycle in these soils, and the absence of alternate acceptors, we hypothesize that AOM in this system is linked to Fe-reduction. We also present genetic data from basin soils to confirm the presence of microorganisms related to known anaerobic methanotrophs. Arctic peatlands are a highly relevant study system for the quantification of the importance of terrestrial AOM to the global climate system, as northern peatlands store vast quantities of soil carbon, and are experiencing rapid change.

**METHODS**

**STUDY SYSTEM: BARROW, ALASKA**

To determine the importance of the AOM pathway to Arctic CH$_4$ cycling, soils from mineral-rich wet meadow tundra with shallow persistent permafrost were incubated using stable isotope pool dilution. Incubated soils were collected from sites located within the Barrow Environmental Observatory research reserve near Barrow, AK (71.2963 N, 156.5891 W) (Hinkel et al. 2003, Lipson et al. 2013b). The Alaskan North Slope landscape is dominated by drained thaw lake basins, which are the footprints of shallow thermokarst lakes that have vegetated since draining (Hinkel et al. 2003). The basins are a successional chronosequence, commonly categorized into four age classes based on time since draining: young (0-50 years), medium (50-300 years), old (300-2500 years), and ancient (2500-5000 years) (Hinkel et al. 2003, Bockheim et al. 2004). Basin succession has been linked to soil productivity and carbon cycling pathways,
including a strong relationship with organic matter content, bulk density, Fe content and soil respiration (Bockheim et al. 2004, Lipson et al. 2010, Friedman et al. 2012, Lipson et al. 2012, Friedman et al. 2013, Lipson et al. 2013a, Lipson et al. 2013b, Sturtevant and Oechel 2013). The shallow, less-developed soils of young and medium age class basins are more closely linked with high rates of methane production and net flux to the atmosphere, as well as thinner organic layers, lower organic matter content, and higher bulk density in the active layer (von Fischer et al. 2010, Zona et al. 2011, Lipson et al. 2012, Sturtevant et al. 2012, Zona et al. 2012). There is also low availability of sulfate and nitrate electron acceptors in in the acidic and anoxic Arctic peat soils, where nutrients are scarce and biological uptake is fast and competitive (Lipson et al. 2010). Basic soil and porewater data from the cores used in this study are presented in Table 2.1, as measured by methods outlined in Lipson et al. (2012).

<table>
<thead>
<tr>
<th>Basin Age Class</th>
<th>Bulk Density (g cm$^{-3}$)</th>
<th>Organic Matter Content (g g$^{-1}$)</th>
<th>Fe$^{3+}$ (µmol cm$^{-3}$)</th>
<th>pH</th>
<th>Dissolved Organic Content (ppm)</th>
<th>CH$_4$ (µM)</th>
<th>CO$_2$ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium</td>
<td>0.42 (0.05)</td>
<td>0.32 (0.04)</td>
<td>30.3 (5.7)</td>
<td>5.54</td>
<td>30.3 (6.1)</td>
<td>1.55 (0.3)</td>
<td>671 (138)</td>
</tr>
<tr>
<td>Young</td>
<td>0.44 (0.06)</td>
<td>0.29 (0.03)</td>
<td>14.0 (3.2)</td>
<td>5.89</td>
<td>42.0 (7.6)</td>
<td>2.36 (0.9)</td>
<td>1019 (378)</td>
</tr>
</tbody>
</table>

Basic soil (21.6 +/- 0.9 cm deep) and water chemistry (0-10 cm depth) for basins sampled for incubations. Values are means and standard errors.

Porewater and pH data represent averages for June 2011 when soils were collected.

POOL DILUTION RATE ESTIMATES

We estimated gross CH$_4$ production and consumption (AOM) rates using an isotope pool dilution model slightly modified from von Fischer and Hedin (2002) and Smemo and Yavitt (2007). This technique is based on a combination of mass balance and isotope mixing model.
equations to simultaneously calculate gross methanogenesis and AOM rates, as illustrated in Figure 2.1.

The isotope pool dilution begins with an initial spike of heavy CH$_4$ into the headspace of the incubation unit. At this point, the amount of $^{13}$CH$_4$ in the headspace can only decrease via the initial diffusive flow of the label into the soil (von Fischer and Hedin 2002), and AOM, which consumes the $^{13}$CH$_4$ and releases $^{13}$CO$_2$ back into the headspace. AOM is the only source of heavy CO$_2$ into the headspace. Methanogenesis, on the other hand, will produce and release $^{12}$CH$_4$ and $^{12}$CO$_2$ into the headspace; anaerobic soil respiration will also contribute $^{12}$CO$_2$ into the headspace. Thus, we expect to see a gradual decline of $^{13}$CH$_4$ with a simultaneous increase of $^{12}$CH$_4$, and an overall increase in total mass of CH$_4$ over time (assuming methanogenesis rates are higher than AOM rates).

Figure 2.1. Conceptual figure of pool dilution technique for the simultaneous measurement of gross AOM and methanogenesis rates.

Over time, the isotopic signature of the headspace CH$_4$ pool will decrease as AOM draws heavy CH$_4$ from the initial headspace spike amount. CH$_4$ mass will also change over time based
on the balance of CH₄ production and consumption (i.e. if P > C, then headspace CH₄ mass will increase over time vs. if P < C, where headspace CH₄ mass decreases over time). Labeled ¹³C was expressed as atom percent (AP%) in all the calculations for this experiment.

CH₄ mass and isotope data were used to calculate an incubation-specific first-order reaction rate constant (k) for gross CH₄ consumption, which we herein refer to as ‘turnover time’ (units of inverse time). In these calculations, we assume a standard isotopic signature of newly produced CH₄ of -60‰, or 1.05 AP% (Quay et al. 1988, von Fischer and Hedin 2002, Smemo and Yavitt 2007). The isotope fractionation of the AOM pathway used in these calculations was set at 0‰, a conservative value for this effect, which has estimates ranging from -2 to -14‰ in terrestrial systems (Alperin and Reeburgh 1987, Martens et al. 1999, Grossman et al. 2002, Smemo and Yavitt 2007). In this way, we cannot overestimate the effect of AOM. All mass values were standardized by dry soil weight using the amount of soil incubated and basin-specific soil moisture measurements. Net CH₄ fluxes were calculated as the rate of CH₄ mass change over time incubated (these values could be negative if C>P). Calculations of gross CH₄ consumption and net CH₄ flux rates were taken for the entire incubation period, allowing for overall rate determinations with minimal bias. Gross CH₄ production rates were calculated for the entire incubation by conservation of mass, as the sum of gross consumption and net CH₄ flux rates, so that “Net Flux = Production – Consumption”.

Incubation data were screened using the time series of measured CH₄ AP%. Given that the only process that should continuously decrease the amount of ¹³CH₄ in the headspace is AOM, the CH₄ AP% should only maintain or decrease throughout the incubation period. Incubations that showed positive net changes in CH₄ AP% were excluded from rate analyses, resulting in a forfeiture of design balance. This was a conservative approach, as most of these
stricken incubations showed only slight increases in AP% that were often within the range of instrument precision (given by UCD SIF as 0.0357%). If process rates were calculated for the incubations with increasing CH₄ AP% over time, the resulting rate calculations of both CH₄ production and AOM in these instances were zero. Because mass data showed overall significant increases in CH₄, and thus refuted the lack of methanogenesis during the incubation period, these jars were disqualified from all statistical analyses.

**SAMPLING AND INCUBATION SETUP**

Soil cores were extracted in June 2011 with a 12 cm diameter SIPRE corer attached to a handheld gas-powered drill. All soils were collected from waterlogged sites, as previous work in these locations have shown soil methane concentrations to be 3 to 5 fold higher in waterlogged areas (as opposed to the more hydrologically mesic soils in micro-topographically raised areas) (von Fischer et al. 2010, Zona et al. 2011, Lipson et al. 2012). Extracted frozen cores were kept on wet ice in the field, and stored at -80°C within 2 hours of collection. In the lab, frozen cores were cut into 1 cm-thick discs with a band saw, weighed and deposited into a 1-liter glass Mason jar with metal ring lids modified to contain a butyl rubber septa and an anaerobic indicator strip (Anaerotest 115112, Merck Millipore). Jars containing the intact ‘pucks’ of frozen soils were sealed, the headspace immediately vacuumed for 2 minutes, and purged with UHP helium gas for 5 minutes. Jars were wrapped in foil and stored in dark incubators at 10°C for one week to allow for full soil thaw and recovery. Jar headspaces were purge-flushed with UHP helium for 2 minutes every other day, to remove any O₂ released or produced upon thaw.

To begin each incubation, an aqueous treatment solution was added to the jar via the septum, the headspace volume was vacuum flushed with UHP helium for 5 minutes and then
allowed to equilibrate to atmospheric pressure through tubing submerged in O₂-free DI water. After the jar reached atmospheric pressure, a CH₄ mixture was injected to create an initial jar headspace of 5% CH₄ with a 5 atom percent (AP%) $^{13/12}$C isotopic composition. Jars were dark incubated at 10°C (range: 8-11°C). All anaerobic indicator test strips signaled anoxia within the incubation chambers before and after incubations began. An additional layer of airtight silicon sealant was applied to the exterior of all sampling port septa after each sampling event. Time series samples at 24, 336, 480, 720 and 960 hours were collected into 10 mL glass serum vials fitted with gastight 10 mm butyl rubber stoppers and stored in dark refrigeration until analysis.

INITIAL ESTIMATES OF AOM ACTIVITY AMONG BASINS

To determine ballpark AOM rates, we conducted a small pilot study using the mass and isotopic signature of CO₂ in the headspace to indicate AOM activity. The pilot study used a set of three soil depths (5, 15 and 25 cm) from one of each of the four basin age class, for a total of 12 jars. No soil amendments were used in the pilot study. Headspace gases were sampled at 24, 48, 120, 216 and 384 hours. Headspace samples were analyzed for the CO₂ concentration (ppmv) and δ$^{13}$C-CO₂ at the University of California-Davis Stable Isotope Facility (Davis, California).

Unlike the pool dilution method, in order to quickly and easily determine if AOM was active, we only followed the appearance of $^{13}$CO₂ over time, which should only be coming from AOM of the $^{13}$CH₄ headspace spike. Jar headspaces were established with 5% of 5.03AP% CH₄. For calculations, the fractionation associated with anaerobic fermentation processes is negligible, so the δ$^{13}$C signature of CO₂ emitted from soil respiration is essentially the isotopic signature of the substrate (which we assign the value of -24‰). Non-methanotrophic anaerobic soil respiration will gradually deplete the headspace $^{13}$C-CO₂ signal, while increasing the overall
concentration of CO$_2$ in the headspace. AOM using $^{13}$C-CH$_4$ should enrich the headspace $^{13}$C-CO$_2$ signal and also positively contribute to the headspace mass of CO$_2$. Hydrogenotrophic methanogenesis could be utilizing some of the $^{13}$C-CO$_2$ resulting from the AOM of the $^{13}$C-CH$_4$ spike in the headspace, thus altering the isotopic signature of the headspace CO$_2$. We ignore this possible feedback here, as our experiments were not long in duration, and assuming null methane production using $^{13}$CO$_2$ is conservative. Using mass balance equations for total CO$_2$ and the known fractionations of $^{13}$CO$_2$ of AOM and anaerobic soil respiration in an isotope mixing model, we calculated the amount of CO$_2$ produced by AOM. AOM rates were calculated as the change in mass of AOM-produced CO$_2$ versus incubation time.

**ELECTRON ACCEPTOR EFFECT ON CH$_4$ PRODUCTION AND AOM RATES**

The primary directive in the full experiment was to determine if the AOM pathway was an important (i.e., influential) aspect of soil carbon cycling in this system. To characterize potential AOM rates more broadly in this system, we incubated a total of 72 soil samples taken from six cores of waterlogged depressions within young and medium age class basins. Twelve depths were sampled from each core, ranging from 1 to 30 cm below the surface.

To determine the effect of electron acceptors on both methanogenic and AOM rates, treatments of DI water, iron (Fe(III)-nitrilotriacetic acid, or Fe(III)-NTA) and sulfate (K$_2$SO$_4$) were used to alter the amount of available electron acceptors present in incubated soils. We created common volume treatment solutions using the average frozen weight of incubated soils and basin-specific soil water content. Treatments were designed to deliver equal amounts of electron accepting capacity, resulting in final soil concentrations of 8 mM Fe(III)-NTA and 1 mM K$_2$SO$_4$. Autoclaved treatment solutions were bubbled with UHP helium for 10 minutes,
capped and stored in serum vials with UHP helium headspaces at 10°C for less than 12 hours before administration. We excluded a nitrate treatment, despite evidence of a nitrate-dependent AOM pathway in other systems (Raghoebarsing et al. 2006, Ettwig et al. 2010). This was done in deference to the low availability of nitrate found in past research on Barrow soils, and the possible inhibitory effect nitrogen can have on methanogenesis rates (Klüber and Conrad 1998, Smemo and Yavitt 2007).

The three treatments were administered in a stratified block design, blocked by core, and stratified into 4 depth classes: top (0-1, 2-3, 5-6 cm), top-middle (8-9, 9-10, 11-12 cm), bottom-middle (14-15, 17-18, 20-21 cm), bottom (23-24, 26-27, 29-30 cm). Electron acceptor treatments were randomly assigned within each depth class. Samples of headspace gas from each jar were drawn after 24, 336, 480, 720 and 960 hours of incubation. Mass and δ^{13}C of CH₄ gas in the headspace were measured at the Stable Isotope Facility of the University of California-Davis in Davis, California, USA using a ThermoScientific PreCon-GasBench system interfaced to a ThermoScientific Delta V Plus isotope ratio mass spectrometer (ThermoScientific, Bremen, DE). Headspace CO₂ mass and isotopic signatures are not needed for rate estimations using the pool dilution method, and were not measured in the full experiment.

MOLECULAR ANALYSIS

We used two datasets (16S rRNA gene sequences and random shotgun metagenomes) to identify gene sequences from Barrow soils that are closely related to microbes capable of AOM. 16S rRNA sequences were obtained from the Earth Microbiome Project, or EMP (Gilbert et al. 2010). This study used soil samples of varying depths from the rims and centers of low-centered polygons of old and ancient basins. Sample processing, sequencing and core amplicon data
analysis were performed by the EMP (www.earthmicrobiome.org) and all amplicon and meta
data has been made public through the data portal (www.microbio.me/emp). Sequences closely
related to those of ANME groups 1, 2 and 3 (Knittel et al. 2005) were identified by local BLAST
of the data set, aligned with known representatives from these groups using the SINA alignment
tool (www.arb-silva.de/aligner/)(Pruesse et al. 2012), and a maximum likelihood phylogenetic
tree was generated using the Fast DNAml application within BioEdit (Hall 1999).

The metagenomic analysis used the libraries described in Lipson et al. (2013a). These
represent soils of varying depths from a single medium-aged basin. MG-RAST was used to
search for mcrA genes, and the relationships of these sequences to mcrA genes from known
ANME clades (Bidle et al. 1999, Hallam et al. 2003, Inagaki et al. 2004) was explored using a
protein maximum likelihood tree (PROml) with amino acid sequences aligned using
CLUSTALW in Bioedit (Hall 1999).

STATISTICAL ANALYSES

Most data were unbalanced due to unequal sample sizes after data screening and were
therefore evaluated using the Type III sum of squares from multi-way ANOVA and ANCOVAs,
with post-hoc determinations by one-way ANOVA and Tukey honest significant difference tests
(both of which are less sensitive to unequal sample size). Soil depths were analyzed as a
continuous variable, while depth class was treated as a categorical variable. We used correlation
analysis to compare quantities and rates of methane produced and consumed. Where appropriate,
data were transformed to conform to test assumptions of normality and homogeneity of variance.
All statistics were performed with R version 3.1.1.
RESULTS

MOLECULAR ANALYSIS OF AOM-ASSOCIATED MICROBES AND GENES

Phylogenetic analysis of 16s rRNA showed the clustering of Barrow basin soil sequences with known methane-cycling archaea, including ANME groups 2 and 3 (Figure 2.2). 3306 (out of 2027920 total) sequences were similar to nitrate-dependent methanotrophs belonging to the phyla NC10. Soils sequenced in this series were exclusively from old and ancient age class basins, and so they are not directly related to the soils incubated for AOM and methanogenesis rates. However, differences in microbial communities between age classes in this ecosystem appear to be more subtle than changes associated with soil depth and microtopography (Lipson et al. 2010, Lipson et al. 2012, Friedman et al. 2013, Lipson et al. 2013a).
Figure 2.2. Phylogenetic tree of 16S rRNA sequences from the Earth Microbiome Project of old and ancient basins, illustrating the relatedness of organisms found in Barrow soils with other known methane-cycling isolates in ANME groups 2 and 3.
Barrow soil isolates related to methanogens capable of Fe(III)-reduction were detected (Bond and Lovley 2002), namely the *Methanosarcina*, *Methanobacterium palustre*, and *Methanococcus* clades (Figure 2.3), grouping closely amongst ANME groups 2 and 3, with greater separation from the ANME group 1.

**Figure 2.3.** Phylogenetic tree of mcrA genes from the metagenomes of a single medium-aged basin. Barrow sequences cluster near mcrA genes known to be associated with the ‘reverse methanogenesis’ pathway of CH₄ consumption.
PILOT STUDY

The relative importance of AOM measured during the pilot study was greatest in young and medium age class basins, and had an unresolved relationship with soil depth. While basin age class and soil depth were most strongly related to total CO$_2$ flux rates ($p=0.018$ and 0.006, respectively), there was also an interaction of soil depth and basin age class with AOM rates (interaction $p=0.078$), supporting a trend of lower AOM rates in the deeper layers of old and ancient basins. In addition, there was a non-significant but qualitatively intriguing relationship seen between the proportions of total CO$_2$ production that was from AOM, relating the importance of AOM on total CO$_2$ flux (Figure 2.4).

![Figure 2.4. Pilot experiment percentage of total CO$_2$ evolution attributed to AOM by basin age class and soil depth. Proportions represent the total amount of CO$_2$ from AOM compared with the total amount of CO$_2$ evolved over the course of the entire pilot study incubation (all 384 hours). There is one incubation represented per age class and depth interaction (n=12).](image-url)
All data in the pilot study showed linear trends in increasing CO$_2$ mass and decreasing $\delta^{13}$CO$_2$ over time. Four of the twelve pilot jars were significantly different than the others in terms of either AOM rates, and/or the ratio of AOM CO$_2$ to total CO$_2$ production. The highest AOM rates were measured in soils from the old basin at 5 cm, the medium basin at 5 cm, and the young basin at 25 cm. The three highest values for the percentage of total CO$_2$ coming from AOM were measured during the incubation of soils from the medium basin at 15 cm, the medium basin at 5 cm, and the young basin at 25 cm. Total CO$_2$ flux rates were also lower in the young and medium basins (independent of depth). The most dynamic (and often the highest) AOM rates measured were found in soils from young and medium basins, thus, we sampled from only young and medium basins for the full experiment.

PRODUCTION vs. CONSUMPTION RATES

The primary directive in the full experiment was to determine if the AOM pathway was an important (i.e., influential) aspect of soil carbon cycling in this system, and the possible drivers of any differences in rates. To evaluate the relative importance of the AOM pathway, we compared the gross CH$_4$ production and consumption rates in our incubations (Figure 2.5). We found a moderate correlation between the two rates, with an overall $R^2$ of 0.5107 upon the removal of the single consumption rate outlier (outlier consumption rate <1 nmol C-CH$_4$/cm$^3$/hr). Consumption rates were higher than production rates in our incubations (overall linear regression slope=0.34, and y-intercept=2.55), demonstrating that AOM can eclipse methanogenesis during closed laboratory incubations. This effect was similar between young and medium basin age classes, which had indistinguishable linear relationships between production and consumption rates.
Figure 2.5. Gross CH$_4$ production versus gross consumption rates from the full experiment ($R^2=0.51$, $p<0.001$), distinguished visually by basin age class. Process rates were calculated for the full incubation period, thus incubations are represented by a single data point, with color and symbol representing basin age class.

BASIN, DEPTH AND TREATMENT EFFECTS

The effects of both basin and basin age class were not statistically significant as related to any CH$_4$ cycling rates (net flux, gross consumption and gross production) during the full experiment. One of the unclear relationships in the pilot study was the impact of depth on AOM rates. In the full experiment, AOM rates did not show a connection with soil depth, but gross CH$_4$ production rates significantly decreased by a factor of 3 with increasing soil depth (Figure 2.6).
Figure 2.6. *Gross CH$_4$ production rate by soil depth measured in the full experiment. Each data point represents an incubation. Depth in soil core is oriented along the y-axis, with soil surface at top and sampling depths delineated along the axis in sequence.*

When soil depth is binned into the four sequential depth classes used as the blocking factor during random treatment assignment, deeper depth regions are related to a decrease in gross production rates (p-value = 0.015), but are not significantly linked with gross consumption rates. There is also not a significant change in net CH$_4$ fluxes by depth class, although there is a visual pattern, shown in Figure 2.7. Despite a lack of overall significance in depth class effect on net CH$_4$ fluxes, there was a significant difference between the Fe(III)-NTA and sulfate treatments within the 7-14 cm depth class, with higher AOM rates and CH$_4$ production rates in these soils as compared to the sulfate-treated soils. If we create a ratio of the gross CH$_4$ production to AOM rates (Table 2.2), we can see that while the Fe and sulfate treatments within
the 7-14 cm depth class are significantly different by Tukey test, the production to consumption ratio (P:C) is not distinct, denoting the dual increase in production and consumption within Fe-treated soils in this depth class. Figure 2.7 also demonstrates the prevalence of net CH$_4$ oxidation in these soils (or the potential thereof), and highlights an important treatment effect. While the effect of treatment is insignificant in the full ANOVA including treatment and depth, if we compare the control fluxes with fluxes from each treatment individually, we find no difference in net fluxes by depth or treatment between the sulfate and control treatments, but significant effects of depth, treatment, and the depth:treatment interaction between the control and Fe(III) average net fluxes (p=0.03, 0.02, and 0.02, respectively). These patterns hold true when using depth class (identical p-values as depth).

![Figure 2.7](image)

**Figure 2.7.** Mean ± SEM net CH$_4$ flux rates by depth class and acceptor treatment. Negative values denote oxidation/consumption. Different letters above each treatment within each depth class denote significant differences by Tukey test (p<0.05).
With the exception of gross production, \( \text{CH}_4 \) cycling rates are mostly insensitive to depth, depth class, and electron acceptor treatment (Table 2.2). Using depth classes, we can see the \( \text{CH}_4 \) cycling rates and turnover time, \( k \), are not significantly altered by electron acceptor treatments. However, the proportion of \( \text{CH}_4 \) cycling rates, represented by the ratio of gross production to gross consumption (P:C), does not significantly vary by depth or treatment.

Average gross production rates within sulfate-treated soils were distinguishable by depth classes (\( p=0.003 \)), with an overall decrease in rates with increasing depth. Within the sulfate-treated soils, the effect of depth class on gross production was significant (ANOVA \( p=0.01 \)). The effect is lost almost entirely in the difference in sulfate-treatment P:C ratio by depth class, with the exception in the 0-6 cm depth, which was marginally higher (ANOVA \( p=0.09 \)).
Table 2.2: Methane cycling rates by depth class and acceptor addition treatment

<table>
<thead>
<tr>
<th>Depth</th>
<th>Gross Production (nmol C-CH₄/cm³ dry soil/hour)</th>
<th>Gross Consumption (nmol C-CH₄/cm³ dry soil/hour)</th>
<th>Production to Consumption Ratio (P:C)</th>
<th>k Turnover Time (day⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>0-6cm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.77</td>
<td>5.85</td>
<td>0.51</td>
<td>0.0019</td>
</tr>
<tr>
<td>Fe(III)-NTA</td>
<td>1.78</td>
<td>31.0</td>
<td>0.10</td>
<td>0.0168</td>
</tr>
<tr>
<td>Sulfate</td>
<td>16.6</td>
<td>32.1</td>
<td>1.6†</td>
<td>0.0083</td>
</tr>
<tr>
<td><strong>7-14cm</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>7.68</td>
<td>14.8</td>
<td>0.61</td>
<td>0.0039</td>
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<tr>
<td>Fe(III)-NTA</td>
<td>8.92</td>
<td>28.3</td>
<td>0.22</td>
<td>0.0072</td>
</tr>
<tr>
<td>Sulfate</td>
<td>2.05</td>
<td>7.28</td>
<td>0.27</td>
<td>0.0024</td>
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<tr>
<td><strong>15-21cm</strong></td>
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<td></td>
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<tr>
<td>Control</td>
<td>3.86</td>
<td>8.91</td>
<td>0.42</td>
<td>0.0038</td>
</tr>
<tr>
<td>Fe(III)-NTA</td>
<td>3.67</td>
<td>10.5</td>
<td>0.51</td>
<td>0.0040</td>
</tr>
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<td>Sulfate</td>
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<td>16.5</td>
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<td>0.0068</td>
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<td><strong>22-30cm</strong></td>
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</tr>
<tr>
<td>Control</td>
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<td>7.60</td>
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<td>0.0035</td>
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<td>0.0028</td>
</tr>
<tr>
<td>Sulfate</td>
<td>1.68</td>
<td>6.68</td>
<td>0.29</td>
<td>0.0023</td>
</tr>
</tbody>
</table>

*ANOVA for depth class vs. gross production p-value: 0.0148
Tukey test for gross production by depth class denoted by small letters in the depth class header rows of 1st data column
† Single outlier removed from within this category (>3 s.d. from mean)

In the full ANOVA, the effects of depth and electron acceptor treatment on turnover time (e.g., the first-order rate constant for AOM, or k) were not significant. However, there is a depth effect nested within the iron treatment group, where an ANOVA of depth class on only Fe-treated soils shows that increasing depth significantly correlated with decreasing turnover times, and thus, lower potential AOM rates (Figure 2.8). To parse out the effects of the Fe-treatment and soil depth on the AOM rate constant, we directly compared the Fe and control treatments (leaving the sulfate-treated soils out of the analysis, as they were not distinguishable from the control group k estimates at any depth, but did contribute a high variance obfuscating the model).
Including all depth classes, we found a marginal effect of the Fe treatment \( (p=0.078) \), and a significant interaction effect between treatment and depth \( (p=0.024) \). Controlling for depth, the direct comparison of Fe and control \( k \) estimates in the first depth class shows a marginal effect of depth \( (p=0.060) \), and an interaction between depth and treatment \( (p=0.060) \). Thus, there is a significant depth x Fe effect, with higher \( k \) rates present in Fe-treated shallow soils.

**Figure 2.8.** Natural log of gross AOM rate constant \( k \), or turnover time, as it relates to depth region and acceptor treatment. No significant differences between treatments, or between overall depth regions, but there was a significant ANOVA result for depth region within the iron treatment group \( (p=0.048) \), and for the depth-by-treatment interaction when directly comparing the control and iron treatments \( (p=0.024) \).
DISCUSSION

EVIDENCE FOR AOM

This study demonstrated that the anaerobic CH$_4$ consumption (AOM) pathway has the potential to greatly influence CH$_4$ cycling in the basin wetland landscapes of the Alaskan North Slope. We found phylogenetic evidence for the presence of anaerobic methanotrophs in soils collected from Barrow, AK. Using in vitro soil incubations, we also quantified AOM rates by two methods: measuring the appearance of $^{13}$CO$_2$ after incubating soils with a $^{13}$CH$_4$ headspace spike; and a stable isotope pool dilution technique to simultaneously measure the rates of methanogenesis and AOM.

Phylogenetic analysis of Barrow soils showed the presence of known methanotrophs using 16S rRNA and mcrA genes associated with the ‘reverse methanogenesis’ pathway, which most closely aligned with ANME groups 2 and 3.

SYSTEM TRENDS

Previous work in this area has shown that soil carbon cycling is strongly linked with basin age class as it relates to successional vegetation and hydrological patterns (von Fischer et al. 2010, Sturtevant and Oechel 2013), and the increased electron acceptor loads and reduction rates in younger basins (Lipson et al. 2010, Lipson et al. 2013b). The pilot study provided our initial estimates of the differences in AOM rates between basin age classes and soil depths, but the lack of replication, and thus statistical power, means we cannot draw defensible conclusions about relationships from the pilot data alone. With this in mind, we focused on the fact that the most dynamic (and often the highest) AOM rates were found in young and medium basins, thus, we sampled from only young and medium basins for the full experiment. Because there was no
discernable pattern seen between higher AOM rates and any of the three soil depths used in the pilot (5, 15, and 25 cm), we sampled extensively throughout the soil profile during the full experiment, spanning 0-30 cm depths for every core. Total CO$_2$ flux rates were also lower in the Young and Medium basins (independent of depth), suggesting that carbon cycling in those soils may have a tendency towards CO$_2$-production pathways, rather than CH$_4$-producing pathways.

The microbial populations capable of Fe-reduction pathways are already present in adequate numbers to potentially inhibit methanogenesis (by either direct or indirect means), as has been shown previously within soils from this system (Lipson et al. 2010, Lipson et al. 2013b). Therefore, the experimental addition of easily-reducible forms of Fe could have hindered CH$_4$ production, while having an unknown effect on AOM.

CH$_4$ PRODUCTION AND AOM RATES

The AOM pathway is still gaining momentum in its recognition as a process of potential importance within terrestrial systems, particularly the peatlands in the high Arctic. AOM is a significant sink of methane in the Barrow soils we tested – in most cases consuming the entire mass of biogenic methane. It is noteworthy that these incubations were conducted on soils that retained their matrix structures – a hybrid of sampling along intact cores, and the soil slurry method often used in anoxic incubations. The complex soil matrix supports high microbial diversity despite the extreme Arctic conditions (Lipson et al. 2013a). By preserving the soil matrix structure, we preserved the soil’s ability to retain CH$_4$ and other gases within the matrix, but also retained the complexity of soil structure that has been linked with methanogenesis (Teh and Silver 2006). Given the conservation of the soil matrix, the high-CH$_4$ concentrations and the strictly anoxic incubations, conditions were likely ideal for AOM.
Our results showed that depth was related to CH$_4$ cycling rates. Basin differences were attributable to differences in vegetation, hydrology and the high spatial heterogeneity of functionality in this wetland landscape, and were therefore more useful as a blocking variable rather than an explanatory variable. More surprisingly, the negative influence of depth on CH$_4$ production was the opposite of expected, however, the decreased CH$_4$ production rates with increasing depth is not a tight relationship despite the statistical significance. The depth distinctions in production rate might also be the result of the natural process rates during *in-situ* soil functioning. The influence of plants (their rooting systems, and their exudates) on the rates of methane production and overall soil respiration in these anoxic soil systems is well-documented (Hines et al. 2008, Laanbroek 2010, von Fischer et al. 2010, Couwenberg et al. 2011, Leppala et al. 2011, Corbett et al. 2013, Sturtevant and Oechel 2013). One manifestation of this is the shift from acetate-splitting methane production in shallower soils where there is easier access to plant roots and their exudates, to CO$_2$-reduction methanogenesis at depth, where substrates are less easily available (Popp et al. 1999). In addition, the mineral-rich peat soils are also capable of supporting high rates of respiration without the availability of direct plant carbon inputs (Popp et al. 1999, Allen et al. 2010). The legacy of plant inputs and the newly-dead plant biomass present in the shallower soil depths sampled, and absence of those influences in the deeper soil depths, all of which were tested in this study, may combine to drive the depth differences in CH$_4$ production rate. If CH$_4$ production is substrate-limited, and there was more labile carbon in the shallower depths (despite the lack of actual active plant growth), this could explain the unexpected higher rates of production in shallower soils.

Also based on the assumption of substrate-limitation, the high levels of CH$_4$ in the headspace of the incubations (5%) was an exaggeration of reality designed to promote AOM
rates, which are substrate-dependent (Smemo and Yavitt 2007). The deviance from reality, however, is less and less clear with increasing soil depth, as soil depth can mean that more CH$_4$ is theoretically present. Within deep anoxic soils, biogenic gas can reach very high concentrations, with published values reaching up to 10% (Comas et al. 2013). Anecdotally, measured concentrations of CH$_4$ up to 0.3% at 20-30 cm soil depth have been measured in Barrow soils (personal communication, Walter Oechel). If the incubation partial pressures of CH$_4$ are, in fact, somewhat realistic within deeper soils *in-situ*, the gross rates of CH$_4$ production and consumption we measured in these layers are also somewhat realistic. The consequences of this assumption is important to understanding the constraints on future CH$_4$ climate forcing deriving from these northern peatland systems. Production to consumption ratios can relate the importance of AOM in anoxic carbon cycling. The consistency in the average P:C, as well as in the measured net CH$_4$ fluxes, show that at least under these experimental conditions, AOM is outstripping CH$_4$ production rates across the board, leading to a decrease in CH$_4$ concentrations over time (and the negative net flux values) under all conditions.

Perhaps the most unexplained aspect of AOM is how it endures in terrestrial systems, despite a marginal energy yield (Cervantes et al. 2000, Hoehler et al. 2001, Larowe et al. 2008, Bethke et al. 2011, Thauer 2011) and uncertain competitive benefits to soil microbes. Given the delicate thermodynamic constraints, the relative insensitivity of AOM to experimental manipulations of the biologically-available electron acceptors is surprising. If AOM is as low-energy yielding as we understand it to be, the simplest rules of thermodynamics should affect functionality. Mechanistically, the shift from AOM to a more favorable form of respiration could also be due to the direct inhibition of these pathways, and/or the result of competitive exclusion by microbial populations respiring using more energetically-favorable electron acceptors.
Despite all this, there has been minimal success in identifying the relevant microbial actors, and even more puzzling, the biogeochemical sensitivities of the process outside of marine systems (Smemo and Yavitt 2007, Smemo and Yavitt 2011, Gupta et al. 2013). The lowest rates for both production and consumption co-vary in our data, and it is not possible to determine from this experiment if this is a causal or simply correlative relationship. Other work does not help to clarify, as the connection between these pathways is somewhat fluid across space (and time?), as there are reports of both strong (Smemo and Yavitt 2007) and weak (Gupta et al. 2013) associations in other terrestrial AOM studies. There is evidence that AOM is an energetically marginal pathway and primarily limited by substrate availability (CH$_4$), further supporting a dependence of the AOM rate on CH$_4$ production rate (rather than the opposite direction). To this end, we also had greater success finding statistically significant relationships between production rates and measured environmental parameters, namely basin, soil depth and electron acceptor presence.

For the Barrow system, we hypothesized that the high native Fe concentrations in the soils and the importance of Fe-reduction respiration in these wetland soils would favor AOM energetically linked with Fe-reduction, as has been seen in some other freshwater systems with high Fe concentrations (Sivan et al. 2011, Amos et al. 2012, Segarra et al. 2013). The lack of linkage (as evidenced by the insensitivity of the process rates to additions of ferric iron) may be explained by the abundance of organic electron acceptors in these complex peat soils. Observational data from numerous Barrow wetlands over multiple seasons has shown that both ferric iron and humic substances are influential and dominant electron acceptors in anaerobic respiration pathways. In fact, there is some evidence to suggest that these soils experience a mid-growing season shift from soil respiration being dominated by humic substances-reduction in the
first part of the summer to ferric iron-reduction for the remainder of the season (Lipson et al. 2013b). The soils tested in these experiments were taken in early June, when humic substances are still oxidized, and are thought to be the dominant electron acceptor. It may be possible that our incubations, which only lasted 960 hours (i.e., 40 days), were not as responsive to electron acceptor additions of ferric iron, and this contributed to the lack of a significant signal.

THINKING FORWARD

Recent shifts in some northern systems from their traditional role as CO₂ sinks to CO₂ sources (Oechel et al. 2014), attributable to alterations in climate and increased globalization (i.e. invasive species introductions), pose an interesting avenue of speculation and inquiry. If changes in local climate are conducive to CH₄ production (e.g. wetter, warmer soils), will AOM rates ‘follow the CH₄’ and likewise accelerate activity? If so, and the P:C ratio remains steady, we would expect a larger quantity of AOM-derived CO₂ released to the atmosphere, positively contributing to the enhanced CO₂ source effect, but diminishing the net release of the more potent greenhouse gas CH₄.

AOM is thermodynamically marginal (Larowe et al. 2008, Sivan et al. 2011, Thauer 2011), and thus the biogeochemistry of the host soil is a significant potential constraint on consumption rates. The lack of response in both the methanogenesis and AOM signals with electron acceptor additions in this study suggests these microbial populations are not adversely affected by more thermodynamically-favorable processes occurring in close proximity. Whether this insensitivity is due to a lack of competition for substrate (Allen et al. 2010), to the abundance of the appropriate electron acceptor for AOM (possible humic substances), or to extensive microsite networks within intact soil structures (Teh and Silver 2006, Sey et al. 2008,
Sachs et al. 2010) is another question for further investigation. Also, despite the correlation between gross production and consumption rates, the relative insensitivity of consumption rates to changes in depth has some interesting potential implications (and explanations) to do with microbial competition and pathway flexibility in some of the major methanogenic clades, which can switch between methanogenesis and Fe-reduction (Potekhina et al. 1990, Bowman et al. 1997, Daniel et al. 1999).

CONCLUSIONS

Laboratory incubations of intact peat showed higher rates of AOM than CH$_4$ production, leading to negative net CH$_4$ fluxes. We used stable isotope tracers to quantify AOM rates, verifying the potential of these soils to perform AOM (a previously unknown result), and corroborated this with genomic data, revealing the phylogenetic relatedness of Barrow soil organisms and known anaerobic methanotrophs of ANME groups 2 and 3. CH$_4$ production rates were related to soil depth and acceptor treatment, yet AOM rates were only sensitive to ferric iron in shallow soils, and insensitive to additions of sulfate treatment additions. This work provides evidence that AOM has the potential to be a local sink of CH$_4$ in northern peatland soils, and that under our experimental conditions we found a promising associations between AOM and ferric iron in this system.

ACKNOWLEDGEMENTS

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Electron acceptor effects on the production and anaerobic oxidation of methane in Finnish peat soils

ABSTRACT

Net fluxes of methane from northern wetlands are mitigated by the anaerobic oxidation of methane (AOM), which converts methane gas into the less potent greenhouse gas carbon dioxide. While we have a decent understanding of the identity and ecology of the organisms that perform AOM in marine systems, we lack those same basic understandings for the terrestrial AOM pathway. Using in vitro incubations, we determined the presence and relative importance of AOM to overall methane cycling in a minerotrophic subarctic fen in northern Finnish Lapland. Measured AOM rates showed potential consumption of between 6-39% of the methane produced, contributing approximately 1% of the total carbon dioxide flux. Treatments of nitrate, sulfate and ferric iron designed to illuminate possible microbial metabolic associations showed that nitrate suppressed methanogenesis. No treatments were positively associated with AOM rates, and methane production and consumption rates were similarly unrelated. Annual in situ methane cycling rates would help clarify the net influence of this pathway to the full landscape, as we have provided evidence that AOM can be an important methane sink in this northern peatland system.

INTRODUCTION

The greenhouse gas methane (CH\textsubscript{4}) is estimated to play an increasingly important role in future climate change, yet controls on net fluxes from terrestrial systems are still not well characterized. Paramount to a better understanding of net methane flux from soil systems is the
quantification of the anaerobic oxidation of methane (hereafter: AOM) consumptive pathway. The majority of work on AOM has been in marine systems where it consumes around 90% of CH₄ produced (Reeburgh 1996, Knittel and Boetius 2009), but less is known about the terrestrial versions of this pathway. In fact, until relatively recently, only the aerobic oxidation of methane was considered important in non-marine systems. There is, however, mounting evidence that AOM can also be an important CH₄ sink in northern peatland systems (Smemo and Yavitt 2007, Kip et al. 2010, Smemo and Yavitt 2011, Zhu et al. 2012, Gupta et al. 2013). Given the importance of northern peatlands as significant global sources of methane (Gorham 1991), a better grasp on the relative impact of, and ecological controls on, the AOM CH₄ sink is invaluable to accurate future climate-carbon modeling.

Our understanding of the prevalence and mechanistic diversity of the AOM pathway has blossomed with recent scientific attention. From work in both marine and non-marine systems, AOM is known to be positively affected by the amount of CH₄ substrate (Smemo and Yavitt 2011), and less consistently related to the presence and identity of alternative electron acceptors (hereafter known as ‘acceptors’). The relationship between AOM and methanogenesis rates is relatively straightforward – both require anoxia, and AOM has been shown to be a substrate-limited process, and so higher AOM rates follow higher rates of methanogenesis (Jugnia et al. 2006, Smemo and Yavitt 2007). In addition to substrate limitation, while AOM can be conducted by bacteria alone (Ettwig et al. 2010), it is much more thermodynamically favorable by bacteria-archaea consortiums (Boetius et al. 2000, Knittel and Boetius 2009, Callaghan 2013, Offre et al. 2013). In the consortium version, AOM is linked with the microbial reduction of a number of acceptors, including nitrate (Hu et al. 2009, Ettwig et al. 2010), sulfate (Caldwell et al. 2008, Hu et al. 2009, Jagersma et al. 2009, Thauer 2011, Segarra et al. 2013, Siegert et al. 2013), and iron
(Fe) (Beal et al. 2009, Crowe et al. 2011, Sivan et al. 2011, Amos et al. 2012, Riedinger et al. 2014) (which is less energy-yielding than nitrate reduction, but more so than sulfate reduction) (Larowe et al. 2008). Contrarily, there is also some evidence that acceptors can have an inhibitory effect on both methanogenesis and AOM, particularly nitrogen acceptors (Le Mer and Roger 2001, Zheng et al. 2013). Adding favorable acceptors may suppress methane production either directly through toxicity of the acceptors or intermediate products of their reduction (Klüiber and Conrad 1998, Cervantes et al. 2000), and/or indirectly via thermodynamic competition. Either mechanism may in turn retard AOM due to substrate limitation. This could happen even if the addition of labile electron acceptors stimulates a syntrophic partner in an AOM consortium, making solid conclusions about the relationships between AOM and acceptors difficult to ascertain (Knittel and Boetius 2009).

Another intriguing aspect of AOM is the potential range of importance this pathway can have on CH$_4$ cycling. Reported AOM rates span an impressive breadth of influence relative to overall CH$_4$ cycling, with very little in the way of explanatory system characteristics. For example, a study contrasting boreal and tropical soils found very little difference in the rates and the relative importance of the AOM pathway between the two systems (Blazewicz et al. 2012), somewhat unexpected given that these are both hot spots of CH$_4$ cycling, and AOM has previously proven temperature-sensitive (Liptay et al. 1998, Nauhaus et al. 2002, Krüger et al. 2005). Since methanogenesis and AOM share obligatory anoxia and thus inhabit the same physical space within soils, quantifications of the ecological constraints on methanogenic and AOM rates could help close the gaps in our understanding of terrestrial methane cycling. If methanogenic conditions exist and methane is being produced, the known conditions for AOM are also extant (Gupta et al. 2013). As methane cycling dynamics in these vulnerable northern
soils have important implications for future carbon-climate feedbacks, a better understanding of sink pathway controls is necessary to accurately predict landscape CH$_4$ source/sink identity and strength.

In this study, we used *in vitro* incubations with stable isotope tracers to quantify the relative importance of AOM within a Finnish Lapland peatland. Peat incubations were treated with additions of different acceptors to determine the effect of acceptor presence and identity on the rates of both AOM and methanogenesis in these soils.

**METHODS**

**STUDY SITE: PETSIKKO, FINLAND**

The field site is a minerotrophic, sub-Arctic wet fen, located near Petsikko in Finnish Lapland (69°29′33″N 27°13′39″E). Petsikko is located approximately 30 km north of the continuous pine forest line at an elevation of 271 m. The snow-free season is from late May to late September, with long-term (1962-2012) mean air temperatures for January and July of -14.5°C and 12.9°C, respectively (data courtesy of the Finnish Meteorological Institute). The Petsikko wetlands are depressions of predominantly sedges and peat mosses with low *Betula nana* shrub hummocks surrounded by gently sloping birch forest. Border zones are characterized by deep organic layers (60-80 cm) (Wayolle 2011), with non-permafrost ice lenses present within the hummocks throughout the year (Kujala et al. 2008).

**INCUBATION SETUP**

Anaerobic incubation units consisted of 610 mL sterile glass jars outfitted with gastight lids equipped with stopcock valve inlets. Jars were wrapped in foil and insulating cloth to
prevent ambient sunlight infiltration. Anaerobic indicator strips (Anaerotest 115112, Merck Millipore, Darmstadt, Germany) were taped into the lid, but not ‘activated’ until jars were filled and sealed in the field.

Peat was collected by extracting partially frozen soil cores using an 8 cm diameter ice auger. All soils were collected from within low, waterlogged wetland depressions, as soil methane concentrations are generally higher in anoxic areas (Moore and Dalva 1993, Bubier et al. 2005). A total of 10 cores were taken from representative portions of the depressed areas; soil samples were composites of material plucked from the top 30 cm of the cores. Soil porewater samples were taken for ion chromatography at the time of core collection, analyzed at the University of Eastern Finland, Kuopio (Table 3.1).

Table 3.1: Ion chromatography of core porewater by transect

<table>
<thead>
<tr>
<th>Concentration (µM)</th>
<th>Eastern Transect</th>
<th>Western Transect</th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Cl</td>
<td>49&lt;sup&gt;a&lt;/sup&gt;</td>
<td>105&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>NO&lt;sub&gt;2&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>NO&lt;sub&gt;3&lt;/sub&gt;&lt;sup&gt;-&lt;/sup&gt;</td>
<td>&lt;0.1</td>
<td>0.24</td>
</tr>
<tr>
<td>PO&lt;sub&gt;4&lt;/sub&gt;&lt;sup&gt;-3&lt;/sup&gt;</td>
<td>3.5</td>
<td>1.8</td>
</tr>
<tr>
<td>SO&lt;sub&gt;4&lt;/sub&gt;&lt;sup&gt;-2&lt;/sup&gt;</td>
<td>8.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.7&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Superscript letters across each row indicate Tukey’s test results, with the same (or no) letters indicating insignificant differences between transects (p<0.05).

To include greater spatial representation across the landscape, jars were filled in sets of four, with approximately equal quarters of each of two cores divided amongst the jars, creating 5 sets of 4 jars. Soil structure was retained, as opposed to creating slurries, as the soil structure may be important to the accurate reproduction of process rates (Teh and Silver 2006, Sey et al. 2008).
Filled jars were immediately sealed and field flushed with ultra-high purity (UHP) N\textsubscript{2} gas to dispel oxygen present in the headspace in preparation for transport to the field station lab facilities. Jars were N\textsubscript{2}-flushed again for 10 minutes each after they reached the field station, approximately 4 hours after initial flushing, and stored in a 10°C dark incubator overnight. Jars were weighed to find wet soil weight (by difference), and were flushed for 15 minutes each prior to refrigerated transport to the University of Eastern Finland in Kuopio, Finland. Jars were flushed upon arrival in Kuopio and stored overnight inside a +4°C dark cold room. All jars were then flush-vacuumed using a switch manifold line equipped with UHP N\textsubscript{2} and a vacuum pump (resulting in air pressure cycles within the jars alternating between +0.3 bar to -1 bar) in 30-second cycles for 10 minutes with light shaking periodically throughout the flushing period. Jars were left slightly over pressurized with N\textsubscript{2} prior to placement in a 10°C dark incubator. Jars were allowed to acclimate for 7 days, with 5-minutes of headspace flush-vacuuming every other day, in order for any remaining O\textsubscript{2} to be consumed and/or removed.

Treatments of iron (FeCl\textsubscript{3}), nitrate (KNO\textsubscript{3}), and sulfate (K\textsubscript{2}SO\textsubscript{4}) were prepared to determine the effect of acceptor chemistry on both methanogenic and AOM rates within the incubations. Using the average wet weight of soils contained within the incubation units and the average soil water content of soils collected previously from this site (Wayolle 2011), treatments were designed to deliver equal electron accepting capacity, while not overwhelming the system with potentially-toxic levels of inorganic acceptors. Common volume treatment solutions resulted in calculated final soil concentrations of 8 mM FeCl\textsubscript{3}, 8 mM KNO\textsubscript{3}, and 1 mM K\textsubscript{2}SO\textsubscript{4} within incubation units. Autoclaved treatment solutions were bubbled with UHP N\textsubscript{2} for an initial 10 minutes, and continuously throughout the aliquot process.
At the end of the acclimation period, the autoclaved and anoxic treatment solutions were introduced into each jar using the stopcock inlets. Jars were then immediately flush-vacuumed for 15 minutes, with slight shaking to allow for the release of any oxygen trapped within the soil and to ensure the treatment solutions spread evenly into the soils. Jars were brought to a consistent slight over pressure by final flushing with the outlet bubbling through O₂-free DI water. Outlets were allowed to bubble vent for 10 seconds after the inflow flushing valve was shut off, leaving a slight and consistent level of over pressure. A common headspace spike gas was mixed using $^{12}$CH₄ and $^{13}$CH₄ with an N₂ balance that was scrubbed for CO₂ with a glassware soda lime filtration unit. A common volume $^{13}$CH₄ isotope spike was then introduced, resulting in an initial headspace concentration of 1.04% CH₄ with an atom percent (AP%) of 5.21%.

GAS SAMPLING AND ANALYSIS

Initial headspace gas sampling occurred approximately 10 minutes after the CH₄ spike was introduced into the incubation jars. Sampling was conducting using dedicated, jar-specific syringes and valves that maintained a closed system throughout the sampling, subsampling, and headspace volume replacement procedure. We are confident that this maintained anoxic conditions throughout the experiment. Samples were collected in flushed and vacuumed Labco glass sampling vials (Labco, UK), with an equivalent volume replaced with UHP N₂ concurrently with sample draw (excepting the initial T₀ sample draw, which was done without replacement). Samples were pulled into jar-specific syringes; prior to sample injection into the Labco vial, a subsample was taken for immediate analysis of CO₂ and CH₄ masses by Shimadzu 14-A gas chromatograph with flame ionization and thermal conductivity detectors. Labco vials
were then overfilled for stable isotope analysis. The $^{13}\text{C}/^{12}\text{C}$ stable isotope ratios of both CO$_2$ and CH$_4$ were measured at the University of Eastern Finland with an isotope ratio mass spectrometer (Delta plus XP, Thermo, Bremen, Germany) interfaced with a gas chromatograph (Trace GC Ultra, Finnigan).

METHANOGENESIS AND AOM RATE CALCULATIONS

We estimated gross CH$_4$ production and anaerobic consumption rates using two different methods: 1) a pool dilution calculation slightly modified from von Fischer and Hedin (2002) and Smemo et al. (2007) for the simultaneous rate estimation of methanogenesis and AOM; and 2) a CO$_2$-based isotope mixing model for calculating the mass of the AOM product (CO$_2$) and thus AOM rates.

![Diagram of pool dilution technique for simultaneous measurement of gross AOM and methanogenesis rates.](image)

Figure 3.1. Conceptual figure of pool dilution technique for the simultaneous measurement of gross AOM and methanogenesis rates.

Both methods were based on how mass and isotopic signatures of CH$_4$ and CO$_2$ in the incubation headspace will evolve within a closed, anoxic system (see Figure 3.1). Methanogenesis will produce $^{12}\text{CH}_4$ and $^{12}\text{CO}_2$, increasing the total masses of CH$_4$ and CO$_2$ in
the headspace, and diluting the headspace isotope signature with $^{12}$CH$_4$ and $^{12}$CO$_2$. On the other hand, AOM will oxidize the $^{13}$CH$_4$ headspace spike and release $^{13}$CO$_2$, increasing the mass of CO$_2$ and decreasing the mass of CH$_4$, and enriching the headspace with $^{13}$CO$_2$. Thus, if both methanogenesis and AOM are occurring, the masses of CH$_4$, CO$_2$ and $^{13}$CO$_2$ should increase, while the mass of $^{13}$CH$_4$ should decrease over the course of the incubation.

Time series samples at 0, 24, 48, 96, 192, 384, 720 and 906 hours were collected from the headspaces of all jars. All rate calculations were made using data collected from 192-hours forward, using the 192-hour time point data as the ‘initial’ conditions in all calculations. We used only these later data because the mass and isotope data collected in the first 96-hours of incubation did not behave consistently, and may have included some noise in the signal due to the loss of $^{13}$CH$_4$ spike during initial diffusion into the soil (von Fischer and Hedin 2002). The data collected after 96-hours showed consistent linear fluxes for all measured variables, and was used in all rate calculations and comparisons.

 USING CO$_2$ AND $\delta^{13}$CO$_2$ DATA

The mass of CO$_2$ produced by AOM was calculated using measurements of the mass and isotopic signature of CO$_2$ evolving from the soil (Figure 1, equation 1). The mass of CO$_2$ in the headspace is affected by methanogenesis, AOM, and anaerobic soil respiration. Methanogenesis and soil respiration will both contribute $^{12}$C-CO$_2$, and thus gradually deplete the CO$_2$ signal while increasing the overall concentration of CO$_2$ in the headspace. Fermentation fractionation is nearly negligible, so the $\delta^{13}$C signature of CO$_2$ emitted by anaerobic soil respiration is the isotopic signature of the substrate (which we assign the value of -24‰). AOM using $^{13}$CH$_4$ will result in the evolution of $^{13}$CO$_2$ into the headspace, thus enriching the headspace $^{13}$CO$_2$ signal.
To determine the total mass of CO$_2$ produced as a result of AOM activity within each incubation, we used the following formula:

$$\text{CO}_2_{\text{AOM}} = \left( \frac{(\text{CO}_2_{\text{total}} + \delta^{13}\text{CO}_2_{\text{total}}) \cdot (\text{CO}_2_{\text{total}} + \delta^{13}\text{CO}_2_{\text{respiration}})}{(\delta^{13}\text{CO}_2_{\text{AOM}} + \delta^{13}\text{CO}_2_{\text{respiration}})} \right)$$ (Equation 3.1)

With $\delta^{13}\text{CO}_2_{\text{total}}$ representing the difference in final and initial CO$_2$ isotope signatures as measured by IRMS, CO$_2_{\text{total}}$ is the difference in final and initial CO$_2$ masses measured by GC, $\delta^{13}\text{CO}_2_{\text{Res}}$ was set at -24‰, and $\delta^{13}\text{CO}_2_{\text{AOM}}$ was set at -12.4 ‰ (Smemo and Yavitt 2007).

Whole-incubation AOM rates were then calculated as the slope of the linear regression of mass of CO$_2$ produced via AOM versus incubation time.

As a methodological consideration, hydrogenotrophic methanogenesis could be utilizing some of the $^{13}$C-CO$_2$ resulting from the AOM of the $^{13}$CH$_4$ spike in the headspace, thus altering the isotopic signature of the headspace CO$_2$. We ignore this possible feedback here, as our experiments were not long in duration, and it produces a conservative AOM estimate when we assume null methane production using $^{13}$CO$_2$.

**Using CH$_4$ and $\delta^{13}$CH$_4$ Data**

To calculate simultaneous rates of methanogenesis and AOM, we use a pool-dilution technique, slightly modified from von Fisher and Hedin (2002) and Smemo et al. (2007), that uses the combination of mass and isotopic signatures of headspace CH$_4$ to create a first-order rate constant for methane consumption. The methane consumption rate constant is known herein as turnover time, or k, in units of inverse time. Turnover times for each incubation were
calculated as the slope of the linear regression between the natural log of $^{13}$CH$_4$ spike mass present in the headspace and the incubation time. In the subsequent calculations of CH$_4$ consumption rates, we assume an isotopic signature of microbially-produced CH$_4$ of -60‰, or 1.05 AP% (Quay et al. 1988, von Fischer and Hedin 2002, Smemo and Yavitt 2007). The isotope fractionation of the AOM pathway used in these calculations was set at 0‰, a conservative value for this effect, which has estimates ranging from -2 to -14‰ in terrestrial systems (Alperin and Reeburgh 1987, Martens et al. 1999, Grossman et al. 2002, Smemo and Yavitt 2007). In this way, we cannot overestimate the effect of AOM. All mass values were standardized by dry soil weight using the amount of soil incubated and location-specific soil moisture measurements.

Calculations of gross CH$_4$ consumption and net CH$_4$ flux rates were made for the entire incubation period. Net CH$_4$ fluxes were calculated as the difference in final and initial total mass measurements over hours incubated. Gross CH$_4$ production rates were then calculated for entire incubations by conservation of mass, as the sum of gross consumption and net flux rates.

STATISTICAL ANALYSES

Methanogenesis and AOM rates were calculated using the last four time points measured during each incubation (from 192-906 hours), when data were consistently linear. Time-series data were analyzed with repeated measures ANOVA with post-hoc one-way ANOVAs. Rate data were analyzed with ANOVA, ANCOVA and Tukey’s pairwise post-hoc tests to determine the effect of both incubation time and treatment solution on the rates of methanogenesis and AOM. Data were transformed where necessary to conform to the assumptions of the statistical methods used. All statistical analyses were performed in R version 3.1.1 (Team 2013).
RESULTS

EVIDENCE FOR AOM AND METHANOGENESIS

Our anoxic incubations captured the increase in total mass and the decrease in δ^{13}C of headspace CH_{4} characteristic of active CH_{4} production. The mass and isotopic signature of incubation headspace CH_{4} were related to incubation time (both p<0.001) and acceptor treatment, with the effect of treatment differing across time (p=0.08). Overall mass of CH_{4} increased by factors of 1.3, 1.5, 1.9 and 2.0 in the nitrate, sulfate, ferric iron and control treatments, respectively. Soils treated with nitrate had final CH_{4} concentrations that were 21% lower than in all other treatments, which produced a significant CH_{4} mass treatment effect.

The headspace ^{13}CH_{4} isotopic signature decreased from an overall average of 5.13 atom percent (AP%) to 3.67 AP% over the full 906-hour incubation (Figure 3.2), although the majority of this decrease occurred after 96 hours, falling from the 96-hour average of 4.91 AP%. A slight common decrease in both CH_{4} mass and AP% over the first 24 hours suggests label diffusion into the soil occurred, but cannot be confirmed as such. Acceptor treatment was significantly related to overall flux rates of CH_{4} AP% (p=0.03), but treatments were not significantly different from one another until soils had been incubated 192 hours. The changes in CH_{4} AP% diverged by treatment after 96 hours, separating the nitrate treated soils from all others (Figure 3.2). CO_{2} follows the same pattern of significant treatment divergence in the CO_{2} AP% signals beginning at the 192-hour time point (Figure 3.2, p<0.001). During this same time period, overall mass of CO_{2} increased by factors of ~6 in the nitrate and sulfate treatments, and ~3 in the ferric iron and control treatments. The nitrate treatment has a significantly lower CO_{2} AP% signal beginning at the 96-hour time point and persisting through the 768-hour time point, but
only the nitrate and ferric iron treatments were significantly different by Tukey’s test at 906 hours (Figure 3.2).

**Figure 3.2.** Time series of the atom percent (AP%) of $^{13}$C in CH$_4$ (a) and CO$_2$ (b) in the headspace, as averaged by treatment (n=5 per treatment) over the full 906 hours. Hours incubated was a significant effect by repeated measure ANOVA, with post-hoc one-way ANOVAs
for treatment only significant for the last four time points for both CH$_4$ and CO$_2$. Nitrate treatment CH$_4$ AP% means were significantly different than all other treatments for all four final time points by Tukey test (p<0.05). Nitrate treatment CO$_2$ AP% was significantly different from all other treatments for 192, 384 and 768 hour time points, but only from the ferric iron treatment during the 906-hour time point by Tukey test (alpha=0.05).

CO$_2$ and CH$_4$ mass and AP% changes became consistently linear after 96 hours of incubation (Figure 3.2). It is from the data shown in Figure 3.3 that all rate constants and calculations were made. Strong treatment effects emerge in this data subset, showing nitrate-treated soils have depressed overall CH$_4$ concentrations while maintaining higher $^{13}$C-CH$_4$ AP% signals (Figures 3.3a and 3.3c). Lower CH$_4$ concentrations signify either increased consumption or decreased production. Higher AP% signifies low rates of consumption (no drawdown of the spike) or low production (no dilution with lighter CH$_4$).

Corresponding statistical treatment effects do not appear in the CO$_2$ mass data (Figure 3.3b), with no significant differences in treatment means overall or within any individual time point. While not statistically significant, there are visual differences in the treatment-averaged time series of CO$_2$ masses (Figure 3.3b), with all acceptor treated-soils depressed relative to the control soils. There is, however, a significant overall treatment effect on the $^{13}$CO$_2$ AP% signal (Figure 3.3d, p<0.001), with the nitrate treatment depressed for three of the final four time points, and significant Tukey tests for the contrast of nitrate from all other treatments for 192, 384 and 768 time points (p<0.05). The average $^{13}$C-CO$_2$ AP% for the nitrate treatment at the 906-hour time point was no longer statistically different from the sulfate and control treatment means, but still remained distinctly lower than the ferric iron treatment mean (Figure 3.3d).
With the exception of the CO₂ mass signal, there is a statistically-significant effect of treatment shared throughout all data, confirmed by post-hoc tests to originate in the nitrate treatment. The CO₂ mass signal is the least transparent of process signals, as it incorporates the products of all anaerobic soil respiration, including methanogenesis and AOM. Accordingly, the CO₂ mass signal cannot be interpreted singly alongside the less-mingled respiration process signals. Thus, both the CO₂ and CH₄ data are consistently showing the same strong treatment effects.

![Graphs showing changes in headspace isotopes over time](image)

**Figure 3.3.** Changes in headspace $^{13}$C-CH₄ mass (a) and AP% (c), alongside $^{13}$C-CO₂ mass (b) and AP% (d) averaged by treatment from 192 to 906 hours of incubation. Common legend above
The graph shows treatment line identifications for all 4 panels. Mass treatment means were not significantly different for either CH$_4$ or CO$_2$, but were significant for both gases by repeated measures ANOVA for AP$\%$ (p<0.001). Post-hoc Tukey tests of CH$_4$ AP$\%$ within each time point show nitrate treatment means are always significantly higher than all other treatments (p<0.05). Tukey tests of CO$_2$ AP$\%$ within each time point show nitrate treatment means are significantly lower than all other treatments until 906 hours, when only nitrate and iron treatment means differ (p<0.05). Treatment has no significant effect on mass of CO$_2$ over time, or within time points.

RATE vs. RATE

Following Smemo et al. (2007), we can evaluate the interactions of time and treatment on the visualized rates of methanogenesis and AOM by plotting the mass and AP$\%$ of headspace CH$_4$ over time, averaged by treatment (Figure 3.4). Using both the full and truncated (192-906 hour) time series, we found a significant effect of time, treatment, and the interaction between the two (all p<0.001). For clarity, we visualized this relationship in Figure 3.4 using the truncated data set, which explains the difference in starting points for the regression lines (starting points demarcated with a ‘1’ for each treatment separately). Assuming an isotopic fractionation of -60‰ for methanogenesis (Quay et al. 1988, von Fischer and Hedin 2002, Smemo and Yavitt 2007), and the conservative value of 0‰ for AOM, the direction of these time series regression lines suggests gross CH$_4$ production is greater than gross CH$_4$ consumption, and that both rates are non-zero (von Fischer and Hedin 2002, Smemo and Yavitt 2007). This corroborates the process rates and treatment relationships found by both the CH$_4$ and CO$_2$ datasets.
Figure 3.4. Isotope space for CH$_4$ mixing model, illustrating the change in mass and AP% of headspace $^{13}$CH$_4$ over time. Data points are labeled with time point identification, representing 192-hour (1), 384-hour (2), 768-hour (3) and 906-hour (4) treatment averages.

Using both the CH$_4$ mass and $^{13}$CH$_4$ AP% data with a stable isotope pool dilution model, we calculated simultaneous rates of gross methanogenesis and AOM. We also calculated an AOM rate based on the evolution of heavy $^{13}$CO$_2$ in the headspace. Gross CH$_4$ production, or methanogenesis, rates showed a strong positive correlation with overall net CH$_4$ flux rates, while there was no real relationship between net CH$_4$ flux rate and either of the AOM rate estimations (Figure 3.5). There was also a single jar that stood out when plotted in Figure 3.5, designated by
the ellipsis enclosing all three rate values. This solitary jar was linear in all measured metrics and was a control-treated soil, but is rather inexplicably seceded from the calculated rate data clouds.

**Figure 3.5.** Relationship of measured net CH$_4$ flux rate by gross CH$_4$ production rate and both estimations of AOM rate (by CH$_4$ and by CO$_2$ data). Data points represent individual soil incubations from all treatments (n=20). Three points contained within ellipsis represent the rate data from a single jar (control treatment). Linear regression equation and $R^2$ value for the production data series are denoted above the line.

The strong relationship between net CH$_4$ flux and gross methanogenesis rates is borne out in the observed treatment effects. Of all rates calculated, gross methanogenesis showed the strongest treatment effect (p=0.01), followed by net CH$_4$ fluxes, which were marginally related to treatment (p=0.09). As seen in Figure 3.5, both calculated AOM rates were unrelated to net
CH₄ flux and methanogenesis rates. There is also no treatment effect in AOM rates calculated from the CH₄ pool dilution technique or the CO₂ data. Thus the strong treatment effect on CH₄ cycling in these soils was driven through the impact on the methanogenesis pathway.

The AOM rates calculated with the CO₂ data were lower on average than the AOM rates calculated by the CH₄ pool-dilution technique by a factor of 2.4 (factor range 0.6-4.6) (rates shown in Table 3.2). Comparisons between both versions of the AOM rate and other rate variables conformed statistically; this is a confirmation that despite the magnitude differences, the relative relationships and treatment effects are uniform between AOM rate calculations. Using the two different datasets also allowed us to calculate the relative importance of the AOM pathway to overall carbon cycling using two independent metrics: how much of the CH₄ produced was consumed, and how much of the total CO₂ produced originated from AOM. The percentage of CH₄ produced that was subsequently consumed varied by treatment, with ferric iron and nitrate-treated soils expressing lesser relative importance of the AOM pathway to net CH₄ cycling. Considering the four treatment conditions in the present study, approximately 6-39% of CH₄ produced is removed from anoxic soils prior to release based on the rates calculated by the pool dilution method (Table 3.2).

Using the isotopic signature and mass measurements of CO₂ in the headspace, we also calculated the percentage of CO₂ flux coming from AOM activities. AOM-derived CO₂ comprise approximately 1% of the molar mass of anaerobic CO₂ evolution. Importantly, this metric includes the direct influence of acceptor additions on anaerobic soil respiration, namely ferric iron-, sulfate- and nitrate-reduction, all of which result in increased levels of ¹²CO₂ in incubation headspaces. Net CO₂ fluxes from soils treated with ferric iron and nitrate were low at the end of
the incubation period, but it is the nitrate and sulfate treatments that resulted in lower proportional influence of AOM (Table 3.2).

**Table 3.2: Rate Averages by Treatment**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Turnover Time, k (day⁻¹)</th>
<th>Net CH₄ Flux (nmol/cm²/hr)</th>
<th>Gross CH₄ Production (nmol/cm²/hr)</th>
<th>Gross CH₄ Consumption (nmol/cm²/hr)</th>
<th>Percent of Production Consumed (%)*</th>
<th>Net CO₂ Flux (nmol/cm²/hr)</th>
<th>Gross CH₄ Consumption via CO₂ (nmol/cm²/hr)</th>
<th>Percentage of Total CO₂ derived from AOM (%)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.0042 (0.002)</td>
<td>1.22 (0.3)</td>
<td>1.65 (0.3)</td>
<td>0.44 (0.2)</td>
<td>39.9%</td>
<td>22.4 (9.4)</td>
<td>0.32 (0.1)</td>
<td>1.4%ab</td>
</tr>
<tr>
<td>Iron</td>
<td>0.0029 (0)</td>
<td>1.17 (0.2)</td>
<td>1.54 (0.2)</td>
<td>0.36 (0.1)</td>
<td>7.2%</td>
<td>9.4 (2.0)</td>
<td>0.15 (0.0)</td>
<td>1.5%a</td>
</tr>
<tr>
<td>Nitrate</td>
<td>0.0025 (0.001)</td>
<td>0.56 (0.1)</td>
<td>0.80 (0.1)</td>
<td>0.24 (0.0)</td>
<td>6.7%†</td>
<td>14.2 (1.6)</td>
<td>0.12 (0.0)</td>
<td>0.8%ab</td>
</tr>
<tr>
<td>Sulfate</td>
<td>0.0028 (0)</td>
<td>1.19 (0.1)</td>
<td>1.60 (0.1)</td>
<td>0.41 (0.1)</td>
<td>18.5%</td>
<td>21.3 (5.8)</td>
<td>0.17 (0.0)</td>
<td>0.9%ab</td>
</tr>
</tbody>
</table>

Whole-experiment averages of treated soils (excluding control jars), with SEM in parentheses. Superscript letters down each column indicate results of Tukey tests, with the same (or no) letter indicating non-significant differences in treatment means (p<0.05). †One outlier removed from this calculation (>3 s.d. from overall mean). *Averages shown are the average of individual incubation calculations for these metrics.

Pre-incubation CO₂ and CH₄ gas fluxes were measured during the week prior to pool dilution experiments, when soils were acclimating within the anaerobic incubation jars; these flux rates provide background soil productivity information. Net CH₄ flux and gross methanogenesis rates calculated during the experiment were related to pre-incubation CO₂ flux rates (p=0.02 and 0.01, respectively), and pre-incubation CH₄ flux rates (p=0.07 and 0.09, respectively), but these variables were not particularly helpful in clarifying any other statistical relationships when used as a covariate in ANCOVA analyses. Pre-incubation fluxes of both CO₂ and CH₄ were also marginally related to the rate constant turnover time, or k (p=0.08 and 0.09, respectively).
DISCUSSION

EVIDENCE FOR AOM IN FINNISH LAPLAND

We found strong evidence for the active anaerobic oxidation of methane (AOM) in subarctic fen soils from Finnish Lapland. Stable isotope and mass balance approaches of both CO\textsubscript{2} and CH\textsubscript{4} provided multiple lines of validation for the conclusion that approximately 6-39\% of CH\textsubscript{4} produced in these soils is anaerobically consumed during anoxic \textit{in vitro} incubations. The CO\textsubscript{2} products of the AOM pathway make up around 1\% of the total net CO\textsubscript{2} flux from these soils.

In all headspace measurements we saw a pronounced lag phase before time series data became linear enough to regress. A part of this lag is undoubtedly the time it took the spike to infiltrate fully into the soil. This may have been somewhat longer in our incubations than in the common slurry-style incubations since we retained the complex soil structure that may retard uniform label diffusion. However, we did not create an additional diffusion boundary by inundating the soils. Microsites and fine-scale variations in soil structure can affect the hydrology and soil chemistry on a super fine scale, which can have a meaningful impact on rates (Teh and Silver 2006, Sey et al. 2008). We wanted to imitate natural conditions as much as possible in order to maximize comparability between lab and field. Methanogenesis rates generated \textit{in vitro} should be analogous to potential \textit{in situ} rates, as the amount of CH\textsubscript{4} present does not impact continuous production rates (Zehnder and Brock 1980). This is unlike AOM, which is sensitive to the amount of CH\textsubscript{4} (substrate) present (Sorensen et al. 2001, Smemo and Yavitt 2007, Smemo and Yavitt 2011, Gupta et al. 2013). The lack of live plants is a major limitation of these incubations, which must be kept dark to prevent oxygenic pathways, thus preventing normal plant growth. Vegetation and root interactions in the soil are major influences
on environmental methane cycling dynamics, providing both a pathway for CH$_4$ atmospheric release that circumvents oxidation zones, as well as a source of simple carbon substrates (Whalen 2005). For the most part, the short duration of the incubations prevents any egregious affronts to reality, and these considerations must be acknowledged when interpreting and comparing results.

The CH$_4$ mass and isotope data presented a pattern of simultaneous methane production and consumption. This is true in all treatment groups, although there is a significant treatment effect on production rates, with nitrate-treated soils expressing depressed methanogenesis, and a concomitant negative effect of nitrate on AOM rate that is not statistically significant. The CO$_2$ mass and isotope data corroborated the pattern of active AOM but higher CH$_4$ production rates that was seen in the CH$_4$ data.

**AOM RATE ESTIMATION**

One of the interesting issues that arose was the discrepancy in the magnitudes of calculated AOM rates made using the CO$_2$ data versus the CH$_4$ data. While it was gratifying that all patterns and statistical relationships were consistent between the two AOM rate estimates and all other measured and calculated variables, the fact remains that the CH$_4$ data yielded AOM rates that were, on average, a factor of 2.4 times greater. One possible explanation for the difference is that there are experimental artifacts differentially affecting the two gases measured. For example, there is an isotope fractionation associated with CH$_4$ gas diffusing through water (Mahieu et al. 2006, Preuss et al. 2013), which was not accounted for in our calculations. We did not include this factor because we were not working with large or uniform depths or soils, thus diffusion distances were small and heterogeneous enough that including a uniform diffusion term
would introduce more error into rate estimates. Another possibility for the difference in CO₂ and CH₄ AOM estimates lies in the choice to use an AOM fractionation factor of 0‰ during the pool dilution CH₄-based rate calculations, but to the published estimate of -12.4‰ (Smemo and Yavitt 2007) during the CO₂-based calculations. Unfortunately, the version of differential AOM fractionation estimates we employed results in the more conservative estimate returned by the CH₄ data, which yielded higher rates. When we use the same fractionation factor (either) in both calculations, the rates are still distinct.

Ultimately, the most likely reason for the discrepancy in AOM rates lies in the mechanism of the AOM pathway itself. It is possible that the oxidation of CH₄ was either not complete or the products were retained in the soil structure, both of which would lead to the disappearance of ^13CH₄ from the headspace, but not in the appearance of headspace ^13CO₂. As the CH₄-based AOM estimation is based on the disappearance of the spike, it does not actually quantify the successful completion of the AOM pathway. Currently, the most favored mechanism for AOM is a form of ‘reverse methanogenesis’, in which the fate of the CH₄ carbon is varied (Caldwell et al. 2008, Moran et al. 2008, Scheller et al. 2010a, Scheller et al. 2010b, Biderre-Petit et al. 2011, Thauer 2011, Callaghan 2013) – it may be retained in the biomass of the associated microorganisms, or end in an intermediate chemical like methanol, neither of which contributes to the ^13CO₂ headspace pool. In contrast, the CO₂-based AOM rate estimates can only account for AOM that results in the evolution of heavy CO₂ into the headspace, and thus might be slightly underestimating the true rates. Fortunately, the two rate estimates were relatively comparable, and more importantly, expressed similar relationships with other metrics.
ELECTRON ACCEPTOR TREATMENTS

We found no evidence that AOM is linked to any particular acceptor reduction pathway. We did, however, find that nitrate-treated soils displayed depressed rates of methanogenesis. The negative effect of nitrate on CH$_4$ production rates is not an entirely surprising result, as nitrogen rides the fine line between beneficial and harmful to CH$_4$ cycling (Haroon et al. 2013). Too much nitrate in anoxic systems and methanogens are outcompeted, and/or the intermediates of denitrification become toxic for methanogens. Too little nitrogen and the system can become nutrient-limited, and the largest supplier of labile substrate, vegetation, is stunted. We included nitrate as an acceptor treatment for two reasons: 1) AOM in other terrestrial systems has been experimentally linked with nitrogen cycling (Raghoebarsing et al. 2006, Ettwig et al. 2010, Zhu et al. 2012, Haroon et al. 2013); and 2) the soils were from a subarctic fen with diverse and thriving vegetation, hallarking a moderate amount of nitrate is available in the soil naturally (Wayolle 2011). Soil survey data from inundated areas show an average of 23.5 micrograms nitrogen as NH$_4$ per g/g dry soil via KCl extracts and flow injection analysis, with nitrogen as nitrate lower than 0.5 microgram per g/g dry soil (Victoria Sloan, personal communication), as we also saw (Table 3.1). We introduced nitrate at a concentration that would mimic moderately high natural levels, thus ideally allowing for the beneficial effects without any of the adverse effects. This is difficult, particularly in anaerobic incubations, where denitrification will be favored, the intermediate products (e.g., nitrite, NO, N$_2$O) of which are highly toxic to methanogens (and possibly methanotrophs) (Klüber and Conrad 1998). The effect of nitrate had less of an impact on the AOM pathway, perhaps due to the consistently high partial pressures of CH$_4$ in the headspace, alleviating any substrate limitation to AOM.
Perhaps more exciting than capturing a significant treatment effect, was the highly synchronized CO₂ and CH₄ datasets confirming the timing and effect of the nitrate treatment. We can see this most clearly in the CO₂ AP% signal, where there is a lag in the nitrate treatment enrichment until hour 384, signaling the beginning of AOM (Figure 2b). For the CO₂ signal, the $^{13}$C label is highly enriched, thus it only takes a small amount of AOM-respired CO₂ to see an increase in the CO₂ AP%. In Figure 3a, b, and d, we see the shift in the nitrate treatment starting at hour 384, indicating the ramping up of AOM in these soils, much like what was seen in all the other treatments beginning at hour 96. There was not a significant treatment effect in the ANOVA of calculated AOM rates, but treatments were significant for the CO₂ AP% signal, including a significant interaction effect of time and treatment, originating in the nitrate treatment. Despite the significant delay of AOM in nitrate-treated soils, average nitrate CO₂ AP% was no longer distinct from the control and sulfate treatments by the end of the experiment. These data relate that the suppression effect of the nitrate treatment was not permanent, nor particularly damaging to potential influence to CH₄ cycling despite the delayed onset.

**CONCLUSIONS**

Our results confirm that AOM is an important part of CH₄ cycling within the soils of a subarctic fen in Finnish Lapland. We found no evidence for syntrophic relationships with the reduction by nitrate, ferric iron, or sulfate, but did induce the temporary inhibition of methanogenesis and net CH₄ flux rates, and a delay in AOM influence in nitrate-treated peat soils. We determined AOM rates using both CH₄ and CO₂ based calculations – the resulting AOM rates differed by an average factor of 2.4, but had overlapping ranges and identical statistical relationships with other rate and explanatory variables. Overall, AOM consumed 6-
39% of methane produced during the incubation, and constituted approximately 1% of the net CO$_2$ flux. We found evidence that AOM can be an important sink of CH$_4$ in anoxic, subarctic fen soils.

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Methane ebullition and year-round diffusive fluxes from a peatland in Northern Lapland, Finland

ABSTRACT

Major ecological controls on the greenhouse gases methane (CH$_4$) and carbon dioxide (CO$_2$) in northern wetland systems are well known, yet estimates of the source/sink magnitudes are often inconsistent with measured flux rates. To determine the intra-annual variability of greenhouse gas contributions, we measured net CO$_2$ and CH$_4$ fluxes from September 2012 to September 2013 within a peatland in northern Lapland, Finland. Average annual fluxes were 6.73 and 175.7 mg C m$^{-2}$ hour$^{-1}$ for CH$_4$ and CO$_2$ respectively, with substantial variation between different depressions within the wetland. We found evidence for both autumnal and spring thaw CH$_4$ bursts, collectively equivalent on a mass basis to 32-55% of the mass of CH$_4$ emitted during the summer growing season. The autumnal burst was more than 5-fold larger than the spring burst. Despite the importance of season to both CO$_2$ and CH$_4$ flux rates, CH$_4$ rates were not related to summertime soil temperatures, or air temperatures outside of the growing season. CH$_4$ ebullition measured throughout the growing season augmented by an average of 49% additional CH$_4$, and was linked with fine-scale spatial heterogeneity within the wetland. Surprisingly, CH$_4$ flux rates were insensitive to Fe(III) and humic acid soil amendments, both of which amplified CO$_2$ fluxes. This study provides insights into seasonal emission variability, including CH$_4$ bursts from winter and shoulder seasons, and the importance of accounting for ebullition in annual CH$_4$ emission estimates.
INTRODUCTION

As climate change accelerates, understanding the ecological controls on carbon release from northern peatland systems become increasingly important to successful quantification of greenhouse gas source/sink magnitude. Coarsely, we can reduce the major physical and biological controls of methane (CH\textsubscript{4}) production and release pathways within peatland systems to three factors: hydrology, temperature, and vegetation (Whalen 2005). Methanogenesis, or the production of CH\textsubscript{4}, is a strictly anaerobic process that occurs in anoxic, saturated soils (Bartlett and Harriss 1993). Methanogenesis is stimulated by increasing temperatures, and is often strongly associated with emergent wetland plants that serve as both a carbon source to the rhizosphere, and an escape pathway via aerenchymous plant tissues (Bridgham et al. 2013).

Despite the pleasant parsimony of these main controlling factors, much of this understanding was built on laboratory studies and/or growing season observations. Recent efforts that offer year-round, uninterrupted flux measurements provide an opportunity to directly quantify annual carbon budgets in Arctic environments, and to examine factors that regulate carbon fluxes beyond the growing season to interannual scales.

The influence of non-growing season contributions is one of the largest sources of uncertainty in estimates of net annual loads of the greenhouse gases carbon dioxide (CO\textsubscript{2}) and CH\textsubscript{4} in the Arctic (Bridgham et al. 2013). As a scientific community, we are continuously adding evidence that suggests the importance of non-summer seasons to annual carbon cycling in northern peatlands, including both point (Bokhorst et al. 2011) and repercussive effects (Morgner et al. 2010, Wipf and Rixen 2010, Callaghan et al. 2011) of the non-summer seasons. The difficulty lies in the unknown reproducibility and universality of these newer ecological patterns – are there patterns or relationships that are pan-Arctic or even site-specific but common inter-
annually? One example is the CH$_4$ flux patterns during autumn when wetland soils are freezing, and during the springtime thaw shoulder seasons. There have been nearly equal numbers of studies presenting the existence (Mastepanov et al. 2008, Jackowicz-Korczynski et al. 2010, Sachs et al. 2010) and the absence (Sturtevant et al. 2012, Tagesson et al. 2012) of shoulder season CH$_4$ bursts, or periods of enhanced landscape-scale CH$_4$ flux. Independent of causation, the variability of these burst events warrants study to determine any spatial and temporal patterns. This is particularly relevant in the context of Arctic climate change. Climate is changing faster in higher latitudes, one consequence of which is the lengthening of the melt season (or snow-free season) (Vaughan et al. 2013). A lengthening of the growing season means the shoulder seasons are particularly vulnerable, which may explain the sporadic nature of CH$_4$ bursts during these periods.

Another source of intrigue is CH$_4$ ebullition, or bubble, events. These episodic contributions are difficult to capture on any scale. An increasing number of studies reveal ebullition often significantly enrich load estimates, although estimates from northern peatlands are still limited (Tokida et al. 2007, Goodrich et al. 2011, Green and Baird 2012, Stamp et al. 2013, Klapstein et al. 2014). In order to determine the effect of season on intra-annual greenhouse gas fluxes, we followed a set of permanent soil collars over an entire year, measuring CO$_2$ and CH$_4$ emissions by closed chamber methods from a wet fen in northern Lapland, Finland. Summer measurements using a closed-circulation method allowed for the measurement of bubble events concurrent with diffusive flux measurements.
METHODS

STUDY SITE AND EXPERIMENTAL DESIGN

We performed all field collections and experiments in a minerotrophic, subarctic wet fen site, located near Petsikko in Finnish Lapland (69º29’33’’N 27º 13’39’’E). Petsikko is located approximately 30 km north of the continuous pine forest line at an elevation of 271 m. The snow-free season is from late May to late September, with long-term (1962-2012) mean air temperatures for January and July of -14.5°C and 12.9°C, respectively (data courtesy of the Finnish Meteorological Institute). The geomorphologic bedrock in this area is gneisses covered by glacial till (Hall et al. 2003). There are non-permafrost ice lenses present within the hummocks comprising the mire border that persist throughout the year (Kujala et al. 2008). The Petsikko wetlands are predominantly sedges and peat mosses with low Betula nana shrub hummocks surrounded by gently sloping birch forest. The mire border zones are characterized by deep organic layers (60-80 cm), with vegetation consisting of Eriophorum spp., Sphagnum spp., Tricophorum caespitosum, Betula nana bushes and dwarf form, Empetrum hermaphroditum ssp. nigrum, Vaccinium vitis-idaea, V. uliginosum, V. myrtillus, Rubus chamaemorus, bryophytes and lichens (Laurila et al. 2001, Van Vliet-Lanoë and Seppälä 2002, Wayolle 2011).
Figure 4.1: Historic weather data for northern Lapland Finland, Kevo meteorological station (1962-2012). Month sequence (January-December) along x-axis, with average air temperature, rainfall and snow depth data on y-axes. Monthly averages for the 2012-2013 study period is presented as single points.

Two transects of soil collars were established along raised boardwalks spanning east-to-west across two, non-adjacent depressed-center polygons within the wetland (Figure 4.2). The eastern boardwalk extended over a wetland depression about twice the width of the depression bisected by the western boardwalk; 48 total collars were distributed equally along the total transect length, so that 16 collars were in the smaller, western transect, and 32 collars were installed in the larger, eastern transect.
**Figure 4.2:** Photos of the Petsikko wetland area and the two experimental transects. Photos were taken in early June (eastern transect), mid-July (Petsikko site), and late August (western transect) of 2013, illustrating the short duration of the peak summer season.

Soil collars were made from 25 cm tall sections of 10 cm-diameter thin-walled polyvinyl-chloride (PVC) pipe buried at a distance of at least 30 cm out from the boardwalks, and to depths of approximately 21 cm. Collars were arranged in pairs (with less than 2 cm between collars within a pairing). There was the possibility that the boardwalk would act as a snow fence or windbreak, and alter the physical conditions enough to affect soil functioning. To account for this potential effect, collar pairs were mirrored along the length of the boardwalk, ensuring that each collar pair had a direct counterpart on the opposite side of the boardwalk (Figure 4.3).
Figure 4.3: Photo of the orientation of one set of collar pairs mirrored across the sampling boardwalk (taken in early October 2012). Treatments were randomized between collar pairs; within each pair, treatments of either Fe(III)-NTA or Sigma Aldrich Humic Acids were randomly assigned to one of the collars, and the remaining collar in the pair received the control (DI water) treatment. Treatments of Fe(III)-NTA and Humic Acids were randomized across all collar pairs, blocking by transect and boardwalk orientation, ensuring design balance within each block.

SOIL CONDITION SAMPLING

Customized probes capable of simultaneously measuring oxidation-reduction potential from 7 cm and 14 cm depths were permanently installed at six locations within the wetland: two along the western transect, and four along the eastern transect, approximately evenly spaced
between soil collar clusters. Oxidation-reduction potential was measured with an internal Ag/AgCl electrode, and converted to redox potential, Eh (relative to standard hydrogen electrode) by applying a correction factor of +212 mV. Once- or twice-weekly measurements of soil conditions were taken using handheld probes and by a Thermo Scientific Orion 5-Star Plus Meter. Data included temperatures at 10 cm and 30 cm soil depths, soil electrical conductivity, pH, and oxidation-reduction potential. Soil metrics were measured from locations within 20 cm of each individual collar pair, excepting redox.

FALL, WINTER, AND SPRING GAS SAMPLING

CO₂ and CH₄ gas samples were collected via static chamber method from a subset of 24 collars a total of twenty-three times from September 11, 2012 to June 14, 2013. Sampling occurred approximately once weekly when soils were thawed, and once monthly throughout the period when soils were fully frozen. A set of opaque PVC end caps were modified with septa, rubber gaskets, and water wells to create an airtight gas sampling chamber when the end caps were seated on soil collars. Gas samples were collected from the chamber headspace immediately following cap placement, and again after twenty minutes elapsed. Winter flux measurements were taken from the surface of the snowpack (thus, representing net fluxes through the snow). Chambers were seated onto the snow surface using a 40-cm square flexible tray into which the chamber top was fitted, which distributed weight and allowed a consistent 2 cm seat into the snowpack. Reflective poles were buried 30 cm directly west of chamber pairs, allowing for the measurement of winter fluxes from the snowpack directly above buried soil collars.
Chambers were pressure-vented with a 21-gauge needle during all gas flux measurements. Over-pressurized samples were stored in pre-evacuated 10 mL glass serum vials fitted with gas impermeable butyl rubber stoppers (Geo-Microbial Technologies, Inc., Oklahoma, USA). Following collection, vials were wrapped in wax film and stored in dark refrigeration until analysis. Gas samples were analyzed for CO₂ and CH₄ contents with an Agilent 6890N gas chromatograph equipped with both flame ionization and thermal conductivity detectors. Data were screened to include only positive fluxes, as negative 2-point flux estimates were likely due to an initial disturbance of the collar, causing early bubbles and inaccurate flux measurements.

SOIL WATER ANALYSES

Soil porewater samplers (Rhizon, type MOM for metal studies, Sunvalley Solutions, Florida, USA) were permanently installed at a depth of 0-10 cm within all 48 soil collars. Soil porewater samples were collected intermittently when soils were thawed and following gas flux measurements. Porewater samples were collected into N₂-flushed and evacuated additive-free vacutainers (Becton Dickinson) with no filtering. Dissolved gases were extracted by equilibrating 1 mL of porewater with an N₂ headspace within an airtight 10 mL glass serum vial fitted with gas-tight butyl rubber stoppers. Vials were slowly shaken for 12 hours, and then allowed to rest in a dark cabinet for 12 additional hours before headspace samples were analyzed by gas chromatograph. CH₄ and CO₂ gas concentrations were corrected for equilibration effects using Henry’s Law. Samples designated for Fe analysis were acidified to pH < 2.0 with 1 M HCl within 1 hour of collection into the air-free sample vial. Total Fe and Fe(II) were quantified from
acidified samples using a 1,10-phenanthroline colorimetric method (Committee 1978, Lipson et al. 2010); Fe(III) content was calculated by difference.

SUMMER GAS SAMPLING

The PVC end caps were modified with brass barb fittings and 45 m of 6.4 mm UV-resistant Tygon plastic tubing (U.S. Plastics, Lima, OH, USA) to create a closed-circulation system with an Ultraportable Greenhouse Gas Analyzer (Los Gatos Research, Mountain View, CA, USA). Gas flux measurements were measured instantaneously in the field every other day from June 20, 2013 to August 27, 2013, for a total of 37 discrete dates. Flux measurements were conducted by fitting the end cap onto a soil collar, creating an airtight seal with rubber gaskets and water wells, and waiting 45 seconds to allow the gas volume in the tubing to flush completely. Concentrations of CO$_2$ and CH$_4$ reported as C (ppmv) were recorded as 2-second averages for a total of 300 seconds, starting from the time when both gas concentrations registered a linear increase. Ambient air was flushed through the system for at least 3 minutes between measurements. Fluxes were calculated as the slope of the best-fit linear regression line. In approximately 8% of measurements, issues with the linearity of the flux curve resulted from mid-run spikes in concentration of CH$_4$, most easily attributable to CH$_4$ ebullition (see Bubble Dataset below). For the estimate of diffusive CH$_4$ flux rates, we used data from periods with linear increases to estimate rates, effectively extracting the ‘bubble’ from the overall flux measurement. The bubble contribution was calculated separately using the methods described below. $R^2$ values for all flux curves were greater than 0.8, with all but one having $R^2$ values greater than 0.95.
Daytime variability in gas flux rates were measured using a subset of 18 collars, each measured once hourly for 12 hours. Using the closed-circulation system, each collar was measured once every hour from 08:00 to 20:00 on a single date, with data collected from August 20 - 24, 2013. Hourly flux rates were compared between collars (ANOVA) and across time (repeated measures ANOVA) to determine the existence and potential impact of diurnal variability.

**BUBBLE DATASET**

Approximately 8% of summer fluxes measured using the closed circulation system showed a jump in the CH$_4$ signal at some point during the 300-second sample collection period. These jumps in the CH$_4$ signal were interpreted as ebullition events if they occurred more than 30-seconds after the onset of dual linear gas fluxes, and if the disturbance appeared in the CH$_4$ signal but not in the CO$_2$ signal. Bubble frequency was calculated by dividing the number of ebullition events over cumulative measurement time. Bubble magnitude was calculated in two ways: as the simple subtraction of the CH$_4$ mass immediately prior to the ebullition event (mass at arrow ‘b’ in Figure 4.4) from the post-ebullition CH$_4$ mass (Figure 4.4, arrow ‘c’), and as the difference in measured and predicted final masses (Figure 4.4, bracket ‘d’). Predicted final CH$_4$ masses were calculated by using the pre-bubble flux rate and extrapolating out to 300-seconds. These two methods for calculating bubble magnitude were highly related, regressing with an $R^2$ value of 0.996. Considering the interchangeability of calculation methods, we chose to continue all analyses using bubble sizes calculated by difference in measured and predicted final mass.
Figure 4.4: \( \text{CH}_4 \) ebullition event, as captured during the closed-circulation system measurement of greenhouse gases from permanent soil collars within the Petsikko mire. Ebullition magnitude was measured by two methods: 1) the simple difference in pre- and post-event \( \text{CH}_4 \) masses (indicated by arrows b and c, respectively); and 2) the difference in measured final \( \text{CH}_4 \) mass value from the predicted final amount of \( \text{CH}_4 \) as illustrated by bracket d. Predicted final \( \text{CH}_4 \) masses were calculated by extrapolating using the pre-event flux rate (measured as the slope of \( \text{CH}_4 \) increase between arrows a and b).

STATISTICS

Collar- and location-specific fluxes and metrics were compared using repeated measures ANOVA and ANCOVA, with post-hoc ANOVA, regression and Tukey’s HSD tests. Seasons were delimited using solar equinox dates: September 22 and December 21, 2012, and March 20 and June 21, 2013. Treatment effects in the manipulated experiment were determined using
repeated measures ANOVA. Data were transformed when necessary to meet test assumptions. All analyses were performed in R-3.1.1.tar.gz. Geometric means for bubble and gas flux data were used in most instances to correct for non-normal distributions of raw data; it is always noted when geometric means are presented.

RESULTS

SPATIAL PATTERNS

Net CH$_4$ and CO$_2$ fluxes varied within the wetland area. Of all site variables, transect had the strongest relationship with flux rates, and sported distinctive soil properties oft related to wetland gas fluxes (Table 4.1). Lower annual CH$_4$ fluxes and higher annual CO$_2$ fluxes were associated with the lower pH and higher soil electrical conductivity found in the western transect (p<0.001 for both gases). Within the summer season, the western transect had higher (less negative) reduction-oxidation potential values within the soil, and higher levels of extractable iron, indicating less-ideal methanogenic conditions.

Table 4.1: Seasonal transect averages for soil properties

<table>
<thead>
<tr>
<th>Western Transect</th>
<th>pH†</th>
<th>Electrical Conductivity (µS/cm)†</th>
<th>Redox Potential 7cm depth (mV)</th>
<th>Redox Potential 14cm depth (mV)</th>
<th>Total Dissolved Iron* (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spring</td>
<td>5.30</td>
<td>56.0$^a$</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Summer</td>
<td>5.23</td>
<td>59.8$^a$</td>
<td>-104.9$^a$</td>
<td>-135.6$^a$</td>
<td>38.1$^a$</td>
</tr>
<tr>
<td>Fall: Pre-Freeze Up</td>
<td>5.67</td>
<td>30.2$^b$</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Eastern Transect</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spring</td>
<td>6.23$^a$</td>
<td>30.8</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Summer</td>
<td>5.09$^b$</td>
<td>35.3</td>
<td>-214.1$^b$</td>
<td>-342.2$^b$</td>
<td>25.3$^b$</td>
</tr>
<tr>
<td>Fall: Pre-Freeze Up</td>
<td>5.33$^{ab}$</td>
<td>26.4</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

†Annual pH and soil electrical conductivity were significantly different between transects (p<0.001)  
*Total extractable iron measurements from porewater samples all taken prior to experimental acceptor treatments introduced into soil  
Superscripts of the same letter (or no letter) down each column within each transect (eastern and western) are not significantly different by Tukey test (p<0.05)
SEASONAL GAS FLUXES

We measured both CO$_2$ and CH$_4$ emissions from 24 to 48 soil collars from September 11, 2012 to August 27, 2013 (Figure 4.5). Flux measurements confirm that significant CH$_4$ and CO$_2$ fluxes do occur throughout the year, with a significant impact of season on flux magnitudes (p<0.001 for both gases). Using air temperature measurements from the Finnish Meteorological Institute’s Kevo weather station, we showed that seasonal variations in the CO$_2$ signal were positively correlated to air temperature (Figure 4.5, $R^2$=0.254, p<0.001). The connection between air temperature and CO$_2$ flux rate was stronger in the western transect than in the eastern transect (p<0.001, $R^2$=0.337 and 0.226, respectively), with increases in temperature translating to 1.6-fold higher CO$_2$ flux rates per degree of temperature change in the western transect as compared to the eastern transect (p = 0.003).
Figure 4.5: Relationship between average daily air temperatures and natural logs of CO₂ and CH₄ carbon fluxes in terms of C. Fluxes were measured from September 11, 2012 – August 27, 2013. Daily mean fluxes averaged for both gases, with error bars representing standard error.

Soil temperature measurements were only taken when soils were adequately thawed to insert handheld probes, constraining the relationships found between soil metrics and flux rates to periods of soil thaw. There was a strong positive relationship between CO₂ flux rate and soil temperatures from both 10 and 30 cm depths (both p<0.001), but this relationship was entirely absent in the CH₄ signal. When using the full annual dataset, CH₄ flux rates were not linearly correlated with air temperature (R² = 0.084), but there is a slight positive relationship in the summer months (Figure 4.6). The effect of transect was the inverse of the CO₂ signal pattern, with stronger relationships between CO₂ flux rate and air temperature in the eastern transect (R²=0.130 for eastern, R²=0.051 for western), and increasing flux rates in the eastern transect 1.6-fold higher per degree of air temperature change (p=0.008). Again, the positive correlation between air temperature and CO₂ flux rates was strongest in the summer months, when air temperatures were all above 10°C (Figure 4.6).
Figure 4.6: Log carbon fluxes by average daily air temperature during the summer growing season, when air temperatures are greater than 10°C. Symbols denote transect (east vs. west), and symbol color represents gas identity (CH$_4$ vs. CO$_2$). Values represent means and standard errors.

The temperature coefficient, or Q10, for CH$_4$ and CO$_2$ flux rates are 1.57 and 2.09, respectively, thus the CH$_4$-temperature connection is only really helpful in predicting fluxes within the summer season, but not between seasons. We also see that the CH$_4$ fluxes are relatively less connected to temperature (than CO$_2$ fluxes are). Transect did not matter to the Q10 for either gas, as the regressions of natural log fluxes on change in air temperature (Figure 4.6) had indistinguishable slopes for the same gas between transects.
**Figure 4.7:** Log CO$_2$ fluxes measured from September 11, 2012 to August 27, 2013. Mean daily air temperature in degrees Celsius shown by heat color map, with red indicating the warmest and blue for the coolest temperatures. Vertical lines connect all measurements made from multiple collars on the same day (collar n=24 for fall, winter and spring, and 48 for summer).

Given the links between seasons and physical conditions (i.e. weather), we break the fall season (originally spanning September 22-December 20, 2012) into two periods: pre- and post-freeze up. Defining a pre-freeze up period (September 22 -October 24, 2012) allows for the capture of the autumnal CH$_4$ burst as a distinct phase. Despite the importance to flux rates, seasons did not trump the effect of spatial heterogeneity, as transect remains a highly significant factor associated with both CH$_4$ and CO$_2$ flux rates throughout the year (p<0.001 and 0.02, respectively). Despite the individual importance of both transect and season, the seasonal effect was common to both transects for CO$_2$, while there was a slight interaction found for the CH$_4$. 
signal (p=0.03). For this reason, we examine CH$_4$ and CO$_2$ flux rates and temperature metrics broken down by transect and seasons in Table 4.2. To examine the balance of carbon cycling pathways, we include the CO$_2$:CH$_4$ ratio data, which were significantly different between transects (p<0.001) and amongst seasons (p=0.01). This interactive term is particularly interesting during the transitional seasons of spring and fall.

Table 4.2: Seasonal transect averages for gas fluxes and temperature metrics

<table>
<thead>
<tr>
<th>Western Transect</th>
<th>CH$_4$ Flux (mg/m$^2$/h) *</th>
<th>CO$_2$ Flux (mg/m$^2$/h) *</th>
<th>CO$_2$:CH$_4$ Flux Ratio *</th>
<th>Soil Temp 10 cm (°C)</th>
<th>Soil Temp 30 cm (°C)</th>
<th>Ground Surface Temp (°C)</th>
<th>Air Temp (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winter</td>
<td>3.7$^b$</td>
<td>82$^b$</td>
<td>36$^{bc}$</td>
<td>-</td>
<td>-</td>
<td>-31.2$^d$</td>
<td>-14.2$^e$</td>
</tr>
<tr>
<td>Spring</td>
<td>4.1$^b$</td>
<td>165$^a$</td>
<td>1710$^b$</td>
<td>11.4$^b$</td>
<td>3.0$^b$</td>
<td>-1.4$^b$</td>
<td>9.0$^b$</td>
</tr>
<tr>
<td>Summer</td>
<td>2.9$^b$</td>
<td>223$^a$</td>
<td>41$^b$</td>
<td>12.9$^a$</td>
<td>11.2$^a$</td>
<td>8.0$^a$</td>
<td>14.1$^a$</td>
</tr>
<tr>
<td>Fall: Pre-Freeze Up</td>
<td>31.5$^a$</td>
<td>120$^c$</td>
<td>108$^a$</td>
<td>3.9$^c$</td>
<td>2.6$^b$</td>
<td>3.7$^a$</td>
<td>4.3$^c$</td>
</tr>
<tr>
<td>Fall: Post-Freeze Up</td>
<td>0.3$^c$</td>
<td>55$^c$</td>
<td>2370$^c$</td>
<td>-</td>
<td>-</td>
<td>-19$^c$</td>
<td>-8.7$^d$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Eastern Transect</th>
<th>CH$_4$ Flux (mg/m$^2$/h) *</th>
<th>CO$_2$ Flux (mg/m$^2$/h) *</th>
<th>CO$_2$:CH$_4$ Flux Ratio *</th>
<th>Soil Temp 10 cm (°C)</th>
<th>Soil Temp 30 cm (°C)</th>
<th>Ground Surface Temp (°C)</th>
<th>Air Temp (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Winter</td>
<td>8.4$^b$</td>
<td>39$^c$</td>
<td>43$^d$</td>
<td>-</td>
<td>-</td>
<td>-31.2$^d$</td>
<td>-14.2$^e$</td>
</tr>
<tr>
<td>Spring</td>
<td>8.9$^b$</td>
<td>155$^a$</td>
<td>266$^b$</td>
<td>10.1$^b$</td>
<td>2.5$^b$</td>
<td>-1.4$^b$</td>
<td>9.0$^b$</td>
</tr>
<tr>
<td>Summer</td>
<td>7.5$^b$</td>
<td>163$^a$</td>
<td>166$^c$</td>
<td>13.0$^a$</td>
<td>10.1$^a$</td>
<td>8.0$^a$</td>
<td>14.1$^a$</td>
</tr>
<tr>
<td>Fall: Pre-Freeze Up</td>
<td>29.1$^a$</td>
<td>159$^a$</td>
<td>73$^a$</td>
<td>3.9$^c$</td>
<td>2.6$^b$</td>
<td>3.7$^a$</td>
<td>4.3$^c$</td>
</tr>
<tr>
<td>Fall: Post-Freeze Up</td>
<td>0.1$^c$</td>
<td>66$^b$</td>
<td>726$^d$</td>
<td>-</td>
<td>-</td>
<td>-19$^c$</td>
<td>-8.7$^d$</td>
</tr>
</tbody>
</table>

*Tukey tests performed on log-transformed values
Superscripts of the same letter down each column within each transect (eastern and western) are not significantly different by Tukey test (p<0.05).

The physical controls on gas emissions alter perceptibly when soil freeze-up occurs and there is measureable snow cover through which the gases must move. There was a seasonal transition period during fall 2012, where snow was accumulating on the wetland surface, but soils had not yet frozen to create a surface ice layer. The snow-wetland interface was slush-like.
from October 8th, as shown in Figure 4.8 at the arrow marked “Freeze-up begins”. Final freeze-up occurred over the following week, with solid ice surfaces present across the entire wetland landscape on October 25th. It was during the five weeks immediately prior to the autumnal freeze-up that CH$_4$ fluxes were approximately 10-fold higher than summer flux rates (Figure 4.8, p<0.001). This ‘burst’ of CH$_4$ emissions in late fall runs counter to the negative temperature effect observed during all other seasons, as soil and air temperatures were decreasing throughout this period. Also seen in Figure 4.8, there were also sporadic elevated flux rates measured during the spring thaw period (e.g. May 2013), with overall CH$_4$ fluxes higher than summer averages by a factor of 1.6, although not as uniformly spread throughout the wetland area.

Figure 4.8: Log annual CH$_4$ fluxes measured from September 11, 2012 to August 27, 2013.

Mean daily air temperature in degrees Celsius shown by heat color map, with red indicating the warmest and blue for the coolest temperatures. Vertical lines connect all measurements made.
from multiple collars on the same day (collar n=24 for fall, winter and spring, and n=48 for summer).

Unlike in permafrost-affected wetland soils, soils in this wetland froze from the surface downward, resulting in an incomplete freeze of soils that persisted throughout the fall, winter and spring seasons. Anecdotally, soil sampling occurred monthly from December to April, allowing us to track soil freeze conditions; within the wetland depressions, only the top 10-15 cm of the wetland surface ever froze, creating a ‘cap’ of frozen water and soil, underneath which soils remained unfrozen. The onset of late-fall and winter conditions is linked with the accumulation of persistent snow on soil surfaces. We found the arrival of measureable snow depths was strongly correlated with a drop in both CO₂ and CH₄ gas emissions (p<0.001), but that this suppression reversed after the December measurement date. The nonlinear relationship between snow depth and CH₄ flux and varied by transect, with a steeper decrease in overall CH₄ flux rates with increasing snow depth within the eastern transect (p=0.03). Average monthly snow depth gradually increased following freeze-up, peaking in March with 77.2 cm, then falling to complete melt by mid-May. Contrastingly, average CH₄ fluxes plummeted coincident with freeze-up and stayed very low through December, but then increased steadily from January to May, except a flux dip during the April measurement when warmer, humid weather conditions on the cusp of snow melt initiation (Figure 4.9).
The collar and sampling configuration was designed to account for any possible differences in physical soil conditions resulting from the presence of the raised boardwalks. This included a ‘snow fence’ effect, where snow piles to one side of the boardwalk structure and persists longer than the snow on the opposite side of the boardwalk. We found no evidence for any effect of boardwalk on CH$_4$ flux rates at any point in the year. The boardwalk was correlated with slightly lower soil temperatures (<0.2°C), most probably attributable to the boardwalk shading soils oriented to the north of the boardwalks, resulting in less direct solar radiation.
While soil temperature was statistically related to CO$_2$ flux rates, the interaction between soil temperature, collar orientation and CO$_2$ flux was not statistically significant (p=0.72).

CH$_4$ EBULLITION

One thing common within all seasons was episodic rogue CH$_4$ flux rates, indicating considerably higher-than-average diffusive fluxes, or alternately, the random capture of CH$_4$ ebullition events, or bubbles. During the summer season, gas fluxes were measured with an in-field analytical instrument, granting us the ability to watch gases increase over the scale of seconds. The real-time nature of the flux measurement method allowed for the collection of CH$_4$ ebullition events, or CH$_4$ bubbles, which occurred in approximately 8% of all fluxes measured. The distribution of bubble magnitudes was right-skewed (Figure 4.10), with the greatest proportion of bubbles contributing less than 2 mg of CH$_4$ m$^{-2}$. As with most other metrics, the two experimental transects had significantly distinct average CH$_4$ bubble influence, with the eastern transect higher than the western transect by a factor of 4 (Table 4.3, p<0.001). The western transect, in addition to having significantly fewer bubbles, also had smaller bubbles, with no single bubble event contributing more than 3 mg m$^{-2}$. This relationship persisted throughout the summer season.
Figure 4.10: Bubble magnitude and frequency distinguished by transect. CH₄ flux and bubble data measured using the closed-circulation gas sampling method throughout summer 2013 (n=186 total events: 172 and 14 from eastern and western transects, respectively).

To better compare transects and determine the relative importance of the contribution of bubble-CH₄, we used geometric means to calculate the average bubble magnitude and contribution. When compared with the geometric mean of summer CH₄ fluxes (rates calculated not including bubble contributions), bubbles add an additional 6.6-52.7% of the mass of CH₄ emitted by diffusive flux (Table 4.3). Thus bubble events contribute significantly to the amount of CH₄ emitted from this wetland, with an overall mean doubling the total amount of CH₄ mass released from the wetland over the season (an additional 49.4%, p<0.001).
Table 4.3: **Bubble metrics by transect**

<table>
<thead>
<tr>
<th></th>
<th>Bubble Frequency (hr⁻¹)</th>
<th>Bubble Magnitude* (mg m⁻²)</th>
<th>Bubble Contribution* (mg m⁻² h⁻¹)</th>
<th>Seasonal Mean CH₄ Flux* (mg m⁻² h⁻¹)</th>
<th>Additional CH₄ due to bubbles (mg m⁻² h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eastern Transect</td>
<td>1.44</td>
<td>1.71ᵃ</td>
<td>2.47ᵃ</td>
<td>4.68ᵃ</td>
<td>52.68%</td>
</tr>
<tr>
<td>Western Transect</td>
<td>0.24</td>
<td>0.43ᵇ</td>
<td>0.10ᵇ</td>
<td>1.57ᵇ</td>
<td>6.60%</td>
</tr>
</tbody>
</table>

*Geometric means presented and used in calculations and analysis. Superscript letters down each column indicate significant differences between transects by Tukey’s test.

The spatial and temporal variability in bubble events, other than the higher frequency consistently found within the eastern transect, was not correlated with any other measured variables. There were, however, some collars that had disproportionate numbers (both positive and negative) of bubbles over the course of the summer season. As an example, we show a single soil collar from the eastern transect and the ‘control’ treatment group for the course of the summer season (Figure 4.11). This collar produced a moderately high quantity of bubbles, with bubble magnitudes ranging from 0.43 to 10.47 mg CH₄ m⁻². The mass of CH₄ (mg CH₄ m⁻²) contributed by diffusive action is the amount of CH₄ emitted in 5 minutes; bubble masses are also in mg CH₄ m⁻² per 5 minutes, so if we limit ourselves to 5 minutes, we can directly compare diffusive and ebullition contributions. Figure 4.11 indicates the mass of CH₄ emitted by diffusive flux. On dates when bubbles occurred, a blue dot was marked to indicate bubble size for that day. The total mass of CH₄ emitted in a 5-minute period on a day with a bubble is the sum of the diffusive and bubble flux masses.
Figure 4.11: The CH$_4$ flux time series of a single (control-treated) soil collar from June 20 to August 27, 2013; daily baseline CH$_4$ flux rates represented by + symbols. If a bubble occurred during the daily measurement, it is indicated by the presence and size of a blue dot centered on the daily flux rate + symbol.

For the collar depicted in Figure 4.11, if only diffusive fluxes were considered, the total contribution of CH$_4$ mass over the season is 9.99 mg m$^{-2}$ season$^{-1}$. If bubbles are included, the seasonal CH$_4$ contribution rises to 54.03 mg m$^{-2}$ season$^{-1}$, increasing the net CH$_4$ load by 5-fold. The seemingly random nature of bubble events begs the question of how diurnal effects might impact rate estimates.
TWELVE-HOUR GAS FLUX TRIALS

Standard gas sampling protocol was kept consistent throughout the year – with fluxes measured first in the western transect (from ~8-12:00), and then in the eastern transect (from ~12-20:00). In order to determine if this sampling scheme correlated with differences seen in measured flux rates by transect, we followed eighteen collars for a single day from 8 am to 8 pm. Transect was a significant factor for CH₄ flux rates (p<0.001), while time of day was not (Figure 4.12a). CO₂ flux rates were related to time of day, with higher rates later in the day (Figure 4.12b, p<0.001), and more pronounced hourly patterns in the higher-flux eastern transect. Twelve-hour trials were conducted in the middle of August, when gas fluxes were comparable with the rest of the growing season.
Figure 4.12: Averaged CH$_4$ (a) and CO$_2$ (b) fluxes by transect and hour during the 12-hour flux trials. Error bars indicate standard error of the mean. A total of 18 collars were measured: 12 from the eastern transect, 6 from the western transect.

DISCUSSION

We found evidence to suggest a significant impact of the shoulder and winter seasons on the annual CH$_4$ and CO$_2$ budgets from this subarctic peatland. Specifically, CH$_4$ ebullition events introduce a substantial amount of CH$_4$ into the atmosphere in addition to diffusive fluxes, and
convey another important control on net emissions. We also found that CH$_4$ emissions were decoupled from soil and air temperatures, especially outside of the growing season, and that fine-scale spatial heterogeneity was an important source of uncertainty in quantifying net emissions and ebullition.

SHOULDER AND WINTER SEASON FLUXES

The CH$_4$ flux rates during the shoulder seasons, when soils are either freezing or thawing, were both significantly higher than average growing season flux rates. The magnitude of the annual CH$_4$ source would have been greatly underestimated if the non-growing season and ebullition event contributions were not considered. Shoulder seasons should be considered when creating annual CH$_4$ budgets, as the timing of freeze-up and snow cover are particularly sensitive to the increased temperatures projected within high-latitude systems (Kattsov et al. 2005). In this system, the bursts in the fall and spring as soils physically change between frozen and unfrozen states could be due to mass flow, or the displacement of gases trapped in lower soil layers as soils contract and swell with phase change. This is more likely to be a factor in the fall in this system, as soils never fully froze, but were just rapidly cooling (Mastepanov et al. 2008), and we saw elevated levels of CH$_4$ in the porewater of soils in the top 10 cm during the periods of greatest net fall emissions (data not shown). The spring thaw-associated burst was more likely related to the thinning ice barrier and the release of gases that had been building up throughout the winter. Another possibility is that spring temperatures encourage the rapid processing of labile organic carbon associated with ice-damaged tissues, and the spring burst is the short-lived exhaustion of this transient carbon pool (Mastepanov et al. 2013).
Winter gas fluxes were a surprisingly strong signal. Net CH$_4$ fluxes plummeted to near-zero during November and December, beginning immediately after freeze-in and snow cover was established. This suppression was reversible, and fluxes from January through May were either equivalent or higher than average growing season values, concurrent with snow depths maintained above 40 cm deep. In other snow-covered systems, the soil-insulating effect of snow pack did not impact gas flux rates until the snow had reached a certain depth (Brooks et al. 1998), suggesting a threshold of snow depth, over which the soils are insulated enough to function decoupled from cold air temperatures. In this dataset, we see the reinvigoration of CH$_4$ fluxes after snow reaches around 40 cm deep, similar to the 30 cm threshold observed in an alpine system by Brooks et al. (1998). The single exception to this insulation effect was the April field date. The April field date had weather conditions that were perhaps not conducive to emissions through snow, most notably near-zero temperatures, high humidity and cloud cover resulting slushy snow cover that could have acted as a physical barrier to gaseous release. Thus, the nonlinear relationship between snow and fluxes in the early and late snow periods might also have to do with the quality of snow, which changes in porosity and therefore diffusive resistance in response to meteorological conditions (Jones et al. 1999, van Bochove et al. 2000). In both the shoulder and winter periods, there was no real relationship between CH$_4$ and air temperature, as some winter and early spring fluxes were equivalent or greater than growing season rates.

The rapid changes in climate already occurring in these northern systems affect the depth and duration of snowpack, and thus soil temperatures (Wrona et al. 2005) and water table in early summer (Mastepanov et al. 2013). For soil carbon cycling, soil temperature is often a main control on winter ecosystem and soil respiration (Wookey et al. 2009, Wang et al. 2011), with possible cascading effects of altered winter functioning (Morgner et al. 2010, Wipf and Rixen
2010, Bokhorst et al. 2011, Callaghan et al. 2011). This includes mid-winter soil exposure following episodic warming events (Bokhorst et al. 2011). In systems like Petsikko where soils freeze from the top-down, and thawed soils persisted throughout the winter beneath a relatively thin cap of frozen water and soil, episodic warming events could have a disproportionate impact on winter-time soil stability.

**TEMPERATURE**

We did not find a relationship between air temperature and flux rate. The CO$_2$ signal shows a more consistent positive relationship with air temperature, but the relationship between temperature and CH$_4$ flux rate is only consistent within individual seasons. Overall, this physical parameter is not a good predictor of CH$_4$ flux outside of the summer season, a pattern that has been seen in other northern systems of high spatial and temporal variability (Leppala et al. 2011, Moore et al. 2011, Mastepanov et al. 2013). The calculated summertime Q10 for CH$_4$ flux (1.57) is on the low end for natural wetland systems, which have an average range from ~2.3 to ~12.1 (with an absolute range from 1-35) (Whalen 2005). One possible explanation for this slight linkage between CH$_4$ and air temperature (when there is none whatsoever with soil temperature) lies in the identity of the transport mechanism. The majority of CH$_4$ release from wetland systems is released from deeper soils via transport through aerenchymous tissue of emergent vascular vegetation (Hargreaves et al. 2001, Le Mer and Roger 2001). The importance of this release pathway has been established in numerous northern wetland systems (Smith et al. 2003, Laanbroek 2010, von Fischer et al. 2010, Leppala et al. 2011). Considering that the water table remained above or within 2 cm of the soil surface at all times throughout the year, expected perturbations to the rate of CH$_4$ production in deeper soil layers are minimal (Kotiaho et al. 2010,
Leppala et al. 2011). Pressure and air temperature changes can draw gases from within vascular plant tissues, thus increasing air temperatures can promote the movement of CH$_4$ out of the soil system. This is not well supported by the diel flux data, where we saw no effect of time of day on net CH$_4$ emissions. It is also possible that the more relevant soil temperature to consider would be soil surface temperature, as the lag between changes in air and deeper saturated soil temperatures may have contributed to the disconnect in these known-to-be-related variables (Crill et al. 1988, Jackowicz-Korczynski et al. 2010, Olefeldt et al. 2013). The CH$_4$-temperature seasonal decoupling is driven in part by the fall and spring bursts, which show that production and release of CH$_4$ can be seasonally decoupled, thus instantaneous temperature may not constitute the most useful predictor of fluxes.

LANDSCAPE VARIABILITY

There was a consistent difference in CH$_4$ emissions between transects, independent of season and temperature. Despite the fact that less than 100 meters separated the two transects, there were distinct transect signatures for net CH$_4$ and CO$_2$ fluxes. The transect specificity also extended to include most of the relationships between environmental variables and gas flux rates, with the notable exception of daytime hourly CO$_2$ flux patterns. Somewhat ironically, the wetland depressions where the two transects were located were chosen specifically because of their similar-looking environments. Within the overall wetland area, these two depressions were visually comparable in terms of general plant cover and identity, soil color and late summer soil pH, thaw depth and water table height. They also both directly abutted mire edges, and so were enclosed on one side by raised hummocks leading into gently-sloped dwarf birch forests, which was a consideration from a hydrological perspective. Despite these good intentions in site
selection, we managed to capture the high spatial heterogeneity in ecological conditions characteristic of northern peatland ecosystems (Bubier et al. 1995). Having observed both transects throughout the year, we saw that there were several periods of visibly-flowing water within the lower-yielding western transect, which may have contributed to more soil oxygenation. This is supported by the less-negative redox potentials measured in the western transect, suggesting less methanogenic conditions. The western transect also had higher native loads of iron concentrations in the porewater, as well as higher amounts of nitrate and sulfate (See Chapter 3), all of which are thermodynamically-favorable terminal electron acceptors and further support lower methanogenesis in this transect.

CH₄ BUBBLES

Another aspect of the sporadic and somewhat unpredictable nature of CH₄ release from northern peatland systems is the importance of CH₄ ebullition events, or bubbles, to annual CH₄ budget estimates. We found evidence that CH₄ bubbles can account for an additional 6.6-52.7% of CH₄ mass (up to approximately 1.5-fold the amount of CH₄ released via diffusion). This can amount to a significant aspect of landscape CH₄ source/sink load. As with all things we measured, there is a clear spatial aspect mediating bubble influence. The strong spatial component to bubble influence followed the same pattern of overall fluxes – much higher bubble frequency and magnitude in the eastern transect. The previously discussed spatial heterogeneity in net emissions is also applicable here; perhaps more CH₄ production is occurring in the eastern transect, increasing the amount of potential release, or creating enough subsurface gas pressure to enhance ebullition by sheer mass. A leading hypothesis regarding ebullition connects bubbling events with drops in atmospheric pressure, whereby drops in barometric pressure cause increased
bubbling (Tokida et al. 2005), but this would not entirely explain the site specificity we found, as changes in atmospheric pressure would have been common to both transects.

In this work, we found that bubble characteristics separated along the transect distinction, with not only smaller sized bubbles, but far fewer of them in the western transect (which also sported lower overall CH₄ flux rates). When considering single collars over time, the presence and magnitude of bubble events were not correlated with diffusive flux rates. In other words, bubbles did not correlate with lower diffusive flux rates, which would have suggested a simple transference of mass from one release pathway to another release pathway. Rather, bubbles appeared during both high and low diffusive flux periods. We also found bubble occurrence and magnitude to be insensitive to landscape temperature metrics, although this could be easily explained by a decoupling in the production and release pathways (Tokida et al. 2009). There were certain collars that had tendencies towards bubbles, but these collars did not share any distinguishing visual, temporal, or process-related features (that just as many collars without bubble tendencies also shared). The likelihood of bubbles also did not significantly depend on time of day. We did notice an anecdotal linkage between the frequency of bubble events and precipitation, especially following hard rains. In these instances, collars subjected to driving rain events prior to flux measurements were subsequently more liable to brandish bubbles during the chamber measurements. These post-rain bubbles could have been associated with the dual influences of decreased barometric pressure during inclement weather, and the increased weight of water on the soil column, which would increase pressure and could cause degassing.
CONCLUSIONS

In conclusion, CO$_2$ and CH$_4$ fluxes were strongly dependent on season, with substantial decreases in CO$_2$ fluxes seen during colder periods. Annual CH$_4$ flux patterns were less closely aligned with changes in temperature compared between seasons, more loyal to fine-scale spatial variability within the wetland, and the physical impediments to release, like snow pack and changes in the soil structure associated with freeze up and thaw. While more data will be necessary to determine the complete set of biotic and physical controls on bubble formation and release, we found evidence that within subarctic peatland systems like Petsikko, bubble emissions have the power to increase the net CH$_4$ contribution to the atmosphere by approximately 49%. In addition to bubbles, the thaw and pre-freeze shoulder seasons were critical to accurate annual estimations of the strength of the CH$_4$ source. While the spring thaw CH$_4$ burst was relatively light compared to the more substantial autumnal burst, the measured mass of CH$_4$ released during these shoulder seasons accounted for 32-55% of the mass of CH$_4$ release measured during the summer growing season. Thus, the effects of climate change are more likely to affect CH$_4$ fluxes via changes in hydrology (including alterations to the water table and freeze-up/thaw), and snow pack and melt dynamics, rather than any direct effect of temperature changes.

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