FINAL REPORT

UNDERSTANDING MECHANISTIC CONTROLS OF HETEROTROPHIC CO_2 AND CH_4 FLUXES IN A PEATLAND WITH DEEP SOIL WARMING AND ATMOSPHERIC CO_2 ENRICHMENT

(DE-SC0014416)

Investigators: Jason K. Keller[1], Scott D. Bridgham[2], and Qianlai Zhuang[3]
[1]Chapman University, [2]University of Oregon, [3]Purdue University

Introduction

This project was funded from August 1, 2016 – July 31, 2019 with a no-cost extension until July 31, 2020 (and built upon the PI-team's previous work supported by DE-SC0008092 (June 2012 – September 2016)). Our project focused on the Spruce and Peatland Responses Under Changing Environments (SPRUCE: https://mnspruce.ornl.gov/) experiment taking place in the S1 Bog in the Marcell Experimental Forest in northern Minnesota, USA. The SPRUCE project is a unique whole-ecosystem experiment where a bog peatland is subjected to warming and atmospheric carbon dioxide (CO_2) enrichment. Deep peat heating of the soil profile (to a depth of at least 2 m) began in June of 2014 and was followed by whole-ecosystem warming in August of 2015. Warming treatments included +0 °C, +2.25 °C, +4.5 °C, +6.75 °C, and +9 °C above ambient temperatures, with 2 experimental chambers for each warming treatment. Half of these chambers received ambient atmospheric CO_2 concentrations, and starting in June of 2016, the other half received elevated atmospheric CO_2 concentrations (~ +500 ppm(v) above ambient). Our primary goal with this project was to explore the mechanistic controls of anaerobic carbon cycling – especially the dynamics of the potent greenhouse gas methane (CH_4) – within the SPRUCE experiment.

The Susceptibility of Deep Peat to Warming

Recent estimates suggest that peatlands may store up to half of the world's soil carbon, with much of this carbon in the deep, permanently anaerobic peat (i.e., the catotelm). The response of this deep peat to warming is a major concern, and the SPRUCE experiment provided a unique opportunity to explore the effects of warming on the stability of this carbon pool over time. Since our initial engagement with the SPRUCE project, we have worked collaboratively with other investigators to explore the effects of warming on peatland carbon cycling. Our measurements of anaerobic CO_2 and CH_4 production were central to two publications in *Nature Communications* (Wilson et al. 2016 and Hopple et al. 2020) showing how the response of deep peat has changed in response to continued warming. While both publications were highly collaborative, Dr. Anya Hopple (as a Ph.D. student with PI Bridgham) served as co-first author on both manuscripts.

From June 2014 through August 2015, deep peat heating (DPH) treatments warmed soils to target temperatures of +0 °C, +2.25 °C, +4.5 °C, +6.75 °C, and +9 °C above ambient. Over this period, surface warming was muted due to loss of heat to the atmosphere. During the DPH phase of SPRUCE, we collected soils through the peat profiles and incubated these soils within ± 1°C of in situ soil temperatures and measured rates of CO_2 and CH_4 production over ~24-96 hours (depending on rates of production). We observed a strong positive effect of temperature on CH_4 production in surface peat. In contrast to the surface peat, CH_4 production in deeper peat exhibited no temperature response during the initial DPH phase of the experiment (Figure 1, Wilson et al. 2016).

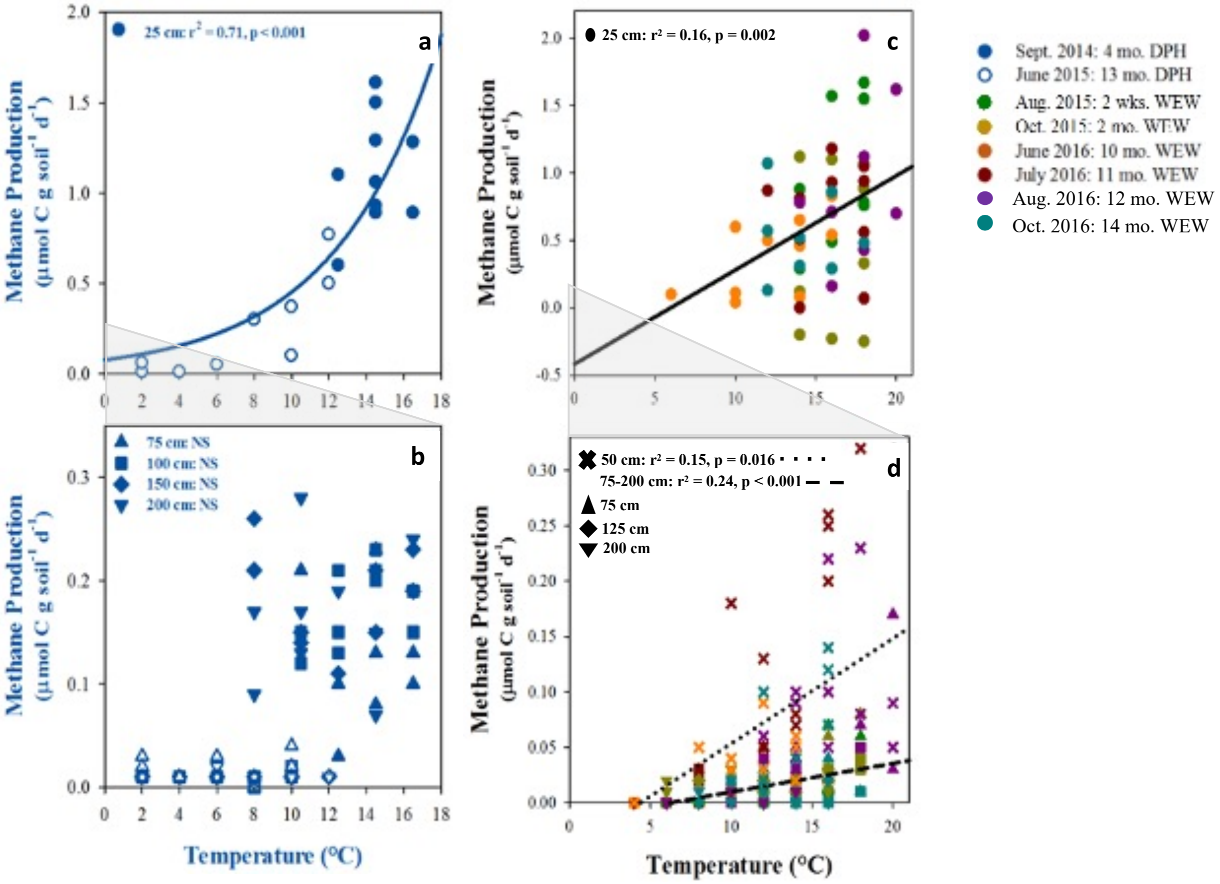

Figure 1. Methane production response to temperature in the initial Deep Peat Heating (DPH; (a) and (b)) and Whole Ecosystem Warming (WEW; (c) and (d)) treatments at SPRUCE. Panels (a) and (c) are in surface peat, and panels (b) and (d) are in deeper peat increments. Samples were in slurries with porewater from the representative depth depths and with headspaces initially flushed with N_2. Data from Wilson et al. 2016 (DPH) and Hopple et al. 2020 (WEW).

Continued measurements of anaerobic CO_2 and CH_4 production as the SPRUCE project moved into the whole-ecosystem warming (WEW) phase (including above ground warming, beginning in August of 2015) revealed a very different response of deep soils to warming. In the WEW phase, there was still a positive effect of temperature on CH_4 production in surface soils, but with much more variation and a decreasing temperature effect over time. Importantly, we observed a significant positive response of CH_4 production to temperature during the WEW phase of the SPRUCE project (Figure 1) This suggests that after an initial lag period, the massive

C pool that has been stored in deep, catotelm peat for centuries has become vulnerable to warming. This suggestion was supported by a trend toward older ^{14}C-dissolved inorganic carbon signature at depth during the WEW phase (Hopple et al. 2020).

Effects of Warming on Microbial Organic Matter Reduction

The microbial reduction of redox-active moieties in solid-phase organic matter (microbial organic matter reduction) can play a key role in aerobic carbon cycling in many northern peatlands. In particular, microbial organic matter reduction can competitively suppress CH_4 production in these soils. The revised manuscript exploring the response of this process to warming at the SPRUCE project is under final consideration for publication in *Geoderma* (Rush et al., *In Review*). Lead author Jessica Rush was an undergraduate student and subsequent laboratory technician for PI Keller.

This manuscript advances our understanding of the connection between microbial organic matter reduction and CH_4 production in a number of important ways. First, we demonstrate that microbial organic matter reduction is faster under warmer temperatures and that this helps explain the faster onset of CH_4 production in response to warming. Moreover, our results show that once solid-phase organic matter has been fully reduced, acetate concentrations generally increase, suggesting that competition for this key microbial substrate could be an important mechanism explaining reduced CH_4 production in peatland soils where microbial organic matter reduction can dominate anaerobic carbon cycling. Finally, we demonstrate that the redox-status of solid-phase organic matter closely tracks the water-table level at SPRUCE (Figure 2), with larger volumes of oxidized organic matter as the water table declines. Taken together, our results suggest that in the zone of fluctuating water-table levels, redox cycling within organic matter will represent a key control over peatland CH_4 dynamics, suppressing the production of CH_4 following rewetting events (although the impact of this suppression will be mediated by temperature).

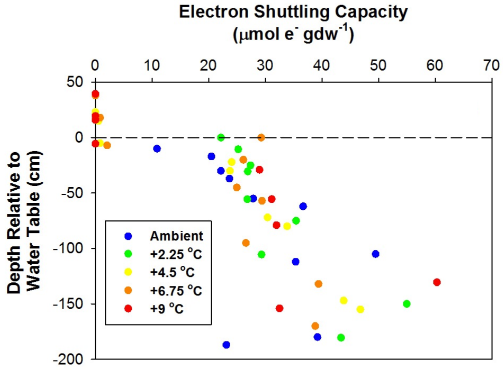

Figure 2. Organic matter collected from the SPRUCE project was oxidized (low electron shuttling capacity, ESC) above the water table, but quickly reduced (high ESC) below the water table. The volume of oxidized OM was generally larger in warmer treatments as a result of lower water-table levels. Data from Rush et al. *In Revision*.

The Role of Homoacetogenesis in Controlling CH_4 Production

We devised a method using the conversion of ^{14}C-HCO_3^- tracer into ^{14}C-CH_4 and ^{14}C-dissolved organic matter to estimate putative homoacetogenesis. Homoacetogenesis is a very understudied process is the anaerobic formation of acetate from single carbon (C1) compounds. Most homoacetogens are chemoautotrophs that convert H_2 and CO_2 to acetate for energy generation (Figure 3). Chemoautotrophic homoacetogens influence CH_4 production by both competing with hydrogenotrophic methanogens for H_2 and by producing acetate as a substrate for acetoclastic methanogens. At SPRUCE, homoacetogens consumed a greater portion of H_2 relative to hydrogenotrophic methanogens with depth (Figure 4), with ~ 50% of the H_2 going to homoacetogenesis at the deepest depths. Hydrogenotrophic methanogenesis often dominates in northern bogs with lower rates of CH_4 production and higher CO_2:CH_4 ratios, and accordingly has often been associated with lower "soil carbon quality" and low pH. Thus, lower rates of homoacetogenesis due to warming, and the associated increase in hydrogenotrophic methanogenesis, may lead to a proportionally lower CH_4 response to increased temperatures.

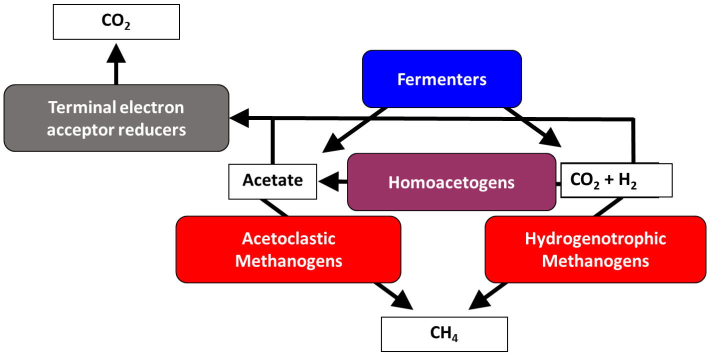

Figure 3. Schematic of the relationship between homoacetogenesis and methanogenesis pathways.

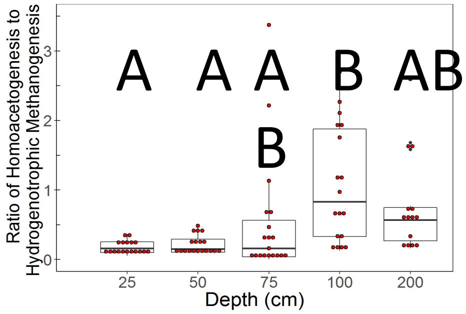

Figure 4. Ratio of homoacetogenesis to hydrogenotrophic methanogenesis in the SPRUCE plots in August and October 2018 *at in situ* temperatures (LeeWays 2019). Since the processes use H_2 in a stoichiometrically equivalent way, this also represents the relative amount of H_2 going to the two processes.

This research comprised the M.S. thesis of Cory LeeWays (LeeWays 2019), a student of PI Bridgham, and will be submitted for publication in 2021.

Pathways of CH_4 Emission

Methane can be transported from a wetland to the atmosphere via diffusion, ebullition (e.g., bubble release), and plant-mediated transport. Transport pathways are an important component of models because ebullition, and to a lesser extent plant transport, bypass methanotrophic bacteria and, thus, maximize CH_4 emissions.

We made extensive measurements of plant-mediated and diffusional transport of CH_4 in S1 Bog outside of the SPRUCE experimental chambers in Sept. 2018 and June 2019 in collaboration with Dr. Jeffrey White (Indiana University) for his sabbatical with PI Bridgham (manuscript in preparation). Results for plants are shown in Figure 5, with eight gas collars randomly placed within the bog and two (7 and 9) intentionally placed on the widely scattered *Eriophorum vaginatum* (cotton grass) tussocks. In September 2018, the small forb *Maianthemum trifolium* (3-leaved false Soloman's seal) had largely senesced, but it was much more abundant in June 2019. In September 2018, generally < 10% of the total CH_4 emissions were from plants except for the chambers over *E. vaginatum* tussocks (the plant contribution can be > 100% because a mean rate per stem with a substantial variability is multiplied by hundreds of stems in a tussock). Plants contributed to a much greater proportion of CH_4 emissions in June 2019 largely because of the greater presence of *M. trifolium*. We measured CH_4 emissions from the two dominant trees in S1 Bog, the conifers *Picea mariana* (black spruce) and *Larix laricina* (larch), in June 2019 and they were relatively low (~ 0.4 mmol CH_4 m^{-1} d^{-1} in the lower boles), but whole tree estimates will require further measurements of how emissions vary over the entire tree. Diffusion of CH_4 through the soil profile made up a substantial component of emissions (>20 %) only in chambers with very low emissions (data not shown). The difference between total CH_4 emissions and the sum of plant and diffusional emissions can be attributed to ebullition. We expect to submit a manuscript from this research within the next year.

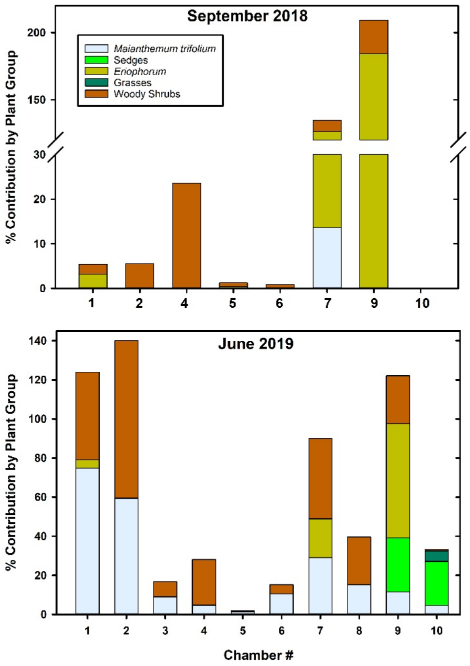

Figure 5. Contribution of different plant groups to total emissions during two sampling periods in S1 Bog (unpublished data). Chambers were placed in random locations in the bog except 7 and 9 were intentionally placed on *Eriophorum vaginatum* tussocks.

Modeling Peatland Carbon Dynamics

Over the project period, PI Zhuang further developed the process-based peatland Terrestrial Ecosystem Model (P-TEM) to explore carbon accumulation in North American peatlands. P-TEM couples a hydrological module, a soil thermal module, a CH_4 module, and a carbon and nitrogen module. This model has been parameterized and used to calculate regional carbon accumulation in a number of areas, including work in Alaska supported by our previous project at SPRUCE. In Zhuang et al. 2020. P-TEM was applied to quantify the carbon accumulation rates and stocks within North America over the past 12,000 years. This study estimates that between 85-174 Pg carbon was accumulated in North American peatlands over this period, with a majority of this carbon in northern latitudes. Rates of peatland carbon accumulation peaked between 10,000 – 8,000 year ago, likely as a result of enhanced plant productivity under warmer and wetter conditions. Rates slowed after this period as a result of warmer temperatures stimulating microbial decomposition.

Over the project period, we also worked iteratively with additional modeling teams at SPRUCE in support of the development and parameterization of Earth System Models of CH_4 biogeochemistry. In particular, we have collaborated with the ELM-SPRUCE modeling team

(Ricciuto et al. *Accepted for Publication*; Yuan et al. *Accepted for Publication*) and the TECO_SPRUCE modeling team (Ma et al. *In Revision*).

Additional Publications

A number of publications resulting from work initiated during our initial funding period at SPRUCE (DE-SC0008092) were completed over the current project period. These included:

- Prior to the initiation of treatments at SPRUCE, we focused on putting the CH_4 and anaerobic carbon dynamics at the S1 Bog in a larger regional context by comparing it to two nearby *Sphagnum*-dominated peatlands. The resulting publication (Zalman et al. 2018a) demonstrated that small changes in degree of ombrotrophy (which coincide with water-table levels) are a major control over anaerobic processes in boreal peatlands.
- Virtually all models of peatland CH_4 cycling assume that CH_4 is produced through the splitting of acetate (acetoclastic methanogenesis) or the reduction of CO_2 using hydrogen as an electron donor (hydogenotrophic methanogenesis). We know, however, that CH_4 can also be produced using a variety of methylated substrates (e.g., methanol, methylamines, and dimethylsulfide) collectively referred to as methylotrophic methanogenesis. Using soils from the same three peatlands used to put S1 Bog in a regional context, Zalman et al. 2018b demonstrated that microbes could convert ^{13}C-labeled methylotrophic substrates into CH_4, raising intriguing questions about the assumption that CH_4 is derived exclusively from acetolactic and hydrogenotrophic pathways in peatland ecosystems.
- We designed a series of laboratory experiment investigating whether dissolved organic matter or solid-phase organic matter fuels microbial CO_2 and CH_4 production in peatland soils. The resulting publication (Hopple et al. 2019) demonstrated that increasing dissolved organic matter concentrations increased CH_4 production (but not CO_2 production) in surface soils from three peatlands. However, the addition of dissolved organic matter did not influence CH_4 or CO_2 production in deeper soils. This suggests that surface CH_4 production can be limited by dissolved organic matter availability but that other factors limit CH_4 production at depth.
- We participated in a manuscript that synthesizes the many nutrient pool and rate measurements at SPRUCE (Salmon et al., *In Revision*). While most of work in this grant revolved around carbon cycling, we also took nitrous oxide emission data in concert with our other efforts that formed an important part of this manuscript.

Publications Resulting from this Grant

Hopple, A.M., L. Pfeifer-Meister, C.A. Zalman, J.K. Keller, M.M. Tfaily, R.M. Wilson, J.P. Chanton and S.D. Bridgham. 2019. Does dissolved organic matter of soil peat fuel anaerobic respiration in peatlands? *Geoderma* 349:79-87.

Hopple, A.M.*, R.M. Wilson*, M. Kolton, C.A. Zalman, J.P. Chanton, J. Kostka, P.J. Hanson, J.K. Keller, and S.D. Bridgham. 2020. Massive peatland carbon banks vulnerable to rising temperatures. *Nature Communications* 11:2373. (* = co-first authors)

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Ricciuto, D., X. Xu, X., Shi, Y. Wang, X. Song, C. Schadt, N. Griffiths, J. Mao, J. Warren, P. Thornton, J. Chanton, J. Keller, S. Bridgham, J. Gutknecht, S Sebestyen, A. Finzi, R. Kolka, and P. Hanson. *Accepted for Publication.* An integrative model for soil biogeochemistry and methane processes I: model structure and sensitivity analysis. *Journal of Geophysical Research: Biogeosciences*.

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Wilson, R.M., M.M. Tfaily, V.I. Rich, J.K. Keller, S.D. Bridgham, C.M. Zalman, L. Meredith, P.J. Hanson, M. Hines, L. Pfeifer-Meister, S.R. Saleska, P. Crill, W.T. Cooper, J.P. Chanton, and J.E. Kostka. 2017. Hydrogenation of organic matter as a terminal electron sink sustains high CO_2:CH_4 production ratios during anaerobic decomposition. *Organic Geochemistry* 112: 22-32.

Wilson, R.M., M.M. Tfaily, C.M. Zalman, M.M. Kolton, E. Johnston, C. Petro, P.J. Hanson, H.M. Heyman, J. Kyle, D.W. Hoyt, E.K. Eder, S.O. Purvine, R.K. Kolka, S.D. Sebestyen, N.A. Griffiths, C.W. Schadt, J.K. Keller, S.D. Bridham, J.P. Chanton, and J.E. Kostka. *Accepted for Publication*. Soil metabolome response to whole ecosystem warming at the Spruce and Peatland Responses Under Changing Environments experiment. *Proceedings of the National Academy of Sciences*.

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Zalman, C., J.K. Keller, M. Tfaily, M. Kolton, L. Pfeifer-Meister, R.M. Wilson, X. Lin, J. Chanton, J.E. Kostka, A. Gill, A. Finzi, A.M. Hopple, B.J.M. Bohannan, and S.D. Bridgham. 2018a. Small differences in ombrotophy control regional-scale variation in methane cycling among *Sphagnum*-dominated peatlands. *Biogeochemistry* 139: 155-177.

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