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Final Report for Wetlands as a Source of Atmospheric Methane: A Multiscale and Multidisciplinary Approach

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Disclaimer

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LDRD FINAL REPORT

Wetlands as a Source of Atmospheric Methane: A Multiscale and Multidisciplinary Approach

Karis McFarlane (14-ERD-038)

Abstract

Boreal peatlands contain large amounts of old carbon, protected by anaerobic and cold conditions. Climate change could result in favorable conditions for the microbial decomposition and release of this old peat carbon as CO_2 or CH_4 back into the atmosphere. Our goal was to test the potential for this positive biological feedback to climate change at SPRUCE (Spruce and Peatland Response Under Climatic and Environmental Change), a manipulation experiment funded by DOE and occurring in a forested bog in Minnesota. Taking advantage of LLNL's capabilities and expertise in chemical and isotopic signatures we found that carbon emissions from peat were dominated by recently fixed photosynthates, even after short-term experimental warming. We also found that subsurface hydrologic transport was surprisingly rapid at SPRUCE, supplying microbes with young dissolved organic carbon (DOC). We also identified which microbes oxidize CH_4 to CO_2 at SPRUCE and found that the most active of these also fix N_2 (which means they can utilize atmospheric N, making it accessible for other microbes and plants). These results reflect important interactions between hydrology, carbon cycling, and nitrogen cycling present at the bog and relevant to interpreting experimental results and modeling the wetland response to experimental treatments. LLNL involvement at SPRUCE continues through collaborations and a small contract with ORNL, the lead lab for the SPRUCE experiment.

Background and Research Objectives

Peatlands cover only about 3% of the Earth's surface (Holden 2005), but contain roughly one-third of carbon stored by the terrestrial biosphere or 500–600 Pg of carbon (Gorham et al., 2012; Yu, 2011). Almost 90% of this carbon is found in northern peatlands, where cold temperatures and anoxic conditions have favored the accumulation of organic matter over the Holocene. These latitudes are already experiencing climate change and future change may be amplified here (Collins et al., 2013). Considering the large amount of carbon stored in peatlands, their response to change may contribute substantially to terrestrial feedbacks to climate (Bridgham et al., 2008) if warmer temperatures and changes in hydrology favor the decomposition of these large, old carbon stocks and the subsequent release of this carbon to the atmosphere as CO_2 and/or CH_4, a much more powerful greenhouse gas than CO_2. Natural wetlands currently account for approximately one-third of global CH_4 emissions (Bridgham et al., 2013), but predictions of future wetland emissions are hindered by a

paucity of ecosystem measurements of C fluxes, environmental drivers, and links to subsurface processes that influence production and transport of these gases.

In this project, we aimed to test whether peatland response to a warmer climate will include enhanced decomposition and release of old peat at a boreal peatland that is the site of a DOE-funded ecosystem manipulation experiment, SPRUCE (https://mnspruce.ornl.gov/). Our specific objectives within the SPRUCE were to:

(1) Link belowground C-sources and processes to atmospheric fluxes of CO_2 and CH_4 through natural abundance observations of ^{14}C and stable isotopes. Of these, the ^{14}C provided the most valuable insights.

(2) Identify microbial species performing key biogeochemical functions using stable isotope probing with nano-scale imaging (Chip-SIP). Successful experiments were performed for CH_4 oxidizers (methanotrophs) and N_2 fixers (diazotrophs), but our anaerobic experiments targeting methanogens were not successful.

(3) Constrain subsurface transport pathways with noble gas profiles and hydrologic tracers. We found the study site has low potential for bubbling and high amounts of dissolved gases. We therefore shifted focus to water dating, recharge, and improved understanding of subsurface hydrologic transport at the study site.

(4) Synthesize our findings with a biogeochemical box model to describe wetland response to warming. Numerous external groups have been working on C cycle models for the SPRUCE site, but not with ^{14}C. We shifted focus to modeling ^{14}C in the peat and in emitted CO_2 with an already existing peat model (Holocene Peat Model, HPM, Frolking et al., 2014). This direction was pursued late in the timeline for the LDRD, but simulations suggest a delayed response of emitted ^{14}C-CO_2 to warming treatments is possible. The results of these simulations will be included in a paper with the isotopic results from 2016.

Success under this project required teamwork and coordination with multiple external collaborators at SPRUCE for the overall benefit of the experiment. Initial plans included both warming and elevated CO_2 treatments, but the implementation of treatments was delayed and required shifting focus to warming only. At SPRUCE, 10 plots are receiving one of 5 levels of warming treatments 0–9°C above ambient temperature. Belowground heaters, which heat deep peat only, were turned on in June 2014. Aboveground heaters, which heat surface peat and aboveground air, were turned on in August 2015. The experiment is planned to continue for ten years.

Scientific Approach and Accomplishments

Isotopic measurements of emitted CO_2 and CH_4

We sampled surface emitted gases at multiple time points during the growing seasons of 2014–2016. Large surface chambers (1.13 m^2) were capped to allow gases to accumulate over 2 hours for CO_2 and ~20 hours for CH_4 to allow for sufficient accumulation of gases for ^{14}C analysis. Air samples were collected into evacuated serum bottles for concentration and stable isotope analyses and into evacuated stainless steel canisters for ^{14}C. CO_2 and CH_4 were extracted and purified and ^{14}C measurements made at LLNL's Center for Accelerator Mass Spectrometry. Additional air samples were collected from 4 m above the bog to provide localized background ^{14}C of atmospheric CO_2 and CH_4. Isotopic end-members were determined using the Keeling plot method if possible; otherwise isotopic mixing models were used.

Measurements were coordinated with external collaborators (not supported under this LDRD) who measured flux rates of CO_2 and CH_4 from these chambers (Hanson et al., 2016), ^{14}C analysis of bulk peat from SPRUCE (McFarlane et al., in revision), and peat porewater samples for concentration and isotopic analysis of DOC and dissolved CO_2 and CH_4. This LDRD supported additional ^{14}C analysis of DOC in porewater sampled at the same time as our gas sampling during 2015. These coordinated measurements have improved the quality of data and interpretations for the SPRUCE project as a whole (e.g., Wilson and Hopple, et al., in press).

During the 2014 growing season, microbes at SPRUCE utilized young carbon sources for CH_4 and CO_2 production (FIGURE 1), consistent with other findings that DOC and dissolved CO_2 in porewater were relatively young throughout the peat profile (Wilson and Hopple, et al., accepted). These results suggested that downward transport of recent photosynthates fuels microbial activity in deeper peat at SPRUCE, i.e., microbes are not utilizing old peat C. On average, CH_4 was 5-10 years older than CO_2, indicative of the time-lag associated with transport of C sources to methanogens and transport of CH_4 from the zone of production to the peat surface. ^{13}C-CO_2 values were similar to fresh photosynthates and ^{13}C-CH_4 indicated that methane at SPRUCE was produced by a combination of acetoclastic and hydrogenotrophic methanogensis (which use acetate and CO_2 as carbon sources, respectively).

During the growing seasons of 2014 and 2015, warming did not alter ^{13}C or ^{14}C of emitted CH_4 or CO_2 (see FIGURE 1 for ^{14}C), despite an increase in CH_4 emissions with warming (Paul Hanson, ORNL, personal communications; Wilson and Hopple, et al., accepted). Therefore, warming treatments increased CH_4 emissions, but did not alter the source of C being used for CO_2 of CH_4 production – microbes utilized young DOC even after short-term warming treatments.

Similarly, in 2016 warming did not effect emitted ^{14}C values, except in spring (DOY 134,

early May) when warming increased ^{14}C of emitted CH_4 ($p = 0.01$). This increase in ^{14}C-CH_4 likely reflects increased microbial utilization of new photosynthates in warmed plots where vegetation was already active rather than increased decomposition of old, deep peat-derived C. This effect was not sustained into the summer.

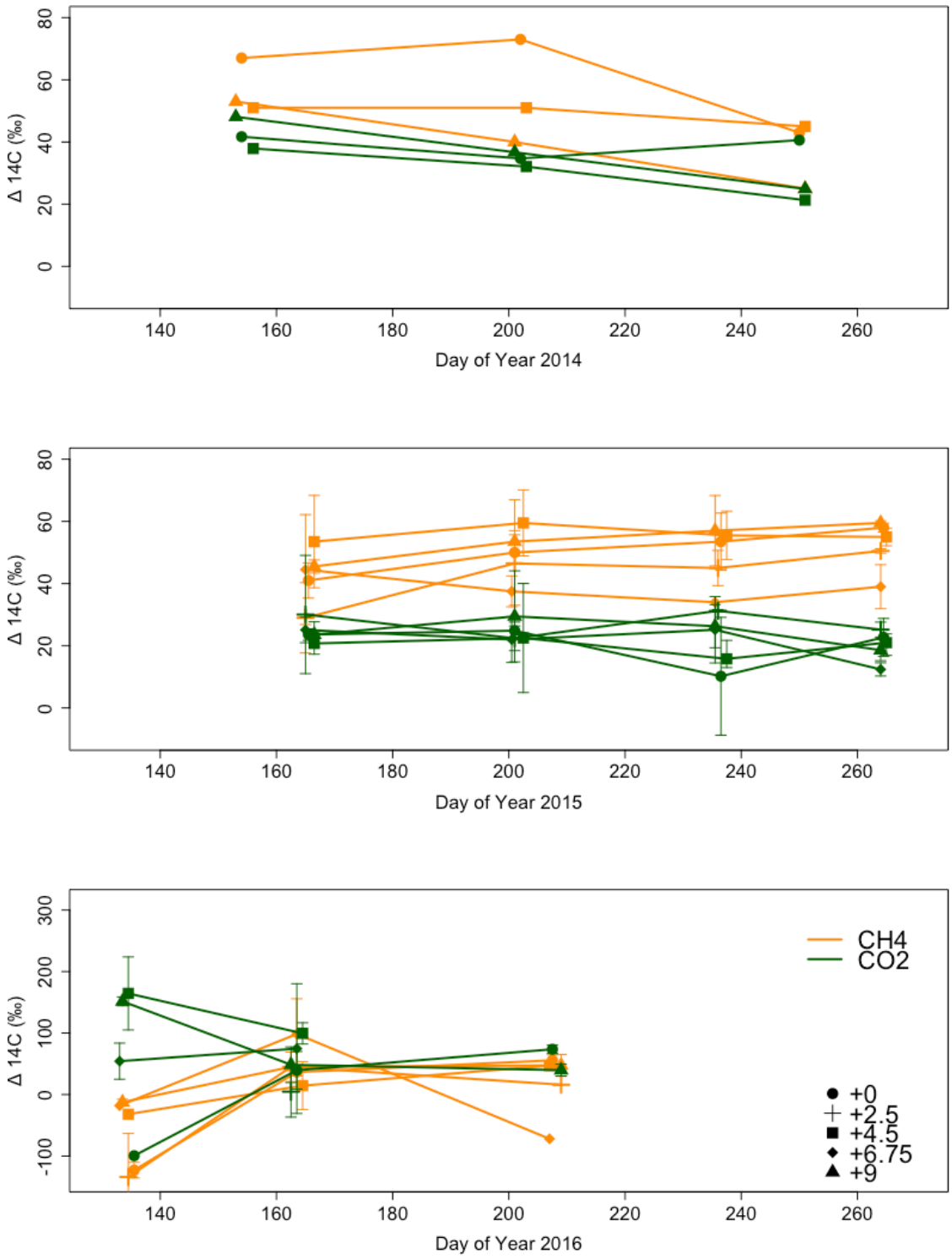

FIGURE 1. ^{14}C isotopic end member for emitted CH_4 (orange) and CO_2 (green) in 2014 (top), 2015 (middle), and 2016 (bottom). Symbols indicate warming treatments. Deep peat heating began on DOY 160 in 2014. Whole ecosystem warming began on DOY 225 in 2015. For reference, atmospheric $\Delta^{14}C$ values were approximately 10-30 permil during this time period.

Microbial Community identity and function

We identified microbial species performing key biogeochemical functions at SPRUCE using stable isotope probing (SIP) and nanoscale imaging with Nano-SIMS (Chip-SIP, Mayali et al., 2012, FIGURE 3). Gene sequencing data from external SPRUCE collaborators (J. Kostka and M. Kolton) were used for microarray construction. We conducted laboratory incubations of peat collected from SPRUCE plots in 2015. Anaerobic incubations to identify methanogens were performed with ^{13}C-enriched acetate or CO_2, but isotopic enrichment was not sufficient for further analysis. Aerobic incubations to identify methanotrophs were performed with ^{13}C-enriched CH_4 and ^{15}N-enriched N_2. Recent indictates that the same archeal species have genes for methanotrophy and for diazotrophy, but it has been unclear whether the same species perform both functions in the field and if this changes with environmental conditions.

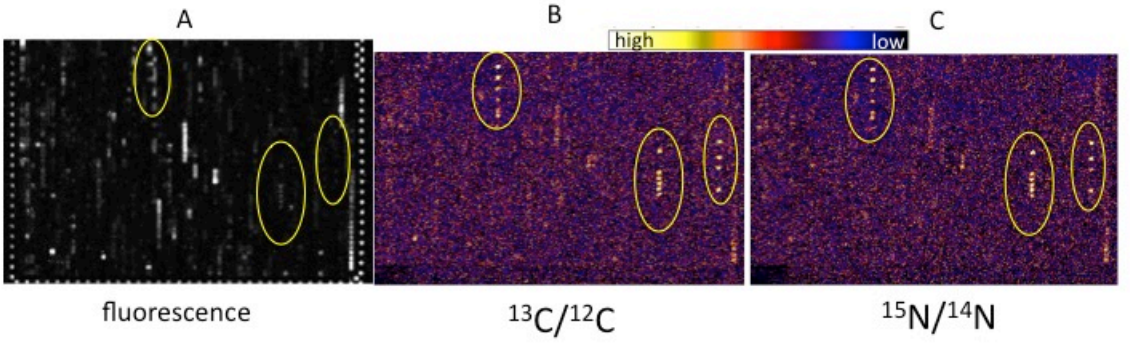

FIGURE 2. RNA extracted from SPRUCE soil incubations with $^{13}CH_4$ and $^{15}N_2$ hybridized to a 16S rRNA gene targeted microarray: A) fluorescence pattern showing locations where the probes are binding the RNA; B) $^{13}C/^{12}C$ isotope ratio image, showing the location of highly enriched RNA molecules from CH_4 incorporation; C) $^{15}N/^{14}N$ isotope ratio image, showing the location of highly enriched RNA molecules from N2 incorporation

The aerobic dual-label incubations yielded enriched RNA in ^{13}C and in ^{15}N (FIGURE 2). We found that the most active organisms were enriched in both isotopes, indicating that they fix N_2 and oxidize CH_4 (FIGURE 3), while some organisms became preferentially enriched in one over the other, indicating that they primarily performed N_2 fixation or methane oxidation (FIGURE 4).

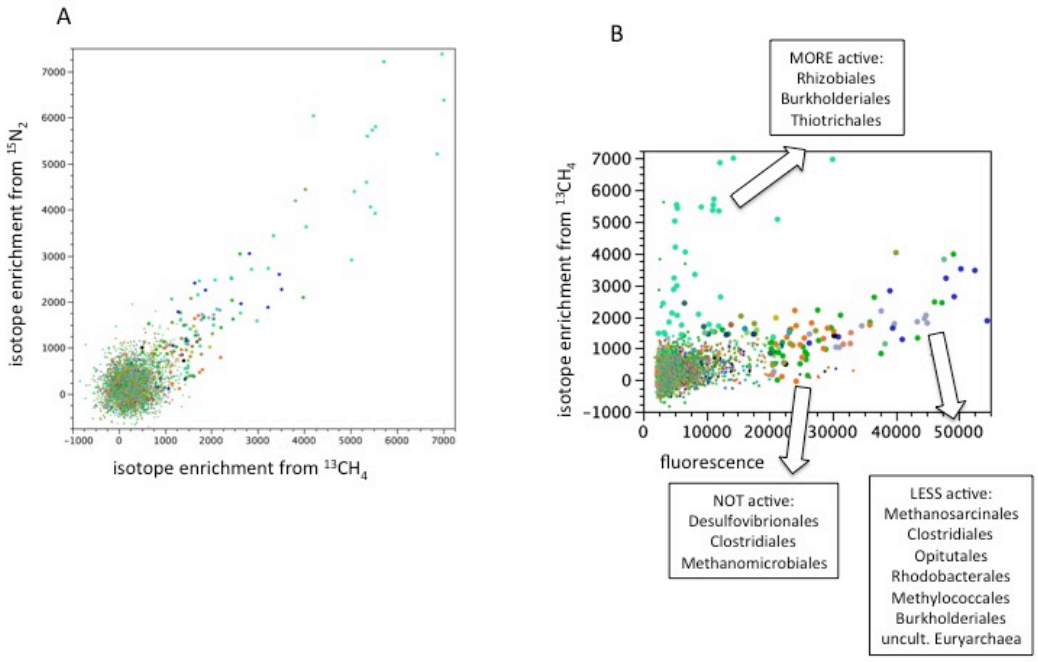

FIGURE 3. A) $^{13}CH_4$ incorporation plotted against $^{15}N_2$ incorporation shows that methanotrophy and nitrogen fixation were tightly linked processes carried out by the same organisms. B) $^{13}CH_4$ incorporation plotted as a function of fluorescence shows that some taxa were highly active in CH_4 incorporation (top), some taxa were less active (middle), and other taxa (bottom) did not incorporate ^{13}C from $^{13}CH_4$.

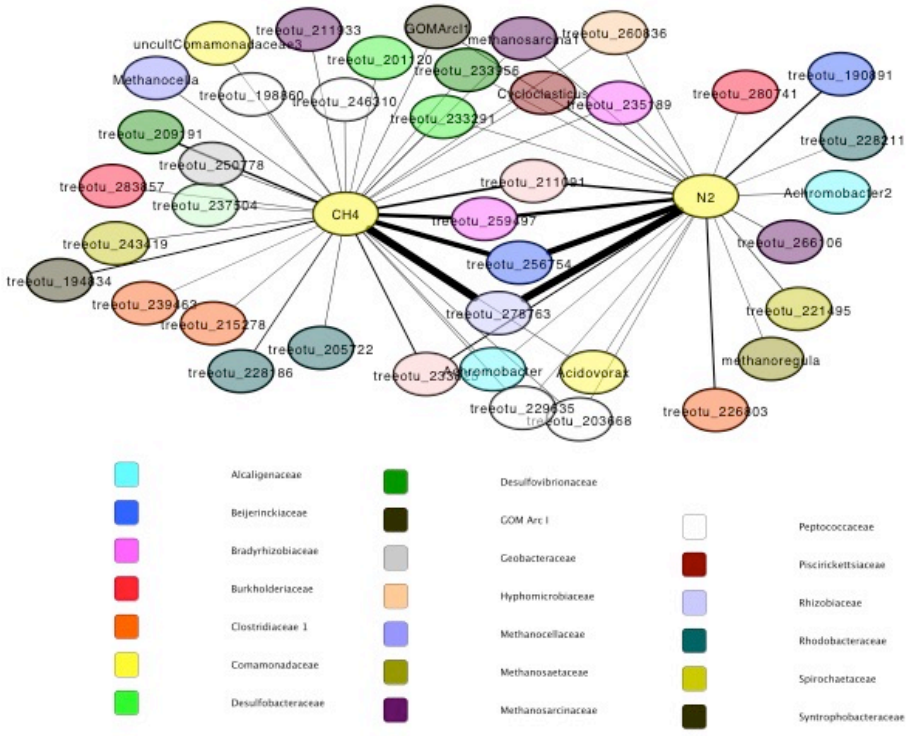

FIGURE 4. Network diagram showing which microbial taxa (colored by family) incorporated C from methane and N from N_2 fixation. Organisms linked to the substrates were significantly enriched according to Chip-SIP analyses (thickness of the lines is quantitatively related to isotope enrichment).

Subsurface Hydrology

We collected porewater from various depths from 3 SPRUCE plots (ambient, +4.5 °C, and + 9 °C). Samples were analyzed for ^{3}H, Helium-3, and noble gases, ^{2}H, and ^{18}O. We found very small amounts of free gases, indicating little potential for bubbling, and high amounts of dissolved gases, suggesting that diffusional transport of CH_4 and CO_2 is likely the dominant mechanism for translocation of gases to the peat surface and emission to the atmosphere. Stable isotopes indicated that the bog receives the majority of its recharge in the spring and summer months. Tritium data show relatively young water at shallow depths with characteristic decay profiles to 1 m depth (FIGURE 5). Below 1 m, pore water remains young (< 5 years old), indicating high lateral mixing (FIGURE 5). We confirmed with recharge testing that shallow wells recharged rapidly, while wells > 1 m depth recharged very slowly. This supports previous hypotheses that subsurface lateral transport of water, DOC, and dissolved N is an important factor at the bog. No changes with warming treatments were observed for subsurface hydrology or porewater chemistry during this project.

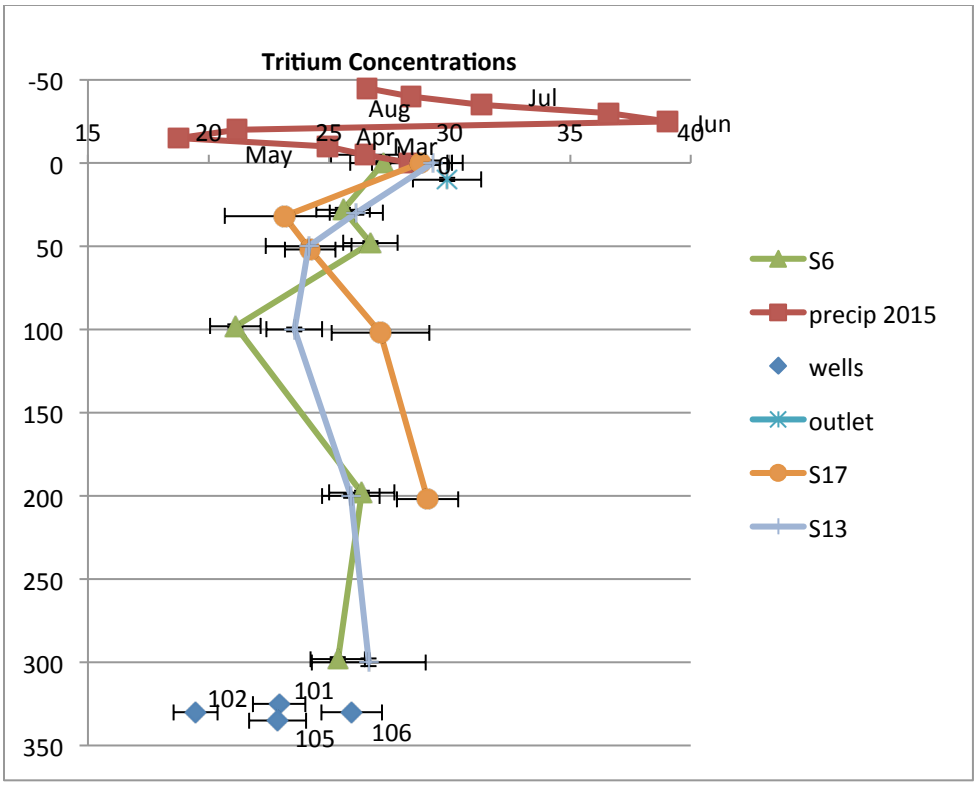

FIGURE 5. Tritium concentrations vs. Depth from wells at SPRUCE in 2015 show relatively young porewater throughout the peat profile. Monthly precipitation is shown in red for reference. US Forest Service monitoring wells are blue diamonds at ~ 325 cm depth also for reference.

Impact on Mission

This project has produced or facilitated numerous peer-reviewed manuscripts including one in press, three in review/revision, four in preparation, and at least two planned for the near future (five of these with LLNL-affiliated first-authors). In addition, the results of this work have been disseminated at professional society meetings, DOE programmatic meetings, and invited seminars, illustrating the invaluable role of this work to the overall SPRUCE experiment and LLNL's contribution to Carbon Cycle Science. Lead-PI McFarlane continues to be involved at SPRUCE through a subcontract from ORNL. ORNL receives funding for maintaining the SPRUCE experiment and its core measurements through a DOE-BER-TES grant, and discussions concerning expansion of LLNL's role into that of funded collaborators at the next funding renewal cycle are ongoing. Future support, particularly for ^{14}C and hydrologic tracer measurements and interpretation to test scientific hypotheses and develop the SPRUCE wetland models are sought from DOE, but could also be attained through collaborations with ORNL and/or university researchers. For example, LLNL team members were co-investigators on two "spin-off" proposals that were led by university investigators also working at SPRUCE and were submitted to DOE proposal calls in FY16.

This project added, developed, and demonstrated analytical capabilities at LLNL, including demonstration of $^{14}CH_4$ and ^{2}H-IRMS analytical capabilities. Additonal support during the final year of this project enabled completion of a unique experiment on microbial community function – dual-label ^{13}C- and ^{15}N-ChipSIP, a capability developed at the lab, but until this project only demonstrated for single isotopic labeling experiments. This experimental method addresses an important question in environmental microbiology and this is the only method currently available to answer this question.

This project provided support for 3 early career scientists at LLNL, including PI McFarlane who was awarded a DOE Early Career Award in FY16.

Conclusion

1. Microbial communities at SPRUCE use recent photosynthates as a carbon source even after short-term warming. A delayed response to warming might result in a shift towards increased use of old peat C and future measurements of ^{14}C of emitted carbon are the only direct test of this hypothesis. There are currently no plans or funding to continue or repeat these measurements.
2. The first dual-label SIP experiment on methanotrophs and diazotrophs showed that some microbes at SPRUCE oxidize methane AND fix N_2, while some appear to preferentially perform one function or the other.
3. Subsurface transport of water (and the C and nutrients dissolved in it) is relatively rapid at SPRUCE in the top meter of peat where most microbial activity occurs. This finding is valuable for understanding (and modeling) the interaction of biogeochemistry and hydrology at the bog. This work highlighted for the DOE community the value that LLNL's capabilities and expertise in water tracers can provide to large experiments like SPRUCE.
4. LLNL involvement in the SPRUCE experiment will continue in the short-term through collaborations established and strengthened by this LDRD project.

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