Final Technical Report
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Title: Methane Oxidation in the Rhizosphere of Wetland Plants
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EXECUTIVE SUMMARY

The main objective of the project was to improve predictions of future CH_4 emissions from natural wetlands. This objective was motivated by the fact that methane (CH_4) is a potent greenhouse gas, and wetlands represent the largest natural source of atmospheric CH_4. Modeling of wetland-methane feedbacks indicates that wetland methane emissions could drive 21st century climate change, with global wetland emissions matching or exceeding anthropogenic emissions by 2100 (Zhang et al., 2017). However, modeled estimates of wetland CH_4 emissions have high variability, which reflects a lack of mechanistic and predictive understanding of the processes and interactions that control methane production, oxidation and emissions from wetlands. The project focused specifically on wetlands within boreal regions that form when permafrost thaws, because warming temperatures have (Poulter et al., 2017) and will continue (Zhang et al., 2017) to increase wetland methane emissions from this region by promoting permafrost thaw and creating new wetland area.

The accomplished aims listed below advanced mechanistic understanding of how vascular vegetation and other factors (such as early spring rainfall and time since permafrost thaw) influence methane production, oxidation and emission from boreal wetlands, and thus, assisted in the successful achievement of the project's main objective of improving predictions of future CH_4 emissions. The project undertook multiple different approaches, including fieldwork, a laboratory plant-growth study, porewater stable-carbon-isotope calculations, and numerical modeling of the wetland methane cycle.

Accomplished Aim 1) Quantified rates of microbial carbon transformation in peatland soils and gained mechanistic understanding of how site factors, such as time since permafrost thaw, influence rates of microbial production and oxidation of methane.

Accomplished Aim 2) Identified two novel roles for rain in the methane cycle — transporting thermal energy into bogs, which increases methane emissions by warming soils and increasing plant and microbial productivity, and transporting oxygen into bogs, which decreases methane emissions by facilitating methane oxidation.

Accomplished Aim 3) Assessed the effects that hallow aerenchyma tissue in vascular plants has on methane production, oxidation and emission in thaw bogs.

Accomplished Aim 4) Determined the impacts that belowground allocation of carbon by plants has on methane production, oxidation and emission in thaw bogs

Accomplished Aim 5) Created and tested a dynamic and climate-sensitive representation of rhizospheric methane oxidation for large-scale wetland models.

The project addressed one of the scientific drivers for the Biological and Environmental Research program at DOE: "discovering the physical, chemical, and biological drivers and environmental impacts of climate change." It did so by both advancing fundamental understanding of the how vascular vegetation and other key factors (e.g., early spring rainfall

and time since permafrost thaw) affect methane production, oxidation and transport within permafrost-thaw bogs and by generating a more realistic representation of rhizospheric methane oxidation for regional-scale wetland models. Project outcomes have progressed knowledge ofr how future shifts in both climate variables (like rainfall amount and timing) and plant behavior (like species composition and productivity) will alter methane emissions in wetlands, improving the scientific community's ability to produce more robust predictions of future methane emissions and climate–methane feedbacks.

PROJECT GOALS

The main objective of the project was to improve predictions of future CH_4 emissions from natural wetlands. This objective was motivated by the fact that methane (CH_4) is a potent greenhouse gas with a global warming potential 32-times larger than that of carbon dioxide on an 100-year timescale (Etminan et al., 2016), and wetlands represent the largest natural source of atmospheric CH_4, contributing 20-40% of global emissions (Denman et al., 2007). Modeling of wetland-methane feedbacks indicates that wetland methane emissions could drive 21st century climate change, with global wetland emissions matching or exceeding anthropogenic emissions by 2100 (Zhang et al., 2017). However, modeled estimates of wetland CH_4 emissions have high variability, indicating both parameter and structural uncertainty in wetland methane models (Ciais et al., 2013), which reflects a lack of mechanistic and predictive understanding of the processes and interactions that control methane production, oxidation and emissions from wetlands. The project focused specifically on wetlands within boreal regions that form when permafrost thaws, because warming temperatures have (Poulter et al., 2017) and will continue (Zhang et al., 2017) to increase wetland methane emissions from this region by promoting permafrost thaw and creating new wetland area.

Over the course of the six year-long project period, the specific goals that were set in order to accomplish the main objective of the project changed from that originally proposed. The original proposal was solely focused on vascular plants and advancing both knowledge and modeled representations of plants within the methane cycle, particularly with regards to methane oxidation. Plants can play a dual role in the methane cycle. Carbon exuded by roots of plants into the rhizosphere (i.e., soil zone surrounding plant roots) can fuel methane production, and hollow aerenchyma tissues inside vascular plants can transport methane from soil directly to the atmosphere. However, aerenchyma tissues also support the diffusion of atmospheric oxygen down to roots where oxygen leaks into the rhizosphere and can oxidize methane. Thus, empirical studies have detected both increases (e.g., Whiting & Chanton, 1993) and decreases (e.g., Sutton-Grier & Megonigal, 2011) in methane emissions as plant productivity increases, and the representation of plants and their role in the methane cycle is a large contributor to uncertainty within modeled estimates of wetland methane emissions (Berrittella & van Huissteden, 2011; van Huissteden et al., 2009; Riley et al., 2011). Given these facts, vascular plants remained a key focus throughout the project, but the investigation identified other important factors and situations (e.g., time since permafrost thaw) that affect methane production, oxidation, and emission from permafrost-thaw wetlands.

Listed below are the specific aims that were accomplished during the project period. The first two aims reflect the expanded scope of the project, while the subsequent aims are solely focused on vascular vegetation.

Accomplished Aim 1) Quantified rates of microbial carbon transformation in peatland soils and gained mechanistic understanding of how site factors, such as time since permafrost thaw, influence rates of microbial production and oxidation of methane.

Accomplished Aim 2) Identified two novel roles for rain in the methane cycle — transporting thermal energy into bogs, which increases methane emissions by warming soils and increasing plant and microbial productivity, and transporting oxygen into bogs, which decreases methane emissions by facilitating methane oxidation.

Accomplished Aim 3) Assessed the effects that hallow aerenchyma tissue in vascular plants has on methane production, oxidation and emission in thaw bogs.

Accomplished Aim 4) Determined the impacts that belowground allocation of carbon by plants has on methane production, oxidation and emission in thaw bogs

Accomplished Aim 5) Created and tested a dynamic and climate-sensitive representation of rhizospheric methane oxidation for large-scale wetland models.

All of these accomplished aims advanced mechanistic understanding of how vascular vegetation and other factors (such as early spring rainfall and time since permafrost thaw) influence methane production, oxidation and emission from boreal wetlands, and thus, assisted in the successful achievement of the project's main objective of improving predictions of future CH_4 emissions from permafrost-thaw wetlands in boreal regions.

PROJECT APPROACHES

The project undertook multiple different approaches to tackle the 5 aims listed above, including fieldwork, a laboratory plant-growth study, porewater stable-carbon-isotope calculations, and numerical modeling of the wetland methane cycle. Details of these five different approaches are described below.

Fieldwork

Aims 2–4 were addressed with a field investigation that spanned three years: 2014–2016.

Fieldwork was conducted in a wetland complex located in Interior Alaska, 30 km southwest of Fairbanks (64.70°N, −148.3°W), which is situated in discontinuous permafrost (Neumann et al., 2019). The site is located within the Bonanza Creek Long Term Ecological Research forest and is part of the Alaska Peatland Experiment. The wetland is classified as a bog because it is recharged only by precipitation and runoff from the surrounding forested peat plateaus. Thaw commenced at the site 50 to 400 years ago and is active along bog margins (Euskirchen et al., 2014; Klapstein et al., 2014).

When ice-rich permafrost thaws, the land subsides and soils are flooded. Fast-growing aquatic plant species such as sedges and hydrophilic *Sphagnum* mosses colonize the area (Finger et al., 2016). With increasing time following thaw, peat accumulates, and hummock-hallow microtopography develops, enabling woody plants to grow in the drier elevated hummocks while hallows remain saturated throughout the growing season. The centers of bogs represent areas where thaw initiated while the edges of bogs represent areas that recently thawed; bog age increases from edge to center.

Project-specific measurements were made at the site during the 2014, 2015 and 2016 growing season, and included :

- static-chamber methane and CO_2 flux (light and dark) from the bog edge and center;
- static-chamber methane flux conducted under N_2-flushed, anaerobic conditions to assess methane oxidation, following method of van der Nat and Middelburg (1998);
- plant metrics (% cover, height, number) within flux collars during peak biomass;
- soil temperature at multiple depths at bog edge and center; and
- optical oxygen measurements (see Figure 1 for more information).

These measurements dovetailed with those made by other site investigators, including landscape-scale fluxes of methane, water and energy collected with eddy covariance measurements stations.

In 2015 and 2016 a vegetation removal experiment was conducted. Collars at the bog edge and center were subjected to one of three experimental treatments: natural-vegetation, simulated-aerenchyma, or *Sphagnum*-only. For natural-vegetation collars, no manipulations were done. For *Sphagnum*-only and simulated-aerenchyma treatments, all vascular plants were hand-pulled (including belowground roots) from inside the collar and within 20-cm of the outer edge. The method of King et al. (1998) was adapted to create the simulated-aerenchyma treatment. After plant removal, gas permeable silicone tubing (1.47mm inner diameter, 0.23 mm wall thickness) was inserted into peat matching site density of *Carex*. Tubes were tied at the bottom to keep water out and inserted to at least 30 cm. Tubing above the peat was held upright with copper wire. Tubes provided a diffusive pathway for subsurface gases to travel to the atmosphere, similar to plant aerenchyma.

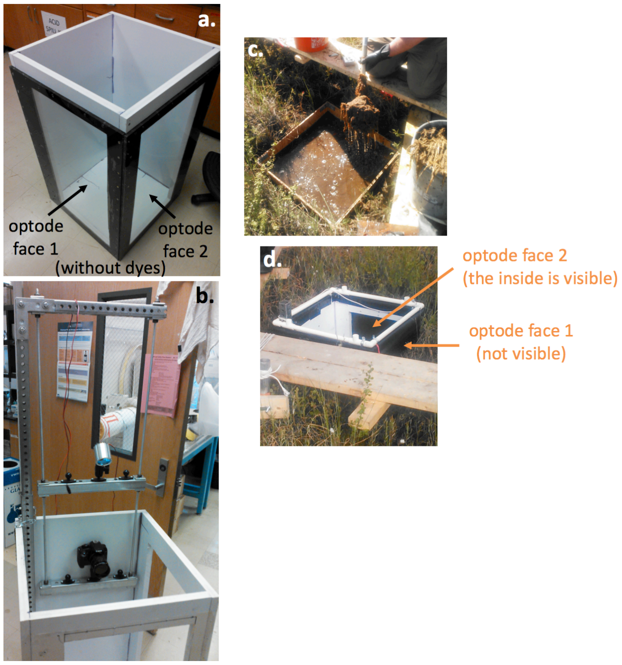

Figure 1. Planar optical oxygen sensors (optodes). Optodes enable quantitative visualization of oxygen concentrations at high spatial resolution across a two-dimensional surface. We adopted and modified the technology of Larsen et al. (2015). Optodes were made from clear polycarbonate sheets coated with an "antenna" dye and a luminophore "indicator" dye. The "antenna" dye, excited by high-intensity blue LEDs, transferred energy to the "indicator" dye whose luminescence was quenched by oxygen. Optode luminescence was captured in 12-bit RAW format with a DSLR camera modified by removing the near-IR blocking filter and outfitted with a yellow filter.

(a) Two optode sensors were attached as windows on separate sides of a hollow box (94.0-cm tall x 55.9-cm long x 55.9-cm wide). Dyes on optodes faced outward. **(b)** This setup enabled visualization of oxygen concentrations and placement of imaging equipment inside the box. **(c)** At the bog, a hole was carefully dug such that adjacent vegetation was minimally impacted. **(d)** The box was placed in the hole and optode windows were pushed against the peat. The box was anchored to deep mineral soil using fence posts and weighted down using steel bricks to offset water displacement.

After installation, vascular vegetation was removed from a ~30-cm strip adjacent to one optode face and continually weeded throughout the season. In addition, along the outer edge of this optode, two silicone tubes matching those used in the simulated-aerechyma treatment were inserted. The other optode face was left unaltered with natural vegetation.

In 2015, standard and anaerobic methane fluxes were performed on individual *Carex* plants to assess methane emissions and methane oxidation using a flux chamber constructed of a 1-m long, 10-cm diameter pipe that isolated and sealed an individual plant within the chamber. Three to six representative plants extending from the bog edge to center were selected for each of the five measurement events.

Laboratory Plant-Growth Study (Addressed Aims 3–4)
Aims 3–4 were addressed with a plant-growth experiment that was conducted using peat collected from the project field site described above.

Mimicking the plant-removal experiment conducted at the field site, the laboratory experiment had three experimental treatments: *Carex aquatilis* plugs purchased from a local nursery grown in peat-filled boxes, unplanted control boxes filled only with peat, and boxes with silicone tubes inserted into the peat instead of plants (Waldo et al., 2019). Boxes were kept at a 30-degree angle during the experiment to encourage roots to grow along the face of the rootbox, which allowed for sample collection across the rhizosphere and visualization of oxygen concentrations. Oxygen concentrations were measured using an optical oxygen sensor that was applied to the face of a subset of boxes (see Figure 1 for information on optical oxygen technology). Boxes without optodes had opaque front panels instead. Boxes were placed in growth chambers with conditions set to imitate central Alaska in the summer (18 hrs of daylight, daytime temperatures of 18 ^{o}C, and nighttime temperatures of 10 ^{o}C).

During the experiment, plant height and fluxes of CH_4 and CO_2 were regularly measured. In weeks 5 and 10 of the experiment, four randomly selected plants were isotopically labeled by placing a clear 6.5 L hood made of extruded acrylic on each box and injecting 15 mL of 99 at.%$^{13}CO_2$ into the headspace every hour for 14 hours each day over a period of five consecutive days. Isotopic composition of fluxes (d^{13}C-CH_4 and d ^{13}C-CO_2) were measured immediately before, during and after labeling. Boxes were destructively sampled within an anaerobic environment 3–5 days after isotopic labeling ended. Soil and root samples were taken from the root–soil surface exposed upon removing the front panel or optode face of the boxes. Soil removed from roots upon sonication was defined as rhizosphere soil (Waldo et al., 2019).

Isotopic composition of carbon within bulk soil, rhizosphere soil and root samples was analyzed at Pacific Northwest National Laboratory using an elemental analyzer coupled to a Thermo Scientific Delta V Plus isotope ratio mass spectrometer (IRMS). Chemical composition of carbon within samples was analyzed at Environmental Molecular Sciences Laboratory using Fourier Transform Ion cyclotron Resonance Mass Spectrometry (FT-ICR-MS) for soil samples (after extraction) and Laser Ablation Electrospray Ionization Mass Spectroscopy (LAESI) for root samples. Microbial composition of bulk soil samples was determined through sequencing of 16S rRNA and whole genomes by the Joint Genome Institute (Waldo, 2019; Waldo et al., 2019).

Porewater Stable Carbon Isotope Calculations (Addressed Aim 1)
Aim 1 was addressed by using porewater stable-carbon-isotope data to estimate *in situ* microbial rates.

A powerful approach for estimating in situ reaction rates involves tracking the temporal change in porewater concentrations and stable carbon isotopes of methane and CO_2 (Corbett et al., 2015; Neumann et al., 2016; Shoemaker & Schrag, 2010; Wilson et al., 2019). Microbes preferentially use isotopically depleted carbon substrates, which causes the carbon product pool to become depleted in ^{13}C and the carbon substrate pool to become enriched in ^{13}C. Different microbial biochemical pathways fractionate the carbon pools to different extents (Whiticar, 1999), which allows for differentiation between the microbial reactions of interest. Thus, by simultaneously tracking both the rate of change of stable carbon isotopes and porewater concentrations of the carbon pools (i.e., CH_4 and CO_2), it is possible to calculate rates of multiple microbial reactions.

This approach was used to estimate *in situ* microbial rates at the field site described in the 'Fieldwork' section above. Porewater was collected once a month in 2013 from the edge and center of the bog complex at multiple depths over the summer growing season and analyzed for concentration and stable-carbon isotopic composition of CH_4 and CO_2. The data were used along with multiple conceptual reaction network schemes the estimate microbial rates of methane production, methane oxidation, CO_2 production, and homoacetogenesis over the season at each sampling location and depth (Neumann et al., 2016). In addition, the approach was used on data from incubations conducted on peat collected from a permafrost thaw gradient in Stordalen Mire, Sweden (68.35° N, 19.05 ° E). The gradient spanned from a recently thawed collapsed palsa to a bog and finally to a fen habitat. The porewater isotope approach was expanded in this second application to include a Bayesian approach to estimating parameters and rates, enabling a probabilistic assessment and comparison of results, and microbial community analyses were used to identify the best conceptual reaction framework among equally mathematically feasible frameworks, facilitating the use of the isotope approach to understand organic carbon transformation and greenhouse gas production along the thaw gradient (Wilson et al., 2019).

Developing a

Aim 5 was addressed by integrating results from root-scale mechanistic model into a process-based landscape-scale wetland methane model.

Most process-based global-scale models assume that when methane travels through aerenchyma in plants from soil to the atmosphere, a constant fraction of methane is oxidized, while in fact, methane oxidation along this transport pathway is not static and changes with plant productivity and plant responses to environmental factors. In order to develop a dynamic and climate-sensitive representation of rhizospheric methane oxidation for large-scale wetland models, this project first developed a root-scale, mechanistic model that was used to explore and understand the relationship between rhizospheric methane oxidation and various plant responses.

The mechanistic model was developed using the Reactive Transport Code MIN3P (Mayer et al., 2002). MIN3P is a FORTRAN code that solves multi-component reactive transport combined with saturated or unsaturated water flow using finite differences. It allows the user to define specific kinetic rates for each reaction in a defined domain with desired initial conditions and boundary conditions. The model in this study was a one-

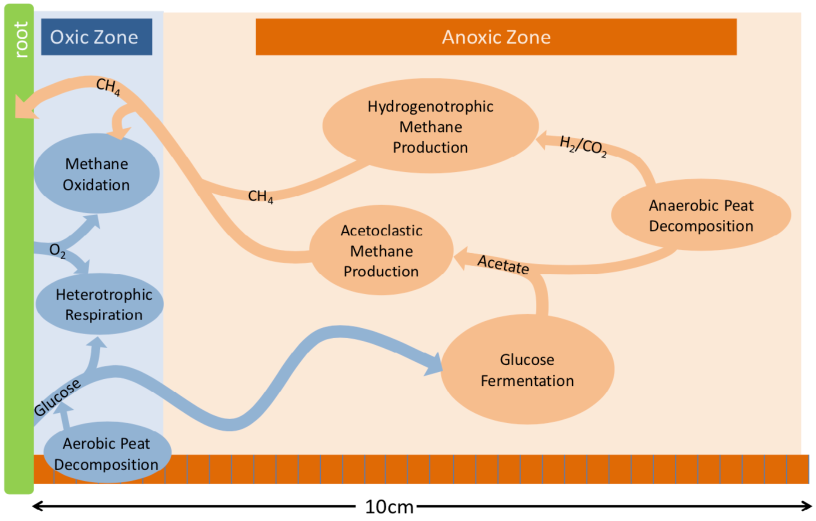

Figure 2. Schematic of processes included in mechanistic rhizosphere model.

dimensional radial model that consisted of a single root of 0.37mm radius and 10 cm of surrounding soil in which microbial reactions and plant-related processes were simulated. Simulated microbial reactions were methane production (acetoclastic and hydrogenotrophic methanogenesis), methane oxidation (methanotrophy), heterotrophic respiration, glucose fermentation and decomposition of organic matter. Simulated plant-related processes were root carbon exudation, root oxygen leakage and plant-mediated methane transport (aerenchyma methane uptake) (Figure 2) (Aslkhodapasand, 2016).

Based on results of the mechanistic modeling exercise, an equation describing the fraction of methane oxidized along the plant transport pathway was developed for use in the Walter & Heimann (2000) wetland methane model that increased the fraction oxidized along the plant transport pathway as root gas transport capacity increased and decreased the fraction oxidized along the plant transport pathway as root carbon exudation increased. The developed equation (outlined and thoroughly described in Aslkhodapasand (2016)) was translated for use in the Walter & Heimann (2000) model. After programming the modified equation into the model, the model was calibrated using measured methane emission rates from four sites with varying vegetation community composition located in West Siberia (provided by Glagolev et al. (2011)). These sites were located within the eastern part of Bakchar Bog in Tomsk area, Western Siberia. Methane emission measurements were available for 1997, 1998, 2006 and 2008 during the growing season (June to August). Calibration was performed by running the modified model for random combinations of calibrated parameter values and calculating of a likelihood score for each parameter combination based on fit to data.

Once calibrated, historic and future methane emissions were simulated using the original model formulation and the modified model formulation. To ensure the results were comparable, both model formulations were run using calibrated parameters for the modified model formulation (as described above). Each historic simulation consisted of a 20 year period (from 1991 to 2010). Thirty different parameter combinations (generated from calibrated parameter distributions) were used to simulate historic methane emissions. Future simulations consisted of a 20 year period (from 2081 to 2100). Future simulations covered 31 different climates models from CMIP-5 climate models (Taylor et al., 2011), which were fed into the UW-VIC model (Bohn et al., 2007) in order to generate time series of Net Primary Productivity (NPP), soil temperature profiles and water-table depth profiles. Parameter combinations used in the future model simulations were the same as the historic simulations.

A sensitivity test was conducted on future modeled methane emissions to assess the effect of vegetation type (as it relates to gas transport capacity) on emissions when both the original and modified frameworks were employed (Aslkhodapasand, 2016).

KEY FINDINGS

Key research finding are organized below based on accomplished aims.

Aim 1) Quantified rates of microbial carbon transformation in peatland soils and gained mechanistic understanding of how site factors, such as time since permafrost thaw, influence rates of microbial production and oxidation of methane.

At the bog outside of Faribanks Alaska, porewater-isotope calculations indicated that *in situ* microbial rates of methane production and oxidation, CO_2 production, and homo-acetogenesis were greater at the edge (i.e., the younger bog area where thaw is actively occurring) than in the center of the thaw bog (i.e., the older bog area where thaw occurred 50 – 400 year ago), that rates at the edge increased more during the growing season than did rates in the center, and that the ratio of acetoclastic to hydrogenotrophic methanogenesis was greater at the edge than in the center. In both locations, calculated rates (excluding methane oxidation) increased with depth. These results indicate that the **bog margin, where permafrost is actively thawing, is a hotspot for microbial production of methane**, particularly through the acetoclastic pathway, and that microbial reactions at this location are more sensitive to seasonal warming. Conceptually, these trends were explained by the proximity of the edge location to the actively thawing permafrost margin; in this location, the proportion of sedges was greater (and therefore providing organic carbon inputs to fuel acetoclastic methanogenesis and aerenchyma transport pathways to facilitate methane emission) and thawing permafrost can provided bioavailable carbon and nutrients needed to support microbial reactions. These results are thoroughly discussed in Neumann et al. (2016).

Along the thaw gradient Stordalen Mire, Sweden, porewater-isotope calculations and microbial community analysis of peat incubations indicated that acetoclasty, hydrogenotrophy, CO_2 production, and homoacetogenesis were the important reactions, with little evidence for anaerobic CH_4 oxidation. There was a distinct transition in the microbial reactions across the thaw gradient. The collapsed palsa stage represent an initial disequilibrium where the abrupt (physically and temporally) change in elevation introduces freshly fixed carbon into anoxic conditions then fermentation products build up over time as the system transitions through the acid phase and electron acceptors are depleted. In the bog, fermentation slows, while methanogenesis increases. In the fully thawed fen, most of the terminal electron acceptors are depleted and the system becomes increasingly methanogenic. As discussed in Wilson et al. (2019), **these results suggest that as permafrost regions thaw and dry palsas transition into wet fens, CH_4 emissions will rise, increasing the warming potential of these systems and accelerating climate warming feedbacks.** These results are thoroughly discussed in Wilson et al. (2019).

Aim 2) Identified two novel roles for rain in the methane cycle — transporting thermal energy into bogs, which increases methane emissions by warming soils and increasing plant and microbial productivity, and transporting oxygen into bogs, which decreases methane emissions by facilitating methane oxidation.

Over the 3-yearlong investigation conducted at the field site outside of Fairbanks, Alaska, we captured an average precipitation year (2015 had 283 mm of rainfall; 30-year normal rainfall for the area is 230– 289 mm; (NOAA, 2017)) and 2 years with abnormally high rainfall (378 mm in 2014 and 345 mm in 2016). Therefore, we had the opportunity to observe and investigate the affect rain has on methane emissions within the thawing bog complex. Notably, the bogs have floating peat mats, which facilitated a consistent water table depth relative to the peat surface all 3 years (Neumann et al., 2019). With no notable change in thickness of unsaturated peat, we avoided the confounding effects that a change in water table depth can have on thermal conductivity of soil (Hillel, 2005) and on production and oxidation of methane (Turetsky et al., 2014).

We discovered that **interactions between rain and deep soil temperatures controlled methane emissions**. To a first approximation, rain has the same temperature as air (Byers et al., 1949), and when air and soil temperatures are mismatched, rainwater inputs can rapidly change subsurface soil temperatures through thermal conduction. In rainy years, recharge from the watershed rapidly altered wetland soil temperatures, warming the top ~80 cm of soil in spring (when air temperatures are warmer than soil temperatures) and cooling it in autumn (when air temperature are colder than soil temperatures). Figure 3 illustrates the rapid penetration of warmer and colder temperatures into soil down to depths ≥75 cm during rain events associated with an temperature offset between the air/rain and soil. Soil warming in the spring did not occur until after cumulative evaporative water loss from the upland watershed area had been met or exceeded by cumulative rainfall (arrow 2, Figure 3). This situation enabled rainwater to move through the upland watershed, enter and recharge the bog rather than remain in upland soils where it is transpired by plants. Notably, **when soils were warmed by spring rainfall, methane emissions across the bog complex increased by ~30% relative to years with either less rain (2015) or similar amounts of rain that fell later in the season (2016)**. The warm, deep soils early in the growing season appeared to support both microbial and plant processes that enhanced emissions — particularly at the edge of the bog where number and percent cover of sedges (*Carex* spp.) and methane emissions more than doubled. These results, thoroughly discussed in Neumann et al. (2019) identify an important and unconsidered role of rain in governing the radiative forcing of thawing permafrost landscapes.

Data collected from the optical oxygen sensors installed in the bog (Figure 1) indicate that **rain not only transport thermal energy into the bog (Figure 3), but it also transports oxygen (Figure 4). This rain-delivered oxygen supports methane oxidation.** Direct measurements of methane oxidation through anaerobic chamber-flux measurements indicate higher rates of methane oxidation during rainy years, exceeding that which can be attributed to warmer soil temperatures (based on Q10 temperature scaling of measured rates). In addition, methane emissions measured with an eddy covariance tower at the site showed a temporary uptake of methane following a series of large rain events that caused cumulative evaporative water loss from the upland watershed area to be met by cumulative rainfall, and thus enabled rainwater to flow from the watershed into the bog (Figure 5). These results identify an unconsidered role of rain in facilitating methane oxidation.

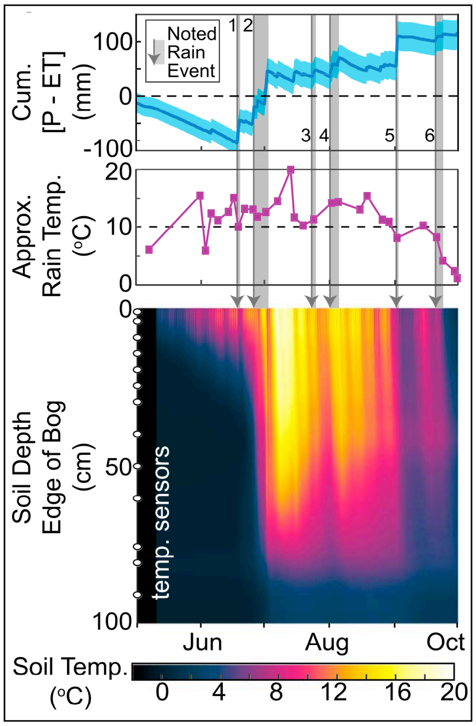

Figure 3. Data collected 2014. **Top panel**: cumulative difference between precipitation (P) and evapotranspiration (ET) for the permafrost forest. Arrows mark rain events, with duration of events highlighted by the gray bars associated with each arrow. Arrow 1 indicates a rain event that did not contribute water to the bog because the cumulative rain amount had not yet offset the cumulative ET demand for the forest. All other arrows mark rain events that did contribute water to the bog. **Middle panel**: estimated temperature of rain, which was calculated as the volume-weighted temperature of the air when the rain fell. **Bottom panel**: soil temperature (indicated by color according to the color bar) as a function of time and depth for the bog edge. Depths of temperature sensors are marked with white circles along y-axis. Arrows 2 and 4 show events that occurred when rainfall was warmer than soil, resulting in rapid penetration of warmer temperatures into soil. Arrows 3, 5, and 6 indicate rain events that occurred when rainfall was cooler than soil, resulting in rapid penetration of colder temperatures into soil.

Northern latitudes are expected to get warmer and wetter, and while there is consensus that warming will increase thaw and methane emissions, effects of increased precipitation are uncertain. **Our results show that precipitation can affect greenhouse gas production by transporting both thermal energy and oxygen into thaw-bogs**. Capturing and correctly accounting for dynamic biosphere-atmosphere interactions and feedbacks, such as those involved with permafrost thaw, requires modeling. However, most biogeochemical models do not include advective heat transport nor oxygen inputs from rain. Our study indicates the impact of rain on soil biogeochemistry and wetland methane emissions could be substantial, representing an important knowledge and modeling gap in advancing environmental predictability.

Aim 3) Assessed the effects that hallow aerenchyma tissue in vascular plants has on methane production, oxidation and emission in thaw bogs.

Field experiments and laboratory plant-growth experiments where methane emissions were monitored from bog locations or plant-growth boxes 1) with vascular vegetation, 2) with no vascular vegetation but with silicone tubes that simulated the aerenchyma transport capacity of vascular vegetation without associated 'biological' influences of plants, and 3) with neither vascular vegetation nor silicone tubes (i.e., controls) indicated that "aerenchyma" (i.e., silicone tubes) increased emissions relative to the controls sporadically and only for brief periods of time (Turner et al., 2019; Waldo et al., 2019). These results signify that **physical aerenchyma transport is important when there is a store of belowground methane, but 'biological' influences of vascular vegetation are necessary for continued methane generation**.

In the field experiment, **simulated aerenchyma did not alter the fraction of methane oxidized relative to control treatments** (Turner et al., 2019), which is explained by the fact that the tubes increased oxic surface area across which methane diffusion and transport occurred. Optical oxygen sensors used both in the field and in the plant-growth experiment detected standing pools of oxygen around simulated aerenchyma (Figure 6). Therefore, the tubes extended the peat-atmosphere interface deeper belowground, which simultaneously increased methane transport and oxidation, leaving normalized oxidation unchanged. Simulated aerenchyma facilitated transport of both methane and oxygen between the peat-atmosphere interface, and thus did not alter the fraction of methane oxidized.

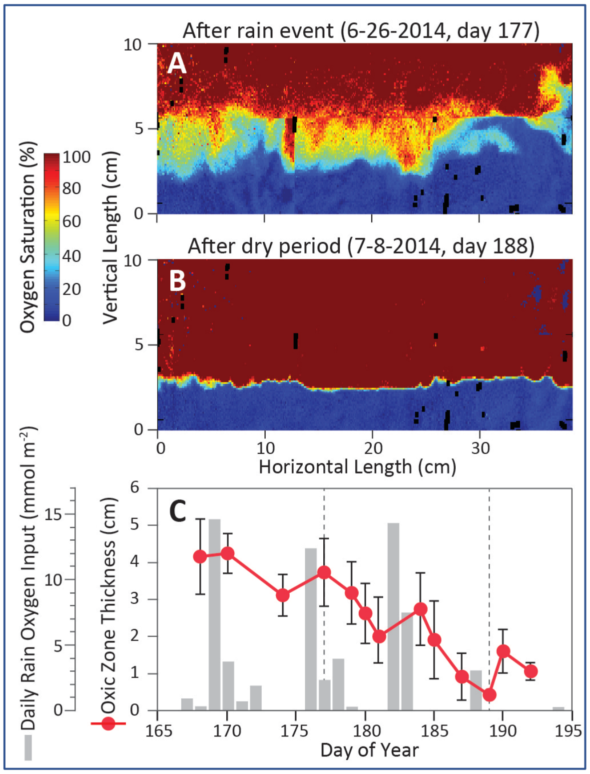

Figure 4. Optical oxygen data collected 2014 from a thaw-bog in Interior Alaska. Top Two Panels: Oxygen concentrations across the air-water interface after **A)** a rain event and **B)** a dry period without rain. **C)** Average thickness of the oxic-water layer at the top of the bog as measured across the optode face over the summer season (red circles) compared against calculated input of oxygen into the bog from rain directly falling on the bog, assuming oxygen concentrations in the rain are in equilibrium with the atmosphere. Thickness of the oxic-water layer decreases over the season, but experiences transient increases after rain events.

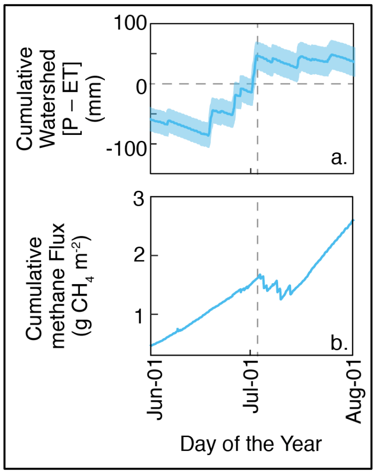

Figure 5. Data from 2014. **A)** Cumulative difference between precipitation and evapo-transpiration in upland watershed. **B)** Cumulative methane flux from bog complex measured using eddy covariance.

Aim 4) Determined the impacts that belowground allocation of carbon by plants has on methane production, oxidation and emission in thaw bogs

Both field and laboratory experiments indicate that the “biological” impact of vascular vegetation (associated with root carbon exudation and/or root-associated microbial communities) increase methane emissions relative to control treatments.

In the field experiment, the **increase in emissions connected with vascular vegetation was associated with an increase in methane production and a decrease in methane oxidation** (Figure 7). Reduced methane oxidation in the presence of vascular vegetation is often attributed to transport of methane through aerenchyma tissues, allowing methane to bypass oxic surface layers where oxidation can occur (Knoblauch et al., 2015; Marushchak et al., 2016; Sebacher et al., 1985; Watson et al., 1997). In our study, measuring methane oxidation in treatments with and without vascular vegetation and in isolated plants enabled partitioning of methane transport and oxidation along plant- versus peat-emissions pathways. Partitioning showed that reduced oxidation associated with vascular vegetation was due in part to aerenchyma transport allowing methane to bypass oxic surface layers, but it was not fully explained by this mechanism. Vascular vegetation did siphon methane away from the peat-transport pathway and minimal methane oxidation occurred along the plant-transport pathway, but vascular vegetation also decreased the fraction of methane oxidized along the peat-transport pathway.

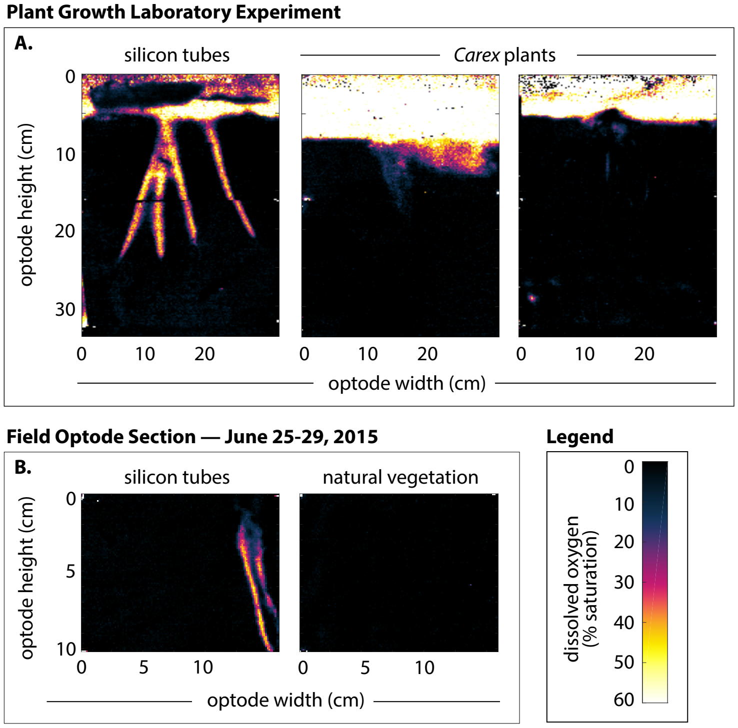

Figure 6. Dissolved oxygen concentrations measured with two-dimensional optical oxygen sensors (optodes). **(a)** Example images from a plant growth laboratory experiment with silicone tubes placed and individual *Carex* plants grown against the optode face. **(b)** Example images from subsections of the optodes installed at the field site (see Fig. 1). Silicone tubes were placed at the far edge of the un-vegetated optode face.

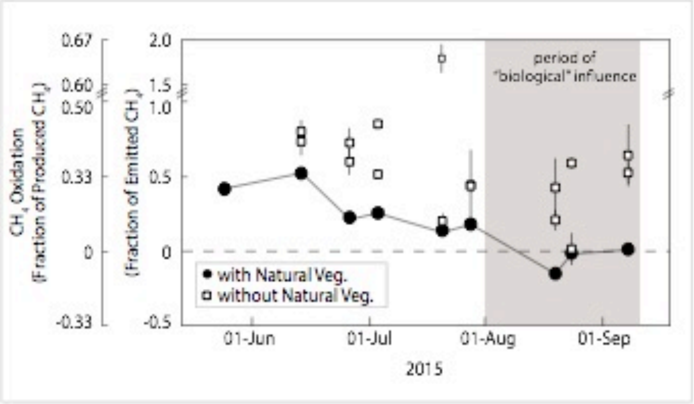

Figure 7. Methane oxidation normalized to emitted and produced methane for treatments with natural vegetation and treatments without vascular vegetation. Brown panel marks the period during which emissions were conceptually controlled by the biological effects of vegetation and when significant differences existed in emissions between vegetated and unvegetated treatments.

The ability of vascular vegetation to reduce methane oxidation along the peat-transport pathway signifies that plants altered the soil environment and/or microbial community such that methane dynamics outside the rhizosphere were impacted. We speculate that at our study site, **decreased oxidation associated with vascular vegetation (Figure 7) was caused by root exudates fueling an increase in other heterotrophic communities that outcompeted methanotrophs for available oxygen and other electron acceptors**. This argument is supported by multiple lines of evidence from our study:

1. Minimal to no oxygen was detected around plant roots, but oxygen was detected around silicone tubes (Figure 6). Assuming plants were transporting and releasing oxygen belowground, this finding implies that root exudates (not released by the silicone tubes) fueled microbial consumption of root-released oxygen.
2. Methane oxidation (Figure 7) and relative abundance of methane oxidizing microbes decreased over the season when plants were present. This seasonal decrease coincided with the time during which ‘biological’ influences of plants dominated physical influences, according to differences in methane emissions between the three treatments. Plant release of carbon belowground is positively associated with productivity (Edwards et al., 2018; Farrar et al., 2003; Nielsen et al., 2017) and known to fuel growth of microbial communities (Robroek et al. 2015).
3. Oxygen injection experiments conducted against the field-installed optical oxygen sensors (Figure 1) suggested that rates of oxygen consumption were fastest in shallow peat in the presence of vascular vegetation (Figure 8). Rates appeared to be slower in shallow peat without vascular vegetation and in deeper peat where conceptually plant roots exert less of an influence. According to Michaelis-Menten kinetics, rates of oxygen consumption are a function of the microbial community and substrate availability (Van Bodegom et al., 2001). The release of root exudates necessarily alters substrate availability, which in turn alters microbial community structure (Galand et al., 2005; Robroek et al., 2015). These results indicate plants facilitated competition among heterotrophic communities for available oxygen.

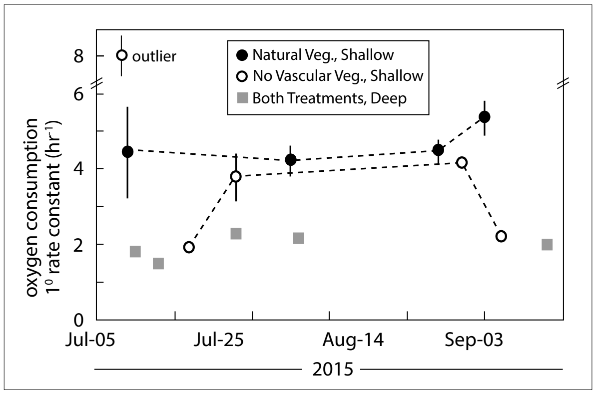

Figure 8. Fitted first-order rate constant from oxygen injection experiments conducted against vegetated and non-vegetated optode faces. Rate constants from 15-cm depth with (black circles) and without (open circles) natural vegetation (black circles), and from 38-cm depth regardless of vegetation (grey squares). Consumption rate was anomalously high for the first shallow-depth injection conducted without vascular vegetation.

Literature often points to root carbon as a source for fueling methane production (Joabsson et al., 1999; Laanbroek, 2010). **Here we have indirect evidence that these same exudates facilitate decreases in methane oxidation** (Turner et al., 2019).

In the plant-growth laboratory experiment, plants were exposed to $^{13}CO_2$ at two timepoints and subsequent enrichment of root tissue, rhizosphere soil, and emitted CH_4 (Figure 9) was used in an isotope mixing calculation to determine the proportion of plant-derived versus soil-derived carbon supporting methanogenesis. Results showed that carbon exuded by plants was converted to CH_4 but also that planted boxes emitted 28 times more soil-derived carbon than the other experimental treatments (Figure 10). At the end of the experiment, emissions of excess soil-derived carbon from planted boxes exceeded the emission of plant-derived carbon (Figure 10, week 10). This result signifies that plants and root exudates altered the soil chemical environment, increased microbial metabolism, and/or changed the microbial community such that microbial utilization of soil carbon was increased (e.g. microbial priming) and/or oxidation of soil-derived CH_4 was decreased (e.g., by microbial competition for oxygen).

Priming is a broad term encompassing any process by which the addition of a different carbon source (e.g., root exudates or leaf litter) increases microbial utilization of soil carbon. Priming can happen by stimulating an increase in microbial biomass, a change In composition of the microbial community, and/or a chan9ge in what metabolisms are active within the microbial community. Priming provides a straightforward explanation of why real plants emitted such a large amount of soil-derived methane relative to the other treatments (Figure 10). Notably, during the week 10 labeling event, more methane was soil-derived than exudate-derived (Figure. 9), indicating a notable priming effect. During the week 5 event there was still priming, but the soil- derived and exudate-derived portions were about equal. Based on rhizosphere enrichment data (Figure 9), we know that there was more root exudation happening during week 10. Together, **the data indicate that more root exudation led to a greater portion of the total emissions being soil-derived** (Figure 10).

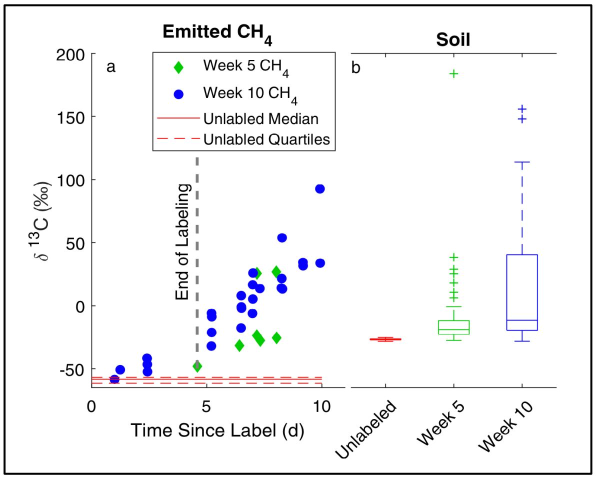

Figure 9. Both panels share the y-axis. **a**) Isotopic enrichment of CH4 emissions over time since labeling for four planted boxes during each labeling event. Red lines are median and interquartile range of unlabeled emissions. During both labeling events emitted CH4 became substantially enriched. **b)** Soil carbon isotopic enrichment. The unlabeled soil includes both rhizosphere and bulk soil (n = 68) while the soil data from the week 5 (n = 43) and week 10 (n = 69) plant harvests is all rhizosphere soil. The rhizosphere became substantially more enriched after the week 10 harvest.

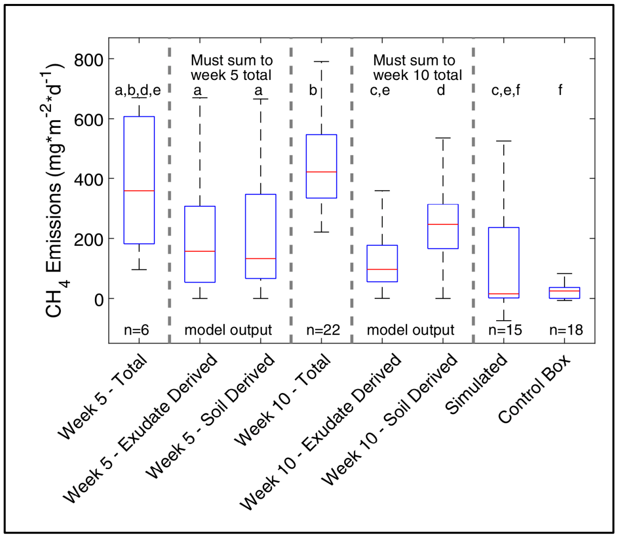

Figure 10. Total measured fluxes from planted boxes (week 5: total and week 10: total), the portion of that total which was calculated to be from each carbon source (soil derived and exudate derived), and measured emissions from the two unplanted box types (simulated plants and control boxes), which were entirely soil-derived. Outliers are not shown. Letters indicate groups that are statistically similar (p >0.05). Any two boxes which do not share a letter are significantly different (p<0.05) from each other

In our experiment, there was an order of magnitude increase in the conversion of soil carbon to methane driven by plant growth (Figure 10). Plant growth is projected to increase in the boreal region under forecasted climate conditions. The presence of such a large priming effect implies that increased plant productivity could potentially lead to increased conversion of soil carbon to CH_4 on climatically relevant scales.

Follow up work characterizing organic carbon in unplanted and rhizosphere soil using FT-ICR-MS indicated that root exudates triggered conversion of large organic compounds into smaller organic compounds and the net removal of nitrogen atoms from smaller compounds (<500 Da) (Waldo, 2019). One theory for why plants release root exudates into soil and trigger 'priming' is related to nitrogen availability. Priming could benefit plants through nitrogen mining (N-mining), a process by which the addition of nutrient-poor carbon compounds causes microbes to degrade existing soil compounds which contain nitrogen (Craine et al., 2007; Ruirui et al., 2014). Wetland types such as bogs and some fens are often nitrogen limited (Aerts et al., 1992) despite the large amount of nitrogen bound in slowly decaying plant matter (Drewer et al., 2010; Moore et al., 2005; Turunen et al., 2004). These wetlands are therefore an environment where it is highly beneficial for plants to access the nitrogen already in the soil, using tactics such as N-mining. Our FT-ICR-MS results (Waldo, 2019), in conjunction with the isotope labeling results (Waldo et al., 2019), **support the nitrogen-mining theory motivating why plants release root exudates into soil and trigger processing of soil organic matter**.

Aim 5) Created and tested a dynamic and climate-sensitive representation of rhizospheric methane oxidation for large-scale wetland models.

As described in the methods section, a mechanistic rhizosphere-scale model was first developed in order to understand the relationship between rhizospheric methane oxidation and the release of carbon and oxygen by plant roots (Figure 2). The mechanistic model results showed that rhizospheric methane oxidation not only can change with availability of carbon from roots and root gas transport capacity, but that it also is a function of microbial competition between microbial populations that live in methanogenic environments (Figure 11 and other figures in Aslkhodapasand (2016)). These modeling results are broadly supported by the laboratory and field results discussed for previous aims.

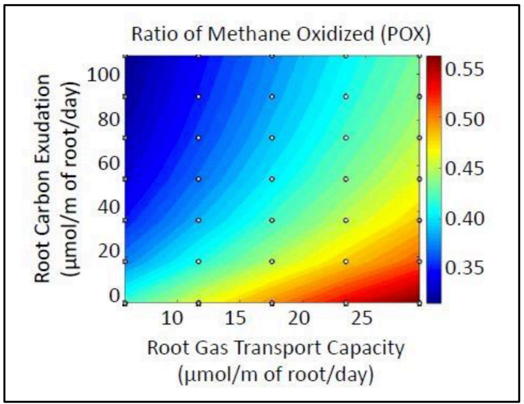

Figure 11. Results from mechanistic model showing response of the fraction of methane oxidized along the plant-transport pathway to release of carbon and oxygen from plant root.

These results were used to develop a numerical representation of rhizospheric methane oxidation that responded to these two variables and could be integrated into the Walter & Heimann (2000) wetland methane model. The Walter & Heimann model uses a variable called Pox, which is the fraction of methane oxidized along the plant-transport pathway. The dynamic Pox equation developed from the mechanistic modeling results was (Aslkhodapasand, 2016):

$$dynPox = \left(A_0 + (A_1 - A_0)e^{-\frac{Q_c}{Q_{c\_ref}}}\right)\frac{Q_o}{Q_{o\_max}} + minPox$$

Where,

Q_o is the root oxygen release rate (Figure 1), which is described in greater detail in section 2.2.8 of Aslkhodapasand (2016).

Q_{o_max} is the maximum root oxygen release rate. This rate was defined as the root oxygen release rate that created a 1 cm layer of oxygen saturation around the root in median condition for all other processes and was the maximum rate used in the mechanistic root-scale model.

Q_o / Q_{o_max} is the normalized root oxygen release rate.

Q_c is the rate of root carbon exudation.

Q_{c_ref} is a fitting parameter for root carbon exudation which represents typical values of root carbon exudation in the growing season. The fitted values of Q_{cref} can be found in Table B-1 in Appendix B of Aslkhodapasand (2016).

Results from the mechanistic root-scale model were fitted to the equation above in order to find values for parameters A_1, A_0, Q_{c_ref} and *min_pox*. A_1 and A_0 represent Pox gradients with respect to low and high root carbon exudation rates respectively. *min_pox* represents the minimum Pox value observed in each MIN3P model simulation. Q_{c_ref} represents the typical value of root carbon exudation in the growing season. Fitting was performed separately for each of the sensitivity tests in the rhizosphere-scale model. Parameter fittings were performed for 351 simulations (shown in Table B-1 in Appendix B of Aslkhodapasand (2016)) which consisted of different scenarios of microbial competitiveness, root oxygen consumption and peat decomposition levels and different time points in the simulation.

As shown in Figure 12 the empirical Pox equation was able to capture the characteristics of the mechanistic model Pox results. Fitting results for one of the simulations is shown in Figure 12 as a reference.

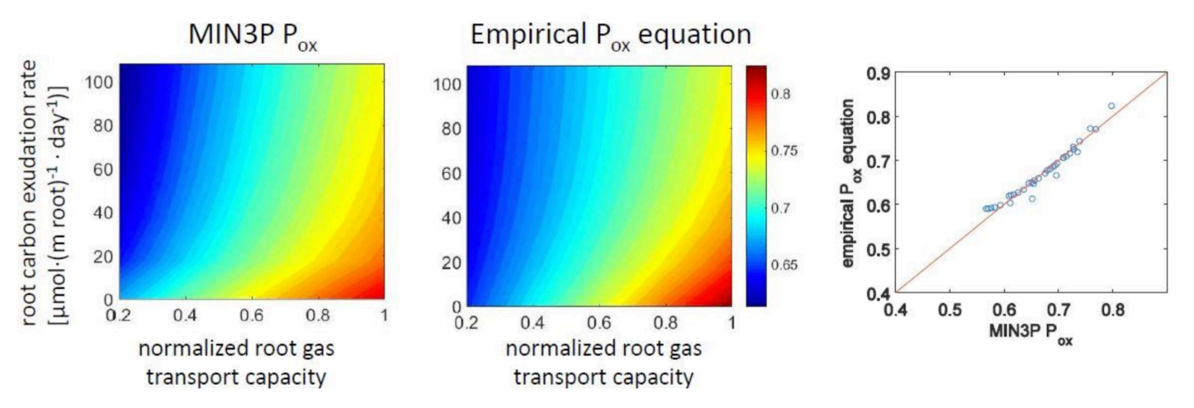

Figure 12. Comparison between fraction of methane oxidized (Pox) resulting from mechanistic model (labeled MIN3P Pox) and Pox resulting from empirical equation after parameter values were fit.

The empirical Pox equation was added to the Walter and Heimann (2000) methane model's internal code after the equation was translated into parameters that are used within model.

Q_o/Q_{o_max} from the empirical equation was replace with *tveg/tveg_max*. *tveg* is a parameter in the Walter and Heimann model that "describes the quality of plant-mediated methane transport at a site which depends on density and type of plant stands" and varies between 1 to 15, 1 representing sites and plants with low methane transport capacity and 15 representing sites and plants with the highest capacity for methane transport (Walter and Heimann, 2000). The *tveg* parameter is similar to the root oxygen release rate (Q_o) because gas transport of methane and oxygen travel through the same hallow internal tissues inside plants. Q_c/Q_{c_ref} from the empirical equation was replace with NPP/NPP_{ref}, where NPP is net primary production. The relationship was supported by the fact that root carbon exudation is known to increase with plant productivity. Typical values of Q_C (chosen as the median of root carbon exudation rates used in the mechanistic model simulations, 50 μmol·(m root)$^{-1}$.day^{-1}) was used to normalize Q_{c_ref} and covert it to NPP_{ref} using typical values of NPP (average of NPP values observed during growing season)

After the modified Pox equation inserted into the Walter and Heimann model, plot-scale model simulations were performed for four study sites located in Western Siberia using the original model formulation and the formulation with the modified Pox equation. Future simulations using both model formulations showed that methane emissions from the study area would increase by a median factor of 1.7 by the end of century (Aslkhodapasand, 2016). Switching from original model formulation to the one with the modified Pox equation resulted in a reduction of total annual methane emissions by 4% and reduction of plant-mediated methane transport by 17% in the four study sites (Aslkhodapasand, 2016). The reduction was more pronounced for sites with higher density of aerenchymatous plants (such as sedges) due to higher root zone methane oxidation. The exercise demonstrated that, counterintuitively, higher coverage of aerenchymatous plants in a wetland can, by increasing methane oxidation along the plant-transport pathway, lead to lower plant-mediated methane transport and lower methane emissions — though we note that this outcome was not seen at the field site in Interior Alaska (e.g., Figure 7)

Ultimately, the results of Aslkhodapasand (2016) showed that **modeled rates of methane emissions are sensitive to the amount of methane oxidized along the plant-transport pathway, particularly at sites with a high density of aerenchymatous plants.** As mentioned previously in this report, most process-based global-scale models assume that when methane travels through aerenchyma in plants from soil to the atmosphere, a constant fraction of methane is oxidized, while in fact, methane oxidation along this transport pathway is not static and changes with plant productivity and plant responses to environmental factors (e.g., Figure 7). Thus, models with the static Pox formulation (like the original formulation of what Walter and Heimann model) could, depending on the context, be over or underestimating methane emissions from sites with high coverage of aerenchymatous plants.

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PARTICIPANTS

Undergraduate Students Trained in Research

Marina Kochuten	2016 – 2017
Ella Fadelye	2016
Jesse Turner	2016
Brianna Hunt	2015 – 2016
Anna Tsai	2014 – 2015
Hunter Brown	2013

Graduate Students with Completed Degrees

Nicholas B. Waldo. P.h.D. 2019.
Now working at Pacific Groundwater Group.
Farnaz Aslkhodapasand. M.S. 2016.
Now working at Cardno.

Postdoctoral Scholars with Faculty Jobs

Colby M. Moorberg. Postdoctoral Researcher 2014–2015.
Now faculty member at Kansas State University.

Established Partnerships

Environmental Molecular Sciences Laboraotry, Richland, WA, USA
Joint Genome Institute, Walnut Creek, CA, USA
In-kind Support, Facilities Support

Dr. Neumann was awarded a EMSL-JGI Collaborative Science Project titled "A Rhizosphere-Scale Investigation of the Relationship Between Plant Productivity and Methane Emissions from Wetlands." This project allowed Nick Waldo, a PhD student on the project, to harness advanced analysis techniques in plant-growth experiment.

Bonanza Creek Long Term Ecological Research (LTER) Program, Fairbanks, AK, USA
Collaborative Research, Facilities Support

The permafrost-thaw bog that was studied as part of this project is located within the Bonanza Creek LTER forest. Association with this organization granted the project access to the fieldsite as well as to lab and office space at the University of Alaska Fairbanks.

Collaborations Fostered

Merritt Turetsky (Associate Professor, University of Guelph) — Merritt established the permafrost-thaw-bog field site where project work was conducted. She and her research group continue to conduct research at this site.

Mark Waldrop (Research Scientist, USGS) — Mark helped established the permafrost-thaw-bog field site where project work was conducted. He and his research group continue to conduct research at this site.

Eugenie Euskirchen (Research Associate Professor, University of Alaska Fairbanks) — Eugenie helped established the permafrost-thaw-bog field site where project work was conducted. She and her research group continue to conduct research at this site.

Jeff Chanton (Professor, Florida State University) — PI on multiple projects investigating the response of soil carbon storage and carbon turnover. Dr. Neumann has and contintues to work with Jeff and his research group, harnessing isotope modeling approach (as described previously in the report) to model porewater stable carbon isotope data collected from different wetland sites. The goal is to estimate in situ microbial rates

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