FINAL REPORT

UNDERSTANDING THE MECHANISMS UNDERLYING HETEROTROPHIC CO_2 AND CH_4 FLUXES IN A PEATLAND WITH DEEP SOIL WARMING AND ATMOSPHERIC CO_2 ENRICHMENT

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Introduction

This project was funded from June 15, 2012 through June 15, 2015, with a no-cost extension until Sept. 15, 2016. Our project focused on a whole-ecosystem warming and enhanced atmospheric CO_2 experiment in the S1 Bog in Marcell Experimental Forest in northern Minnesota, USA called "Spruce and Peatland Responses Under Climatic and Environmental Change" (SPRUCE; http://mnspruce.ornl.gov). Construction of substantial infrastructure required for these treatments was beyond our control and led to a staggered initiation of experimental treatments at this site. Deep peat heating (DPH) was instituted in June 2014, whole-ecosystem warming began in August 2015, and the CO_2 enhancement began in June 2016. Prior to the initiation of the experimental treatments, we completed a large amount of research to better understand factors controlling anaerobic carbon (C) cycling, and particularly methane (CH_4) dynamics, in northern peatlands in an effort to put the SPRUCE project in a broader context. We additionally focused extensively on the DPH treatment, which provided a unique opportunity to isolate warming effects on the vast reservoir of permanently anaerobic C stored in peatlands below the water table.

How Representative is S1 Bog of Other Northern Bogs?

Prior to treatment implementation in the SPRUCE plots, we initially focused on putting the CH_4 and anaerobic C dynamics in S1 Bog in a larger regional context for *Sphagnum* moss-dominated "bogs" by comparing it to two other nearby peatlands. This research was done in collaboration with SPRUCE investigators Joel Kostka (Georgia Institute of Technology) and Jeff Chanton (Florida State University), and a manuscript from it is in the final stages of preparation for submission for publication (Medvedeff et al., in preparation). We led this collaboration, and the first author of the resulting manuscript is a postdoctoral associate in PI Keller's lab.

At first glance, bogs look similar in that they are dominated to varying degree by *Sphagnum* mosses, ericaceous shrubs, and black spruce, with sparse cover of graminoids and forbs. However, in terms of anaerobic biogeochemistry, these systems act quite differently with some producing a great deal of CH_4 and others producing virtually no CH_4 based upon our

extensive previous experience working on peatlands in Upper Midwest. We chose three *Sphagnum*-dominated peatlands that span this gamut of CH_4 production. A variety of evidence from other SPRUCE investigators suggests that S1 is very weakly minerotrophic (i.e., has weak and sporadic groundwater influence in the upper layers), despite the fact that it has a surface pH of ~4 and the moss layer is dominated by *Sphagnum* species (*S. magellanicum*, *S. angustifolium*, *S. fallax*) with a large component of ericaceous shrubs and scattered black spruce typical of more ombrotrophic systems (i.e., entirely precipitation-dependent systems). It also has extensive areas of both hummocks and hollows and cover of the herb *Maianthemum trifolia* (Three-leaf False Solomon's Seal), both of which may be associated with higher CH_4 fluxes. The nearby Bog Lake peatland in Marcell Experimental Forest is considered a poor fen with a pH of ~ 4.5, and it is primarily a lawn dominated by *S. papillosum* and graminoids. Extensive research by others has shown very high CH_4 fluxes from Bog Lake. The Zim Bog is part of a large peatland complex that is a highly ombrotrophic bog dominated by the mosses *S. fuscum*, *S. capillifolium*, and *S. magellancum* with a pH of 3.8. Its vascular vegetation is relatively similar to S1 but it is essentially a large hummock with only scattered hollows. Our previous research has shown that the peat from this site has very low rates of CH_4 production under even optimal laboratory conditions for reasons that are unclear. We have extensively surveyed *Sphagnum*-dominated peatlands in the Upper Midwest and have found these three sites reasonably well encompass their vegetation, hummock-hollow development, anaerobic C chemistry, and CH_4 dynamics.

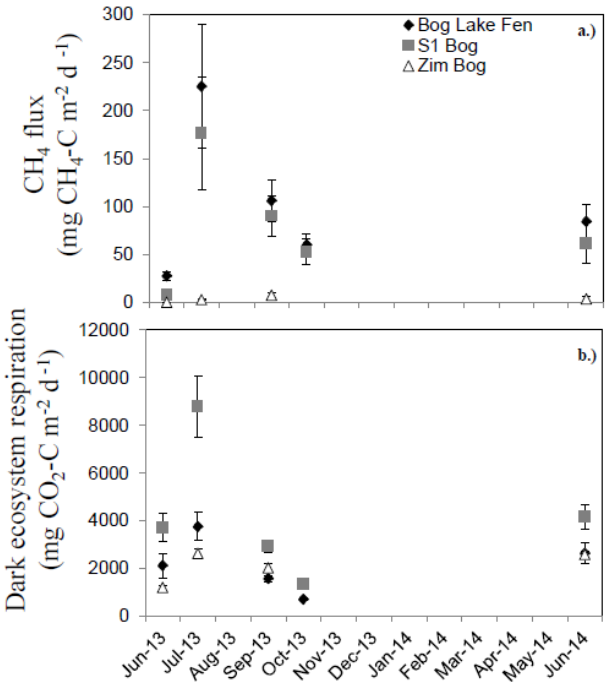

Fig. 1. Methane and CO_2 flux in three northern Minnesota peatlands (Medvedeff et al., in preparation).

In 2013-2014, we installed chamber bases for net flux measurements at each site (5 in hollows at each site and 5 in hummocks at S1 and Zim). We also installed 5 nests of porewater piezometers at depths of 25, 50, 100, 150 and 200 cm below the soil surface in each peatland. We measured a wide array of response variables seasonally that together comprise one of the most thorough assessments of C cycling dynamics and microbial communities in any peatland. These response variables included net CO_2 and CH_4 flux and the $\delta^{13}C$-CH_4 and δD-CH_4 of emitted CH_4; porewater depth profiles of low molecular weight fermentation products, SO_4^{2-}, NO_3^-, and soluble phenolics; depth profiles of potential anaerobic carbon mineralization in peat; extensive microbial community analysis of both the total (using DNA extraction) and active (using RNA extraction) components of the community, including abundance using quantitative polymerase chain reaction, high-throughput sequencing of community composition and diversity, and the expression and abundance of the functional genes methyl coenzyme M reductase (mcrA) for

methanogenesis and the α-subunit of CH_4 monooxygenase (*pmoA*) for methanotrophy; and microtopography (hummock/hollow extent and magnitude).

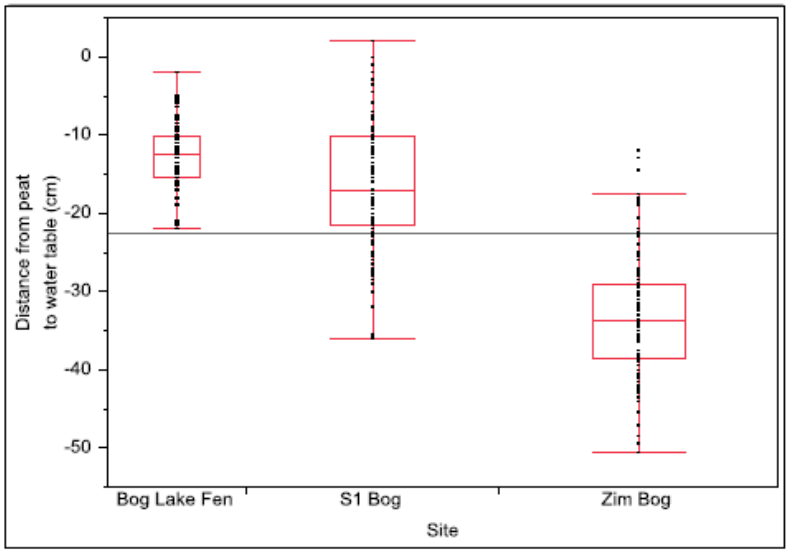

Fig. 2. Mean and variation in microtopography in three northern Minnesota peatlands (Medvedeff et al., in preparation).

Pronounced differences were observed in anaerobic C dynamics and resultant CH_4 and dark CO_2 emissions among the peatlands studied (Fig. 1). Net CH_4 flux was lowest in the peatland with well-developed hummocks (i.e., Zim Bog), and the isotopic composition of the CH_4 along with quantitative data on the gene expression of methanotrophs indicate a strong role for CH_4 oxidation in controlling net CH_4 flux, in line with the lower water table at this site. In contrast to net CH_4 flux, there were limited site differences in porewater chemistry (CH_4 and dissolved inorganic C concentrations) or microbial community composition among sites. Potential CH_4 production was also similar among the sites. The site with the highest CO_2 flux (S1 Bog) had the lowest potential heterotrophic CO_2 production, highlighting the importance of autotrophic respiration in ecosystem respiration. High variability in seemingly similar peatlands over such a small geographical region suggests a "one peatland represents all" approach is inappropriate - even among *Sphagnum*-dominated peatlands - and caution must be used when extrapolating data from a single site to the landscape scale. In particular, the development of continuous hummocks in the succession from weakly minerotrophic to ombrotrophic peatlands, with its concomitant effect on water table depth, appears to be a central control over CH_4 emissions (Fig. 2). Thus, the ombrotrophic-minerotrophic gradient of peatland successional development and hydrogeochemistry provides the appropriate regional context to examine the role of peatlands in C cycling dynamics and trace gas emissions.

How Susceptible Is Deep Peat to Climatic Warming?

From June 2014 through August 2015, DPH treatments to > 2 m depth were established within ten, 12 m diameter plots (0, +2.25, +4.5, +6.75 and +9 °C, relative to ambient, in duplicate) within the S1-Bog. Because of the loss of heat to the atmosphere, surface peat experienced only muted warming, whereas deeper peat was effectively warmed at the target temperature differentials. This experiment provided a unique opportunity to examine if this globally significant reservoir of deep, permanently anaerobic peat (i.e., the "catatelm") is susceptible to climatic warming. This deep peat is posed at approximately the regional mean temperature, so

it is expected to warm proportionally in the future. The revised manuscript describing the results from the DPH experiment is under final consideration for publication in *Nature Communications* (Wilson et al., in review a). In the collaborative spirit of SPRUCE, many investigators contributed data for this manuscript, but the co-first author, Anya Hopple, is a Ph.D. student of PI Scott Bridgham.

Our team focused on measuring CH_4 and CO_2 production seasonally throughout the peat profile under relatively in situ conditions within 1 °C of the soil temperature at the time of sampling. We found that the observed increases in CH_4 emissions due to warming were entirely due to the subdued warming effect of the DPH treatment on surface peat (i.e., 20-30 cm depth). There was no temperature effect on CH_4 production in deeper peat, even after one year of DPH (Fig. 3). Similar results were found for anaerobic CO_2 production. Moreover, the CO_2:CH_4 ratio in anaerobic respiration was also negatively correlated with temperature in surface peat, suggesting that anaerobic respiration will become increasingly methanogenic with warming in the future. No changes in dissolved organic matter concentrations (DOM), methanogenesis pathways, or microbial community composition with the DPH treatments substantiated the laboratory incubation findings that warming had little effect on catotelm peat. Moreover, ^{14}C dating indicated that CH_4 and CO_2 are predominantly derived from relatively young DOM and not from the bulk peat, even at great depth and with prolonged warming.

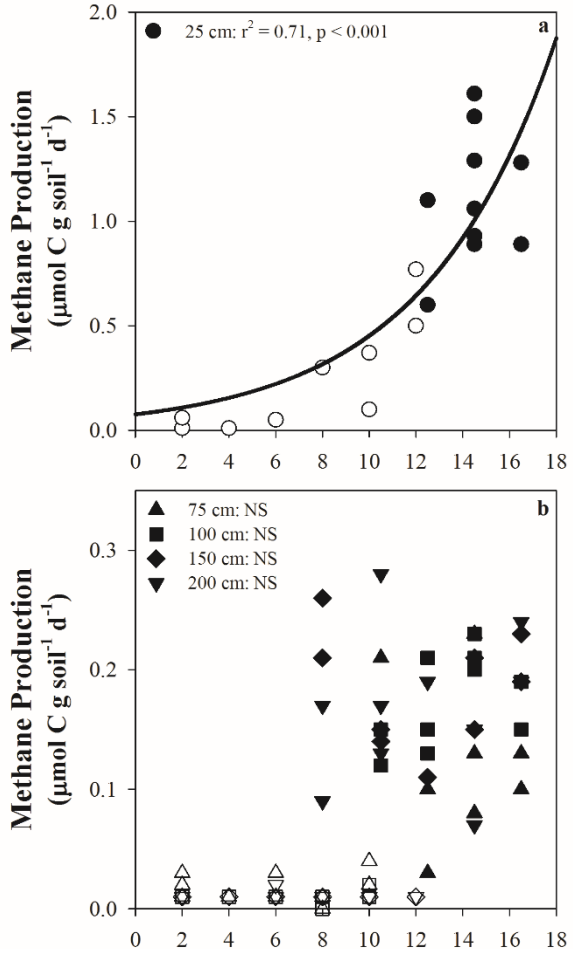

Fig. 3. Temperature response of CH_4 production from surface **(a)** and deep **(b)** peat samples that were anaerobically incubated within 1ºC of in-situ temperatures after approximately 4 (closed symbols, September 2014) and 13 (open symbols, June 2015) months of deep peat warming at SPRUCE. Temperatures reflected in situ temperatures at time of collection. (Wilson et al. In revision)

Our results have profound implications. Approximately 1/3 of the world's soil C is contained within the catotelm of peatlands, and the response of this C to climatic warming is of major concern. While the increase in CH_4 emissions due the effects of warming on surface peat does not provide complete solace, if future results from SPRUCE verify those from the DPH experiment, the positive feedbacks of anthropogenic warming on CH_4 emissions from northern peatlands is a much more tractable problem than originally thought. However, results from permafrost peatlands provide opposite results, probably reflecting the permanently frozen state of the carbon before melting that leaves it in a relatively chemically labile state. The lack of temperature response of catotelm peat from non-permafrost peatlands is apparently due to

chemical recalcitrance of this peat that has undergone slow decomposition over millennia. The SPRUCE experiment is uniquely set up to test if these preliminary results will hold over a planned decade of warming.

Role of *Sphagnum* Chemistry in Controlling Anaerobic Carbon Dynamics

Despite being diminutive, ground-layer plants, *Sphagnum* mosses are true keystone species in peatlands and are responsible for approximately half of the peat accumulation globally. They are known to contain inhibitory organic compounds, and one possible explanation for differences in anaerobic C cycling among peatlands is the presence of different *Sphagnum* communities. To test for the role of *Sphagnum* chemistry in controlling anaerobic C cycling, soils from the three northern Minnesota peatlands described above (i.e., S1 Bog, Bog Lake Fen, and Zim Bog) were incubated anaerobically for 40 days with *Sphagnum*-derived organic matter (S-DOM) extracted using distilled water at 25 or 60 °C in a fully-crossed experimental design (Medvedeff et al. 2015). S-DOM extracted at 25 °C had a minimal effect on decomposition, but S-DOM extracted at 60 °C increased CO_2 production in all soils, indicating that it was a source of labile C for microbial decomposition. However, the magnitude of the increased CO_2 production in response to S-DOM depended on the source of the S-DOM. The response of CH_4 production to additions of S-DOM extracted at 60 °C was more complex. Soils from one peatland produced no CH_4 during the incubation, regardless of S-DOM source. The same S-DOM additions led to an increase in CH_4 production in a second soil, but a decrease in CH_4 production in the third soil. Stable isotopic evidence suggests that these patterns were driven by the selective inhibition or stimulation of acetoclastic methanogenesis. Taken together, these data suggest S-DOM alone does not explain differences in anaerobic decomposition in peatlands, but may play a role in regulating CO_2 and CH_4 production.

Role of Dissolved Organic Matter in Controlling Anaerobic Carbon Dynamics

As described above in the DPH results, ^{14}C dating of CH_4, CO_2, solid peat, and DOM in S1 Bog indicates that most of anaerobic C mineralization is derived from relatively young DOM rather than the old peat, even at great depth in the peat profile. Based upon the work of our SPRUCE collaborator, Dr. Chanton, this results appears to be relatively consistent across ombrotrophic northern peatlands. This has vast implications given that most of the C in peatlands is stored in the solid peat matrix and not DOM, and most past studies (including ours) have focused on solid peat rather than DOM.

This past work has been based upon inferences derived for ^{14}C dates of various C pools, so we directly tested the role of DOM in anaerobic mineralization of C in the three northern Minnesota peatlands described above (Hopple et al., in preparation). Two experiments were conducted with peat that was collected in June 2013 and frozen and with peat collected in July 2014 and used within two weeks of collection. We meticulously removed all DOM from peat

collected from depths of 25-50, 75-100, and 150-200 cm in the June 2013 experiment, and 0% and 50% DOM collected from the appropriate depth in the field was added back to the peat (the highest treatment was 50% DOM because the peat contained distilled water from the pre-treatment rinsing). In the July 2014 experiment, native DOM was removed in peat collected from 25-50 and 75-100 cm and 0%, 25%, and 50% DOM was added back. These treatments were also compared to a control (hence 100% DOM), and 50% surface DOM was added to deep peat to see if anaerobic C mineralization was limited by labile C, assuming the DOM collected from deeper depths has been more microbially processed.

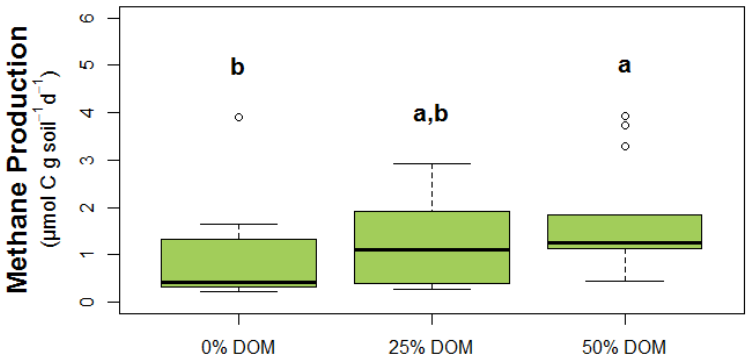

Fig. 4. Methane production rates determined from peat samples collected at 25 cm across three northern Minnesota peatlands anaerobically incubated for two weeks at 18°C with increasing concentrations of DOM in July 2014. (Hopple et al. in preparation)

Results from both experiments showed a substantial stimulation of CH_4 production by addition of DOM in surface peat only, with no effect on CO_2 production. For example, surface CH_4 production averaged across the three sites increased by 27% and 49% as experimental treatments increased from 0 to 25 and 50% DOM (Fig. 4). The control (100% DOM) treatment was not different than the 50% DOM treatment, suggesting a C availability threshold of DOM for the native microbial community. Also, surface-derived DOM did not stimulate CH_4 or CO_2 production in deep peat. Our results generally support the ^{14}C data collected by others for surface peat, indicating that a substantial portion of CH_4 production is derived from DOM, even with relatively young surface peat. The lack of a discernable response of DOM addition on anaerobic C mineralization in deeper peat is more quizzical, but it does support the DPH experimental results that this peat is highly recalcitrant and its resident microbial communities are relatively insensitive to both warming and labile C addition.

Modeling of Peatlands

We developed a process-based peatland model, P-TEM, by coupling and revising a core carbon and nitrogen dynamics module of an extant Terrestrial Ecosystem Model (TEM), a soil thermal module (STM), a CH_4 dynamics module (MDM), and a hydrological module (HM) (Wang et al., 2016). We evaluated 1) hydrological dynamics using observed data of soil moisture and water table depth at peatland sites in Alaska and Canada; 2) soil temperature estimates using data collected in Alaskan peatlands; and 3) CH_4 emission estimates using CH_4 flux data of S1 Bog (i.e., the SPRUCE site) in Minnesota. We then applied the model to four peatlands sites on the Kenai Peninsula in Alaska driven with paleoclimate data from ECBilt-CLIO model output to evaluate peat C accumulation rates and depth profiles by comparing them to peat core data.

Finally, we tested the P-TEM model sensitivity to various controls and factors as a way to identify the main factors that influence peatland C dynamics.

We found that P-TEM simulations matched the observed C accumulation rates at fen sites during the Holocene (R = 0.91, 0.90, 0.85 and -0.26 using comparisons in 500-year bins). The simulated (2.04 m) and observed peat depths (on average 1.98 m) also compared well (R = 0.91). The early Holocene C accumulation rates, especially during the Holocene thermal maximum (HTM) (35.9 $\mathrm{g\ C\ m^{-2}\ y^{-1}}$), were estimated to be up to 6-times higher than the rest of the Holocene (6.5 $\mathrm{g\ C\ m^{-2}\ y^{-1}}$). Our analysis suggests that high summer temperatures and the lengthened growing season resulting from the elevated insolation seasonality during the HTM in Alaska might be a major factor causing the rapid C accumulation. Our sensitivity tests indicate that, apart from climate, initial water table depth and vegetation canopy are major drivers of C accumulation in peatlands. When the modeling framework is evaluated for various peatland types in the Arctic, it can quantify peatland C accumulation at regional scales. Also, in the DOE renewal, P-TEM is being applied to peatlands in the Marcell Experimental Forest in Minnesota, including S1 Bog.

Other Activities

Other Publications

We published a major review of controls over CH_4 dynamics at the microbial, biogeochemical, and global scales in wetlands, along with the potential impact of climate change, in *Global Change Biology* (Bridgham et al. 2013). This article was one of the 20 most downloaded articles in the Wiley Online Library in 2013. The PIs have additionally written several textbook chapters relating to C dynamics in wetlands that were informed by this grant (see publication list).

Anaerobic CH_4 Oxidation

One of the major objectives of our renewed DOE grant at SPRUCE is to better quantify the role the role of anaerobic oxidation of CH_4 (AOM). The development of the methodology for this task and the initial measurements were conducted under the grant that is the focus of this report. While AOM is the dominant mechanism of CH_4 consumption in marine systems, it was long thought to be relatively unimportant in freshwater wetlands because of low sulfate concentrations that act as a terminal electron acceptor in a coupled syntrophic reaction. However, the handful of studies that have examined it in freshwater wetlands recently have found it to ubiquitous, but the relative importance of this process varies drastically among these studies.

We did some initial experiments with soil from the three northern Minnesota peatlands described previously to develop techniques using a $^{14}CH_4$ tracer to measure this process (with $H^{14}CO_3$ as the product of the reaction). We found definitive evidence that anaerobic CH_4 oxidation is occurring in all three sites, but the method was relatively laborious to perform. We have since switched to a method using C^3H_4 as a tracer (with ^3H_2O as the reaction product).

After extensive method development to quantify background abiotic isotopic exchange between the labeled CH_4 and H_20, this method is providing robust results in seasonal sampling in the SPRUCE plots in 2015 and 2016. While we are still finalizing our data, we have found AOM to occur at all depths, although rates are highest in surface peat, which is expected if it is tied to a terminal electron acceptor. AOM also appears to be an extremely important process to the extent that we often see net CH_4 consumption throughout the peat profile.

Gross In Situ Rates of CH_4 Production and Consumption at SPRUCE

Purchase of Picarro G2201i analyzers by the Bridgham and Keller labs have allowed us to use a $^{13}CH_4$ isotopic tracer method to estimate gross rates of CH_4 production and aerobic oxidation in real time in the SPRUCE plots. This is again a major objective of our DOE renewal that was set up with extensive preliminary work under the former grant reported herein. This preliminary work included determining that the ^{13}C tracer had only a transient signature in porewater CH_4 (< 24 hrs) and was not detectable in the peat, DOM, and plants. Determining this was essential because of the larger amount of ^{13}C work being done in the SPRUCE plots related to the signature of the elevated CO_2 treatment, which our experiments could not interfere with.

This research will provide the first in situ estimates of gross CH_4 production and consumption in any ecosystem to our knowledge, and it will be even more fascinating in the context of the SPRUCE experiments.

Publications Resulting From this Grant

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