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BIOGEOCHEMICAL RESPONSE TO VEGETATION AND HYDROLOGIC CHANGE IN AN ALASKAN BOREAL FEN ECOSYSTEM

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BIOGEOCHEMICAL RESPONSE TO VEGETATION AND HYDROLOGIC CHANGE IN AN ALASKAN BOREAL FEN ECOSYSTEM

By

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Preface

This body of work includes multi-authored papers in various stages of publishing. Details regarding publish status, copyright, and author contributions are detailed below for each chapter.

Chapter 2, *Plant functional group effects on peat carbon cycling in a boreal rich fen*, is previously published in the journal Biogeochemistry, © 2019 Springer Nature Switzerland AG. The reprint RightsLink® license no. is 4680250496895 for use in this dissertation. The following details co-author contributions: Danielle Rupp, Catherine Dieleman, and Evan Kane conceived and designed the mesocosm experiment. Merritt Turetsky provided advice on experimental design. Danielle Rupp and Catherine Dieleman performed the experiments. Jason Keller conducted the electron shuttling assays. Danielle Rupp analyzed the data, created figures, and wrote the manuscript; other authors provided editorial advice.

Chapter 3, *The rhizosphere responds: rich fen peat and root microbial ecology after 11 years of water table manipulation*, is planned for submission to the journal Applied Environmental Microbiology in the near future. The following details co-author contributions: Merritt Turetsky and Evan Kane conceived and designed the long-term study-site. L. Jamie Lamit designed the core sampling and DNA isolation method protocols and ran statistics requiring the Primer® software. Stephen Techtmann and L. Jamie Lamit provided protocols and guidance for bioinformatics processing. Erik Lilleskov assisted in interpretation of fungal data. Danielle Rupp collected samples, isolated and cleaned DNA, analyzed the data, created figures, and wrote the manuscript. Other authors provided editorial advice.

Chapter 4, *Trace gas ^{13}C and pore water chemistry response to vegetation community and long term water table manipulation in an Alaskan rich fen*, is planned for submission to the journal Oecologia in the near future. The following details co-author contributions: Merritt Turetsky and Evan Kane conceived and designed the long-term study site. Evan Kane assisted and provided guidance with pore water sample collection. Danielle Rupp collected field samples and analyzed the data, created figures, and wrote the manuscript; other authors provided editorial advice.

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List of abbreviations

APEX: Alaska Peatland Experiment

BIX: biological index

CH_4: methane

CO_2: carbon dioxide

DOC: dissolved organic carbon

ESC: electron shuttling capacity

HIX: humification index

NDVI: Normalized Difference Vegetation Index

PFG: plant functional group

Sr: spectral ratio

SUVA: specific ultraviolet absorbance

TI: tryptophan index

TN: total nitrogen

Abstract

Boreal peatlands store approximately one third of the earth's terrestrial carbon, locked away in currently waterlogged and frozen conditions. Peatlands of boreal and arctic ecosystems are affected increasingly by shifting hydrology caused by climate change. The consequences of these relatively rapid ecosystem changes on carbon cycling between the landscape and the atmosphere could provide an amplifying feedback to climate warming. Alternatively, the advancement of terrestrial vegetation into once waterlogged soils could uptake carbon as a sink. Previous work suggests that fens will become an increasingly dominant landscape feature in the boreal. However, studies investigating fens, their response to hydrologic and vegetative change, and their carbon cycling dynamics are relatively few compared with other peatland types. This research investigates the biological and geochemical controls over carbon dioxide and methane cycling in a central Alaskan rich fen. The research concentrates on how these processes react to changes in water table and vegetation composition. The objectives of this body of research were to 1) Gain insights on how water table change affects carbon dioxide and methane transformation in a boreal rich fen from the pore water to the atmosphere; 2) Assess the mechanistic controls of specific boreal rich fen plant functional groups on carbon cycling; and 3) Profile the microbial community of a boreal rich fen and report on its response to water table change and specific plant functional groups. Although the oxidation of methane is prevalent in the studied rich fen, a raised water table and associated root exudates from greater sedge abundance fuels greater methane production than oxidation, for a net effect of greater methane production. However, the net methane that is released from the fen site is likely diminished compared with expected emissions due to the oxidizing nature of sedge, grass, and horsetail rhizospheres. Methanogens may also be in competition with other microorganisms for metabolic resources in this fen, which is recharged by the cyclic rewetting characteristic of these ecosystems. Overall, fens as a peatland type appear to have a resilience buffer in their carbon cycling response to hydrologic change more so than other peatland types.

1 Introduction

1.1 Socioecology of climate change in Alaska and northern latitudes

The boreal and arctic ecosystems of the north hold some of the most pristine, wild places left on Earth. Relatively unaffected by the developed and agricultural land that dominates much of the landscape in the United States, Europe, and Asia, these lands are dominated instead by coniferous forest, tundra, and wetlands. In the United States specifically, boreal and arctic wetland accounts for 43% Alaska's land area, as opposed to 5% of the land area of the lower 48 (Hall et al. 1994). Much of the area's pristine nature is due to the harsh conditions that make northern latitudes difficult for survival. The plants, fungi, microorganisms, and animals—including humans—that call the boreal and arctic home have adapted, biologically and/or behaviorally, to the conditions that be. Frigid temperatures, waterlogged soil, and the presence of permafrost—perennially frozen ground—limit winter survival, rooting activity, and building potential. As climate change affects northern latitudes at a disproportionately faster rate than the rest of the world (Stewart et al. 2013), these key natural conditions characteristic of the north are changing—and the landscape with it.

The consequences of climate-related change are widespread and their impact felt from the landscape to the people. Warming mean annual temperatures, precipitation regime shifts, rising sea levels, and more frequent extreme weather events are well documented in Alaska (Hinzman et al. 2005; Sulikowska et al. 2019). This summer, July 2019, saw the warmest month recorded in Alaska's history. Wildfire occurrence and intensity is increasing, affecting air quality, shifting the ecology of boreal forests, and speeding permafrost thaw (Kasischke and Turetsky 2006; Wolken et al. 2011). Homes and entire villages are being relocated in response to sea level rise and the collapse of once-frozen ground (Anisimov and Reneva 2006; Bronen and Chapin 2013; Perreault and Shur 2016). Fisheries—the core economy of much of coastal Alaska—are facing declines and failures due to warmer sea water (Welch et al. 1998; Farley et al. 2011; Loring 2013). Permafrost, a key structural and hydrologic component of boreal and arctic ecosystems, is thawing. The thaw of permafrost has the potential to release an immense volume of carbon, currently stored in frozen and waterlogged soil, to the atmosphere in the forms of carbon dioxide and methane (Oechel et al. 1993; Lawrence and Slater 2005; Dutta et al. 2006; Hinzman et al. 2006; Schuur et al. 2008). The thaw process also alters landscape hydrology, which is rapidly transforming the wetland-dominated landscape of Alaska.

1.2 Landscape transformation of boreal peatlands and the question of greenhouse gas implications

Peatlands, organically rich wetlands, are on the front lines of hydrologic change in Alaska and are often the ecosystem most directly affected by permafrost thaw. Boreal peatlands also store approximately one third of the earth's terrestrial organic carbon,

locked away in currently waterlogged and frozen conditions. The drying or further wetting of Alaska's peatlands leads to ecosystem change – ponding will lead to peatlands acting more like lake ecosystems, whereas drying will spur the advance of more terrestrial, upland vegetation. The consequences of these relatively rapid ecosystem changes on carbon cycling—greenhouse gases carbon dioxide and methane—between the landscape and the atmosphere could provide an amplifying feedback to climate warming (Martikainen et al. 1995; Komulainen et al. 1998; Johansson et al. 2006; Waddington and Day 2007). Alternatively, the advancement of terrestrial vegetation into once waterlogged soils could uptake carbon as a sink. There is evidence that, in the initial stages of landscape change, fens—peatlands that receive water from surface or groundwater flow—will become an increasingly dominant landscape feature (Lara et al. 2016). However, studies investigating fens, their response to hydrologic and vegetative change, and their carbon cycling dynamics are relatively few compared with other peatland types.

1.3 Dissertation overview

The increasing land area dominated by fens in Alaska, caused by permafrost thaw, calls for research examining carbon cycling dynamics in these hydrologically dynamic ecosystems. In this dissertation, my research investigates the biological (microbial and plant-driven) and geochemical (site conditions and molecular character) controls over carbon dioxide and methane cycling in a central Alaskan rich fen. The research concentrates on how these processes react to changes in water table and vegetation composition. Specifically, the objectives of this body of research were to:

1. Assess the mechanistic controls of specific boreal rich fen plant functional groups on carbon cycling
2. Profile the microbial community—the biological producers of carbon dioxide and methane—of a boreal rich fen and report on its response to water table change and specific plant functional groups
3. Gain insights on how water table change affects carbon dioxide and methane transformation in a boreal rich fen from the pore water to the atmosphere

In Chapter 1, *Plant functional group effects on peat carbon cycling in a boreal rich fen*, I intensively study carbon cycling dynamics of four dominant rich fen plant functional groups in a mesocosm experiment. Key findings of this study revealed that sedges, grasses, and horsetail plant functional groups create an oxidizing environment in the rhizosphere (the soil surrounding the roots). This leads to favorable conditions and evidence of methane oxidation—which converts geochemically produced methane, a more potent greenhouse gas, to carbon dioxide before release to the atmosphere. Sedges had a strong effect on the character of pore water carbon, depleting their rhizospheres of total dissolved organic carbon and introducing low quantities of simple carbon—likely in the form of root exudates. Marsh cinquefoil, an obligate wetland shrub and the fourth plant functional group, had higher pore water methane concentrations but suppressed

decomposition (which releases carbon dioxide) compared with all other treatments, including an unplanted treatment.

In Chapter 2, *The rhizosphere responds: rich fen peat and root microbial ecology after 11 years of water table manipulation*, I present a profile of the bacterial, archaeal, and fungal community in a boreal rich fen. Highlighting specifically the communities associated with dominant plant functional groups, I investigate how these communities and the bulk peat respond to long-term shifts in water table change. Key findings include the identification of two distinct groups of methane-producing archaea, one associated with deep peat and another associated with the rhizosphere and iron-cycling bacteria. Further, clades of bacteria associated with the breakdown of complex carbon were some of the key responders to water table change in this experiment, highlighting the need for further research on fen bacteria as carbon degraders. Additionally, findings highlight the need for further investigation of fungal endophytes and their role in plant relations and carbon cycling in these understudied ecosystems.

In Chapter 3, *Trace gas ^{13}C and pore water chemistry response to vegetation community and long term water table manipulation in an Alaskan rich fen*, I investigate the geochemical conditions in a boreal fen water table manipulation experiment to gain insights into the in-situ conditions surrounding carbon cycling. Using natural abundance $^{13}CH_4$ and $^{13}CO_2$, this study profiles the likely fate of trace greenhouse gasses from pore water to the atmosphere. The characterization of dissolved organic carbon gives insight to the molecular differences in the pore water caused by water table change over time, and the implications for trace gas production. The key finding from this study is that methane oxidation is prevalent in this fen site. However, a raised water table and resulting vegetation community shift to greater sedge presence—increasing root exudates as simple carbon sources for methane-producing archaea—outweighs the effects of methane oxidation in a flooded scenario.

2 Plant functional group effects on peat carbon cycling in a boreal rich fen*

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2.1 Abstract

Dominant plant functional groups (PFGs) found in boreal rich fens include sedges, grasses, horsetails, and cinquefoils (obligate wetland shrubs). Precipitation regime shift and permafrost thaw due to climate change will likely trigger changes in fen plant community structure through shifts in these PFGs, and it is thus crucial to understand how these PFGs will impact carbon cycling and greenhouse gas dynamics to predict and model peatland-climate feedbacks. In this study, we detail the above and belowground effects of these PFGs on aspects of carbon cycling using a mesocosm approach. We hypothesized that PFGs capable of aerating the rhizosphere (sedges, horsetails, and grasses) would oxidize the belowground environment supporting higher redox potentials, a favorable environment for decomposition, and higher CO_2:CH_4 in pore water and gas efflux measurements than PFGs lacking aerenchyma (cinquefoil, unplanted control).

Overall, sedges, horsetail and grasses had an oxidizing effect on rhizosphere pore water chemistry, producing an environment more favorable for methanotrophy during the growing season, as supported by an approximate isotopic enrichment of pore water methane ($\delta^{13}CH_4$) by 5‰, and isotopic depletion in pore water carbon dioxide ($\delta^{13}CO_2$) by 10‰, relative to cinquefoil treatments. Cinquefoil and unplanted control treatments fostered a reducing environment more favorable for methanogenesis. In addition, cinquefoil appeared to slow decomposition in comparison with the other PFGs. These findings, paired with PFG effects on oxidation-reduction potential and CO_2 and CH_4 production, point to the ability of rich fen plant communities to moderate

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biogeochemistry, specifically carbon cycling, in response to changing climatic conditions.

2.2 Introduction

Peatlands are common landscape features in boreal North America. Cool, hypoxic and acidic conditions as well as the input of plant litter that is difficult to decompose have resulted in the accumulation of soil organic matter in these ecosystems, with peatlands storing an estimated one-third of the terrestrial soil carbon (Gorham 1991; Yu 2012). As climatic change disproportionately affects northern landscapes (Stewart et al. 2013), changes in temperature and hydrology (Hinzman et al. 2005) will likely cause plant community shifts in northern peatlands, as has been demonstrated by numerous studies (Minkkinen et al. 1999; Weltzin et al. 2001; Laiho et al. 2003; Brancaleoni and Gerdol 2014; Churchill et al. 2014; Pedrotti et al. 2014; Potvin et al. 2014; Dieleman et al. 2015; McPartland et al. 2019). These vegetation community shifts may have consequences for belowground carbon cycling linkages (Jassey et al. 2013; Radu and Duval 2018). In fact, recent studies have demonstrated that plant community structure alone can have a stronger effect on belowground carbon cycling than established environmental drivers like temperature (Ward et al. 2015; Dieleman et al. 2017).

A growing number of studies have found plant functional groups (PFGs) to be an effective predictor of carbon cycling dynamics in northern peatlands (Chapin III et al. 1996; De Deyn et al. 2008; Ward et al. 2009). Mosses, particularly *Sphagnum* spp., are a widely studied PFG in peatland science (Crum and Planisek 1992; Asada et al. 2003; Basiliko et al. 2004; Turetsky et al. 2008a; Turetsky et al. 2012; Dieleman et al. 2015). Two main vascular PFGs have been widely studied in peatland literature, namely ericaceous shrubs (e.g., *Vaccinium* spp., *Chamadaphne* spp., etc.) and sedges (e.g., *Carex* spp.). Shrubs in the Ericaceae family are characterized by recalcitrant leaves and shallow roots with mycorrhizal associates. Belowground linkages to carbon cycling in ericoid shrubs include the capability to suppress decomposition in the rhizosphere due to antagonistic relationships between soil saprotrophs and ericoid fungal mycorrhizal associates (Gadgil and Gadgil 1975; Read et al. 2004; Romanowicz et al. 2015; Wiedermann et al. 2017). Sedges are monocots with a graminoid form and are known to contain aerenchymous tissue that can passively aerate the soil rhizosphere. The oxidized rhizosphere can increase the availability of energetically favorable electron acceptors needed to support relatively rapid, aerobic decomposition and methanotrophy in an otherwise anoxic environment (Holzapfel-Pschorn et al. 1986; Whiting and Chanton 1992; King et al. 1998; Strack et al. 2017). Conversely, sedges also release labile root exudates into deep peat horizons, which stimulate trace gas production as a form of rhizosphere or microbial priming (Mary et al. 1993; Chanton et al. 1995; Glaser and Chanton 2009). The net effect of both the addition of electron acceptors (O_2) and electron donors (labile carbon) by sedges appears to have different consequences for carbon cycling depending on site, experiment, and other environmental variables.

Understanding the differential impacts of ericoid shrub and sedge PFGs has helped us better understand the complex controls of carbon cycling in many northern peatlands (Schaepman-Strub et al. 2009; Kuiper et al. 2014; Noyce et al. 2014; Potvin et al. 2014; Wiedermann et al. 2017). However, past studies of these PFGs have largely taken place in bogs and poor fens at the ombrotrophic end of the peatland gradient. In contrast, few studies have explored the importance of PFGs in more minerotrophic rich fens. Rich fens are one of the most common boreal peatland types in western North America (Vitt et al. 2000), and are an increasingly dominant component of the Alaskan landscape (Lara et al. 2016). Moreover, carbon cycling in these systems has been shown to be more sensitive to vegetation than in other types of peatlands (Turetsky et al. 2014).

Rich fens are commonly dominated by sedges, but understudied PFGs like horsetail (e.g., *Equisetum fluviatile*), marsh cinquefoil (e.g., *Comarum palustre* L.), and grasses (e.g., *Calamagrostis* spp.) (Rydin and Jeglum 2013) can also make up significant percentages of the plant community. Horsetail is part of an ancient phenology of plants belonging to the family Equisetaceae that reproduces by spores. The silica-containing stems of the plant are divided into a series of hollow segments that rise perpendicularly from a horizontal subterranean rhizome which maintains the hollow, segmented structure (Hauke 1979). Marsh cinquefoil is an obligate non-ericoid wetland shrub in the family Rosaceae (USDA 2018). It is characterized by sprawling stems that run above and below the water table, often parallel to the earth, rooting shallowly along the length of the stem and rising up to produce small clumps of stems and leaves. In contrast, grasses such as *Calamagrostis* spp. form dense root clumps and have been purported to contain aerenchymous tissue (Landhäusser and Lieffers 1994). The effects of these widely prevalent yet understudied PFGs on carbon cycling dynamics may be key for understanding indirect effects of climate change on rich fens through changes in plant community composition.

By understanding the effects of PFGs we can better understand the large degree of variation in anaerobic carbon cycling observed in many peatland soils, perhaps via impacts on organic terminal electron acceptors. The ratio of CO_2:CH_4 production should be 1:1 under anaerobic conditions that support methanogenesis (Conrad 1999); however, multiple studies report CO_2:CH_4 values in excess of 1:1 under these conditions (Valentine et al. 1994; Vile et al. 2003; Keller and Bridgham 2007; Estop-Aragonés et al. 2013; Fan et al. 2013; Kane et al. 2013; Olefeldt et al. 2017). A growing body of literature indicates that dissolved and solid-phase organics may be responsible in part for these trends by functioning as regenerable electron acceptors in peats with fluctuating oxidation-reduction (redox) conditions (Keller and Takagi 2013; Klupfel et al. 2014). In fact, a recent study by Agethen et al. (2018) demonstrated that in-situ regeneration of dissolved organic electron acceptors in peatlands, caused in part by cyclic rewetting and root activity of various aerenchymous plants, suppressed methane production. However, exactly how different PFGs mediate these redox conditions controlling trace gas production is poorly resolved in rich fen ecosystems.

Here, we investigate PFG effects on trace gas production, decomposition, dissolved and solid phase organic matter, pore water and litter leachate chemistry, and peat redox dynamics in a mesocosm experiment. Specifically, we hypothesized that PFGs capable of aerating the rhizosphere via aerenchyma or hollow rhizomes (sedges, horsetails, and grasses) would oxidize the belowground environment supporting higher redox potentials, a favorable environment for decomposition, and higher CO_2:CH_4 in pore water and gas efflux measurements than in PFGs lacking aerenchyma (cinquefoil, unplanted control). Conversely, we hypothesized that PFGs without these traits would be associated with lower redox potentials, an unfavorable environment for decomposition, and lower CO_2:CH_4 ratios.

2.3 Materials and Methods

2.3.1 Mesocosms

To test our hypotheses, 30 mesocosms were created using four dominant PFGs typical of rich fens in central Alaska. The PFGs included sedge (*Carex atherodes* Spreng.), grass (*Calamagrostis* spp. Michx), horsetail (*Equisetum fluviatile* L), and cinquefoil (*Comarum palustre* L.). Unplanted mesocosms were also used as controls (n=6 for all treatments). A mesocosm approach allowed us to control for peat microform (hummocks, hollows, and lawns), which strongly influences carbon cycling in a natural peatland setting (Strack et al. 2006a; Hribljan et al. 2017). In this study, all mesocosms consisted of peat collected from lawns. Peat and plants for the mesocosm experiment were collected in June 2017 from Pixie Fen (WGS 84: 64°42'04" N 148°16'50" W), a rich fen with pH 5.7-6.1 just outside of the Bonanza Creek Experimental Forest southwest of Fairbanks, Alaska. Pixie Fen is situated in a narrow alluvial plain within the floodplain of the Tanana River, and is characterized by *Sphagnum* and brown moss species in addition to the dominant plant species listed above. There are no trees within the fen except for a central island of birch and aspen. Interior Alaska experiences large temperature fluctuations, with a mean annual temperature of -2.9 °C and 269 mm of rain per year (Hinzman et al. 2006). The growing season is short, ranging from around mid-May to mid-August. In June, daylight reaches >21 hours/day.

Peat was collected from within one area of the fen with an expanse of lawn microtopography (25 m x 10 m) to a depth of approximately 30 cm, transported to Fairbanks, and homogenized by hand to remove large roots. Individual plants from the same area were carefully removed from the fen with a shovel in order to maintain the health of the fine roots. These were transported in water back to Fairbanks and randomly assigned to the relevant mesocosm treatment. Two to five individual plants of the same species were planted in homogenized peat in 3.8 L (roughly 17 x 26.5 cm) glass jars (approximately 1.2 kg peat dry mass equivalent per jar) with a spigot 3 cm from the base of the jar. The number of plantings in each mesocosm was based on the visually assessed consistency of biomass going into each jar. For example, horsetails and sedges occupy much less space than cinquefoils or grasses, so more individuals were planted in those

mesocosms to attain a similar plant density per jar as the other treatments. Measured differences in biomass were accounted for in statistical analyses (described below). Peat was gently but firmly packed around the plants to a constant height within the jar, and leveled to control for microtopographical effects (i.e., creating a "lawn" environment in each mesocosm as opposed to hummocks and hollows). Approximately 40 cm of vinyl tubing was secured around the spigot using plumber's tape covered with electrical tape. The tubing was wrapped around the jar and secured 3 cm below the location of the peat surface within the jar to maintain the water level within the jars at a constant, as conducted by Dieleman et al. (2017). This design also allowed for water flow through every time more water was added, mimicking fen system hydrology (constant inflow and outflow from surface/groundwater). Each jar was wrapped with a white canvas shroud to exclude light. The water levels within the mesocosms were checked throughout the week, depending on the weather (more often on hot, dry days). Water for the mesocosms was collected approximately bi-weekly from a rich fen with standing water about 1 km from the source site and stored in opaque plastic jugs. Mesocosms were randomly assorted in a fenced-in open area on the University of Alaska (Fairbanks) campus, exposed to all the natural elements that would be present at Pixie Fen. The mesocosms were established in early June 2017 and the experiment was ended in September 2017 to coincide with senescence in the field.

Two weeks after the initiation of the mesocosm experiment, decomposition bags consisting of a pre-weighed Whatman 0.2 μm cellulose filter inside a 1 x 1 mm screen 8.5 x 9.5 cm mesh bag were installed into each mesocosm to a depth of 10 cm. Bags were removed at the close of the mesocosm experiment (after 13 weeks of decomposition), gently rinsed to remove peat, and dried at 65 °C before weighing the remains of the filter to determine mass loss by decomposition. An overview of measurements collected throughout the course of the study is summarized in Table 2.1.

2.3.2 Pore water collection and analysis

Pore water samples were collected on three dates during the growing season after allowing one month for plant acclimation (July 14, 20, and 27, 2017) and once during fall senescence (September 2017). A stainless steel "sipper" (0.52 cm diameter tubing with 2 cm slotted region at the end), was carefully inserted into the peat to the bottom of the mesocosm (a depth of 20 cm below the peat surface), about 5 cm from the main plant stems. Pore water was drawn up through the sipper into a syringe that was rinsed first with deionized (DI) water, then with sample prior to aliquot collection. The first aliquot was used to measure the oxidation-reduction potential of the water using an oxidation-reduction (Eh) probe (Hach Co., Loveland, Co., USA, Intellical MTC301) connected to a sealed and sample-purged flow-through cell, following Romanowicz et al. (2015). All Eh values were normalized to a pH of 7 (Eh7), based on pH – Eh relationships for Quinhydrone (Bier 2009). The second aliquot was injected into a 125 mL dinitrogen gas flushed and evacuated Wheaton® glass vial capped with a butyl rubber septa via a 0.45 μm Whatman syringe filter and needle. The needle hole was immediately covered with

vacuum grease and wrapped in parafilm upon removal of the needle. A third aliquot was filtered into 60 mL high density polyethylene (HDPE) Nalgene bottles for analysis of ions and organic acids (Dionex® ICS-2000 ion chromatograph with an IonPac AS11 separator column; Thermo Fisher, Sunnyvale, CA). The final pore water aliquot was run immediately on a field spectrophotometer using SpectraWiz® spectroscopy software (StellarNet Inc., Tampa, FL). Ultraviolet absorbance at λ=195-1000 nm were determined using a 1 cm quartz cuvette averaged over 5 reads.

Headspace gases were collected from the glass pore water vials within 2 hours of field collection. Bottles were shaken vigorously for one minute, after which two aliquots of 12 mL of gas were removed and injected into dinitrogen gas flushed and pre-evacuated 12 mL Exetainer^{TM} vials. Isotopic composition ($\delta^{13}C$) and concentrations of CO_2 and CH_4 in gas samples were measured at the UC Davis Stable Isotope Facility, CA, USA.

The remaining gas-stripped pore water samples were stored upside down on ice packs and shipped to the Northern Research Station in Houghton, MI, USA for further processing. Pore water was extracted from each of the Wheaton vials and run on a fluorometer (Horiba-Jobin Yvon Aqualog C; Horiba Co., Edison, NJ) to simultaneously collect ultraviolet-visible absorbance and florescence spectra. Run parameters were excitation: 240 to 600 nm in 3-nm increments; emission: 212 to 608 nm by 3 nm bandpass; integration time = 0.25 s. Samples with absorbance greater than 0.6 at λ 254 ($A_{254} > 0.6$) were diluted with DI water, such that they were $0.20 < A_{254}(\text{sample}) < 0.6$ to satisfy the assumption of detector linearity required by modeling (Stedmon and Bro 2008; Lawaetz and Stedmon 2009). Data post-processing and correction for inner filter effects were as described in detail by Veverica et al. (2016), following Stedmon and Bro (2008) and Lawaetz and Stedmon (2009). Absorbance and fluorometric indices were calculated to characterize dissolved organic matter in all pore water samples. The remaining pore water was acidified to pH 2 with concentrated HCl acid and analyzed for dissolved organic carbon and total dissolved nitrogen (TDN) on a Shimadzu® Total Organic Carbon Analyzer with a TDN module (Shimadzu Scientific Instruments, Columbia, MD).

2.3.3 Trace gas flux measurements

Chambers for dark CO_2 and CH_4 efflux measurement were constructed from 10.2 cm inner diameter x 61.5 cm opaque polyvinyl chloride (PVC) pipe to create an exact fit between the top of the mesocosm and the chamber. Due to the small size of the chambers, no internal fans were installed. Chambers were lined with closed cell foam weather stripping to ensure a complete seal. Dark static chamber CO_2 efflux measurements (Carroll and Crill 1997) were collected using an Infrared Gas Analyzer (IRGA; EGM-4; PP-Systems International, Amesbury, MA). Changes in CO_2 concentrations were measured in the headspace of each chamber for ~3 minutes. Methane efflux was measured by taking a syringe of gas from a collection tube with a stopcock attached to the chamber every 5 minutes for 30 minutes. Gas was mixed within the collection tube and chamber prior to final sample using a syringe. Methane was analyzed within 24 hours on a gas chromatograph (Varian 3800 FID detector; Varian Analytical Inc., Palo Alto,

CA) calibrated with three concentration standards daily (0, 10.22, and 100 ppm). Standards were run every 14 samples to ensure read accuracy. Each flux was examined visually for linearity and none were discarded ($R^2 \geq 0.50$).

2.3.4 Peat core electron shuttling assay

Small sharpened PVC corers 1.9 cm in diameter and 20 cm long were used to core the mesocosms twice within the time period of the experiment, once on July 24 and again on September 25. Sharpened PVC corers were rotated as inserted, to cut the peat. Inserted corers were capped, allowing suction to remove intact core, and upon extraction the bottom end was flushed with pore water extracted from the bottom of the hole from whence the core came and capped (PVC end caps). Closed PVC tubes were placed within the holes created by core collection to minimize further impact to the mesocosms. Cores were shipped on ice to Chapman University (Orange, CA, USA) where electron shuttling assays were performed on the peat, as described in detail by Keller & Takagi (2013). Briefly, capped PVC cores were brought into an anaerobic chamber (Coy Laboratory Products, Grass Lake, MI) for processing. The cores were opened, pore water was decanted into 50 mL centrifuge tubes and peat was transferred to plastic weigh boats where it was gently mixed with forceps. Approximately 3 g of field-moist peat was added to the 50-mL centrifuge tubes containing pore water. Tubes were removed from the anaerobic chamber and centrifuged at 4100 rpm for 5 minutes. The centrifuge tubes were returned to the anaerobic chamber, pore water was decanted and filtered (Whatman GF/F) and 0.25 mL of filtered pore water was added to 1 mL of 5 mM ferric iron complexed with nitriloacetic acid (Fe(III)-NTA). The resulting formation of Fe(II) was measured using buffered ferrozine (0.1% ferrozine in HEPES buffer, pH = 7.0) to quantify dissolved electron shuttling capacity (ESC). Fifteen mL of 5 mM Fe(III)-NTA was added to the centrifuge tubes still containing peat, tubes were shaken, removed from the anaerobic chamber and centrifuged at 4100 rpm for 5 minutes. A 0.10 mL aliquot of the supernatant was used to measure Fe(II) using ferrozine to quantify ESC. The centrifuge tubes were dried to constant mass and solid-phase ESC was corrected for dissolved ESC by the pore water in the peat sample.

2.3.5 Plant biomass and ancillary measurements

Stem counts, plant heights, and depth to peat from top of mesocosm jars were measured twice during the experiment, once on July 9 and again on July 27. Dimensions were used to calculate air volume for gas efflux measurements. Upon take-down of the mesocosm experiment, aboveground biomass and belowground biomass were separated from the peat and dried to constant mass at 65 °C. The remaining peat was bagged and shipped to the Northern Research Station in Houghton, MI (USA) where it was frozen. This peat was used to calculate peat bulk density and moisture content. A subset of peat samples was analyzed using Fourier-transform infrared spectroscopy (Nicolet iS5 Series FTIR; Thermo Fisher Scientific) to test if inherent properties of the peat changed throughout the experiment, using the wavelength ratio 1060 cm^{-1}:1620 cm^{-1} (polysaccharides/lignin) to

calculate a substrate quality index (Basiliko et al. 2007; Hribljan et al. 2017). Leaf extracts were made from the aboveground biomass of each treatment by soaking 5 g dry mass equivalent of aboveground material (leaves and stems) in 50 mL of DI water for 72 hours at 7°C. Extracts were filtered through 0.45 µm Whatman filters. Leaf extracts were characterized for dissolved organic carbon quality, as described below.

2.3.6 Pore water and leaf extract characterization

A suite of absorbance and fluorometric indices were calculated to describe dissolved organic matter in pore water samples and leaf extracts. Specific ultraviolet absorbance (SUVA) was calculated by dividing absorbance at λ=254 by DOC concentration (SUVA 254) to evaluate changes in pore water aromaticity (Weishaar et al. 2003). Absorbance index E2:E3 (absorbance at λ250 / absorbance at λ365) was calculated, along with SUVA 254, using both the fluorometer and the field spectrophotometer to evaluate the character of DOM. E2:E3 expresses an inverse relationship to molecular size of DOM (De Haan and De Boer 1987; Avagyan et al. 2014). Fluorometric indices of statistical importance to this study include the humification index (HIX) (Ohno 2002), biological index (BIX) (Huguet et al. 2009), and tryptophan index (TI) (Fellman et al. 2009), and were calculated as described in detail by Veverica et al. (2016).

2.3.7 Statistical analysis

We monitored Eh7 conditions for three campaigns in June to track experiment equilibration, which was reached in July (Appendix Figure 2.A.1). All statistical analysis and graphing was completed in R version 3.4.1 open source software, using packages nlme (Pinheiro et al. 2014), lsmeans (Lenth 2016), and ggplot2 (Wickham 2016). Spearman's correlations were run among all variables to identify potential relationships. Type II mixed effects models (nlme function lme) were run on all measurements to test differences among plant functional type treatments for a given response variable (CH_4 and CO_2 efflux; concentration and isotopic fractions of CH_4 and CO_2 in pore water; oxidation-reduction potential (Eh7); fluorometric indices BIX, HIX, TI, and E2:E3; DOC and TDN; and ions). All mixed effects models used belowground biomass and plant treatment as fixed effects, and individual mesocosm I.D. as a random variable to account for repeated measurements. Changes in belowground biomass co-occurring with PFG treatment were accounted for by running belowground biomass as the first effect in the fixed effects models. Residuals were visually inspected for normal distribution and data were log transformed when necessary to meet model assumptions. Models were run separately by season; July data (3 campaigns) were pooled for the growing season model, and September data (1 campaign) were run separately for the end-of-experiment (late season) model. Multiple comparisons of means and Tukey Honest Significant Difference Test (HSD) post-hoc analysis was used to compare treatments using the R package lsmeans (adjust = "tukey"), the results of which are found in Appendix Table 2.A.1. Statistical significance was accepted at $p<0.05$, and trending relationships $0.05<p<0.07$.

2.4 Results

Belowground biomass, which varied by PFG, strongly affected CO_2 efflux, CH_4 concentration in pore water, $\delta^{13}CO_2$ in pore water, SUVA 254, BIX, E2:E3, TDN, C:N ratio, phosphate, and chloride concentrations throughout the entire season (Table 2.2). Eh7 was significantly lower (more reduced) in the unplanted and cinquefoil treatments (-100± 100 mV and -50 ± 60 mV, respectively) than in the sedge treatment in September (202mV ± 7; Table 2.2; Figure 2.1; Appendix Figure 2.A.1).

Across all treatments, there was a negative relationship between Eh7 and pore water CH_4 (Figure 2.2). There were significant PFG effects on Eh7 and pore water CH_4 (Table 2.2). When adjusted for PFG treatment, there was still a significant effect of Eh7 on CH_4 (ppmv) in analysis of covariance ($F=12.99$, $p<0.001$). The slopes between Eh7 and CH_4 (ppmv) for PFGs that transport gases via aerenchyma or hollow stems ranged from -4.5 to -6.7, while the cinquefoil treatment had a slope of -1.2 (Figure 2.2), although there was no significant interaction between PFG and Eh7 in determining CH_4 concentrations ($F=0.47$, $p=0.76$). Most varied were the sedge treatment, which produced lower concentrations of pore water CH_4, and the unplanted treatment, which produced higher concentrations of CH_4 over a similar Eh7 range—suggestive that CH_4 concentrations were not caused strictly by environmental conditions but likely by the presence/absence of PFGs (Figure 2.2). Sedges had a significantly lower concentration of pore water CH_4 than the unplanted treatment ($p=0.0048$) and trended lower than the cinquefoil treatment ($p=0.0576$) after accounting for changes in Eh7 (mixed effects model, $F = 4.28$, $p = 0.009$). The cinquefoil and unplanted treatments trended toward higher and more erratic CH_4 concentrations and lower Eh7 (Figure 2.2).

After the effects of belowground biomass were accounted for, the plant treatments had a significant ($p<0.0005$) effect on pore water $\delta^{13}CO_2$ (sedges and horsetail significantly more depleted than the cinquefoil and unplanted treatments, grasses falling in between); pore water $\delta^{13}CH_4$ (sedges, horsetail, and grasses less depleted than cinquefoil and No Plant treatments; Figure 2.3); TDN (plant treatments had lower TDN than the unplanted treatment); and pore water C:N ratios (all plant treatments had higher ratios than the unplanted treatment) throughout the entire season (Table 2.2; Appendix Table 2.A.1).

Although surface chamber-based measurements yielded no significant differences in gaseous CO_2:CH_4 ratios, sedges did produce significantly higher rates of CO_2 efflux during the growing season than cinquefoil and unplanted treatments (Appendix Table 2.A.1). Additionally, sedges produced a pore water CO_2:CH_4 ratio larger (July mean 30 ± 20 and September mean 200 ± 200) than expected (1:1). This ratio was significantly larger than cinquefoil and unplanted treatments in both peak growing season (means 7 ± 4, 8 ± 6 respectively) and at the end of the experiment (means 7 ± 3, 10 ± 5 respectively).

Methane efflux was not significantly related to biomass or treatment. However, belowground biomass and treatment (during the mid-season measurements) influenced pore water CH_4 concentrations (Table 2.2). The highest pore water CH_4 concentrations

were seen consistently in the unplanted and cinquefoil treatments, whereas sedges generally exhibited the lowest pore water gas concentrations throughout the experiment but for CO_2 concentration in September.

Dissolved organic carbon concentration (DOC) paired with SUVA 254 results suggest that sedges and horsetail presence resulted in lower concentrations of DOC with less aromatic carbon, whereas cinquefoil and unplanted treatments resulted in high concentrations of DOC composed of more aromatic carbon (Figure 2.1, relationship between DOC and SUVA 254 by plant type). This was supported by humic index (HIX) (Ohno 2002) data showing low humification in sedges, mid-range values in the grass and horsetail, and high humification in the unplanted and cinquefoil treatments. Biological Index (BIX) and Freshness Index (Parlanti et al. 2000) values were significantly higher in the sedge, horsetail, and grass treatments than the cinquefoil and unplanted treatments, and the spectral ratio (Sr) (Helms et al. 2008) was significantly higher in sedges than in cinquefoil and unplanted treatments (Appendix Table 2.A.1). No significant differences by treatment were detected for the redox-related indices examined, e.g. the Ca:Cc (Kothawala et al. 2012) and Cory Index (Miller et al. 2006).

Dissolved Organic Carbon (DOC), Eh7, $\delta^{13}CO_2$ in pore water, and SUVA 254 were strongly correlated ($R^2>0.6$, $p<0.01$), with variations among plant types in the slopes/intercepts of these relationships (Figure 2.1). Belowground biomass also exhibited tight trends with these variables, separated by plant type. The leaf tissue extracts also exhibited significant differences between DOC character in the BIX, TI, and Freshness indices, which were higher in horsetail than in all other treatments. Leaf extract spectral ratio (Sr) was relatively low in the sedge (not significant) and significantly lower in horsetail ($p=0.0054$) compared to all other treatments (Table 2.3).

Solid phase peat used in this experiment exhibited polysaccharide:lignin (1060 cm^{-1}:1620 cm^{-1}) ratios from 1.807 to 2.495 (mean of 2.091) from FTIR analysis, and were not different between the treatments at the end of the experiment ($p = 0.91$). Electron shuttling assays of solid phase peat did not statistically change by treatment, but in July the lowest electron shuttling capacity (ESC) reflecting high oxidation was observed in the sedge treatment. Conversely, the unplanted treatment had the highest mean ESC, showing a more reduced character of the solid-phase peat in July (Appendix Table 2.A.1). Values from the September ESC assay were highly variable across all treatments (i.e., cinquefoil had mean 10± 10 μmol e^- g^{-1} and grass had mean 3± 3 μmol e^- g^{-1}) and there were no significant differences in electron shuttling capacity across treatments in the end-of season cores ($p = 0.541$, data not shown).

There were no significant variations among treatments regarding the decomposition of cellulose ($F = 2.26$, $p=0.09$; Figure 2.4). However, a marginal trend was driven by changes in cellulose mass loss between the unplanted (mean 92.71% mass loss) and cinquefoil treatments (mean 68.79 mass loss; $p = 0.06$ with Tukey post-hoc comparison).

2.5 Discussion

Over the course of this four-month experiment, PFGs, including several understudied in peatlands, drove biogeochemistry in rich fen mesocosms. In particular, carbon cycling was highly influenced by the plant community's rhizosphere and functional traits—especially seen in changes in redox (Eh7). As we hypothesized, plants that had aerenchyma or hollow rhizomes (sedges, horsetail, grasses) tended to produce oxidized environments favorable for methanotrophy when we controlled for microtopographical effects. Sedges were particularly effective at oxidization of the belowground environment, followed by horsetails and grasses, as demonstrated by redox and isotope data (Table 2.2; Figure 2.3; Appendix Figure 2.A.1). Conversely, the non-aerechymal cinquefoil fostered a highly reduced belowground environment more suitable for methanogenesis, and showed signs of slowed decomposition (Figure 2.4). Peat by itself produced conditions relatively more favorable for methanogenesis, yet showed a higher decomposition rate than plant treatments. In this study, it was apparent that PFG had significant or trending effects on redox, trace gas production, character of the dissolved belowground environment, and decomposition.

2.5.1 Rhizosphere biogeochemical effects of PFGs

Pore water gas concentrations and isotopes showed PFG effects on rhizosphere redox and C cycling. Sedges, for example, had a significant oxidizing effect on pore water CH_4. Although low concentrations of pore water methane under sedges could point to methane venting to the atmosphere (Strack et al. 2017), no increase in CH_4 efflux was detected. Furthermore, a depletion of pore water $\delta^{13}CO_2$ in the sedge, horsetail, and grass treatments points to an enhancement of CO_2 production in these treatments; in this case we suggest the conversion of CH_4 to CO_2 by means of methanotrophy (Figure 2.3; Berger et al. 2018). Methanotrophy produces CO_2, which would explain the isotopic evidence of enhanced CO_2 production (Chasar et al. 2000; Knorr et al. 2008; Throckmorton et al. 2015; Zalman et al. 2018).This observation was further supported by a corresponding $\delta^{13}CH_4$ enrichment indicative of preferential consumption of ^{13}C-depleted CH_4. Conversely, cinquefoil and the unplanted treatments exhibit depleted $\delta^{13}CH_4$ paired with enriched $\delta^{13}CO_2$, suggesting relatively higher rates of methanogenesis by conversion of CO_2 to CH_4 (Galand et al. 2010). This finding is in agreement with Koelbener et al. (2009) who found that peat cores under cinquefoil (the shrub form) *Comarum palustre* L. produced higher CH_4 than other plant treatments (barring sedges, in their study). Therefore, it appears that in this mesocosm setting, either methanotrophy or a hindrance to methanogenesis is at play under sedges, horsetail, and possibly grasses, which possess aerenchyma or hollow rhizomes that allow air transfer deeper into the peat. These data suggest horsetails may have similar oxidizing effects as sedges in rich fens, though this plant functional group is relatively underrepresented in the literature (Marsh et al. 2000). Methanotrophy is very likely the driving force behind the observed trends in this study, which support observations by Agethen et al. (2018) and offer a partial explanation for the anomalous high CO_2:CH_4 ratios observed in northern boreal peatlands.

Dissolved organic matter responds faster to PFG-mediated changes in redox conditions than does solid phase organic matter. Dissolved and solid phase organic matter have been shown to transfer electrons in peatlands, with resulting consequences for redox-related reactions (Bauer et al. 2007; Heitmann et al. 2007; Blodau et al. 2009; Keller et al. 2009; Keller and Takagi 2013; Lau et al. 2015; Walpen et al. 2018). As variability in our solid-phase electron shuttling capacity assays was high, it may be that our treatments were too short in duration to manifest consistent changes in solid phase redox state. In contrast, pore water character responded to PFG and Eh7, and represented a rapid and non-destructive way to assess changes in redox conditions.

Contrary to expectation, all plant treatments tended towards slower cellulose decomposition rates than the unplanted treatment, suggesting that root priming effects either through plant-mediated oxygen delivery or through rhizodeposited carbon were not significant in the decomposition of the simple cellulose substrate. Because dissolved nitrogen concentrations were significantly lower (and C:N ratios significantly higher; Appendix Table 2.A.1) in the plant treatments than the unplanted treatment, it is likely that the plants were taking up nitrogen at a rate that limited saprotrophic microorganisms, depressing decomposition (Kaye and Hart 1997; Cheng and Kuzyakov 2005). Interestingly, decomposition was the most suppressed within the cinquefoil treatment (Figure 2.4), even though this plant group had the lowest root biomass, highest pore water dissolved nitrogen, and lowest pore water C:N ratio of the plant treatments by the end of the experiment. Paired with the observation that cinquefoil and unplanted treatments generally produced similar results except for in the decomposition assay (see Figure 2.1), this suggests a departure from decomposition dynamics as observed in the other treatments. As a potential explanation, there are documented reports of the inhibition of free-living saprotrophic decomposers by mycorrhizal communities (Gadgil and Gadgil 1971). Since mycorrhizal communities can obtain their carbon from host plants, it is thought that their enzyme systems focus more on nutrient mining rather than carbon uptake (Treseder et al. 2007; Hobbie et al. 2013; Lindahl and Tunlid 2015). Mycorrhizal associates of *Comarum palustris* have been documented (Schütte et al. 2019), along with several other plants of the family Rosaceae and genus *Comarum* (Kytoviita and Ruotsalainen 2007; Sudová and Vosátka 2008). If such mycorrhizal fungi were associated with the cinquefoil rhizospheres in this experiment, this may explain the slowed decomposition (Gadgil and Gadgil 1975; Wiedermann et al. 2017). Further investigation of the mycorrhizal effects on decomposition of this extensive rich fen shrub is warranted.

The findings of this experiment suggest that the sedge-dominated PFG resulted in DOM with higher redox potential and lower aromaticity than that of shrub dominated or bare peats, in agreement with Chanton et al. (2008), Corbett et al. (2013), and Dieleman et al. (2017). Taken together, our results indicate that increases in low-aromaticity DOC is likely driven by higher rhizodeposition in the sedge, grass and horsetail treatments than in the cinquefoil treatment. In contrast, the cinquefoil treatment DOM pool was dominated by large, aromatic compounds supported by high HIX and SUVA 254 indices (Appendix Table 2.A.1). While rhizodeposition has been reported to stimulate methanogenesis

(Chanton et al. 1995), we suggest that rhizodeposition and rhizosphere oxidation capabilities belong to the same PFGs in this study, with the oxidizing effect likely outweighing methanogenesis stimulation in the mesocosms.

2.5.2 Aboveground C inputs

Dissolved organic carbon analyzed from leaf extracts suggests that water-soluble leachates from aboveground biomass of horsetail is different from the other plant treatments. First, horsetail leaf extracts demonstrated higher values in the BIX (Huguet et al. 2009), Freshness (Parlanti et al. 2000), and Tryptophan Indices than in other treatments (Table 2.3, Appendix Table 2.A.2). It is possible that horsetail possesses leaf traits that distinguish its chemical character in leaf extracts. For example, horsetail is known to be enriched in silica, calcium and potassium. One possibility could be that the additional base cations present preferentially adsorb to certain fractions of the DOM (Ali et al. 2014; Sowers et al. 2018); however, this is yet to be tested. The elevated indices could also speak to circumstances under which the aboveground biomass was harvested. In all of the horsetail treatment mesocosms, young shoots grew up around mid-season to replace the older shoots, producing younger, fresher tissue likely composed of relatively undamaged amino acids and proteins as opposed to the older, senescent leaves of the other treatments. Leaf nutrient content was not analyzed in this experiment, but this could be a possible explanation for the elevated Freshness and Tryptophan Index values for horsetail (Fellman et al. 2009). Horsetail has also been shown to act as a "nutrient pump" in Alaskan peatlands due to their deep rooting nature, bringing nutrients such as phosphorus to the surface in elevated concentrations within their tissue (Marsh et al. 2000). Although HIX, a measure of the degree of humification of DOM, was not significantly different between horsetail and other treatments, the spectral ratio (Sr) of the sedge and horsetail treatments was low compared with other treatments. This suggests higher molecular weight DOM derived from leaf litter of these species. Interestingly, the belowground pore water DOM of sedge and horsetail showed the opposite trend, with a high spectral ratio (low molecular weight DOM), comparatively. The juxtaposition of more labile root-derived DOM with recalcitrant leaf litter inputs highlights the differences between surface and subsurface carbon inputs to the system by the same plant. In the case of this short-term mesocosm experiment, plant litter did not have much time to accumulate on the surface of the peat. Based on this fact and the aforementioned C cycling effects, it appears that the belowground inputs had a greater impact on carbon cycling than aboveground inputs. Future work of interest could include a comparison of dissolved organic carbon in long-term horsetail peat vs. sedge peat vs. grass peat (cinquefoil does not usually form a litter- dominant peat).

2.5.3 Aboveground/belowground biogeochemical linkages

In conclusion, it is apparent that plant functional groups affect both above and belowground carbon quantity and quality, with resultant effects on our conceptual understanding of carbon cycling pathways in rich fen ecosystems (Figure 2.5). Root

structure, biomass, and chemical activity can impact how PFGs interact with and affect their environment. Root biomass in particular drove significant changes in trace gas concentrations and effluxes, and has the potential to affect ecosystem respiration. In this ecosystem, root respiration accounts for approximately 40% of soil respiration, with additional variation in ecosystem respiration being controlled by water table position and vegetation composition (McConnell et al. 2013). Climate change could lead to higher precipitation and higher water tables in central Alaska, which would likely lead to higher abundances of gas-transporting PFGs such as sedge and horsetail (Churchill et al. 2014). While prior work suggests this could increase CH_4 efflux to the atmosphere (King et al. 1998; Strack et al. 2006; Strack et al. 2017), we suggest that upon controlling for microform there may be an oxidizing effect of sedge/horsetail proliferation, which could attenuate CH_4 efflux, as was found in Strack et al. (2006)'s drier sites (see also Dinsmore, 2009). These plants' ability to oxidize CH_4 under anaerobic conditions, in addition to their unique effects on pore water redox status, may explain in part the elevated CO_2:CH_4 ratios observed in central-Alaskan rich fens (Fan et al. 2013). As the landscape changes with the changing climate, vegetation community shifts will affect carbon cycling; whether it be oxidation of the belowground environment by hollow or aerenchymous plants like sedges, horsetail, and grasses, or via slowing of decomposition and lack of methanotrophy, as seen with obligate wetland shrubs such as marsh cinquefoil.

2.6 Acknowledgements

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2.7 Tables & Figures

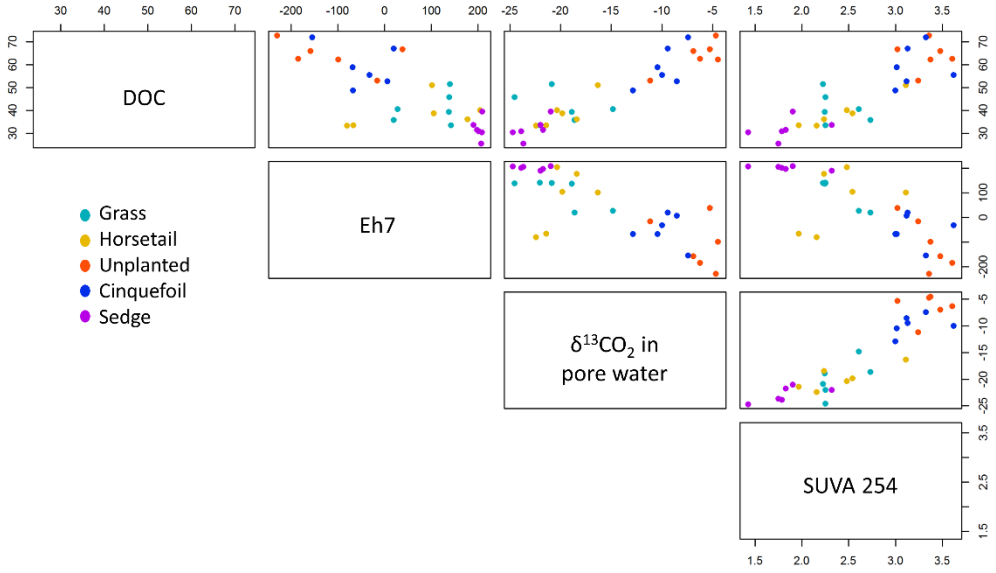


Fig. 2.1 Correlations between carbon quantity (DOC) and quality (SUVA 254), which influence environmental conditions (Eh7) and carbon reaction pathways ($\delta^{13}CO_2$). DOC is correlated with Eh7 (R^2=-0.53, p<0.01), $^{13}CO_2$ (R^2=0.83, p<0.01), and SUVA 254 (R^2=0.73, p<0.01). Eh7 is correlated with $^{13}CO_2$ (R^2=-0.62, p<0.01) and SUVA 254 (R^2=-0.59, p<0.01), and SUVA 254 is correlated with $^{13}CO_2$ (R^2=0.83, p<0.01).

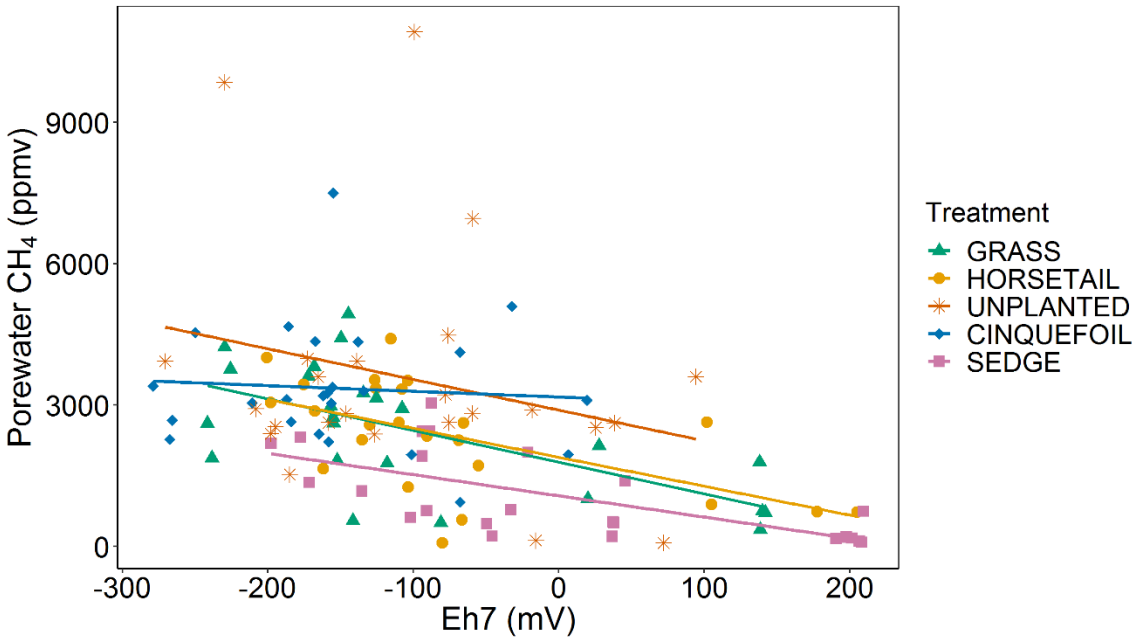


Fig. 2.2 Negative relationships between pore water Eh7 and [CH_4] by plant functional type. (overall $R^2 = 0.23$, $p < 0.001$). Sedges: y= -4.504x (SE=1.068) + 1073.447 (SE=142.690), $p<0.05$. Grass: y= -6.724x (SE=1.771) + 1788.319 (SE=275.627), $p<0.05$. Horsetail: y= -6.139x (SE=1.850) + 1892.782 (SE=243.595), $p<0.05$. Cinquefoil: y= -1.207x (SE=3.544) + 3171.111 (SE=601.967), $p=0.74$. Unplanted: y= -6.513x (SE=5.260) + 2893.146 (SE=736.769), $p=0.23$. Shown are data from all four campaigns

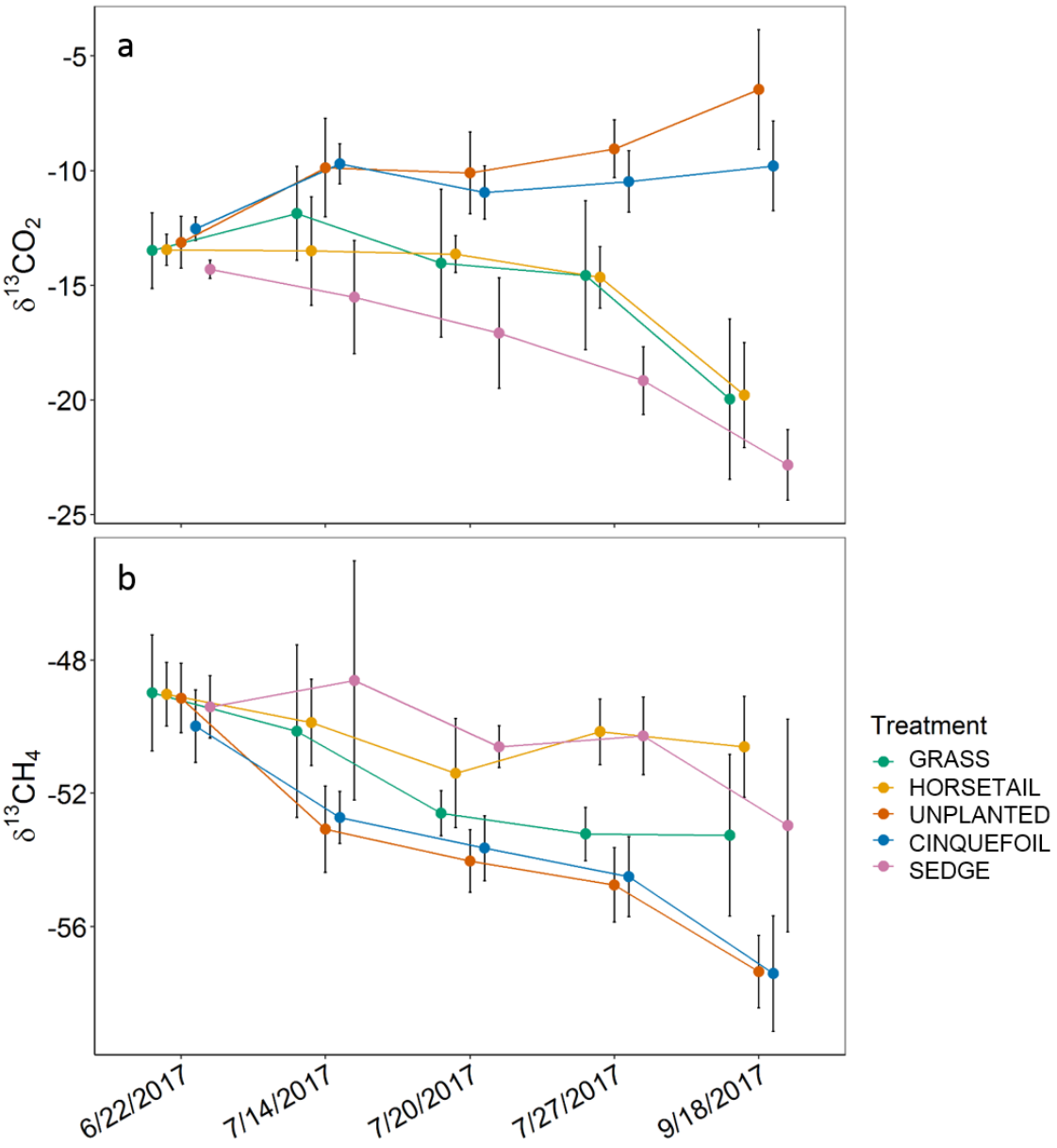


Fig. 2.3 ^{13}C natural abundance in pore water. (a) ^{13}C in methane was more enriched in the sedge, grass, and horsetail treatments than the cinquefoil and unplanted treatments ($p<0.05$), suggesting selective methanotrophy. (b) Enrichment of $^{13}CO_2$ in the unplanted and cinquefoil treatment compared to the other plant treatments ($p<0.05$) suggests more methanogenesis in these treatments, whereas the depletion of the sedge, grass, and horsetail further support the preferential conversion of light methane to carbon dioxide

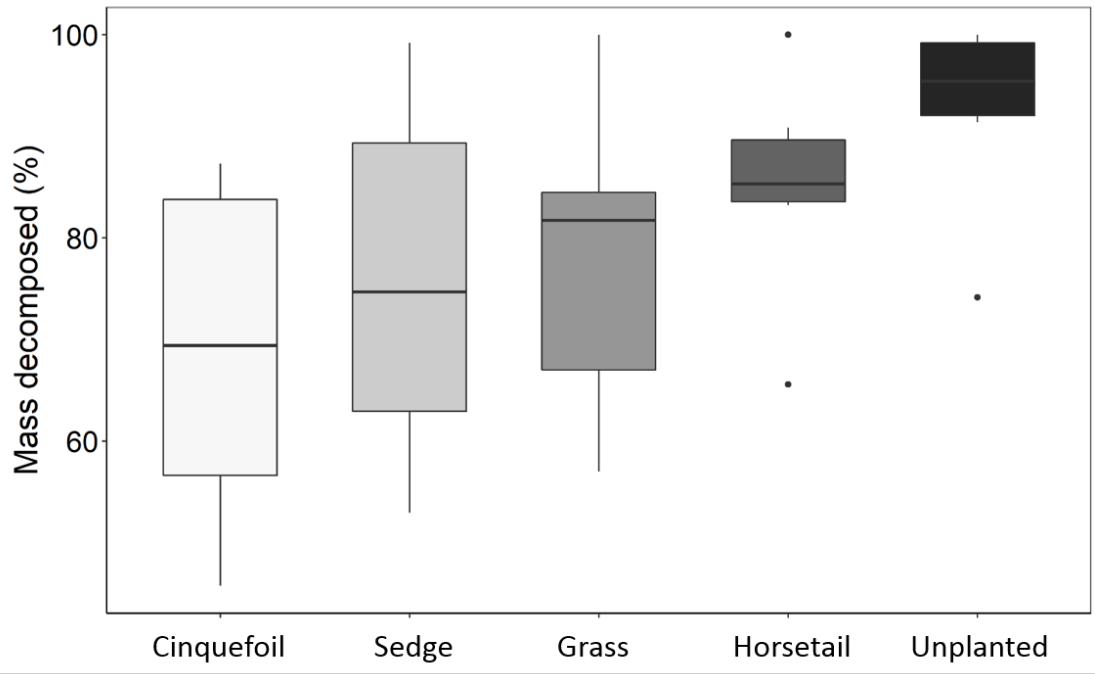


Fig. 2.4 Percent mass decomposed of a cellulose filter after 8 weeks of field incubation ($F = 2.26$, $p = 0.09$). Boxplot shows 25th and 75th percentiles and 95% confidence interval

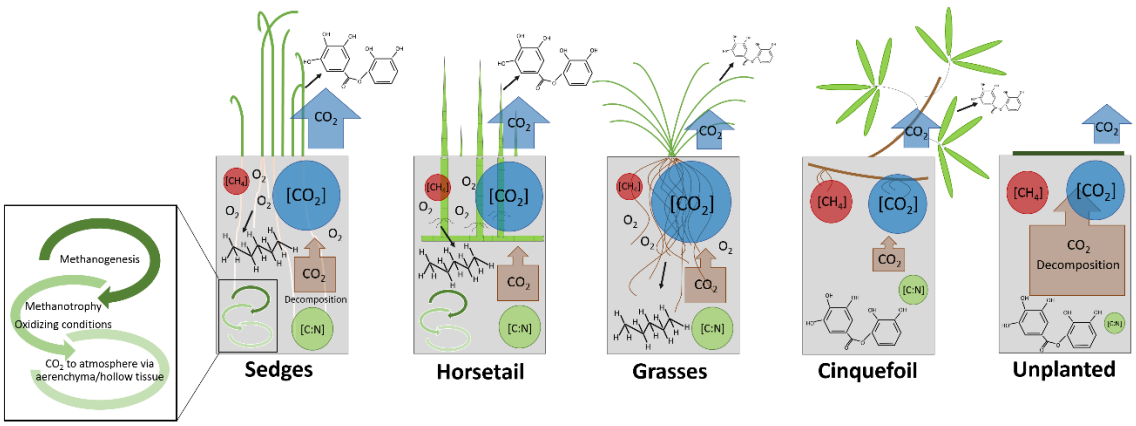


Fig. 2.5 Our conceptual understanding of C cycling in this rich fen as informed by mesocosm results. Sedges provided an oxidizing environment and root exudates (as represented by simple sugar in the diagram), but had recalcitrant above-ground inputs (as represented by aromatic compounds in the models). Horsetail acted like a sedge in the fen

environment. Grasses were the hardest to predict when it came to carbon cycling, but for the most part acted like sedges and horsetail with respect to Eh; this is likely due to their highly variable root mass and facultative aerenchymous rooting nature. Cinquefoil acted similarly to the unplanted control. This is likely due to its minimalist rooting structure; however, its association with mycorrhizae may slow decomposition in the rhizosphere (as represented by brown decomposition boxes in the diagram). In the unplanted treatment, microbes did not have to compete with plants for nitrogen (lower pore water C:N), enhancing the decomposition of organic matter and the model substrate cellulose; this resulted in poor quality (highly aromatic) carbon left behind

Table 2.1 Summary of measurements collected during the duration of the study

Activity/Measurement	Data obtained	# Campaigns
Chamber measurements	CH_4, CO_2 efflux	4
Porewater collection	$^{13}CO_2$, $^{13}CH_4$, DOC, TN, Spectral DOC characterization, Ions, Organic Acids	4
Oxidation-reduction potential	Eh7, monitoring treatment separation	7
Peat cores	Electron shuttling capacity of peat	2
Decomposition assays	Mass decomposed over study period	1
Biomass measurements	Aboveground and belowground biomass	1
Leaf extract characterization	Spectral character of leaf tea extracts	1
Peat mass	Bulk density	1
FTIR	Inherent peat characterization	1

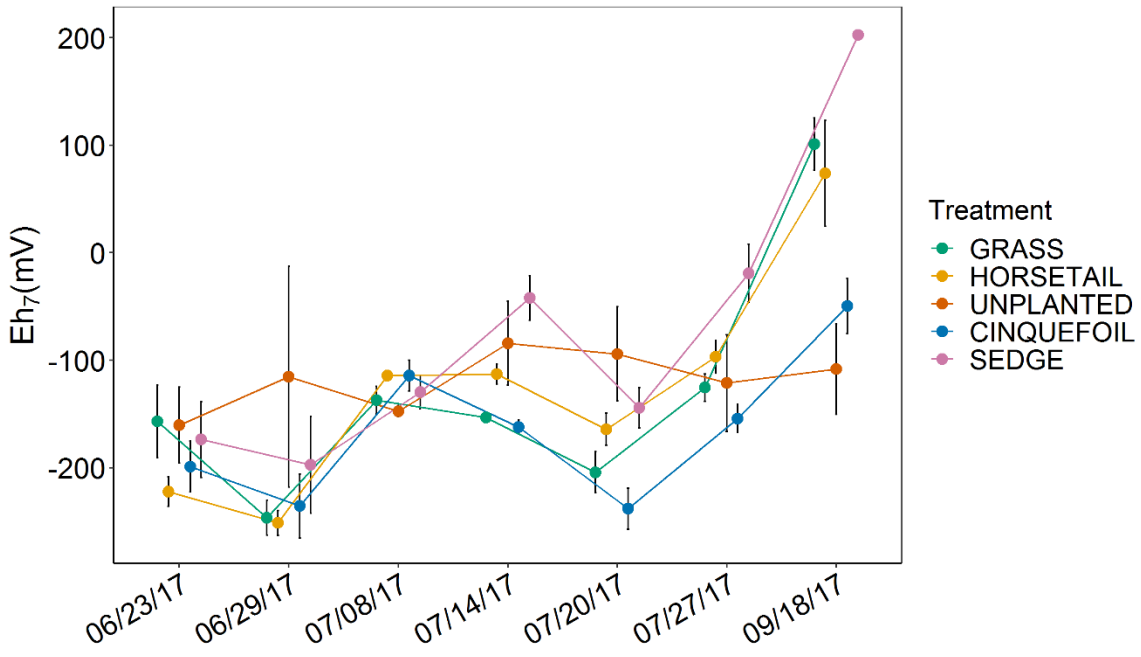
Table 2.2 Results of mixed effects modeling investigating plant functional type controls on gas and chemistry measurements from July (3 campaigns) and September (1 campaign) mesocosms. Shown first for each variable is the response (F-Value and p-value) to below-ground biomass; second is the response of the variable to the PFG treatment after accounting for below-ground biomass

	Mid-season		Late-season	
Predicted variable	***F-Value***	***p-value***	***F-Value***	***p-value***
CH_4 Efflux	0.165	0.6941	0.452	0.5184
	1.876	0.1989	1.105	0.4113
CO_2 Efflux	46.63	**<0.0001**	126.58	**<0.0001**
	1.401	0.2842	8.67	**<0.0001**
CH_4:CO_2 Efflux	2.346	0.1599	1.067	0.3287
	0.61	0.6659	1.112	0.4085
ppmv CH_4 in porewater	8.482	**0.0076**	7.495	**0.0115**
	5.439	**0.0029**	2.08	0.115
ppmv CO_2 in porewater	9.819	**0.0045**	0.308	0.5842
	2.63	0.0594	0.711	0.5927
$\delta^{13}CH4$	15.96	**0.0005**	2.079	0.1623
	17.69	**<0.0001**	20.361	**<0.0001**
$\delta^{13}CO2$	48.071	**<0.0001**	152.994	**<0.0001**
	14.801	**<0.0001**	34.177	**<0.0001**
Eh_7	0.287	0.597	25.86	**<0.0001**
	7.616	**0.0004**	7.223	**0.0006**
SUVA (254)	6.748	**0.0158**	61.815	**<0.0001**
	1.111	0.3742	16.751	**<0.0001**
BIX	5.91	**0.0229**	12.908	**0.0015**
	2.32	0.0856	12.223	**<0.0001**
HIX	1.74	0.1996	12.307	**0.0018**
	0.271	0.894	26.048	**<0.0001**
TI	3.143	0.0889	3.907	0.0597
	6.692	**0.0009**	2.826	**0.0472**
E2:E3	6.994	**0.0142**	14.473	**0.0009**
	1.013	0.4202	2.654	0.0578
DOC	0.782	0.3854	25.679	**<0.0001**
	1.095	0.3816	20.84	**<0.0001**
TDN	12.463	**0.0017**	34.037	**<0.0001**
	7.502	**0.0005**	19.763	**<0.0001**
Pore water C:N Ratio	27.032	**<0.0001**	60.17	**<0.0001**
	16.388	**<0.0001**	28.97	**<0.0001**
Phosphate	3.509	**0.0733**	No data	
	4.516	**0.0073**	No data	
Chloride	6.682	**0.0162**	No data	
	6.641	**0.001**	No data	

Table 2.3 Dissolved organic carbon characterization of pore water carbon and dissolved carbon made from leaf extracts. Shown first for each variable is the response (F-Value and p-value) to below-ground biomass; second is the response of the variable to the PFG treatment after accounting for below-ground biomass

	Late-season		Leaf teas	
Predicted variable	***F-Value***	***p-value***	***F-Value***	***p-value***
HIX	12.3074	**0.0018**		
	26.0482	**<0.0001**	2.435	0.0947
BIX	12.908	**0.0015**		
	12.223	**<0.0001**	14.029	**<0.0001**
Freshness	8.76	**0.0068**		
	9.624	**0.0001**	13.055	**<0.0001**
TI	3.9066	0.0597		
	2.8255	**0.0472**	11.591	**<0.0001**
Sr	9.207	**0.0057**		
	4.716	**0.006**	5.723	**0.0054**

2.8 Appendix 2.A: Supplemental Materials



Appendix Fig. 2.A.1 Oxidation-reduction potential measured over the seven campaigns of the mesocosm experiment with standard error, demonstrating that the treatment effects did not equilibrate until early July, whereupon it was decided to use only data from campaigns 4-7 for statistical analysis and comparison

Appendix Table 2.A.1 Mean values of all measurements throughout the course of the experiment. First section (green) represents July measurements, whereas the second section (yellow) represents measurements taken at the end of the experiment. E2:E3 and E4:E6 refer to pore water characterization ratios at given absorbance wavelengths. E2:E3 is inversely related to size of DOC (dissolved organic carbon). SUVA 254 is absorbance of DOC at λ 254 divided by the concentration of DOC in the sample. TDN is total dissolved nitrogen; Eh_7 refers to oxidation-reduction potential (higher values more oxidized); ESC is electron shuttling capacity (lower values are more oxidized); Sr has an inverse relationship to molecular weight; HIX, or humification index, is directly related to humification, with higher values indicating more humified DOC. BIX, Freshnass index, and TI in this context refer to the freshness of the material (higher values = more recently produced)

MEASUREMENT	Mid-season (July)														
	SEDGE			GRASS			HORSETAIL			CINQUEFOIL			UNPLANTED		
	Mean	SD	Post-hoc	Mean	SD	Post-hoc	Mean	SD	Post-hoc	Mean	SD	Post-hoc	Mean	SD	Post-hoc
Temperature (C)	28.11	3.05	A	27.07	3.62	A	25.37	2.06	AB	28.04	2.84	A	24.04	3.39	B
CH_4 Efflux	1.31	0.36	A	1.24	0.99	A	0.94	0.42	A	0.90	0.56	A	0.85	1.01	A
CO_2 Efflux	5.07	2.03	A	4.12	3.67	AB	2.81	1.20	AB	2.45	1.56	B	1.65	0.98	B
CO_2:CH_4 Efflux	4.23	1.94	A	9.17	13.87	A	3.33	1.78	A	3.84	3.82	A	6.94*	251.19	A
ppmv CH_4 in pore water	1353	896	A	2865	1223	B	2824	838	B	3210	817	B	3205	1341	B
ppmv CO_2 in pore water	28070	12409	AB	39510	14697	A	32239	15762	AB	22009	13641	B	22234	13055	B
CO_2:CH_4 in pore water	27.93	17.67	A	19.56	23.72	AB	11.66	5.22	B	7.12	4.26	B	8.40	5.92	B
$\delta^{13}CH_4$	-49.83	2.17	A	-51.98	1.99	B	-50.47	1.37	AB	-53.62	1.16	C	-53.95	1.23	C
$\delta^{13}CO_2$	-17.25	2.47	A	-13.49	2.86	B	-13.94	1.55	B	-10.38	1.15	C	-9.67	1.65	C
E2:E3 (A λ 250:365)	4.10	0.44	A	4.06	0.37	A	3.77	0.24	A	3.81	0.28	A	3.49	0.13	A
SUVA (λ 254)	3.52	0.24	A	3.68	0.50	AB	3.71	0.30	AB	3.68	0.53	AB	3.93	0.34	B
DOC (mg C/L)	72.35	10.09	A	77.92	12.54	AB	82.41	12.24	B	79.77	8.92	AB	80.96	8.42	AB
TDN (mg N/L)	2.16	0.50	A	2.88	1.09	A	2.50	0.55	A	2.78	0.42	A	4.84	1.36	B
C:N ratio (pore water)	34.21	3.98	A	28.94	5.66	B	33.53	3.42	A	29.14	4.18	B	17.65	3.88	C
Eh_7 (mV)	-68.40	76.04	A	-160.98	45.73	BC	-124.52	42.82	ABC	-184.76	50.38	C	-99.77	99.43	AB
ESC (µmol e/gwd)	3.22	6.24	A	8.10	5.16	A	8.17	6.50	A	8.39	7.98	A	11.79	10.23	A
Sr (λ 275/295:350/400)	0.81	0.06	A	0.81	0.03	A	0.83	0.05	A	0.84	0.08	A	0.80	0.03	A
HIX	20.15	4.39	A	18.71	2.80	A	20.12	4.21	A	20.08	2.90	A	20.90	3.79	A
Freshness index	0.47	0.02	A	0.48	0.02	A	0.47	0.02	AB	0.46	0.01	B	0.45	0.01	B
BIX	0.48	0.02	AB	0.48	0.02	A	0.48	0.02	ABC	0.46	0.02	BC	0.46	0.02	C
TI	1.46	0.16	A	1.88	0.40	B	1.62	0.25	AB	1.68	0.34	AB	1.77	0.28	B
Fluoride (ppm)	0.17	0.05	A	0.15	0.03	A	0.16	0.03	A	0.15	0.02	A	0.16	0.04	A
Chloride (ppm)	2.61	1.46	A	1.29	0.43	B	1.12	0.42	B	2.42	0.69	A	3.00	0.83	A
Sulphate (ppm)	0.14	0.04	A	0.13	0.04	A	0.16	0.04	A	0.15	0.04	A	0.14	0.03	A
Oxalate (ppm)	0.10	0.05	A	0.08	0.01	A	0.08	0.02	A	0.08	0.02	A	0.08	0.01	A
Nitrate (ppm)	0.06	0.03	A	0.08	0.04	A	0.08	0.05	A	0.07	0.04	A	0.08	0.04	A
Phosphate (ppm)	0.32	0.20	A	0.61	0.49	A	0.53	0.24	A	0.54	0.22	A	1.10	0.52	A
Formate (ppm)	0.11	0.05	A	0.14	0.07	A	0.11	0.05	A	0.12	0.04	A	0.10	0.04	A

MEASUREMENT	Late-season (September)														
	SEDGE			GRASS			HORSETAIL			CINQUEFOIL			UNPLANTED		
	Mean	SD	Post-hoc	Mean	SD	Post-hoc	Mean	SD	Post-hoc	Mean	SD	Post-hoc	Mean	SD	Post-hoc
Temperature (C)	12.73	0.54	B	18.03	1.91	A	10.87	1.69	BC	12.35	1.06	B	8.95	2.67	C
CH_4 Efflux	0.13	0.05	A	0.72	1.10	A	0.06	0.04	A	1.67	2.48	A	0.23	0.20	A
CO_2 Efflux	1.79	0.26	A	1.05	1.88	A	1.16	0.41	A	0.38	0.16	A	0.39	0.17	A
CO_2:CH_4 Efflux	16.11	6.06	A	79.84**	138.28	A	23.95	13.97	A	1.01	0.90	A	648.61**	1122.07	A
ppmv CH_4 in pore water	248	249	A	1127	689	AB	938	879	AB	3785	2348	AB	4613	4579	B
ppmv CO_2 in pore water	30822	15908	A	34502	11593	A	25326	6295	A	22766	14137	A	28152	16101	A
CO_2:CH_4 in pore water	218.78	236.92	A	38.07	19.14	AB	75.50	110.68	AB	6.99	3.17	B	9.86	4.95	B
$\delta^{13}CH4$	-52.96	3.04	A	-53.26	2.31	A	-50.60	1.44	A	-57.41	1.65	B	-57.35	1.04	B
$\delta^{13}CO2$	-22.83	1.46	A	-19.97	3.33	A	-19.79	2.18	A	-9.79	1.85	B	-6.47	2.49	B
E2:E3	5.80	0.44	A	5.32	0.37	A	5.35	0.24	A	4.98	0.28	A	4.81	0.13	A
SUVA (254)	1.83	0.29	A	2.38	0.22	B	2.41	0.40	B	3.20	0.24	C	3.35	0.20	C
DOC (mg C/L)	31.93	4.56	A	41.13	6.58	A	38.82	6.55	A	59.13	8.79	B	63.85	6.49	B
TDN (mg N/L)	0.98	0.147	A	1.27	0.24	A	1.12	0.17	A	2.45	0.80	B	4.62	1.28	C
C:N ratio (pore water)	32.53	1.41	A	32.76	3.13	A	34.81	2.64	A	25.31	4.48	B	14.48	2.90	C
Eh_7 (mV)	202.37	7.28	A	101.06	59.83	A	73.85	120.93	AB	-49.48	63.22	BC	-108.31	103.26	C
ESC (µmol e/gwd)	6.79	7.14	A	3.44	7.51	A	13.89	14.95	A	10.49	21.44	A	3.72	4.86	A
Sr (λ 275/295:350/400)	0.94	0.06	A	0.85	0.03	AB	0.87	0.05	AB	0.80	0.08	B	0.80	0.03	B
HIX	12.42	1.27	A	16.13	3.10	B	14.53	1.98	AB	20.59	1.71	C	20.48	1.13	C
Freshness index	0.55	0.02	A	0.53	0.02	A	0.55	0.03	A	0.48	0.02	B	0.48	0.02	B
BIX	0.57	0.02	A	0.54	0.02	A	0.56	0.04	A	0.48	0.02	B	0.49	0.02	B
TI	0.82	0.13	A	0.89	0.08	AB	0.90	2.55	AB	1.08	0.23	AB	1.11	0.12	B

* outlier removed

**large standard error due to 2 of 3 measurements being zero or very small, and the third being erratically large. Because so few measurements were taken, we decided to leave these values in to demonstrate the large variability in these treatments

Appendix Table 2.A.2 Mean values of post-experimental analysis, including leaf extracts. Fourier-transform infrared spectroscopy (FTIR) was performed to ensure no significant differences between cellulose and lignin content in peat between treatments. HIX, or humification index, is directly related to humification, with higher values indicating more humified DOC. BIX, Freshness index, and TI in this context refer to the freshness of the material (higher values = more recently produced). Sr has an inverse relationship to molecular weight. In post-processing peat material from the mesocosms, leaf extracts were not significantly different according to fluorometric redox activity (Ca:Cc and RI indices). E2:E3 is inversely related to size of DOC (dissolved organic carbon)

MEASUREMENT	*SEDGE*			*GRASS*			*HORSETAIL*			*CINQUEFOIL*			*UNPLANTED*		
	Mean	*SD*	*Post-hoc*	*Mean*	*SD*	*Post-hoc*	*Mean*	*SD*	*Post-hoc*	*Mean*	*SD*	*Post-hoc*	*Mean*	*SD*	*Post-hoc*
Decomposition (% mass loss)	75.78	18.54	A	77.98	15.74	A	85.04	11.32	A	68.79	17.16	A	92.71	9.70	A
Total biomass	21.08	7.45	AB	24.88	18.48	A	8.29	1.72	BC	6.52	2.26	BC	0.00	0.00	C
Above-ground biomass	4.76	1.63	A	4.90	2.48	A	0.96	0.27	BC	3.07	1.36	AB	0.00	0.00	C
Below-ground biomass	16.32	5.92	AB	19.98	16.16	A	7.33	1.51	ABC	3.45	1.02	BC	0.00	0.00	C
Cellulose in peat (FTIR λ 1160)	2.37	0.03	A	2.44	0.06	A	2.39	0.04	A	2.38	0.06	A	2.37	0.12	A
Lignin in peat (FTIR λ 1265)	1.51	0.04	A	1.49	0.10	A	1.51	0.04	A	1.51	0.02	A	1.50	0.09	A
Bulk density (g/cm^3)	0.43	0.11	AB	0.41	0.09	AB	0.51	0.05	A	0.35	0.03	B	0.41	0.08	AB
HIX (tea)	2.39	1.27	A	3.52	1.43	A	2.89	0.29	A	2.19	1.00	A	N/A	N/A	N/A
BIX (tea)	0.33	0.02	A	0.27	0.06	A	0.41	0.04	B	0.28	0.03	A	N/A	N/A	N/A
Freshness (tea)	0.33	0.02	A	0.26	0.06	A	0.40	0.03	B	0.27	0.03	A	N/A	N/A	N/A
TI (tea)	11.38	0.13	AB	9.46	2.64	BC	14.65	2.55	A	5.92	1.53	C	N/A	N/A	N/A
Sr (λ 275/295:350/400) (tea)	0.17	0.07	AB	0.28	0.20	B	0.09	0.08	A	0.34	0.07	B	N/A	N/A	N/A
Ca:Cc (tea)	1.20	0.06	A	1.07	0.33	A	1.34	0.50	A	1.20	0.37	A	N/A	N/A	N/A
RI (tea)	0.13	0.02	A	0.15	0.05	A	0.17	0.06	A	0.17	0.05	A	N/A	N/A	N/A
E2:E3 (tea)	7.61	0.44	A	8.06	1.86	A	7.56	0.92	A	6.32	0.85	A	N/A	N/A	N/A

3 The rhizosphere responds: rich fen peat and root microbial ecology after 11 years of water table manipulation

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3.1 Abstract

Hydrologic shifts due to climate change will affect the cycling of the immense volume of carbon (C) stored in boreal peatlands. C cycling in these systems is carried out by microorganisms and plants in close association. This study investigated the microbiome of a boreal rich fen, to understand how root and bulk peat communities are influenced by experimentally lowered (drought scenario) and raised (flooded scenario) water tables. All samples were sequenced and processed for bacterial, archaeal (16S rDNA—V4), and fungal (ITS2) DNA. Rooting zone (10-20 cm) bacterial and archaeal populations were most affected by water table treatments in this study. There were two main methanogen communities: rooting zone (dominated by the archaeal family Methanobacteriaceae) and deep peat (dominated by family Methanomicrobiaceae). Iron cyclers, particularly members of the family Geobacteraceae, were enriched around the roots of plants that transport oxygen to the rhizosphere—sedges, horsetails, and grasses—and may associate with methanogens in close proximity to the roots. The shrub marsh cinquefoil had significantly lower bacterial and archaeal diversity in its roots than the other plants, including a low relative abundance of methanogens. Some bacteria associated with the hydrolysis of complex C (creating precursor compounds for methanogenesis) were enriched by a raised water table in the rooting zone. The fungal community was affected largely by plant functional group, especially cinquefoils. Fungal endophytes (particularly *Acephala* spp.) were enriched in sedge and grass roots, which may have underappreciated implications for organic matter breakdown and cycling.

3.1.1 Importance

This study demonstrated that root-associated methanogens, iron cyclers, and fungal endophytes are overlooked in boreal fen biogeochemistry, and are likely directly or indirectly affecting carbon cycling in these ecosystems. These taxa, all associated with grass and sedge roots, should be investigated in future research as projected higher precipitation rates may lead to an increased abundance of sedges and grasses in boreal fens.

3.2 Introduction

The boreal region is disproportionately affected by climate change, where precipitation and temperature changes are occurring at a faster rate than in other areas of the world (Hinzman et al. 2005; Kunkel et al. 2013). Peatlands, which store up to 1/3 of soil carbon (Gorham 1991), are particularly susceptible to water table and vegetation shifts (Minkkinen et al. 1999; Frolking et al. 2006; Walker et al. 2006; Churchill et al. 2014; Dieleman et al. 2015). There is a developing body of literature highlighting mechanisms of peatland C cycling change with water table position (Moore and Roulet 1993; Megonigal et al. 2003; Blodau et al. 2004; Chivers et al. 2009a; Knorr et al. 2009; Kane et al. 2013; Ballantyne et al. 2014). There are fewer studies examining the role of plant functional groups (PFGs) as drivers of peatland biogeochemistry (Ward et al. 2009; Dieleman et al. 2017; Radu and Duval 2018; Rupp et al. 2019). Rich fens, which are typically characterized by surface- or groundwater-fed hydrology, are especially sensitive to plant community shifts as the climate changes (Turetsky et al. 2014). One of the many reasons that plants are effective drivers of biogeochemistry is their intimate and complex relationship with microorganisms, which are understudied in northern rich fens (Fisk et al. 2003; Jaatinen et al. 2008; Winsborough and Basiliko 2010; Lin et al. 2012) and are directly involved in carbon transformation processes.

Studies of northern peatland microbiomes have gathered baseline microbial community data in different peatland types and biogeochemical gradients (ombrotrophic bog, poor fen, rich fen, etc.)(Lin et al. 2012; Serkebaeva et al. 2013). Because of these studies, we have learned that microbial activity in many northern fens may be dominated by bacteria as opposed to fungi (Winsborough and Basiliko 2010; Lin et al. 2012; Myers et al. 2012). Bacterial and archaeal phyla commonly present include the Proteobacteria, Acidobacteria, Actinobacteria, Chloroflexi, Planctomycetes, Euryarchaeota, Bacteroidetes, and Verrucomicrobia (Juottonen et al. 2017; Potter et al. 2017), whereas fungi are generally overrepresented in the Ascomycota, often dominated by the order Helotiales (Wang et al. 2019). Fungal taxa in many previously studied northern peatlands is driven by the presence of ericoid shrubs and associated ericoid mycorrhizal fungi (Read et al. 2004; Lamit et al. 2017); however, ericoid shrubs are generally absent from rich fens. Many of the phyla present show signs of adaptation to the harsh environment that peatlands have to offer: cold, acidic and anaerobic ecosystems that accumulate recalcitrant organic matter. For example, members of the bacterial phylum Acidobacteria

generally thrive in soil and wetland environments and have been shown to possess functional genes and traits related to the degradation of recalcitrant organic matter, such as cellulose, lignin, and chitin (Kielak et al. 2016; Wegner and Liesack 2017; Belova et al. 2018). In rich fens, the highest expression of genes associated with cellulose breakdown are found in the Proteobacteria and Bacteroidetes (Woodcroft et al. 2018), which also happen to be the most drought-responsive phyla in previous water table experiments (Potter et al. 2017). The few research observations like these highlight the unknowns surrounding the effects of changing hydrology on microbial communities in climate change-sensitive ecosystems, such as boreal fens (Myers et al. 2012).

Anaerobic metabolic processes dominate the livelihoods of rich fen microorganisms. Of these processes, methanogenesis has been widely studied in wetlands due to its implications for climate change. Initial and/or rate limiting steps to methanogenesis in peatland systems involve the hydrolysis and/or fermentation of complex organic materials (i.e polysaccharides, cellulose, lignin) to organic acids (i.e. acetate) or CO_2 and H_2, whereby methanogenic archaea metabolize these molecules to methane (Valentine et al. 1994; Glissmann and Conrad 2002; Megonigal et al. 2003; Tveit et al. 2015). Under conditions favorable for methanogenesis, the anaerobic breakdown of organic matter using H_2 and CO_2 or acetate should produce a 1:1 ratio of CO_2:CH_4 (Conrad 1999); however, this is not the case in many northern peatlands. There have been a number of recent studies examining the interconnectedness of anaerobic microbial carbon and iron cycling as an explanation to often larger than expected CO_2:CH_4 effluxes from these systems (Yavitt and Seidman-Zager 2006; Hines et al. 2008; Lipson et al. 2010; Juottonen et al. 2017; Kügler et al. 2019). The complex relationships between organic matter, iron, and the microbial community have the ultimate potential to alter CH_4 and CO_2 efflux (Miller et al. 2015; Miller et al. 2019). Research to date has suggested that irregularities or shifts in precipitation regimes driven by climate change could make microbe-iron-organics interactions even more influential in peatland carbon cycling due to their collective tendency to create an environment of re-generable redox activity (Küsel et al. 2008; Klupfel et al. 2014).

The majority of studies investigating organo-metallic complexes in peatland anaerobic metabolism involve the analysis of bulk peat and/or take place in the laboratory. However, there are a select number of studies that have demonstrated that iron cyclers (i.e. family Geobacteraceae), fermenters (i.e. Clostridiaceae, Opitutae) and some methanogens (i.e. Methanosaetaceae, Metanobacteriaceae) have an affinity not for bulk peat, but for the roots of wetland plants. Many wetland plants such as sedges contain aerenchyma, air-filled tissue capable of transferring gasses between the atmosphere and rhizosphere, aerating the roots (Colmer 2003). Most of these studies have examined rice plant roots and paddy rhizospheres in an agricultural setting in Asia (Lu et al. 2006; Zhu et al. 2014). Laboratory-based studies have revealed unusually high rates of iron cycling in the rhizospheres of other wetland plants as well, alongside the dominance of iron reducing bacteria in the rhizosphere compared with the surrounding bulk peat (Weiss et al. 2003; Weiss et al. 2004). There is an underrepresentation of field-based studies

examining links between iron cycling and methane producing organisms in the rhizospheres of pristine peatland ecosystems.

Despite the rapidly increasing number of datasets procured by next generation sequencing (Knief 2014), much is yet unknown about the environmental function of most northern fen microorganisms and how these bacteria, archaea, and fungi are affected specifically by shifts in water table and plant species composition. Peat depth and depth to water table have been directly linked to effects on fungal and bacterial distribution, which bears consequences for carbon cycling (Lamit et al. 2017; Potter et al. 2017; Wang et al. 2019). Likewise, numerous studies highlight plants as drivers of peatland carbon cycling, yet few look specifically at the microbiomes of individual PFGs save studies on mycorrhizal relationships (Weishampel and Bedford 2006; Hines et al. 2008). For example, given previous research, we know that dicots (e.g. cinquefoils) tend to host arbuscular mycorrhizal fungi, whereas monocots (e.g. sedges, cattails) are more likely to form relationships with dark septate endophytes in rich fens (Thormann et al. 1999; Cornwell et al. 2001; Weishampel and Bedford 2006).

In this study, we examine in depth the microbiomes of rich fen bulk peat and four dominant plant fine root systems. Based on previous work in Rupp et al. (2019) examining the same plant functional groups as in this study, we also know that dominant fen PFGs sedges, grasses, and horsetail have been observed to create environments favorable to methane oxidation. Marsh cinquefoil, on the other hand, produced higher concentrations of pore water methane. The next step in this line of research is to identify how PFGs and water table dynamics 1) independently affect the microbial community and 2) interact to influence microbial activity in boreal rich fens, and the collective impact on carbon cycling.

To address this knowledge gap, the goal of this research was to:

1) Characterize the microbial community of a boreal rich fen in the bulk peat at four depths (0-10 cm, 10-20 cm, 30-40 cm, and 60-70 cm), and report shifts in community due to long-term water table manipulation, taking into account the influence of plant root microbiomes in the rooting zone (0-20 cm)
2) Characterize the bacterial, archaeal, and fungal community associated with the roots of four dominant rich fen plants in central Alaska—sedges (*Carex atherodes* Spreng.), grasses (*Calamagrostis* spp. Michx), horsetail (*Equisetum fluviatile* L.), and cinquefoil (*Comarum palustre* L.)—and identify the ways in which these communities reacted to long-term water table manipulation
3) Report on potential in-situ relationships between organisms (plant, bacterial, or fungal) associated with methanogenesis—initial hydrolysis of polymers and fermentation, secondary fermentation to organic acids, methanotrophy, and finally methanogenesis (Bridgham et al. 2013)—in response to PFG and long-term water table manipulation, with a specific focus on interactions with iron cyclers

To address the water table-PFG effect in tandem, we isolated microbial DNA from peat and plant fine root systems from a long-term experiment with three water table treatments

(raised, lowered, control) in a rich fen. The rhizosphere samples could then be compared to microbial DNA isolated from the surrounding bulk peat to elucidate which environmental shifts triggered specific responses from the microbial community.

3.3 Methods

3.3.1 Field site

The Alaska Peatland Experiment (APEX) rich fen (pH 5.6-5.9) is located southwest of Fairbanks, Alaska in the floodplain of the Tanana River. APEX is a long term wetland monitoring and research project examining how climate change will affect carbon cycling via water table and plant community shifts (Olefeldt et al. 2017). As such, experimental water table manipulation plots (lowered, raised, control) have been in place since the initiation of the project in 2005, and have been maintained every year since except for years in which the fen has flooded. The fen is a dynamic ecosystem, and experiences both years in which the water table remains below the surface of the peat *and* years of heavy flooding, with the water table persisting up to a meter above the peat surface. Since the initiation of the project in 2005, the fen has experienced heavy flooding during 5 seasons (2008, 2014, post-July 2016, 2017, and 2018) (Euskirchen 2019 in press). During the time of sample collection (June 2016), the experimental water table treatments had been maintained for an entire growing season the year prior (2015) and the first half of the growing season of 2016. For context, the order of the water table manipulations in space is: control, lowered, raised, separated by approximately 50 meters in between each. Therefore, some of the differences in the results (i.e. differences between control and raised) may be influenced more so by spatial location/distance than water table treatment if little variation is seen between the lowered vs. raised, which are located next to each other. The site is rich in calcium (~14 mg/L pore water) (Kane et al. 2010) and iron (Fe), concentrations of which are dominated by organically-bound Fe (2,700-6,200 mg/kg peat) (Herndon et al. 2019). Dominant plant species include *Carex atherodes* Spreng. (a sedge), *Calamagrostis* sp. Michx (a grass), *Equisetum fluviatile* L. (a horsetail), and *Comarum palustre* L. (marsh cinquefoil) (Churchill et al. 2014).

3.3.2 Peat core collection

In June of 2016, four peat cores were taken from each of the three water table treatment plots using a sharpened 6 cm diameter stainless steel corer fit with an adapter to a power drill (Nalder and Wein 1998). A visual estimate of percent cover composition of vegetation in a 1 m^2 plot surrounding each core was recorded as ancillary data. In the field, cores were placed on a fresh sheet of aluminum foil for further processing. Each core was subsampled at four depth intervals: 1-10 cm, 10-20 cm, 30-40 cm, and 60-70 cm. Nitrile gloves were replaced with fresh gloves between cores, and the distinct depth segments were only touched using the inside of separate pre-labeled Whirl-Pak® bags. Samples were immediately transferred to a cooler and stored on ice until transportation to

the lab. All samples were frozen at -20°C and shipped to the Northern Research Station in Houghton, MI, where they were stored at -20°C until laboratory processing.

3.3.3 Fine root collection

Three replicates of each dominant plant species (horsetail, sedge, cinquefoil, and grass; see above) were collected from each of the three water table treatment plots. To avoid disturbance to the long-term experimental plots, plants were collected outside of the main long-term study area, where the cores were taken, but still in the area influenced by the water table treatments. Collection was performed as follows: a 10 cm^2 square was cut around the desired plant to a depth of 20 cm. The resulting volume of peat was removed from the wetland with gloved hands and the peat removed as best as possible from the roots. No rinsing was performed in the field. The above-ground portion of the plant was severed and the remaining root system was placed into a Whirl-Pak® bag and placed in a cooler for transport back to the lab. Roots were refrigerated, then shipped on ice to the Northern Research Station in Houghton, MI where they were stored at -20°C until laboratory processing.

3.3.4 Molecular methods for sampling bacteria, archaea, and fungi

3.3.4.1 Peat Cores

Approximately 10 ml of peat from a sample was placed in a 50 ml tube, followed by twenty 3.2 mm chrome-steel beads, and pulverized with a modified mini-beadbeater-96 (BioSpec Products, Bartlesville, OK, USA) bead beater for 2 minutes. DNA was then extracted from a 0.5 g subsample of the pulverized peat using a PowerSoil® DNA (MoBio Laboratories Inc., Carlsbad, California, USA) Isolation Kit following the manufacturer's instructions, with the addition of a 30 minutes incubation at 65° C following the addition of the C1 lysis buffer and 10 minutes of vortexing. DNA was cleaned with a MoBio PowerClean® Pro DNA Clean-Up Kit and quantified with a Qubit Fluorometer (Invitrogen, Life Technologies, Carlsbad, CA, USA). DNA was then subjected to a test PCR (polymerase chain reaction) to ensure that it could be amplified, and products from the PCR were examined on an agarose gel.

3.3.4.2 Fine Roots

Each frozen root sample was removed from the freezer and placed between freezer packs in an ice box. Using flame-sterilized scissors and gloved hands, sections of the fine root system were snipped from the root mass and placed immediately in 95% ethanol in a petri dish. Fine roots were manually cleaned of peat using a dissecting microscope and flame-sterilized forceps and sorted into a second "clean roots" petri dish with 95% ethanol. Only fine roots that had been living at the time of collection were selected for further processing, based on color and turgor. Depending on the species and size of the root system, three to nine 2-3 cm lengths of fine root from different parts of the fine root

system were isolated into 2 mL centrifuge tubes with 0.5 mL 95% ethanol. An additional small subset of sedge and grass roots were surface sterilized using 30% H_2O_2 for 1 minute, then rinsed thrice with nano-pure autoclaved water.

Ethanol was evaporated from the samples using a CentriVap, then immediately freeze dried for 72 hours. After drying, ten 3.2 mm chrome steel beads (BioSpec Products, Bartlesville, OK, USA) were added to each tube, and samples were subjected to bead beating for 45 seconds. DNA was extracted and purified from the pulverized roots using the Qiagen DNeasy® Plant Minikit and Mo Bio PowerClean® DNA Clean-Up kits, respectively, following the manufacturer's protocol. The following methods were the same as for those of the peat cores, above.

3.3.4.3 Sequencing

Community amplicon sequencing was conducted at the US Department of Energy Joint Genome Institute (JGI, Walnut Creek, CA, USA). Sample and library prep were performed as documented in detail by Lamit et al. (2017). Briefly, the sequencing protocol followed the sample preparation protocol of Caporaso et al. (2012). The fungal ITS2 region was targeted using the forward primer sequence fITS9 (Ihrmark et al. 2012) and the reverse primer ITS4 (White et al. 1990), and the V4 region of the prokaryote (bacteria and archaea) 16S ribosomal DNA gene was targeted using the forward primer sequence 515f and reverse primer 806r (Caporaso et al. 2011). A PNA clamp was used to exclude plastids and mitochondria in the root samples (Lundberg et al. 2013). Primers were fitted with Illumina sequencing adaptors and the reverse primer contained an 11bp index unique to each sample. Samples were pooled into equimolar aliquots and sequenced on an Illumina MiSeq platform (Illumina, Inc., San Diego, CA) using 2 x 300 bp chemistry. Data are available through the JGI genome portal (project IDs 1141768 and 1127271, http://genome.jgi.doe.gov/).

3.3.5 Bioinformatic processing

Bioinformatic processing of the resulting microbial sequence data was conducted with the JGI BBtools suite (sourceforge.net/projects/bbmap/) and the Quantitative insights into Microbial Ecology pipeline (QIIME1) (Caporaso et al. 2010). BBMap 37.58 (https://sourceforge.net/projects/bbmap/) was used to perform adapter trimming and to filter PhiX 174 from raw interleaved .fastq files using bbduk.sh. Primers were trimmed using Cutadapt 1.14 (Martin 2011). Primer trimmed sequences were merged using BBMerge (bbduk.sh, minimum overlap = 30 bp, max error rate 0.3. Merged sequences were quality filtered in QIIME 1.9.1 with VSEARCH 2.4.2, using a minimum bp length of 100, maximum expected errors 0.5, and maximum number of Ns 0. After barcode extraction, demultiplexing was completed with a quality parameters set so there was no filtering at this step. Data are available through the JGI Genome portal (project IDs 1127271 and 1141768, http://genome.jgi.doe.gov/).

3.3.5.1 V4 region of 16S rDNA (bacteria and archaea)

Reference-based chimera detection was completed with USEARCH61 (Edgar 2010) trained to the SILVA 128 (Quast et al. 2012) database at 97% sequence similarity. Open reference OTU picking was performed using the UCLUST (Edgar 2010) clustering tool at 97% sequence similarity. Representative sequences were assigned taxonomy with the Ribosomal Database Project (RDP) Classifier 2.2 (Wang et al. 2007) with confidence set at 0.8 (Werner et al. 2012) trained against the SILVA 128 QIIME release reference dataset. Mitochondria, chloroplasts, unclassified sequences, and underrepresented sequences (<0.005 % across the entire dataset) were filtered from the final OTU table. The final OTU table was rarefied to 25,037 sequences per sample, the size of the smallest sample, using the Phyloseq package in R 3.5.1 (Team 2013), where the rest of the analyses were completed. Functional groups for prokaryotes were assigned to the most abundant taxa via literature review.

3.3.5.2 ITS2 (fungi)

The ITS region of the sequences was extracted using ITSx 1.1 (Bengtsson-Palme et al. 2013). Additional non-fungal sequences were identified and removed using a closed-reference OTU picking method at 100% similarity against a hand-curated NCBI dataset of non-fungal ITS2 adapted from a shell script found here, containing 145,169 sequences: https://github.com/gzahn/Format_NCBI_QIIME. OTUs identified as plants, animals, and protists were removed using filter_fasta.py. OTU clustering was completed using an open reference approach (Halwachs et al. 2017) using UCLUST with 97% sequence similarity (Bokulich et al. 2018). Taxonomy was assigned using the RDP Classifier trained with the UNITE 7.2 species hypothesis dynamic clustering dataset (released Dec. 1, 2017). Any additional OTUs classified as non-fungal and unidentified were filtered from the dataset, and sequences classified only to a fungal phylum were put through BLASTn searches in the NCBI nucleotide database. If tested OTUs were clearly of fungal origin with an E-value $\leq 1 \times 10^{-20}$, they were retained. Low-occurrence OTUs (<10) were filtered from the dataset, and tentative functional group assignment was completed using FUNGuild (Nguyen et al. 2016). The final OTU table was rarefied to 4,098 sequences per sample, the size of the smallest sample, in R using the Phyloseq 1.24.2 package (McMurdie and Holmes 2013). Percent similarities between sequences in this study and published or documented species hypotheses of interest to further elucidate functionality were aligned and compared with the MUSCLE (Edgar 2004) plugin in UGENE 1.32 (Okonechnikov et al. 2012).

3.3.5.3 Statistical analysis

All statistical analysis was completed in R statistical software (Team 2013) and PRIMER®(Anderson 2001). Statistical significance for all tests was accepted at $p < 0.05$. All reports and comparisons regarding abundance were calculated relative abundance values. Core peat from the main rooting zone (10-20 cm and, where shown in the figures

and mentioned in the text, 0-10 cm) was used to compare PFGs to "bulk" peat in the presented analyses and figures. One way analysis of variance (aov function) was used to compare specific microbial taxa between treatments at specific depths, using Tukey's Honest Significance Difference post-hoc test. The DESeq 1.34.1 package (Anders 2010) was used to calculate differential abundances of OTUs between treatments, with threshold cutoffs of|log2 fold change|>2 and $p<0.01$. Canonical analysis of principal coordinates (CAP) was performed with the Bray-Curtis dissimilarity metric in Phyloseq to visualize differences in community structure and composition. PERMANOVA tests strictly between water table treatments at specific depths and between PFGs were run and pairwise results reported using the mctoolsr 0.1.1.2 package. For bulk peat, PERMANOVA models included water table, peat depth and their two-way interaction as fixed effects, with core as a random effect. For rhizosphere communities, PERMANOVA included water table, plant species and their two-way interaction. These more complex PERMANOVA analyses were run with Bray-Curtis dissimilarity in PRIMER® multivariate analysis software (Auckland, New Zealand)(Anderson et al. 2008), using type III sums of squares and p-values obtained by permuting reduced models lacking the specific factor being tested. Between-taxa correlations and p-values at the same phylogenetic level (i.e. genus, class, family) were obtained from a correlation matrix created using the package Hmisc 4.2-0 (Harrell Jr and Harrell Jr 2019). Multivariate tests for the effects of percent vegetation cover in plots surrounding the cores and water table treatments were run using the adonis function in the vegan 2.5-4 package (Oksanen et al. 2010).

Diversity metrics (observed richness and Shannon index) were calculated by averaging from 100 rarefication iterations. Mixed effects models were run in lmerTEST 3.1-0 (Kuznetsova et al. 2017) using water table treatment and depth (or plant) and their interactions as fixed effects, and core as a random effect. Residuals were visually checked for normality, and models were analyzed using Type III analysis of variance. Between-treatment differences were calculated with lsmeans 2.30-0 (Lenth and Lenth 2018), with false discovery rate (p.adjust = "BH") (Benjamini and Hochberg 1995) corrected p-values reported, computed as defined in Storey 2002.

3.4 Results

3.4.1 Bulk Peat

3.4.1.1 Dominant taxa

The top 10 prokaryote phyla in the bulk peat were the Acidobacteria, Actinobacteria, Aminicinantes, Bacteroidetes, Chloroflexi, Euryarchaeota, Ignavibacteriae, Proteobacteria, and Verrucomicrobia. The top 20 prokaryote OTUs found in each control environmental category are listed in **Table 3.A.1a.** The fungal samples in general were dominated by the Ascomycota; specifically order Helotiales. Other dominant orders

included the Thelebolales, Pleosporales, Agaricales, and Tremellales. The top 20 fungal OTUs found in each control environmental category are listed in **Table 3.A.1b**.

3.4.1.2 Community responses to water table and depth treatments in bulk peat

Both the prokaryote and fungal community were affected by water table treatment and depth. The prokaryote community from the bulk peat responded significantly to water table ($p=0.001$, $F=2.96$, $df=2, 9.17$) and depth ($p=0.001$, $F=26.89$, $df=3, 26$), but not their interaction ($p=0.138$, $F=1.31$, $df=6,26$). Bacterial and archaeal communities differed significantly between all peat depths ($p<0.01$, pairwise PERMANOVA). Observations regarding prokaryote community change with depth include a decrease of the Proteobacteria and increase in the Chloroflexi relative abundance with depth. Driving the differences were individual taxa which were significantly affected by the water table treatment. In general, if bacterial and archaeal OTUs were significantly affected by the water table treatment, it was seen primarily at the 10-20 cm depth, where the greatest fluctuations and water table differences occurred (**Table 3.1c**). The bacterial order Rhizobiales, largely composed of root associates, had a lower relative abundance at the 10-20 cm depth in the lowered water table treatment compared to the control, yet extended deeper, perhaps mimicking a shift in rooting behavior driven by the water table treatments. Conversely, the order Clostridiales, containing possible acetogens, fermenters, and nitrogen fixers—was significantly more abundant in the raised water table treatment than the control or the lowered. Members of the Betaproteobacteria (i.e., Nitrosomonadaceae, Comamonadaceae, Methylophilaceae, Neisseriales, Rhodocyclales, *Uliginosibacterium*, etc.) tended to be overrepresented in the OTUs significantly affected by water table with a large log2 fold change, with significant higher relative abundances in the raised water table treatment. When percent composition of plant functional groups in the plot surrounding the core was accounted for before water table treatment, the prokaryote response to water table treatment was still significant (e.g. $p=0.001$, $F=3.44$ for water table treatment response at 10-20 cm after considering sedge abundance in the plot, permutational multivariate AOV), but the fungal community response was not (e.g. $p=0.11$, $F=1.90$ for water table treatment response at 10-20 cm after considering sedge abundance in the plot, permutational multivariate AOV).

The fungal community from the bulk peat also responded significantly to water table treatment ($p=0.025$, $F=1.60$, $df=2, 9.9$) and depth ($p<0.001$, $F=3.27$, $df=3, 14$), but not their interaction ($p=0.500$, $F=0.99$, $df=6, 14$). Fungal community composition differed significantly only between the uppermost samples (0-10 and 10-20 cm) and the deepest samples (60-70 cm) (pairwise PERMANOVA). Main differences included the relative increase in abundance of Thelebolales with depth as the Helotiales decreased. Of the fungi, five taxa were significantly different at the 0-10 cm depth between the lowered and raised water table treatments ($p<0.01$, differential abundance). Of these taxa, the order Polyporales of the Basidiomycota, containing saprotrophs able to degrade linocellulosic organic matter, was enriched in the lowered water table treatment compared with the raised. Genus *Venturia*, containing plant pathogens, was enriched in the raised water

table treatment, as was the family Helotiaceae. Numbers of significantly different OTUs ($p<0.01$, differential abundance) between treatments are found in **Tables 3.1a-3.1c**.

3.4.1.3 Diversity

Alpha diversity for both prokaryotes and fungi was affected by depth but not water table (linear mixed effects). For prokaryotes, bulk peat alpha diversity metrics were affected by depth ($p<0.001$, $F=37.845$, $df=3, 32.06$), but not water table ($p=0.338$, $F=1.121$, $df=2, 32.06$) or their interaction ($p=0.142$, $F=1.748$, $df=6, 32.05$). In the bacteria, the 60-70 cm samples had lower diversity than all other depths, and the 30-40 cm depth differed significantly from the 0-10 cm depth as well. For the fungi, bulk peat alpha diversity metrics were also affected by depth ($p=0.036$, $F=3.503$, $df=3, 18.29$), but not water table ($p=0.426$, $F=0.946$, $df=2, 8.51$) or their interaction ($p=0.654$, $F=0.699$, $df=6, 17.76$). Specifically for fungi, observed diversity and Shannon's diversity were higher at the 0-10 cm sampling depth than at 60-70 cm ($p<0.01$, least squared means, false discovery rate). Richness was also higher at the 0-10 cm depth than the 10-20 cm depth in the fungi.

3.4.2 Plant rhizospheres

3.4.2.1 Dominant taxa

The top 20 prokaryote and fungal OTUs found in each control environmental category varied by depth and PFG (**Table 3.A.1a** and **Table 3.A.1b**). The top 10 bacterial/archaeal phyla in the PFG rhizospheres were the same as the bulk peat (**Figure 3.A.1**), substituting out Aminicenantes and Ignavibacteriae for Planctomycetes and Tenericutes, the latter of which was classified as *Candidatus phytoplasma,* or Brinjal little leaf phytoplasma—a possible disease dominating several of the cinquefoil samples. The rhizosphere fungal samples, as in the bulk peat, were dominated by the Ascomycota; specifically order Helotiales. Other dominant orders included the Thelebolales, Agaricales, Tremellales, and Cantharellales (in the rhizosphere of cinquefoil) (**Figure 3.A.2**).

3.4.2.2 Community responses to PFG and water table in the rhizosphere

The microbial communities associated with the roots of PFGs were affected by PFG and water table. Plant root prokaryote communities significantly differed by PFG ($p<0.001$, $F=6.12$, $df=3, 23$) and by water table treatment ($p=0.031$, $F=1.80$, $df=2, 23$). There was no interaction between PFG and water table in the prokaryotes ($p=0.210$, $F=1.19$, $df=6, 23$, PERMANOVA).

The plant root fungal community also differed by PFG ($p<0.001$, $F=5.45$, $df=3, 23$) and by water table treatment ($p<0.001$, $F=2.50$, $df=2, 23$). There was an interaction between PFG and water table treatment in the root samples for fungi ($p<0.001$, $F=1.96$, $df=6, 23$, PERMANOVA).

Numbers of significantly different OTUs ($p<0.01$, differential abundance) between treatments are found in **Tables 3.2a-3.2c**. Overall bacterial and fungal community differed significantly between all PFGs and the bulk rooting zone (0-10 and 10-20 cm depths) peat ($p<0.02$ for all relationships, pairwise PERMANOVA) (**Figure 3.2**). Particularly of note is that the cinquefoil rhizosphere and bulk peat had the greatest number of significantly different OTUs both in the bacteria and in the fungi ($p<0.01$, differential abundance, **Table 3.1a**). The bacterial clades that were enriched in cinquefoil in regard to the bulk peat belonged to the Erysipelotrichaceae (also enhanced in other plant roots), the previously mentioned little leaf phytoplasma, family Methylophilaceae and genus *Paludibacterium* of the Betaproteobacteria (also enhanced in other plant roots), with log 2 fold change >10 for all. Significantly diminished in the cinquefoil were the Anaerolineae, Bacteroidetes vadin HA17, Ignavibacteria and Deltaproteobacteria (log 2 fold change <-20).

Several taxa of key biogeochemical and functional importance responded to WT or appeared to have an affinity for specific PFGs, and many associated with iron or methane cycling. The bacterial genera *Geobacter* (organic compound oxidizing/iron reducing), *Methylomonas* (methanotrophy), and family Gallionellaceae (iron oxidizing, organic compound cycling) had a greater relative abundance in the rhizosphere communities of horsetail, sedge, and grass roots than in cinquefoil roots or bulk peat; *Geobacter* also exhibited a marginally significant preference for the raised water table treatment compared to the lowered ($p=0.058$). The presence and relative abundance of family Gallionellaceae appeared to be correlated significantly ($p<0.001$) with the presence and abundance of green sulfur bacteria in the orders Chlorobiales and Ignavibacteriales within the rhizosphere. Iron reducing genus *Albidiferax* was more abundant in horsetail, cinquefoil, and sedge roots than in grass roots and bulk peat.

Other taxa associated with plant function, infection, and carbon breakdown responded to water table and PFG. Abundances of genus *Duganella*, associated with antifungal/antiviral properties, were significantly enhanced in the sedge rhizosphere in comparison to the bulk peat. Sedge and horsetail roots hosted a greater abundance of sequences related to the Enterobacteriaceae, many of which fix nitrogen, and sequences related to family Veillonellaceae (Firmicutes), which contains cellulose- and chitin-degrading organisms. These families were also enhanced by the raised water table treatment at 10-20 cm. Cinquefoil roots, on the other hand, hosted significantly greater abundances of genus *Steroidobacter*, many of which are steroid degraders, than all other root treatments and the bulk peat. The bacterial family Chitinophagaceae appeared multiple times in association with roots to be significantly enhanced in the lowered water table treatment versus the raised water table treatment (log 2 fold change = 6.92, 7.74 in horsetail and grass). Statistical details, descriptions of taxa, and references can be found in **Table 3.A.1**.

In the fungi, the most notable differences between PFGs and the bulk peat were associated with cinquefoils. The saprobic/pathogenic families Ceratobasidiaceae and Tremellaceae (*Cryptococcus neoformans*), saprobic Saccharomyces, and saprobe

Lachnum were all enhanced in cinquefoil versus bulk peat (log 2 fold change >10). Possible root endophyte Sebacinales, the reads of which were most closely related to ericoid and orchid associates, were also enriched in cinquefoil roots. The *Mortierella*, *Phaeogalera*, and *Hypholoma* were all diminished in the cinquefoil rhizosphere (log 2 fold change <-10).

3.4.2.2.1 Methanogens and methanotrophs

One of two main methanogenic genera, *Methanobacterium*, was associated with the roots of sedge, grass, and horsetail but not cinquefoil, and was most abundant in the raised water table plot. In fact, cinquefoil roots have a smaller relative abundance of genus *Methanobacterium* than even the deeper rooting zone bulk peat (10-20 cm, p=0.01; 0-10 cm, p=0.42). The other most abundant methanogenic order, Methanomicrobiales (Rice Cluster II), was most abundant at the 60-70 cm depth and in the lowered water table, unassociated with roots (**Figure 3.3**).

Root associated methanogen abundance was correlated with the relative abundance of the iron-cycling family Geobacteraceae. A positive relationship exists between these taxa in the rhizosphere of the PFGs ($p<0.001$), but not in the bulk peat ($p = 0.38$; **Figure 3.4**). This relationship is strongly driven by grass roots, with the highest abundances of both methanogens and iron cyclers, and cinquefoil roots, in which methanogens and Geobacteraceae are found in very low abundance. Conversely, abundances of methanogens in the bulk peat more strongly correlated with the families Syntrophaceae, which only grow in the presence of an H_2 scavenging partner (Kuever 2014); Anaerolineaceae, acetogens found to pair with methanogens (Liang et al. 2015; Yamada and Sekiguchi 2018); and the order Ingavibacteriales, fermentative green sulfur bacteria with relatives that produce and utilize acetate and sometimes reduce iron (Iino et al. 2010; Podosokorskaya et al. 2013).

Bacteria in the genera *Methylosinus*, *Methylomonas,* and *Roseiarcus*, of the Alphaproteobacteria, contain known methanotrophs found in peatlands, and were present at the site (Kip et al. 2011; Kulichevskaya et al. 2014). Additionally, one genus of the Verrucomicrobia, *Candidatus Methylacidiphilum*, was present at the site and contains organisms which are capable of methanotrophy (Erikstad and Birkeland 2015). The bulk peat at 10-20 cm depth, which is most influenced by the rhizosphere, contained the largest ratio of possible methanogens:methanotrophs, averaging from 4.3 in the control to 6.3 in the lowered water table treatment. Bulk peat from 0-10 cm contained lower ratios (0.6 control – 2.0 lowered). Sedge, grass, and horsetail roots had ratios approximating 1 in the control and lowered treatments; however, ratios dropped to around 0.5 (more methanotrophs) in the raised water table treatment. Cinquefoil consistently had the lowest methanogen:methanotroph ratio, averaging 0.07 across all treatments (**Figure 3.5**).

3.4.2.2.2 Possible pathogens

Cinquefoil samples also contained a number of DNA sequences classified as bacterial pathogen Brinjal little leaf phytoplasma, especially roots in the lowered and raised water table treatments; bacterial family Eryipelotrichaceae, more abundant in the lowered water table treatment; and fungal pathogens *Filosporella exilis,* family Tremellaceae (*Crypotococcus neoformans*), and family Ceratobasidiaceae (Cannon and Kirk 2007). The presence of the possible bacterial disease phytoplasma appears to coincide with lower relative abundance of other bacterial clades—the proteobacteria overall, and in particular the order Sphingimonadales.

3.4.2.2.3 Possible fungal-plant associates

There were several fungal lineages that appeared to be associated with specific PFG rhizospheres. The fungal community within the horsetail and grass rhizospheres was significantly dominated by Ascomycota class Leotiomycetes, order Helotiales—an order which contains many endophytes (Zijlstra et al. 2005)—whereas this order was less abundant in the bulk peat (10-20 cm) and cinquefoil roots. The saprotroph *Pezoloma ciliifera* had a significantly greater relative abundance in the horsetail and cinquefoil rhizospheres than the other PFG rhizospheres or the bulk peat. The dark septate endophyte genera *Mollisia* (Order Helotiales, Family Dermataceae) and *Acephala* (Order Helotiales, Family *Incertae sedis*) were each identified as >20% of the sequence reads in surface sterilized grass roots. *Acephala* was also more abundant in the sedge rhizosphere compared with horsetail, cinquefoil, and bulk peat samples. Genus *Mortierella* was significantly more abundant in sedge root samples than any other PFG. Overall, much of the fungal response to water table treatment seems in part due to long-term vegetation shifts caused by the water table treatments, as fungi were sensitive to vegetation composition surrounding the peat cores (Churchill et al. 2014). For example, over time, sedges have become more abundant in the raised water table treatment, and generally the aforementioned fungi associated with sedges were more abundant in the raised water table treatment as well.

Arbuscular mycorrhizal fungi (AMF),mainly associated with the grass and cinquefoil roots, were dominated by Archaeosporales of the Glomeromycota. Reads of the AMF genus *Glomus* were also present in cinquefoil roots (Redecker et al. 2013). AMF were unaffected by water table treatment in both the cinquefoil and grass roots.

3.4.2.3 Diversity

Diversity for both prokaryotes and fungi was affected by PFG, whereas only fungal diversity was affected by water table (linear mixed effects). For prokaryotes, rhizosphere alpha diversity metrics were affected by PFG ($p<0.001$, $F=12.015$, $df=5, 40$), but not water table, although the relationship was marginally significant ($p=0.054$, $F=3.143$, $df=2, 40$). The interaction between PFG and water table was not significant ($p=0.175$,

F=1.500, df=10, 40). Bacterial and archaeal alpha diversity were affected by PFG, with cinquefoil roots exhibiting a significantly lower observed richness than the other PFGs and bulk peat. Shannon's diversity index-was also significantly different between cinquefoil roots and bulk peat (**Figure 3.1**).

For fungi, rhizosphere alpha diversity metrics were affected by PFG ($p<0.001$, $F=6.440$, $df=5, 35$) and water table ($p=0.008$, $F=5.513$, $df=2, 35$) but not their interaction ($p=0.680$, $F=0.743$, $df=10, 35$). Specifically, sedge, grass and horsetail observed fungal richness and Shannon's diversity were lower than the 0-10 cm bulk peat. Grass and horsetail also had lower diversity than the 10-20 cm bulk peat; horsetail had the lowest Shannon's diversity compared with all plant groups and bulk peat. Additionally, grass and horsetail had lower observed fungal diversity than cinquefoil. Cinquefoil Shannon's diversity but not observed richness was lower than the bulk peat at 0-10 cm, but otherwise did not show variation in diversity metrics from the bulk peat for the fungi. In the root data, the control water table treatment had higher observed and Shannon's diversity than the raised ($p<0.01$ observed, $p<0.01$ Shannon's) and lowered ($p=0.01$ observed, $p=0.02$ Shannon's) in the fungi.

3.5 Discussion

The bacterial, archaeal, and fungal communities had different relationships with each PFG and differences in how they responded to water table position (summarized in **Figure 3.6**). The most compelling results from this study included:

First, water table response is seen in the rooting zone (10-20 cm depth). This vertical zone in the profile will likely be most immediately affected by hydrologic shifts, as has been suggested by others (Asemaninejad et al. 2017). Here, plant and microbial reactions to water table change will likely be interdependent in nature. Of the bacteria that were significantly enriched by the raised water table, the Clostridiaceae and Veillonellaceae have all been shown to be important polysaccharide hydrolysers, as well as utilizers of root exudates (Lu et al. 2006; Hernández et al. 2015; Juottonen et al. 2017). Complex carbon hydrolysis is often the rate limiting step in wetland methanogenesis. In this study, Veillonellaceae was also significantly enhanced in the sedge roots, indicating that a shift in plant composition away from sedges could diminish an important step in methane production. Conversely, sedge, horsetail, and grass rhizospheres average a smaller ratio of methanogens:methanotrophs in the raised water table plot. A significantly higher abundance of bacterial family Chitinophagaceae was found in roots associated with the lowered water table. Hydrolysis of cellulose and chitin are carried out by some members of this family (Rosenberg 2014), which suggests accelerated breakdown of complex organics in drought and/or plant ingrowth scenarios to the fen. Interestingly, the bacterial disease little leaf phytoplasma, affecting some of the cinquefoil rhizospheres, co-occurred with a decreased relative abundance of the order Sphingimonadales, to which Chitinophagaceae belongs. Should climate change bring about enhanced stress and

disease to plants, it appears that the breakdown of complex organics by these bacteria could be slowed in this specific case.

Second, there appears to be a relationship between PFG rhizospheres and bacterial communities associated with iron cycling. Added together, the microorganisms likely associated with iron cycling made up anywhere from roughly 1-20% of the amplicons from PFG roots, compared with 2% or less of the reads associated with bulk peat. *Geobacter* has been associated with the rhizospheres of rice plants and other freshwater and marine plants, due to the consumption of root exudates and the availability of oxygen as an electron donor/acceptor, delivered by aerenchyma (King and Garey 1999; Cabezas et al. 2015). Other iron cycling taxa, such as the Gallionellaceae, were also significantly enriched in the rhizosphere in comparison with the surrounding bulk peat.

Third, there was a distinction of two groups of archaeal methanogens: one "deep peat" community (Class Methanomicrobia), of greatest amplicon abundance at the 60-70 cm depth, and one "rhizosphere" community (Class Methanobacteria), of greatest amplicon abundance in the rooting zone, from 1-20 cm in depth. Methanobacteria were of greatest abundance in the raised water table treatment at all depths. Stimulation of methanogenesis by root exudates in vascular plants, including sedges, is substantial in fens (Mary et al. 1993; Chanton et al. 1995; Franchini et al. 2014). Additionally, rhizosphere methanogens, dominated by Methanobacteria, demonstrated a positive relationship with family Geobacteraceae abundance in the PFG root samples but not bulk peat (**Figure 3.4**). Previous research has revealed that not only do methanogens sometimes use Fe (III) as an electron acceptor (Reiche et al. 2008), but are capable of developing symbiotic relationships with iron reducers (Morita et al. 2011; Kato et al. 2012; Zhou et al. 2014). Methanobacteria and Methanomicrobia both have been shown to at times participate in syntrophic acetate oxidation, in which an acetate oxidizing bacterium (e.g *Geobacter*) partners with another organism, in this case a hydrogenotrophic methanogen (Petersen and Ahring 1991; Schnürer et al. 1997; Karakashev et al. 2006; Zhou et al. 2014). This suggests that there may be a complex relationship between iron cyclers and methanogens in this iron-rich fen, facilitated by the plant rhizosphere environment but not the bulk peat environment.

Perhaps because there are significantly fewer Geobacteraceae in the bulk peat, the rhizosphere-associated and bulk peat-associated methanogens appear to co-occur with different bacteria. In the bulk peat (10-20 cm), methanogens co-occur with potential symbionts within the families Syntrophaceae, Anaerolineaceae, and order Ignavibacteriales. Family Syntrophaceae of the Deltaproteobacteria contains species that oxidize organic substrates into acetate or CO_2, and require symbiotic relationships with H_2 scavengers for survival, making them ideal partners for hydrogenotrophic methanogens. Anaerolineaceae of the Chloroflexi has been documented to couple with acetoclastic methanogen family Methanosaetaceae (present in these samples) as an acetogenic partner (Liang et al. 2015). Cocultivation with hydrogenotrophic methanogens is also known, likely using hydrogenotrophs as an electron scavenging systems (Yamada and Sekiguchi 2018). Less is known about the green sulfur bacteria of the

Ignavibacteriales—members of this order are documented fermenters that could produce or use acetate or potentially reduce iron (Iino et al. 2010; Podosokorskaya et al. 2013).

Fourth, dark septate endophytes (DSE) are likely important in this rich fen, particularly in the sedges and grasses, and decline in relative abundance with a low water table (**Figure 3.6**). Species of fungal genera *Mollisia, Acephala,* and *Mortierella*, were significantly enhanced in the sedge and surface sterilized grass root samples. *Mollisia* has previously been reported as associated with grass roots (Luo et al. 2017). Members of *Mortierella* can also act as saprotrophs and/or provide plants with resistance to pathogens. However, all three of these genera have also been documented to act as root endophytes (Narisawa et al. 1998; Zijlstra et al. 2005; Porras-Alfaro and Bayman 2011). Some endophytes have the ability to foster disease resistance for their hosts (Narisawa et al. 2002). Furthermore, *Acephala* isolated from peat—with 98% similarity to one of the dominant OTUs in this study—have been shown capable of Fenton chemistry/quinone redox cycling as a biodegradation tool, which utilizes iron cycling to ultimately create powerful oxidants and CO_2 (Krueger et al. 2016). The production of oxidants in the rhizospheres of grasses and sedges could help explain the oxidized nature of the rooting zone associated with these plants, aside from their possession of aerenchyma, or spongy air-filled tissue (Rupp et al. 2019). The Fenton reaction carried out by *Acephala* in the rhizosphere could help oxidize methane in addition to that done by bacterial methanotrophs, resulting in a relatively high ratio of CO_2:CH_4 efflux seen at the site (e.g. Kane *et. al* 2013). Further research questions should address whether or not this biogeochemical reaction is occurring in this rich fen, or if naturally present fungal symbionts are capable of creating such an environment.

Finally, the rhizosphere of the shrub marsh cinquefoil, documented to enhance methane production (Koelbener et al. 2009; Rupp et al. 2019), may be inhospitable to certain bacterial and archaeal taxa—inferred from the reduced bacterial and archaeal diversity and low relative abundances of certain taxa found there. Both bacteria and archaea show reduced diversity, and surprisingly, certain clades such as the *Methanobacteria* are reduced in relative abundance in these samples, even in comparison with the bulk peat (in the 10-20 cm peat but not significantly different from the 0-10 cm peat). In part, this may be due to the innately shallow rooting habit of the shrub, growing in a more oxidized zone generally hostile to methanogens. This shrub is also apparently under pressure from fungal and bacterial disease in this wetland, which could be an early response to actual (not simulated) climate change (Schütte et al. ; Anderson et al. 2004).

Taken together, these findings suggest that the rhizosphere is arguably the most important and dynamic biogeochemical zone in carbon and iron cycling, which appear to be linked in this rich fen ecosystem. Suites of bacteria, archaea, and fungi associated with complex organic matter breakdown, methanogenesis, and iron cycling significantly prefer the rhizospheres of sedges, grasses, and horsetail, but not the shrub cinquefoil or bulk peat. These same taxa are among those that are also the most influenced by shifts in the water table. Future research should examine 1) specific rhizosphere interactions of iron reducers and rhizosphere-associated methanogens in iron-rich fens, 2) in-depth

interactions of root endophytes with iron and carbon cycling, especially examining the Fenton cycle, with a focus on CO_2:CH_4 ratios, and 3) the biology of the marsh cinquefoil rhizosphere regarding documented enhanced methane production, yet shown inability to host methanogens.

3.5.1 Implications for a changing climate

This study has shown that PFG has a stronger influence over peat microbiology than water table in this boreal fen, yet hydrology often governs what species of plants can live in an ecosystem. Long term hydrologic manipulation has already brought changes in vegetation community composition to this long term research site, with abundance of sedges and grasses higher in the raised water table treatment (Churchill et al. 2014). An increase in graminoid abundance in Alaskan fens with projected increases in precipitation in central Alaska (Stewart et al. 2013) further highlights the need for a deeper understanding of rhizosphere methanogens, iron cyclers, and fungal endophytes that are vastly underrepresented in the literature, yet likely govern—directly or indirectly—carbon cycling within the rhizosphere.

3.6 Acknowledgements

The University of Alaska-Fairbanks Institute of Arctic Biology (Fairbanks, AK), Bonanza Creek Long Term Experimental Research station (Fairbanks, AK), and USDA Northern Research Station (Houghton, MI) provided both lab space, equipment, and time to this project. Natalie Zwanenberg and Samuel Knapp contributed invaluable fieldwork. NCBI database curation methods relied on code and ideas provided by Geoffrey Zahn. Funding for this project was provided by in-kind support of the Global Peatland Microbiome Project by the Environmental Molecular Sciences Laboratory (EMSL) Proposal ID Number 48292, US National Science Foundation grant DEB 1146149 and National Science Foundation grant DEB LTREB 1354370. The processing of DNA samples was funded in part by the US Forest Service Northern Research Station, Houghton, MI. Sequencing was supported by the U.S. Department of Energy Joint Genome Institute under grant #xxx. The work conducted by the U.S. Department of Energy Joint Genome Institute, a DOE Office of Science User Facility, is supported by the Office of Science of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231. The APEX site has also been supported by National Science Foundation grants (DEB-0425328, DEB-0724514 and DEB-0830997).

The authors declare that they have no conflict of interest.

3.7 Tables & Figures

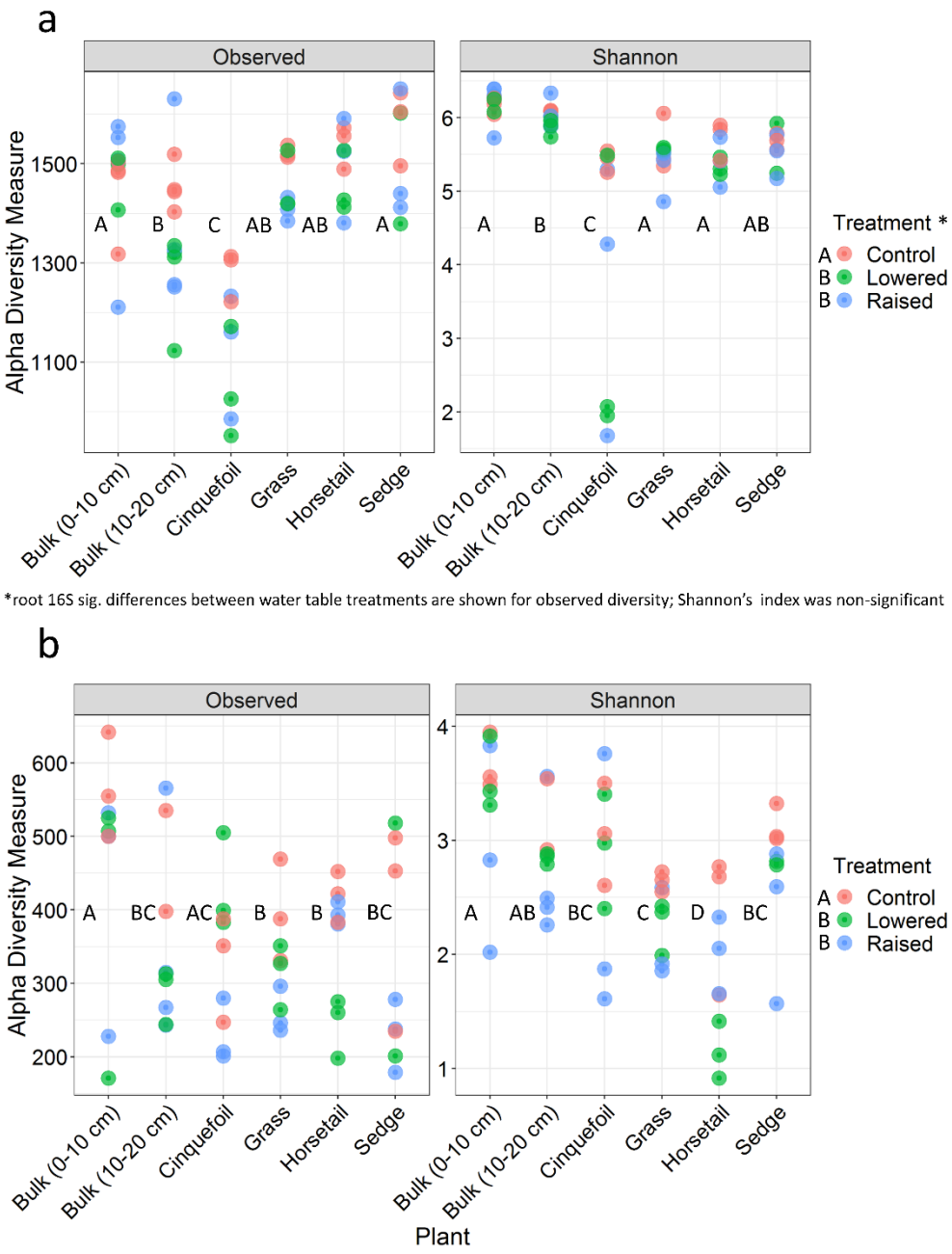


Fig. 3.1 Observed (OTU richness) and Shannon index alpha diversity in a) bacterial/archaeal communities and b) fungal communities by treatment (plant functional group or water table treatment). Post-hoc significant differences are denoted with letters

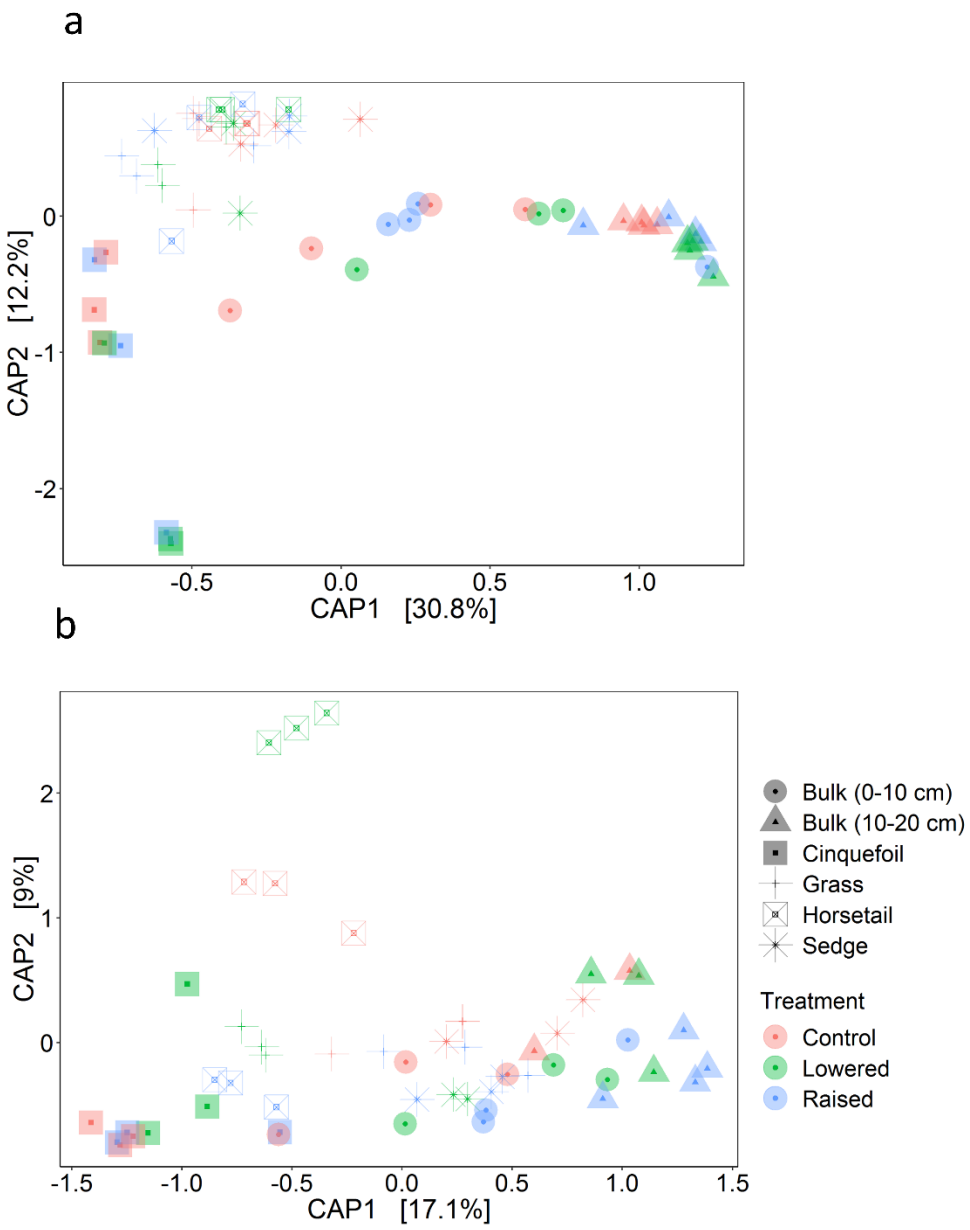


Fig. 3.2 Canonical analysis of principle coordinates (CAP) using Bray-Curtis dissimilarity. Ordination is constrained by water table treatment and PFG in a) bacterial/archaeal communities and b) fungal communities

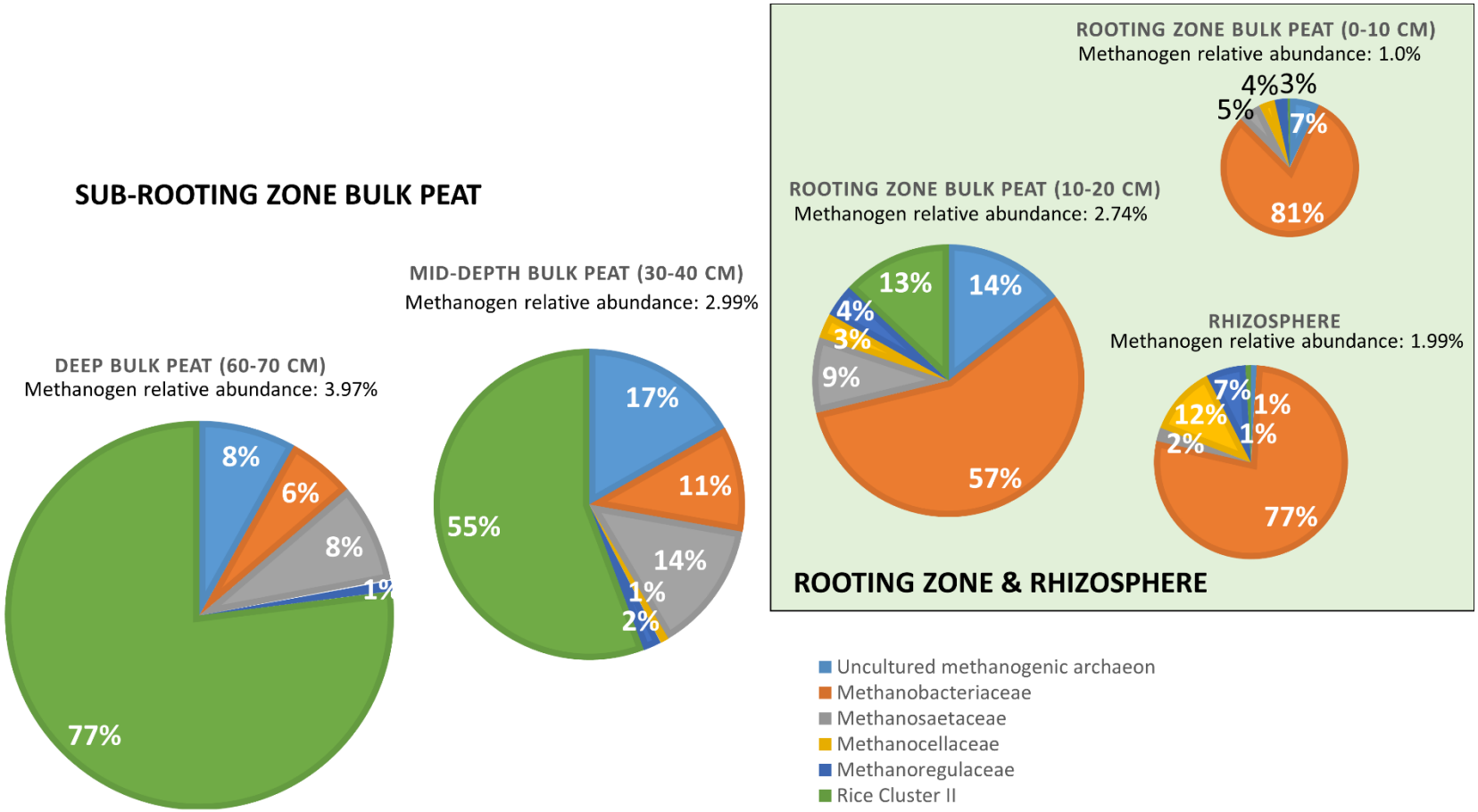
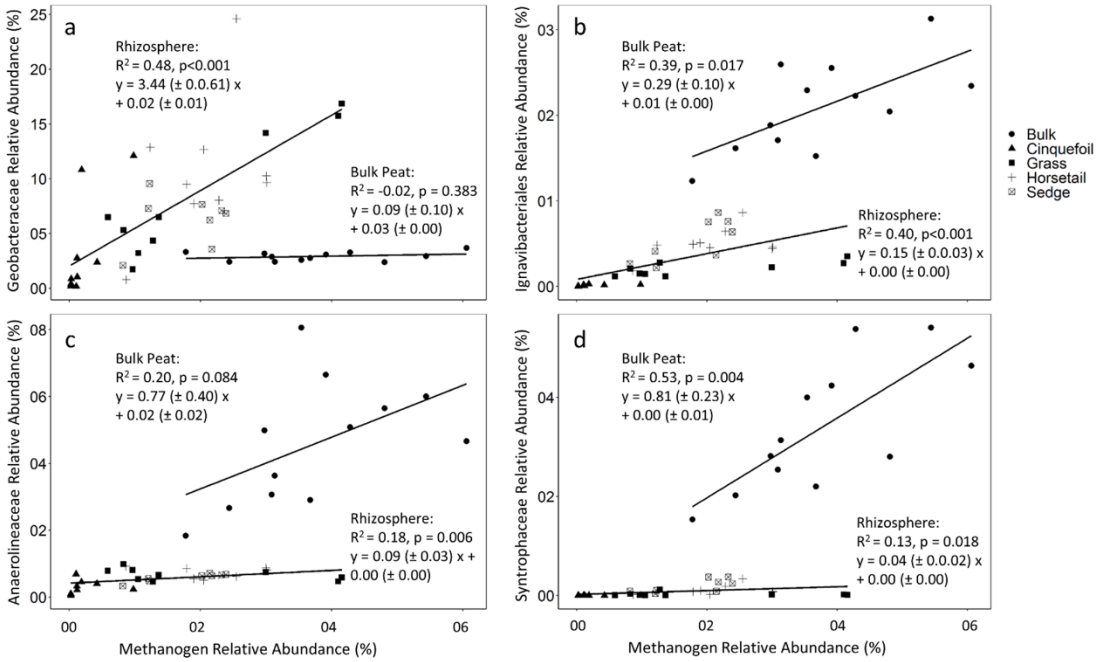


Fig. 3.3 Methanogen community composition by family in bulk peat by depth and the root-associated or rhizosphere community


a
Rhizosphere:
R² = 0.48, p<0.001
y = 3.44 (± 0.0.61) x
+ 0.02 (± 0.01)
Bulk Peat:
R² = -0.02, p = 0.383
y = 0.09 (± 0.10) x
+ 0.03 (± 0.00)
Geobacteraceae Relative Abundance (%)
b
Bulk Peat:
R² = 0.39, p = 0.017
y = 0.29 (± 0.10) x
+ 0.01 (± 0.00)
Rhizosphere:
R² = 0.40, p<0.001
y = 0.15 (± 0.0.03) x
+ 0.00 (± 0.00)
Ignavibacteriales Relative Abundance (%)
Bulk
Cinquefoil
Grass
Horsetail
Sedge
c
Bulk Peat:
R² = 0.20, p = 0.084
y = 0.77 (± 0.40) x
+ 0.02 (± 0.02)
Rhizosphere:
R² = 0.18, p = 0.006
y = 0.09 (± 0.03) x +
0.00 (± 0.00)
Anaerolineaceae Relative Abundance (%)
Methanogen Relative Abundance (%)
d
Bulk Peat:
R² = 0.53, p = 0.004
y = 0.81 (± 0.23) x
+ 0.00 (± 0.01)
Rhizosphere:
R² = 0.13, p = 0.018
y = 0.04 (± 0.0.02) x
+ 0.00 (± 0.00)
Syntrophaceae Relative Abundance (%)
Methanogen Relative Abundance (%)

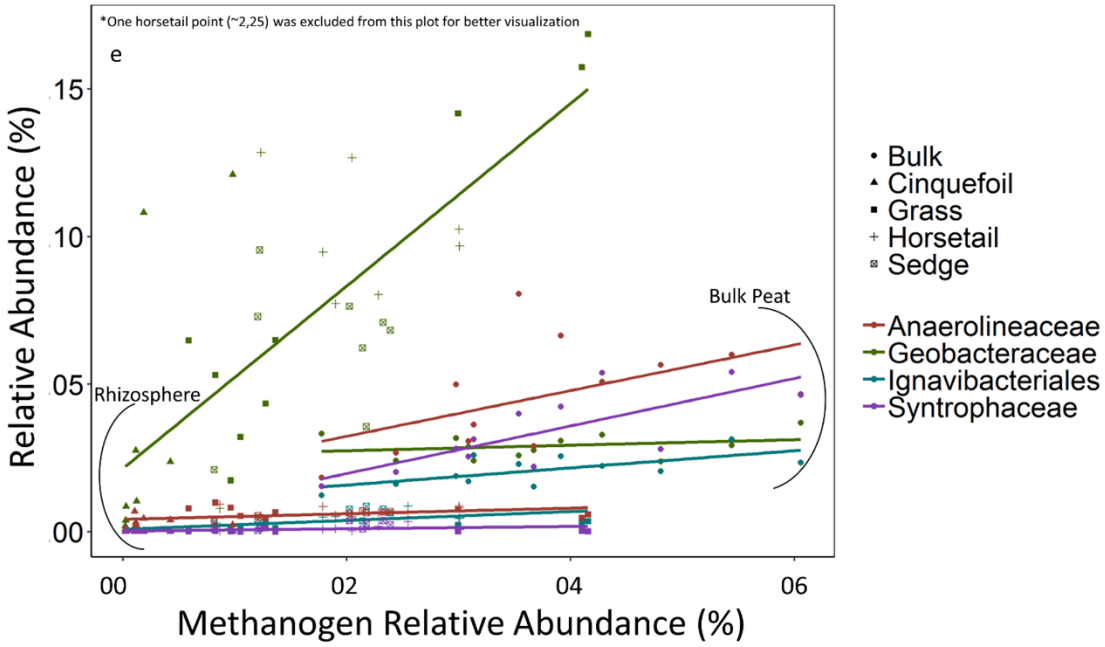

*One horsetail point (~2,25) was excluded from this plot for better visualization
e
Rhizosphere
Bulk Peat
Relative Abundance (%)
Methanogen Relative Abundance (%)
Bulk
Cinquefoil
Grass
Horsetail
Sedge
Anaerolineaceae
Geobacteraceae
Ignavibacteriales
Syntrophaceae

Fig. 3.4 Significant co-occurrences of methanogens with other bacterial clades, several of which have documented symbiotic relationships with methanogens, based on environment (bulk rooting zone peat (10-20 cm) or rhizosphere-associated). Co-occurrences between relative abundance of methanogens and a) Geobacteraceae, b) Ignavibacteriales, c) Anaerolineaceae, d) Syntrophaceae, and e) all plotted on the same graph for comparison

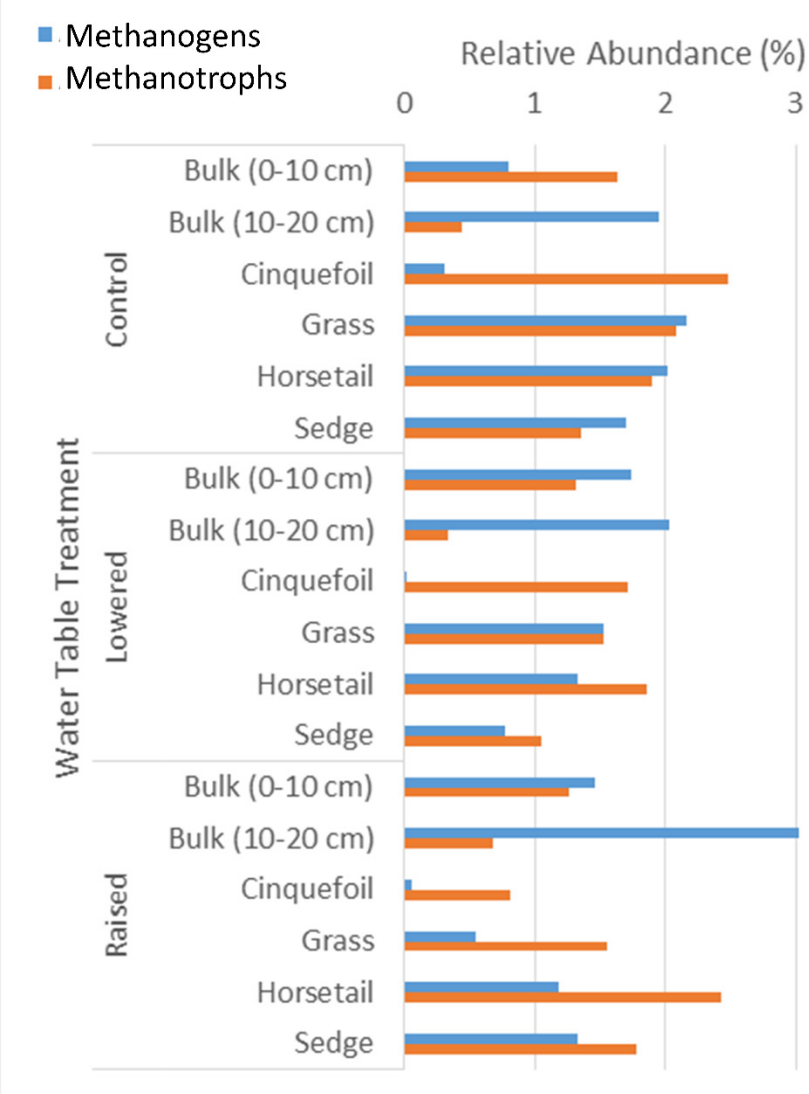


Fig. 3.5 Relative abundances of methanogens and methanotrophs in the rhizosphere

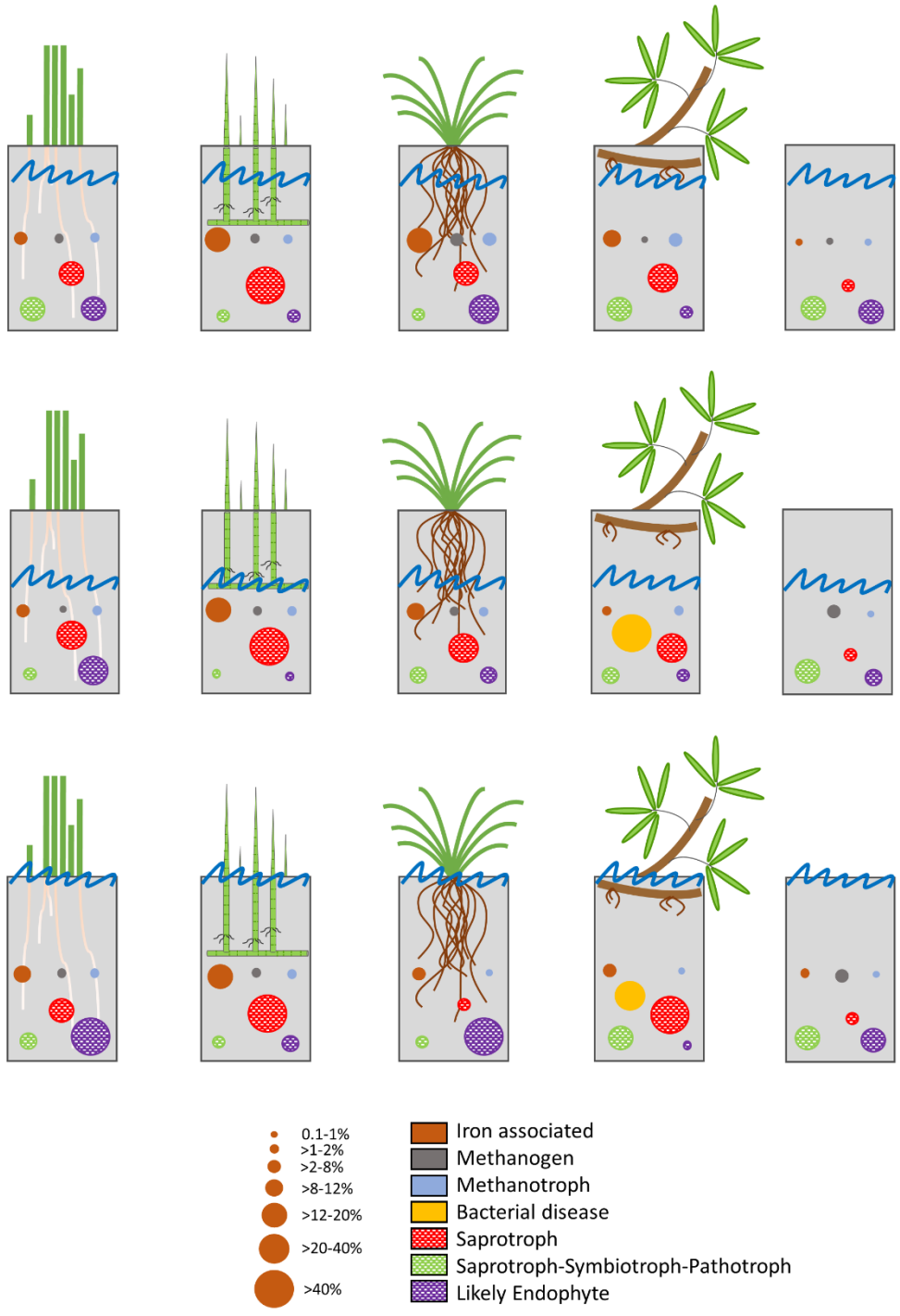


Fig. 3.6 Generalized summary of findings by relative abundance (functional groupings of the top 20 OTUs plus taxa of statistical interest mentioned in the results). The top row represents the control, middle represents the lowered water table treatment, and bottom represents the raised water table treatment, which is accurate to how the treatments occur

in space. The plant representations from left to right are as follows: sedges, horsetail, grasses, cinquefoil, bulk peat (10-20 cm). Represented genera for iron associates are: *Geobacter* (Geobacteraceae), *Albidiferax* (Comamonadaceae), *Sideroxydans* (Gallionellaceae), *Geothrix* (Holophagaceae), and *Ferribacterium* (Rhodocyclaceae). Methanogens: *Methanobacterium.* Methanotrophs: *Methylomonas, Methylosinus, Roseiarcus, Candidatus Methylacidiphilum.* Bacterial disease: Brinjal little leaf phytoplasma (Tenericutes). Fungal functional groups are derived from literature mentioned in the text and assignments by FunGUILD

Table 3.1 (a-c). The number of significantly different OTUs between groups for bacteria/archaea (bottom diagonal, green) and fungi top diagonal, orange).

Table 3.1a. Comparing between-PFG differences

	sedge	grass	horsetail	cinquefoil	bulk
sedge	0	28	21	76	62
grass	103	0	24	88	73
horsetail	83	122	0	63	53
cinquefoil	780	525	712	0	122
bulk (10-20 cm)	769	996	839	1261	0

Table 3.1b. Comparing by-plant effect of water table manipulation

sedge	control	lowered	raised	**horsetail**	control	lowered	raised
control	0	5	9	control	0	1	8
lowered	11	0	9	lowered	14	0	12
raised	6	24	0	raised	3	65	0
grass	control	lowered	raised	**cinquefoil**	control	lowered	raised
control	0	4	5	control	0	5	9
lowered	7	0	8	lowered	12	0	5
raised	24	61	0	raised	10	49	0

Table 3.1c. Comparing by-depth effect of water table manipulation

0-10 cm	control	lowered	raised	**10-20 cm**	control	lowered	raised
control	0	2	6	control	0	3	3
lowered	1	0	5	lowered	148	0	0
raised	3	4	0	raised	92	36	0
30-40 cm	control	lowered	raised	**60-70 cm**	control	lowered	raised
control	0	1	2	control	0	7	8
lowered	2	0	3	lowered	18	0	4
raised	4	1	0	raised	11	0	0

3.8 Appendix 3.A Supplemental Materials

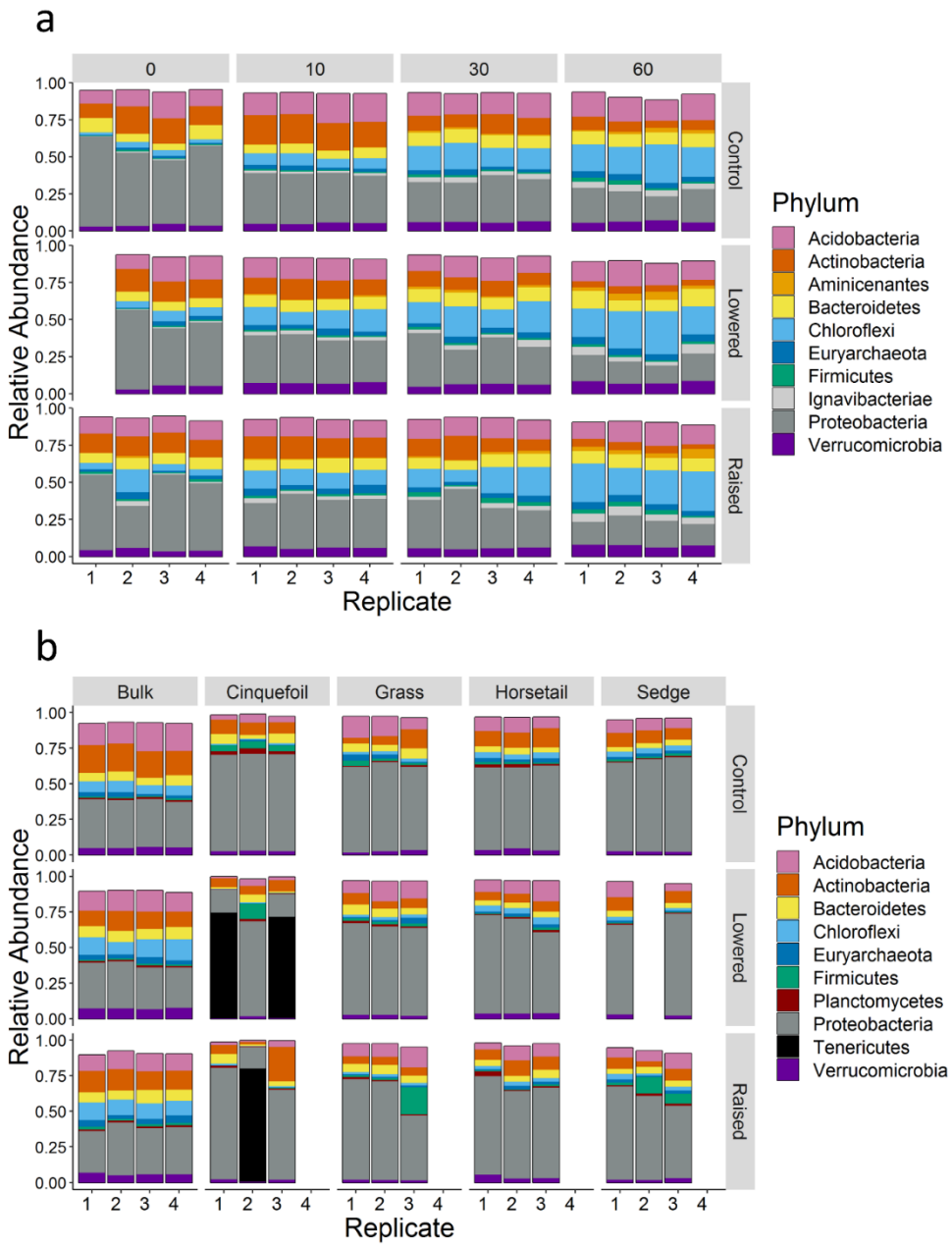


Fig. 3.A.1 Top 10 prokayrote phyla for a) bulk peat per depth (cm) and water table treatment and b) PFG and water table treatment

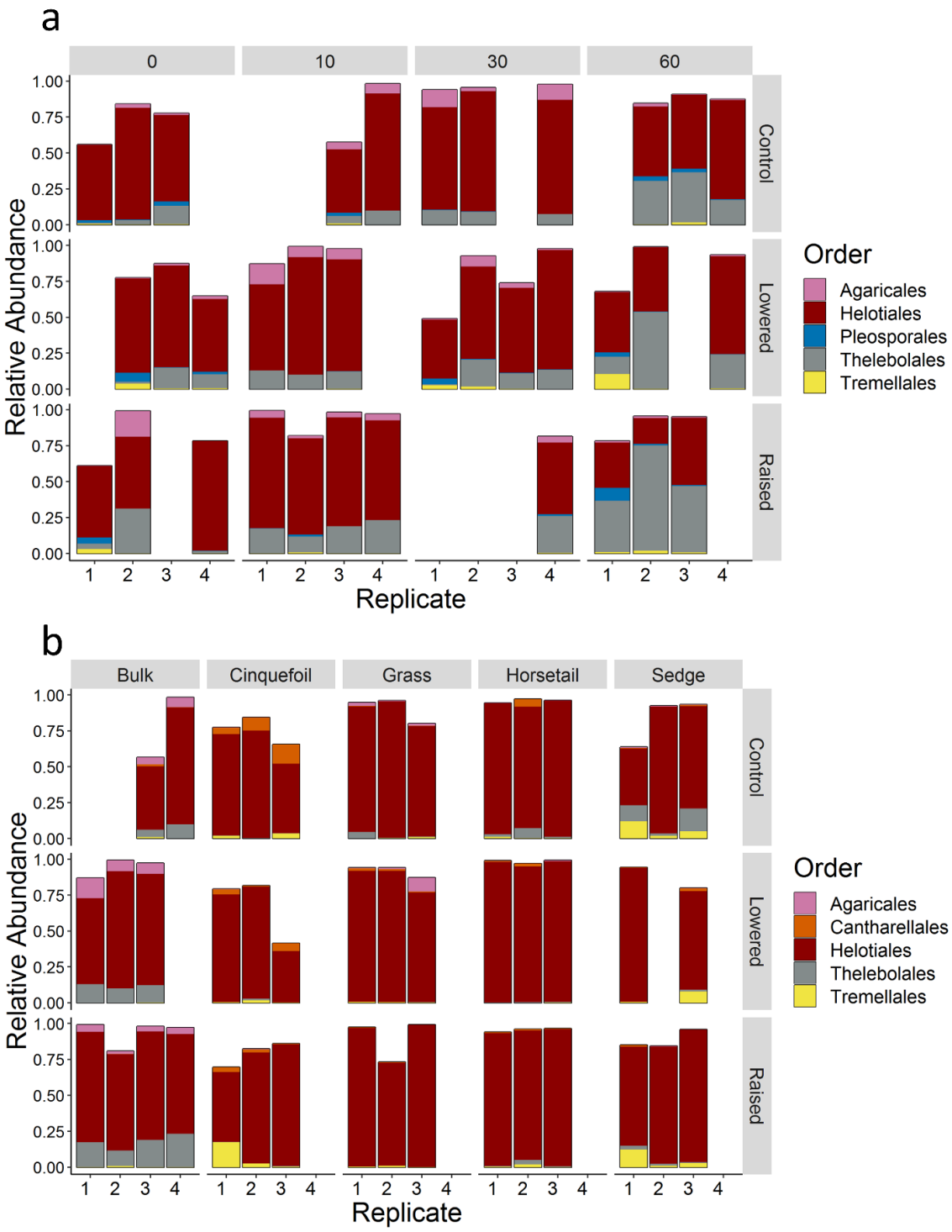

a
0
10
30
60
Control
Lowered
Raised
Relative Abundance
1.00
0.75
0.50
0.25
0.00
1 2 3 4
Replicate
Order
Agaricales
Helotiales
Pleosporales
Thelebolales
Tremellales
b
Bulk
Cinquefoil
Grass
Horsetail
Sedge
Control
Lowered
Raised
Relative Abundance
Replicate
Order
Agaricales
Cantharellales
Helotiales
Thelebolales
Tremellales

Fig. 3.A.2 Top 5 fungal orders for a) bulk peat per depth (cm) and water table treatment and b) PFG and water table treatment

Table 3.A.1(a-b). Top 20 OTUs by relative abundance in each control treatment >1% in at least one treatment. Relative abundances correspond to the *most specific* taxa to which they were classified. There is no overlap. For example, if several OTUs were identified to order but not finer resolution (family, genus), these were grouped in one line/dot labeled with that order (Helotiales, for example). That line/dot does not include those OTUs that are of the same order but identified to a finer resolution (OTUs of genus *Acephala* of the order Helotiales, for example, are included on a different line). If an OTU was identified to genus, everything of that genus appears on the same line/dot, but is not included in the line mapped only to the corresponding order

Table 3.A.1a Top 20 bacterial & archaeal OTUs, by control condition (depth, PFG)

Orders (or higher) of bacteria and archaea represented in top 20 OTUs	Family/Genus/Species, if available	0-10 cm	10-20 cm	30-40 cm	60-70 cm	Sedge	Grass	Horsetail	Cinquefoil
Acidobacteriales (Acidobacteria/Subgroup 18)	Acidobacteriaceae (Subgroup 1)	•	●	●	●				
Anaerolineales	Anaerolineaceae			●	●				
Anaerolineales (Bacteroidetes vadinHA17)	*Leptolinea*		●	●	●				
(Betaproteobacteria)			•	●					
Burkholderiales	*Albidiferax*					●	•	●	●
Burkholderiales	Comamonadaceae	•				●			●
Burkholderiales	*Duganella*					●			●
Burkholderiales	*Rhizobacter*							•	●
Caldisericales	*Caldisericum*				●				
(Chloroflexi/KD4-96)			●	●	●	•		•	
Corynebacteriales	*Mycobacterium*							•	●
Desulfuromonadales	*Geobacter*		•			●	●	●	●
Enterobacteriales	Enterobacteriaceae					●			
Erysipelotrichales	Erysipelotrichaceae								●
Frankiales	*Nakamurella*	•	●						
Gaiellales		•	•		●				
Holophagae Incertae Sedis	*Thermoanaerobaculum*				●				
Holophagae Subgroup 7			●	●	●				
Holophagales	*Geothrix*	•				•	●	●	
Kineosporiales	*Kineosporia*								●
Kineosporiales	Kineosporiaceae							●	
Methanobacteriales	*Methanobacterium*	•	●			●	●	●	
Methanomicrobiales	Rice Cluster II			●	●				
Micrococcales	Microbacteriaceae						●		
Myxococcales	*Haliangium*	•				●	●		
Myxococcales	Blrii41						●		
Neisseriales	Neisseriaceae					●			
Nitrosomonadales	*Sideroxydans*					●		•	
Pseudomonadales	*Pseudomonas fluorescens*					●			●
Rhizobiales	*Bradyrhizobium*	●	●	●		●	●	●	●
Rhizobiales	Hyphomicrobiaceae					●			
Rhizobiales	*Rhizobium*								●
Rhizobiales	*Rhodomicrobium*	●	●			●		●	•
Rhizobiales	*Roseiarcus*	•					●	●	●
Rhizobiales	Xanthobacteraceae		●	●	●				
Rhodospirillales	Acetobacteraceae	●							
Solirubrobacterales	YNPFFP1	•	●						
Syntrophobacterales	Syntrophaceae			•	●				
(Verrucomicrobia/OPB35 soil group)				•	●				
Xanthomonadales	*Steroidobacter*								●

Table 3.A.1b Top 20 fungal OTUs, by control condition (depth, PFG)

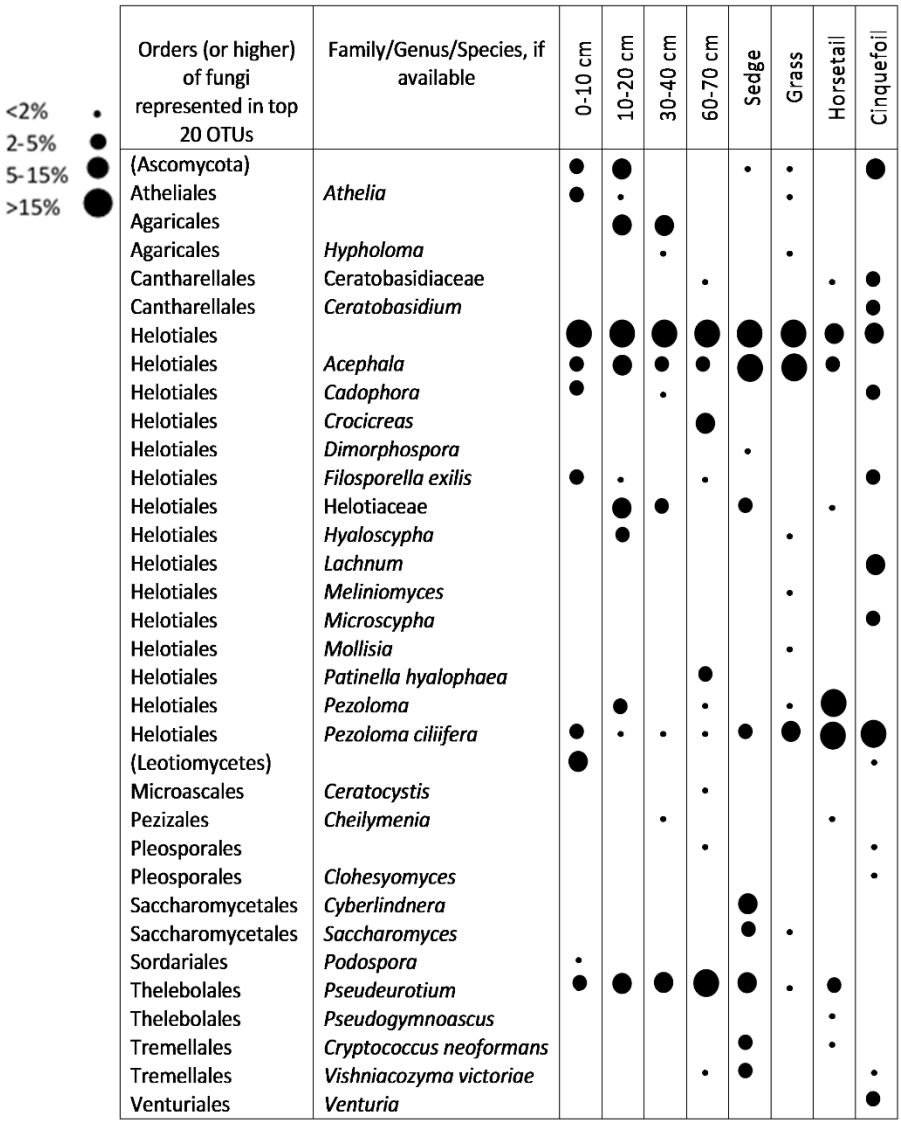


Table 3.A.2 Microbial clades affected by treatment (water table or PFG; non-exhaustive). All relationships listed in this table are significant unless stated otherwise (p<005); .where specific PFGs are grouped in significance, post-hoc letters are given.

Group affected by treatment	+ or - rel. abundance treatment conditions	Functional hypothesis
Genus *Geobacter*	(+) horsetail^{A}, grass^{A}, and sedge^{AB} roots (-) bulk peat^{B}, cinquefoil^{B} Trend (+) in raised compared to lowered (p=0.058)	Coupled oxidation of organic compounds (i.e. acetate, fatty acids, aromatic compounds, etc.) with reduction of Fe(III) and Mn(IV) oxides (Coates et al. 2001; Lovley et al. 2011)
Genus *Methylomonas*	(+)horsetail^{A}, sedge^{B} and grass^{B} roots (-) bulk peat^{C}, cinquefoil^{C} roots	Many species of this genus are methanotrophs (King and Adamsen 1992; Kalyuzhnaya et al. 1999; Ogiso et al. 2012; Hoefman et al. 2014)
Genus *Albidiferax*	(+) horsetail^{A}, cinquefoil^{A}, and sedge^{AB} roots (-) bulk peat^{B}, grass^{B} roots	Iron reducer (Willems 2014)
Genus *Duganella*	(+) sedge^{A}, horsetail^{AB}, grass^{AB}, cinquefoil^{AB} roots (-) bulk peat^{B}	Some species produce antifungal/anti-tumor/antiviral compounds (Jiang et al. 2010; Aranda et al. 2011)
Order Rhizobiales	less abundance at 10-20 cm in lowered compared to control	Rhizosphere associates, many of which fix nitrogen in peatlands (Leppänen et al. 2015)
Family Gallionellaceae	(+) sedge^{A}, horsetail^{AB}, and grass^{BC} roots (-) cinquefoil^{C} roots, bulk peat^{C}	Aerobic ferrous iron-oxidizing bacteria (Hallbeck and Pedersen 2014)
Family Enterobacteriaceae	(+) sedge^{A}, horsetail^{AB} roots, (-) bulk peat^{B}, cinquefoil^{B}, grass^{B}, roots	Root-associated members of this family include endophytic nitrogen fixing symbionts. This family also forms N-fixing root nodules reported on horsetail (Haahtela et al. 1981; Ladha et al. 1983; Fuji et al. 1984; Yaish 2016; Defez et al. 2017)
Genus *Steroidobacter*	(+) cinquefoil roots^{A} (-) bulk peat^{B}, sedge^{B}, grass^{B}, horsetail^{B} roots	Some species exhibit steroid degradation by nitrate reduction; may be enhanced by metabolites of order Rhizobiales (Sakai et al. 2014)

Family Veillonellaceae	(+) raised WT (10-20 cm), sedges[A], horsetail[AB] roots (-) control and lowered WT, bulk peat[B], cinquefoil[B] and grass[B] roots	Contains cellulose and chitin degrading species (Dai et al. 2016), likely carries out the rate-limiting step of methanogenesis (polysaccharide hydrolysis) (Glissmann and Conrad 2002; Tveit et al. 2015; Juottonen et al. 2017)
Family Erysipelotrichaceae	(+) cinquefoil roots[A], lowered WT vs. control (10-20 cm) (-) bulk peat[B], sedge[B], horsetail[B], grass roots[AB] (-)raised WT vs. control	Associated with mammalian pathogens, it is also closely related to the plant pathogenic Tenericutes (see Brinjal little leaf phytoplasma)(Verbarg et al. 2014), which, at this site, infects cinquefoil roots.
Order Clostridiales	(+) raised WT vs. lowered and control (10-20 cm)	Contains acetogens capable of symbiotic relationships with methanogens (Koesnandar et al. 1990; Cheryan et al. 1997; Ramirez 2018), likely carries out the rate-limiting step of methanogenesis (polysaccharide hydrolysis) (Glissmann and Conrad 2002; Tveit et al. 2015; Juottonen et al. 2017)
Genus *Methanobacterium*	(+) sedge[A], grass[A], horsetail[A] roots and bulk peat[A]; (+) most abundant in 10-20 cm, sig. more than 30-40 & 60 -70 cm (-) cinquefoil roots[B]	Methanogen, of the Methanobacteria (Oren 2014)
Family Rice Cluster II	(+) lowered 10-20 cm and 60-70 cm than control; most abundant in deep peat (60-70 cm)	methanogen, of the Methanomicrobia (Watanabe et al. 2006)
Brinjal little leaf phytoplasma	(+) cinquefoil roots in lowered and raised	bacterial plant pathogen (Jones 2001)
Filosporella exilis	(+) cinquefoil roots	aquatic hyphomycete/saprotroph (Baschien et al. 2013; Bärlocher 2016)
Pezoloma ciliifera	(+) horsetail[A] and cinquefoil[B] roots	saprobe (Stenroos et al. 2010)

	(-) bulk peat^{C}, grass^{C}, sedge^{C} roots	
Genus *Acephala*	(+) grass^{A} and sedge^{A} roots (-) bulk peat^{B}, cinquefoil^{B}, horsetail^{B} roots	potential dark septate endophyte, symbiotroph (Porras-Alfaro and Bayman 2011)
Genus *Mortierella*	(+) sedge^{A} roots (-) bulk peat^{AB}, horsetail^{B}, grass^{B}, cinquefoil^{B} roots	saprobe, members may provide plant resistance to pathogens (Eroshin and Dedyukhina 2002; Thormann 2006)
Order Helotiales	(+) horsetail^{A}, grass^{AB}, sedge^{ABC} roots (-) cinquefoil^{C} roots, bulk peat^{BC}	widely root associated order (Tedersoo et al. 2009)

4 Trace gas ^{13}C and pore water chemistry response to vegetation community and long term water table manipulation in an Alaskan rich fen

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4.1 Abstract

The boreal landscape of central Alaska is predicted to shift toward a greater prevalence of fen ecosystems due to changing hydrology. Fens are relatively understudied in regard to climate-driven shifts in carbon storage dynamics. The effects of hydrologic change on a boreal fen ecosystem are studied here via a long-term experimental water table manipulation (raised, lowered, and control) in a rich fen near Fairbanks, Alaska. Using natural abundance stable isotopes ($^{13}CH_4$ and $^{13}CO_2$) within peat pore water, the inner stems of aerenchymatous plants, and evaded gases, we studied carbon dioxide production, and methane production and oxidation dynamics, within the water table treatments. The most enriched $^{13}CO_2$ and $^{13}CH_4$ within the profiles were in the inner stem gasses of sedge and horsetail. Overall, methane oxidation was not as prevalent at the raised water table treatment as in the lowered or control treatments. This may be in part due to root exudate-stimulated methanogenesis, as sedges have become more prevalent in the raised treatment. Conversely, pore water CO_2 production was positively related to dissolved organic carbon concentrations, which were lowest in the raised treatment. This leads to overall greater uptake of CO_2 (lower net ecosystem exchange) in trace gas measurements from the raised water table plot. We suggest that methane oxidation is an important biogeochemical process in the fen, but the oxidation in the raised plot—which produced conditions indicative of both methane oxidation and methanogenesis—may be overwhelmed by the saturated nature of the plot and/or methanogen stimulation by labile sedge root inputs.

4.2 Introduction

Northern boreal ecosystems are experiencing changes in climate at an accelerated rate in comparison to other areas of the world (Hinzman et al. 2005; Stewart et al. 2013). In particular, hydrologic regime shifts are expected to occur in central Alaska as the timing and amounts of precipitation change and the rates of permafrost (perennially frozen soil) thaw increase (Hinzman et al. 2006; Avis et al. 2011). Especially in wetland-heavy landscapes such as Alaska, changes in hydrology exert direct controls over carbon exchange with the atmosphere, as demonstrated in numerous studies (Moore and Roulet 1993; MacDonald et al. 1998; Turetsky et al. 2008b; Chivers et al. 2009a; O'Donnell et al. 2012; Kane et al. 2013; Klein et al. 2013). In this setting, the prevalence of fens, or wetlands that receive water in part from surface and groundwater sources, is largely present and predicted to increase in area (Vitt et al. 2000; Lara et al. 2016). However, the dynamic nature of fen hydrology poses challenges for in-situ field studies; therefore, these increasingly important components of the boreal landscape are understudied in comparison with other types of wetlands (Lund et al. 2010; Turetsky et al. 2014).

To further our understanding of carbon cycling in boreal fens, a long-term experimental water table manipulation (raised water table scenario, lowered water table scenario, control) was established in a rich fen near Fairbanks, Alaska in 2005, coined the Alaska Peatland Experiment (APEX). Through 14 years of experimental manipulation and research, key findings for carbon cycling in response to hydrologic change at the fen include a) warmer, wetter conditions lead to enhanced methane production (Turetsky et al. 2008b); b) the long-term low water table scenario resulted in lower carbon dioxide uptake, but yet unresolved effects on ecosystem respiration (Olefeldt et al. 2017); and c) water table manipulation led to vegetation shifts resulting in more sedges in the raised water table experimental plot (Churchill et al. 2014). Sedges, and other gas-transporting plants such as horsetails, have demonstrated capabilities of oxidizing the soil around their roots, creating an environment favorable to methane oxidation to CO_2 (Popp et al. 2000; Agethen et al. 2018; Rupp et al. 2019). On the other hand, sedge rhizospheres have been shown to have more labile dissolved organic carbon (DOC) than those of other rich-fen plants (Rupp et al. 2019), which can also foster methanogenic microorganisms (Chanton et al. 1995; Glaser and Chanton 2009). Sedges also possess air-filled (aerenchymal) tissue capable of transporting atmospheric gasses to the rhizosphere, oxidizing the peat there. However, the extent of methane oxidation and, conversely, the conversion of CO_2 to methane (methanogenesis)—processes mediated by changes in plant and microbial communities in response to altered hydrology at APEX—have not been directly investigated.

Stable isotopic measurements of pore water gases and gas effluxes to the atmosphere can elucidate mechanisms of methane oxidation and production (Inglett et al. 2013). Briefly, an enrichment of $^{13}CO_2$ in pore water coupled with a depletion of $^{13}CH_4$ could point to the occurrence of methanogenesis via CO_2 conversion to methane (Galand et al. 2010). Acetoclastic methanogenesis was exluded in consideration due to the general lack of microorganisms using this pathway in Alaskan peatlands, including nearby rich fens

(Rooney-Varga et al. 2007). Conversely, an enrichment in $^{13}CH_4$ coupled with a depletion of $^{13}CO_2$ could point to the conversion of methane to CO_2, or methane oxidation (Chasar et al. 2000; Popp et al. 2000). Additionally, little to no research, to our knowledge, has explored the natural abundance and/or transformation of gaseous ^{13}C inside the stems of sedges and other plants, such as horsetail, that serve as gas conduits between the soil and the atmosphere (Armstrong and Armstrong 1991; Chanton et al. 1993; Brix et al. 1996; Grosse et al. 1996; Colmer 2003).

To investigate the mechanistic controls of methane oxidation and production in boreal fen peatlands in response to changes in hydrology and dominant plant functional groups, we leveraged the APEX framework to test our hypotheses. In this experiment, we hypothesized that 1) methane oxidation, as demonstrated by natural abundance ^{13}C in pore water, would be enhanced in the raised water table plot because it has adapted over time to contain more sedges in the vegetation community. As such, sedge-mediated methane oxidation could be mediating C export dynamics in the raised water table treatment owing to greater sedge abundance. In addition, we expected that 2) the character of dissolved organic carbon (DOC) would be indicative of higher degradation in the lowered plot. Conversely, we would expect to find preserved, less degraded carbon as well as aliphatic compounds indicative of rhizodeposition from sedge roots in the raised water table plot; the increased labile substrate could override any oxidizing effects of sedge rhizospheres in mediating methane oxidation. An additional goal of this research was to map a profile of ^{13}C from the soil profile to the atmosphere, including gasses from inside sedge and horsetail stems, during a year in which the entire fen was flooded, creating a natural growing-season long "flood treatment" in the entire fen.

4.3 Methods

4.3.1 Field collection

4.3.1.1 Pore water isotopes and chemistry from water table treatments

Two pore water samples were taken from 20cm and 40 cm below peat surface in each water treatment plot on May 26, July 1, and August 2 of the 2016 growing season. Pore water was collected using a stainless steel 0.52 cm diameter tube with a 2 cm slotted region at the end, equipped with a three-way stopcock at the top of the device. For each sample, water was first drawn up to purge the tube, wasted through the stopcock so as to not introduce ambient air, and then 60 mL of pore water were collected with the syringe. Immediately after extraction, a 0.45 µm polyethersulfone syringe filter equipped with a borosilicate glass fiber prefilter (Whatman™) and needle were attached to the syringe and the pore water sample was injected into a pre-evacuated (<-3 milletorr) Wheaton vial sealed with a butyl septum. Vials were stored inverted and refrigerated until lab processing.

4.3.1.2 CO_2 and CH_4 gas efflux sampling

In 2005, metal collars, measuring 60 cm x 60 cm, were permanently installed in each of the three water table treatment plots to accommodate static chambers with the same footprint for use in determining volumetric fluxes of carbon dioxide and methane (Turetsky et al. 2008b). Seven campaigns of gas efflux measurements from four collars within each treatment were taken between June 10 and July 29, 2016, after which flooding prevented further gas efflux data collection. Peat temperature (10 cm depth) and oxidation-reduction potential (Eh; 20 cm depth) were taken coincidentally using a soil thermometer and Hach Co. (Loveland, Co., USA, Intellical MTC301) probe connected to a sealed and sample-purged flow-through cell. All Eh values were normalized to a pH of 7 (Eh7), based on pH–Eh relationships for quinhydrone (Bier 2009).

Carbon dioxide flux data were collected using a PP Systems EGM-4 infra-red gas analyzer (IRGA) paired with a PP Systems compatible photosynthetically active radiation (PAR) probe to monitor light-induced changes in photosynthetic activity as previously described by Chivers et al., 2009. The PAR probe was installed directly into a 60 x 60 x 60 cm Plexiglas static chamber with a metal frame. At each site, the chamber was placed in the metal collar with care taken to ensure a tight seal. Fans installed within the chambers allowed mixing of air, and were powered via a 12V external battery. Measurements were logged using PP Systems Transfer® software. A minimum of 120 measurements over a span of approximately 3 minutes were collected at each collar in lighted (ambient) conditions. Dark chamber measurements were taken immediately following ambient measurements to compare primary productivity and ecosystem respiration. Dark chamber measures were accomplished by placement of a large opaque white shroud over the static chamber to block sunlight. PAR probe output was monitored throughout the dark measurement to ensure no PAR was entering the chamber. Carbon dioxide static chamber measurements were generally taken once a week from late May-mid July 2016, when flooding at the site prevented further efflux measurements. Net ecosystem exchange was calculated and reported as light - dark flux measurements.

4.3.1.3 Methane (CH_4)

Static chambers were placed into installed collars and covered with an opaque white shroud to prohibit photosynthetic influence and solar degradation to gas samples and to keep the chamber from overheating. Internal fans were powered by an external 12V battery to ensure mixing of the air within the chamber. Gas from inside the chamber was extracted through a tube with a stopcock into 20 mL syringes equipped with stopcocks at minutes 0, 5, 10, 15, 20, 25, and 30. Gas line was purged prior to sample collection by pulling and compressing the syringe several times before filling. Gas samples were stored in the syringes overnight before processing, as described below.

4.3.1.4 Gaseous $^{13}CH_4$ isotopes released to the atmosphere

Gaseous samples for $^{13}CH_4$ were collected following 30 minutes of gas accumulation the same darkened chamber used for flux measurements from each water table treatment on two dates. Gas was extracted from the chamber using a 20 mL syringe and stopcock, then injected into a 12 mL Exetainer® vial and sent for analysis as described below.

4.3.1.5 Pore water, surface water, and inner stem gas profile

An exploratory investigation of CH_4 concentrations of inner stem gasses of sedges was undertaken during the growing season of 2016. On July 15, 2016, six gas samples each were extracted from the inner tissues of in situ, live sedge plants in the control, raised, and lowered plot. Gas was extracted from the sedges using a 20 mL syringe equipped with a 3-way stopcock and 0.4 mm x 13 mm BD PrecisionGlide™ needle as described below. One ambient air sample was also collected from each site during that time. All samples were analyzed for methane concentrations as described below.

On June 28th of the 2017 growing season, when the APEX fen was flooded, a profile of CO_2 and CH_4 concentrations and isotopes, including interior stem gases of horsetail and sedge, were collected. From each of 5 sites along a transect outside of the treatments, two pore water samples (20 cm below peat surface) and one standing water sample (20 cm below water surface) were collected as described above. At each site, six sedge and six horsetail stems were sampled for gasses using a needle and syringe, puncturing the stem walls into the hollow/aerenchymous interior and withdrawing 10 mL of gas. These samples were analyzed for CH_4 concentrations. An additional inner gas sample from each plant species was taken at each site and injected into a 12 mL Exetainer® vial for later processing for natural abundance isotopes. Oxidation-reduction potential was measured at each site at 20 cm below peat surface and 20 cm below water surface.

4.3.2 Laboratory analysis

4.3.2.1 Headspace gas collection for $^{13}CO_2$ and $^{13}CH_4$ isotope analysis

Pore water collection bottles were shaken vigorously for one minute, after which two aliquots of 12 mL of gas were removed and injected into dinitrogen gas-flushed and pre-evacuated (<-3 milletorr) 12 mL Exetainer® (Labco) vials. Isotopic composition (δ13C) of CO_2 and CH_4 in gas samples were analyzed at the UC Davis Stable Isotope Facility, CA, USA using a continuous flow Isotope Ratio Mass Spectrometer (IRMS). Data returned also included CO_2 ad CH_4 concentrations (ppmv) within the samples.

4.3.2.2 Porewater chemistry

Pore water was extracted from each of the Wheaton® vials, and analyzed for absorbance and fluorescence spectroscopy using a Horiba® (Horiba-Jobin Yvon Aqualog C; Horiba

Co., Edison, NJ) fluorometer. Samples were diluted as needed such that absorbance values were in the range $0.1<A_{254\ (sample)}<0.6$ to optimize spectral signal and to prevent the detector from losing linearity. Data were normalized to an internal standard (integral of the Raman peak of the sample), relativized to an external standard (Raman peak of Starna® water), corrected for inner filter effects, and scrubbed of Raman and Rayleigh scatter, as described in detail in Veverica et al. 2016. Absorbance and fluorometric indices for $SUVA_{254}$, Humification Index (HIX), Spectral ratio (Sr), E2:E3 (De Haan and De Boer 1987; Avagyan et al. 2014), and Ca:Cc (Kothawala et al. 2012) were calculated, which express size and character of dissolved organic carbon. The remaining porewater was acidified to pH <2 and analyzed for total dissolved organic carbon and total dissolved nitrogen (TDN) on a Shimadzu® (Shimadzu Scientific Instruments, Columbia, MD) Total Organic Carbon Analyzer with a TDN module.

4.3.2.3 Methane

Methane samples were processes within 24 hours of collection using a gas chromatograph (Varian 3800 FID detector; Varian Analytical Inc., Palo Alto, CA) calibrated with three concentration standards daily (0, 10.22, and 100 ppm). All fluxes were inspected visually for evidence of ebullition events during measurements; these fluxes were discarded along with negative effluxes, resulting in the deletion of 4 of 84 data points.

4.3.3 Statistics

Relationships between variables were tested using general linear models, using function lm in R statistical software (Team 2013). Analysis of variance (aov function) with Tukey Honest Significant Difference Test (HSD) post-hoc analysis were run to determine between-treatment differences. Gas efflux measurements between water table treatments were tested using mixed effects models using water table treatment as the fixed effect and sampling campaign/date as a random effect, using the lmer and eemeans (Tukey adjustment) functions for modeling and post-hoc analysis in the lme4 and eemeans packages (Bates et al. 2007; Lenth and Lenth 2018). CH_4 efflux data were square root transformed to meet model assumptions for mixed models. Pore water gas concentrations and inner stem gasses were log transformed to fit model assumptions. Significance was accepted at $p=0.05$, with marginal significance $0.05<p<0.07$ Summary statistics reported in-text are mean ± standard deviation. Graphics were created using ggplot2 (Wickham 2016).

4.4 Results

4.4.1 Background environmental measurements

Trace gas efflux, oxidation-reduction potential, temperature, and depth to water table fluctuated throughout the experimental period. Generally, the water table treatments most affected the minimum depth to water table, with the raised water table never falling below 1.5 cm beneath the peat surface, as opposed to the lowered water table falling to 33 cm beneath the peat surface within the experimental period (**Table 4.1**). Depth to water table between water table treatments (cm) was significant ($p=0.01$ between raised and lowered water table treatments). Oxidation-reduction potential decreased throughout the season and was not significantly different between treatments at 20 cm and 40 cm. Net ecosystem exchange (light + dark CO_2 efflux measurements) trended lowest (greatest CO_2 uptake) in the raised treatment plot ($p=0.09$ compared with the control), in continued agreement with Olefeldt et al. 2017 (**Fig. 4.A.1**). Overall, the fen was a small net source of CO_2 during the relatively wet, cold summer of 2016 taking into account fluxes collected during midday (light and dark) only. Ecosystem respiration (ER) was lower in both the lowered and the raised water table treatments than the control, but ER in the lowered and raised plots were not different from one another ($p=0.191$). Gross primary productivity was not different between treatments. Methane efflux was higher in the raised water table treatment than in the control ($p<0.0001$) and lowered ($p=0.001$) plots (**Fig. 4.A.2; Table 4.1)**.

4.4.2 Isotopes and chemistry from water table treatments

Isotopes from all depths and 2016 sampling campaigns (effluxed CH_4 gas from chamber measurements, 20 cm, and 40 cm pore water) differed between pore water gas and efflux samples, but generally not between treatments except for the raised water table treatment (**Fig. 4.1 and Fig. 4.2)**. The raised water table treatment $^{13}CH_4$ efflux (δ -58.9 ± 1.351), after chamber accumulation, was isotopically depleated (closer to the signature of the pore water) than that of the lowered (δ -53.4 ± 1.257, $p=0.017$) and control plots (δ -51.6 ± 1.027, $p<0.001$, **Fig. 4.1**). Lowered and control treatment chamber accumulated $^{13}CH_4$ was isotopically enriched compared with pore water counterparts (lowered pore water δ -59.1 ± 0.971, $p=0.030$; control pore water δ -57.7 ± 0.971, $p=0.019$). In the raised plot, δ $^{13}CO_2$ in pore water increased throughout the season as the fen flooded, but this was not observed in the control or lowered plots (**Fig. 4.2**). Treatment differences in pore water isotopic signatures for $^{13}CO_2$ and $^{13}CH_4$ were not significant except for $^{13}CH_4$ at 40 cm, which was more depleted in the raised water table (δ -60.37 ± 2.80) than in the control (δ -56.37 ± 2.84; $p=0.035$). There were positive relationships between $^{13}CO_2$ and pore water $[CH_4]$ at 20 cm ($p<0.001$, **Fig. 4.3a**), and $^{13}CH_4$ and pore water $[CO_2]$ at 40 cm ($p=0.021$, **Fig. 4.3b**). There was no interactive effect of pore water gas concentration and treatment on ^{13}C in either case. There was a marginal treatment effect on $^{13}CH_4$ at 40 cm when $[CO_2]$ was also accounted for ($p= 0.058$).

Pore water [CO_2] was positively related to DOC at all depths (**Fig. 4.4**). Water table treatment explained a marginal amount of variation in pore water [CO2] (p= 0.06, F= 3.09), with the raised water table harboring both the smallest concentrations of DOC (46.76 ± 11.18 mg C L^{-1}) and pore water [CO_2] (**Fig. 4.4**). There was no interactive effect of DOC and treatment effect on pore water [CO_2]. At the 20 cm depth, the raised water table treatment had lower concentrations of pore water CO_2 (11494 ± 7787 ppmv) than both the lowered (26967 ± 8908 ppmv; p=0.001) and control (31737 ± 12965 ppmv; p<0.001). At 40 cm, the relationship was the same, but weaker: the raised water table treatment had lower concentrations of pore water CO_2 (14388 ± 4906 ppmv) than both the lowered (27607 ± 12819 ppmv; p=0.060) and control (25931 ± 11978 ppmv; p<0.031).

Generally, differences in absorbance and fluorescence spectroscopic characteristics of the pore water between treatments were more pronounced at the shallower depth sampled (20 cm). Of the parameters measured, the following were significantly different by water table treatment: HIX was lower in the raised treatment (0.94 ± 0.01) compared with the lowered treatment at 20 cm (0.96 ± 0.01; p=0.003), but not at 40 cm (**Fig. 4.5a**). Sr, inversely related to oxidation status and molecular weight, was higher in the raised plot (0.80 ± 0.04) than lowered (0.74 ± 0.03; p=0.008) and control (0.76 ± 0.02; p=0.047) plots at 20 but not 40 cm. (**Fig. 4.5b**); TDN was lowest in the raised water table (1.47 ± 0.19 mg N L^{-1}; p<0.001 vs. lowered 3.1 ± 0.71 mg N L^{-1}; p=0.003 vs. control 2.6 ± 0.63 mg N L^{-1}) at 20 cm but not 40 cm. DOC was lowest in the raised water table plot at 20 cm (46.33 ± 11.34 mg C L^{-1}; p=0.027 against control 80.73 ± 24.17 mg C L^{-1}; p=0.009 against lowered 87.5 ± 23.33 mg C L^{-1}), but not at 40 cm. $SUVA_{254}$ was also lowest in the raised plot at 20 cm (4.11 ± 0.31; p=0.029 against control 4.77 ± 0.36; p=0.014 against lowered 4.86 ± 0.50), but not at 40 cm. When the water table treatments were the most separated (7/1), the raised plot had higher Ca:Cc in both 20 and 40 cm, with a legacy effect of this trend persisting in the 20 cm depth when the site flooded in August. Wet conditions in general appear to make Ca:Cc go up in this fen (from approximately 1.35 to 1.5). E2:E3 did not differ by water table treatment, but was significantly higher, linked to more oxidation, at the 20 cm depth (4.66 ± 0.21) as opposed to 40 cm depth (4.38 ± 0.36; p=0.007).

4.4.3 Pore water, surface water, and Inner stem gas profile

The distribution of gaseous [CH_4] (13C and ppmv reported with stable isotope data) in pore water, surface water, inside stems of sedge and horsetail, and ambient air from the 2017 profile sampling campaign are displayed in **Fig. 4.6**. No significant differences were found in gas chromatograph-obtained [CH_4] (ppm) between inner stem gasses of sedges from the water table treatments in 2016, but the spread of these data can be viewed in **Fig. 4.A.3**. The concentrations of CH_4 found within the stems of horsetail (179.78 ± 351.77 ppm) were higher than those found within the sedge stems (60.96 ± 87.18 ppm) on the day of sampling in 2017 (p=0.037).

Interestingly, the least depleted $^{13}CO_2$ (isotopically heaviest) was found inside horsetail stems (δ -9.72 ± 1.18), and the least depleted $^{13}CH_4$ (isotopically heaviest) gas was found inside sedge stems (δ -40.24 ± 2.09). The highest concentrations of CO_2 (31547 ± 14798 ppmv) and CH_4 (588 ± 428 ppmv) were found in the pore water. Both plants had elevated concentrations of CH_4 in comparison with the surface water and atmosphere (**Fig. 4.6**). Environmental properties at the time of profile collection are found in **Table 4.A.1**.

4.5 Discussion

Contrary to our first hypothesis, the persistence of a high water table (raised treatment) precluded a higher abundance of sedges in moderating methane oxidation. In fact, methane oxidation was more prevalent in the lowered water table and control treatments than in the raised treatment as evidenced in $^{13}CH_4$ effluxes (**Fig. 4.1**). In agreement with our second hypothesis, water chemistry reflected more dissolved organic carbon of a degraded nature in the lowered water table treatment than the raised, which had lower DOC concentrations and DOC of a more labile nature. Overall, the water table treatments have affected the pore water chemistry and character in the upper 20 cm, but not at the deeper 40 cm sampling depth. In general, the raised water table treatment has seen the greatest divergence from the control and lowered plots in character of pore water gas and chemistry composition. The results of our exploratory isotopic profiling, vertically in the peat and pore water and within stems of different plant functional groups, revealed patterns that can guide future isotopic work in rich fen ecosystems.

4.5.1 Depth driven C transformation relationships in water table treatments

There appear to be depth-determined relationships between pore water concentrations of CO_2 and CH_4 and their respective isotopic enrichments. At the 40 cm pore water sampling depth, there was a positive relationship between δ $^{13}CH_4$ and pore water [CO_2], with the raised water table plot samples concentrated on the lower end of the relationship closest to the axis (most depleted $^{13}CH_4$ and lowest concentration of [CO_2]). This is suggestive of higher rates of hydrogenotrophic, or CO_2-driven methanogenesis occurring in the raised treatment at 40 cm, which has been suggested based on an accumulation of acetate in this treatment plot in previous findings (Kane 2013). Additionally supporting this argument is the relative abundance of a hydrogenotrophic but not acetoclastic methanogen community present in this fen (unpublished data).

At the 20 cm pore water sampling depth, there is a positive relationship between $^{13}CO_2$ and pore water [CH4], and the raised treatment falls on both ends of the graphical spectrum, representing both the most enriched and most depleted samples. The raised water table also encompasses some of the lowest and highest pore water [CH_4] concentrations. It appears that the nature of the raised water table treatment can promote both conditions indicative of methane oxidation (likely caused by oxidizing effects of the sedge/horsetail rhizospheres) and methane production. In saturated conditions geochemically favorable for methanogenesis, it is likely that we would find anaerobic

microsites of pore water where carbon dioxide is being converted to methane further up in the peat profile. It is of interest to note that the oxidation-reduction potential at 20 cm did not significantly differ between water table treatment plots, indicative of similar geochemical conditions. This could suggest that the shift in plant community toward more sedges, and resultant aerenchymal delivery of oxygen, precluded a deterministic relationship between ORP and methane efflux rates, at least at the depths measured in this study. The very plants that oxidize the rhizosphere could also be contributing labile root exudates (as also indicated by lower HIX) to the methanogen community, which could be stimulating methanogenesis (Murray et al. 2019).

In the raised plot, $\delta\ ^{13}CO_2$ increased throughout the season as the fen flooded in August 2016, indicative of increasing methanogenesis (**Fig. 4.2**); however, this was not observed in the control or lowered plots. One explanation for this could be that the raised plot is already depleted of alternate electron acceptors leading to methanogenesis occurring at the site at a higher rate. The control and lowered plots, on the other hand, may have a built-up reservoir of rechargeable alternate electron acceptors due to greater water table fluctuation and upper layer peat drying, thereby suppressing methanogenesis via a lag effect when the site flooded (Estop-Aragonés and Blodau 2012; Klupfel et al. 2014).

$^{13}CH_4$ gas efflux samples, taken after 30 minutes of accumulation in a static chamber, were relatively enriched from the lowered water table and control plot pore water, but not from the raised water table pore water (**Fig. 4.1**). This is indicative of methane oxidation occurrence within the top layer of peat, before diffusion to the atmosphere (Chasar et al. 2000; Knorr et al. 2008). Taken together, these findings suggest that methane oxidation is an important biogeochemical process in the fen, but less so in the raised plot. Future work examining these isotopic changes in experimental manipulations of the vegetation communities is necessary to elucidate the relative effects of hydrology and sedge abundance on methane oxidation and production.

4.5.2 Character of dissolved carbon and pore water chemistry

Overall, the greatest differences in pore water chemistry were seen in the raised water table treatment. The raised treatment contained lower DOC and TDN compared to the other treatments, likely owing to a reduction in the water soluble products of decomposition, rather than any potential dilution effects (Kane et al. 2010). The nature of the DOC in the raised water table treatment was of a relatively low molecular weight—low $SUVA_{254}$ and HIX; high Sr (Ohno 2002; Weishaar et al. 2003; Helms et al. 2008)—or labile in nature compared with the lowered and control treatments. Given that the raised water table treatment contains a greater abundance of sedges (Churchill et al. 2014), these findings are in agreement with those of Rupp et al. 2019, who found lower concentrations of DOC, of lower aromaticity and molecular weight, in sedge-only mesocosms than other plant treatments. In that case, we suggested the lower molecular weight organics could be root-derived inputs such as simple sugars. Another possible explanation for a more labile DOC pool in the raised water table treatment is a greater presence of algae due to the higher water table and persistence of a photic zone (Wyatt et

al. 2012). Sedge-associated microorganisms in a relatively aerated rhizosphere could be more effective in the raised water table treatment at mineralizing DOC. As we see from the positive relationships found in this study between DOC concentration and pore water [CO_2], DOC likely provides a substrate for CO_2 production, though production and consumption relationships for DOC are difficult to disentangle (Michaelson et al. 1998; Wickland et al. 2007). Future work examining the complex interplay among sedge rhizosphere oxidation, sedge rhizodeposition, algal inputs, and altered hydrology is needed to fully appreciate the importance of DOC in carbon cycle processes at this site.

4.5.3 Isotopic profile & inner stem gasses

The most enriched $^{13}CO_2$ and $^{13}CH_4$ within the profiles were found in the inner stem gasses of sedge and horsetail, suggesting that these plants are accumulating $^{13}CO_2$ and $^{13}CH_4$ (**Fig. 4.6**). Plant accumulation of isotopically heavier compounds in tissues, such as Zn (Caldelas and Weiss 2017) and radioisotopes (Mahon and Mathewes 1983) is documented. The isotopic skew within the plant stems could be caused by the photosynthetic process (Farquhar 1983). Cycling of gases could be occurring within the stem itself; for example, there is evidence that plants may recycle the CO_2 held within their stems for photosynthesis (Billings and Godfrey 1967), which could lead, over time, to isotopic enrichment if $^{12}CO_2$ is favored in those reactions. Abiotic oxidation of methane within the stems as CH_4 comes in contact with O_2 (plant-produced or diffused) could cause an enrichment of $^{13}CH_4$ within stems. There are few studies examining inner stem gasses, but those that do conclude that diffusion (from the rhizosphere or atmosphere) is the primary actor on inner stem gas concentrations (Teal and Kanwisher 1966; Brix 1988). Diffusion of trace gas from the rhizosphere to the atmosphere is in agreement with the CO_2 and CH_4 gas concentrations found within the sedge and horsetail stems in our study, as the concentrations are midway between pore water concentrations and atmospheric concentrations (**Fig. 4.6**).

4.5.4 Implications for future hydrologic change

Altogether, the findings from this study demonstrated that methane oxidation is likely an important biogeochemical process in this rich fen, and is likely more prevalent in the lowered and control plots. By maintaining a higher water table in the raised water table treatment plot, we significantly reduced the variation in water table fluctuations within that treatment (cf. Kane et al. 2010), which likely reduced the regeneration of alternate electron acceptors with the capacity to suppress methanogenesis. Over time, this may have decreased the raised plot's capacity to oxidize methane despite the prevalence of rhizosphere-oxidizing sedges. Additionally, the stimulation of methanogenesis by sedge root exudates and aerenchymal transport of methane could be more influential than any oxidizing effects of the sedges. Although carbon dioxide recycling and methane oxidation could still be occurring within the above-ground stems of sedges and horsetails, the net effect of a raised water table was higher methane efflux coupled with higher uptake of

CO_2. As the boreal region moves toward a wetter landscape, this may suggest a degree of resilience in these rich fen ecosystems, from a carbon cycling perspective.

4.6 Acknowledgements

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Conflict of Interest: The authors declare that they have no conflict of interest.

4.7 Figures & Tables

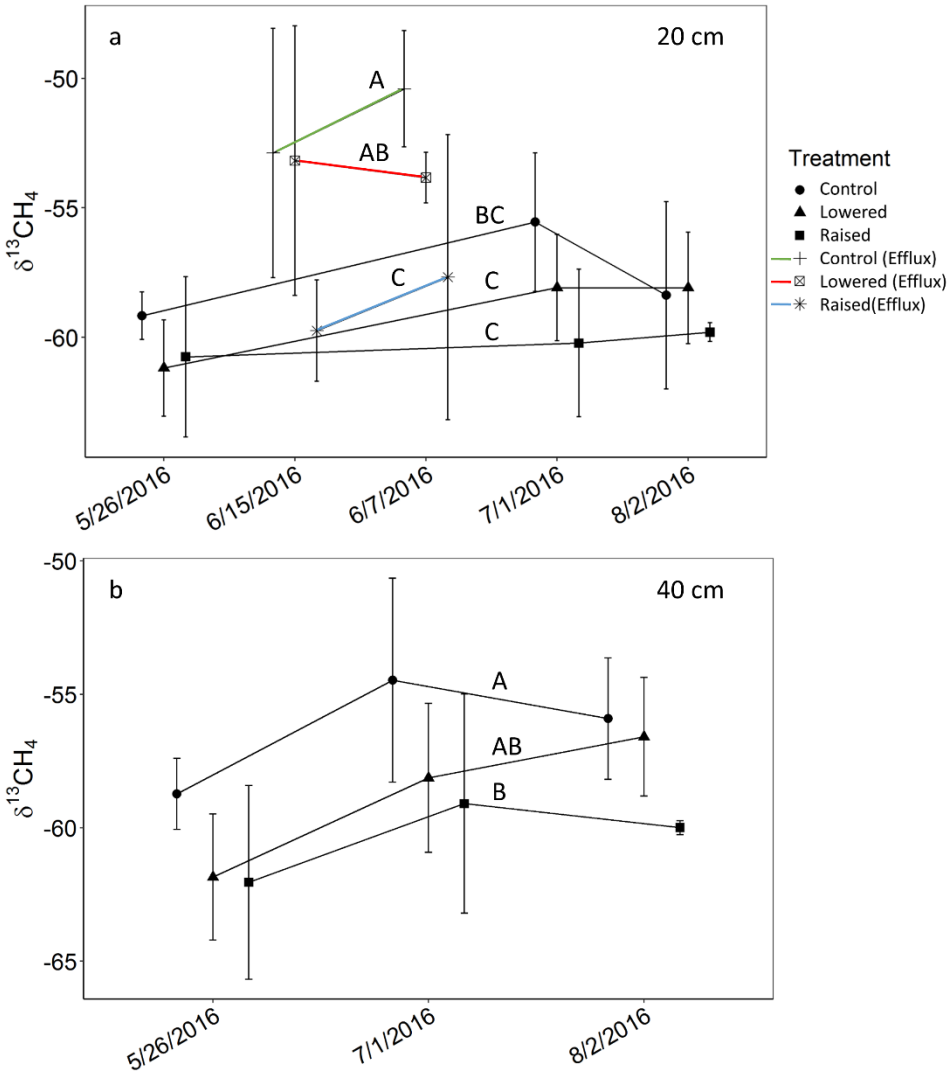

Fig. 4.1 $^{13}CH_4$ in pore water gas (3 sampling campaigns throughout the growing season) and after 30 minutes of efflux chamber accumulation (2 sampling campaigns). Shown are samples from a) air above peat surface, within chambers (efflux) and 20 cm below peat surface (solid shapes, pore water), and b) 40 cm below peat surface (pore water). Significant differences from post-hoc tests are denoted with different letters comparing treatments across the entire season. Treatments were sampled on the same date for each campaign; data is offset for ease of interpretation

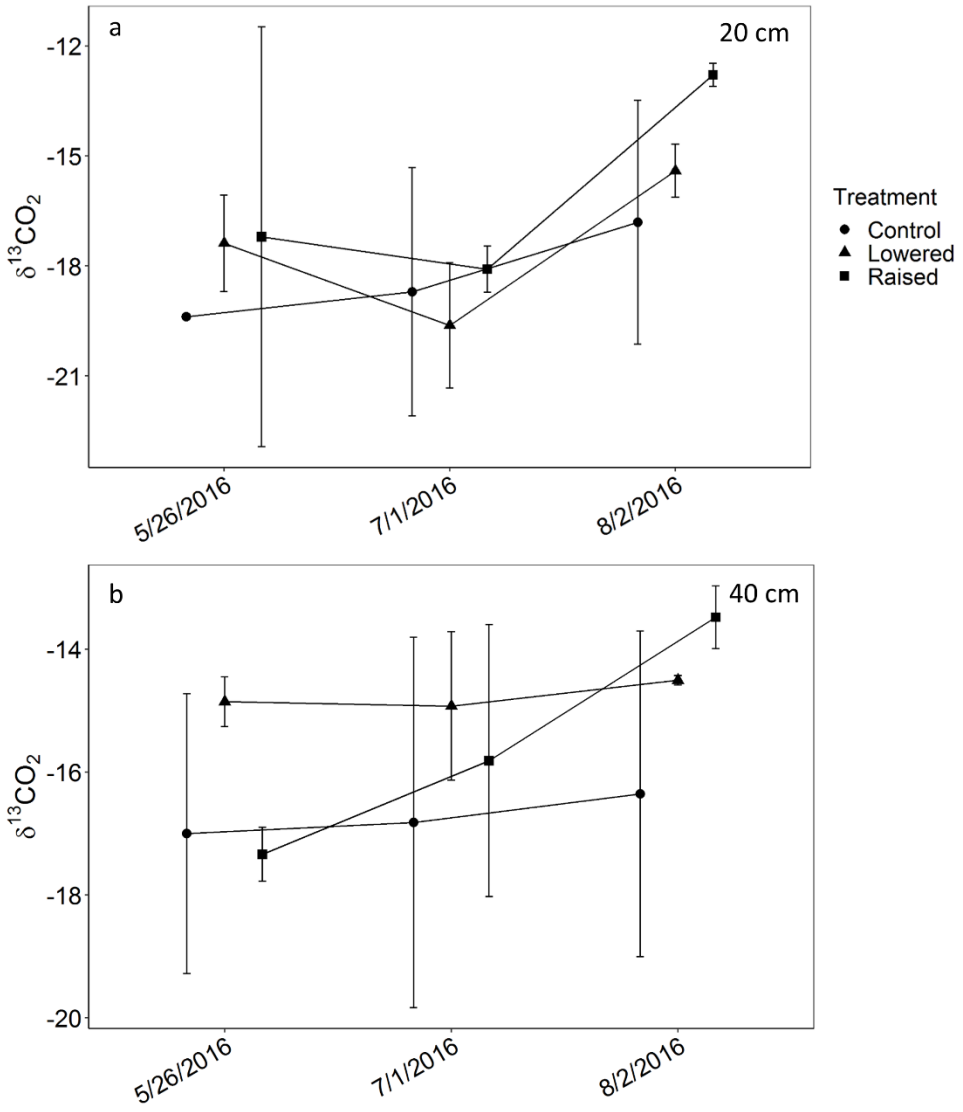

Fig. 4.2 $^{13}CO_2$ in pore water gas (3 sampling campaigns throughout the growing season). Shown are samples from a) 20 cm below peat surface, and b) 40 cm below peat surface (pore water). Treatments were sampled on the same date for each campaign; data is offset for ease of interpretation

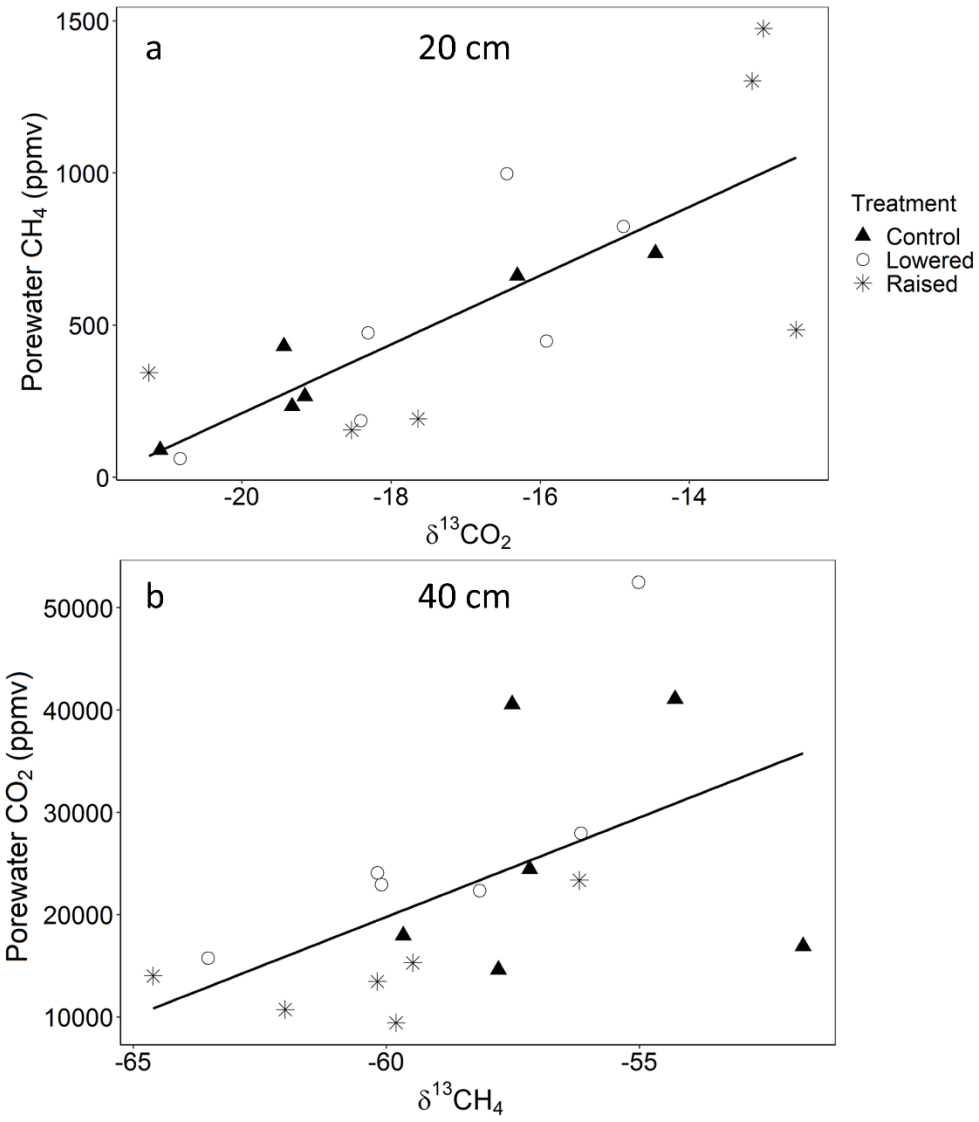

Fig. 4.3 Positive relationships between a) $^{13}CO_2$ and [CH_4] at 20 cm (y=113.01(±23.09)x + 2471.75(±403.66); p=0.0001; F=23.95) and b) $^{13}CH_4$ and [CO_2] at 40 cm (y=1943(±757)x + 136373 (±44374); p=0.017; F=7.630) in pore water gasses from water table treatment plots. When accounting for treatment in ANCOVA, neither treatment (p=0.182; F=1.957) nor the interaction between treatment and [CO2] (p=0.306; F=1.308) were significant.

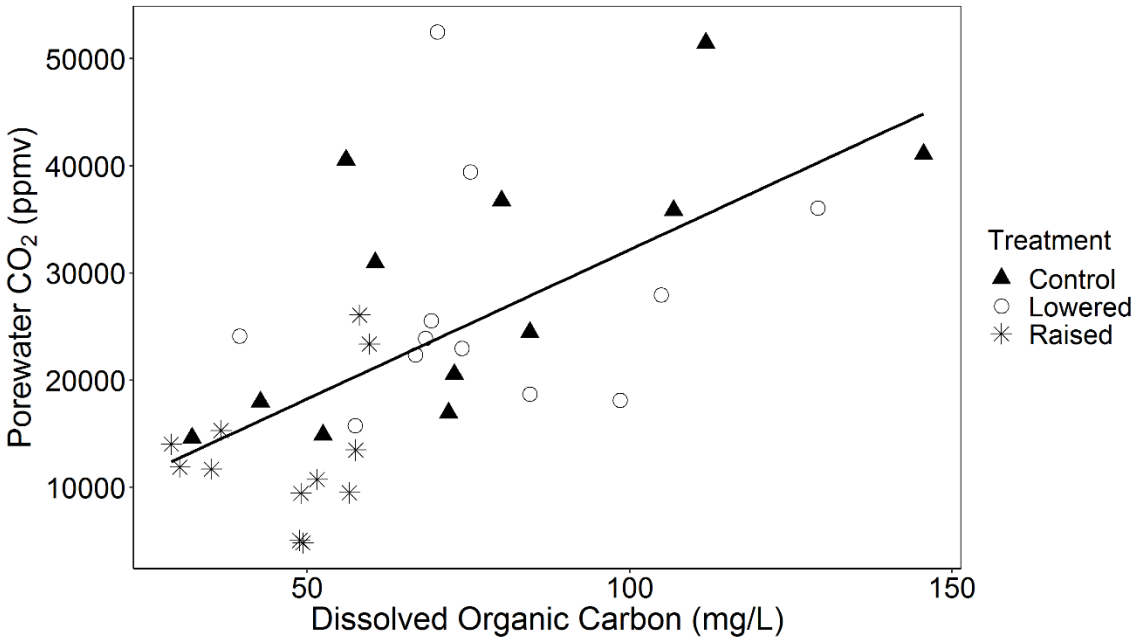

Fig. 4.4 Positive relationship between pore water dissolved organic carbon [DOC] and [CO_2] across all depths and treatments ($y=0.001(\pm0.0003)x + 34.4(\pm7.8)$; $p<0.001$; $F=24.712$). When accounting for water table treatment in ANCOVA, there was no interactive effect between DOC and water table treatment in explaining [CO_2] ($p=0.439$; $F=0.846$)

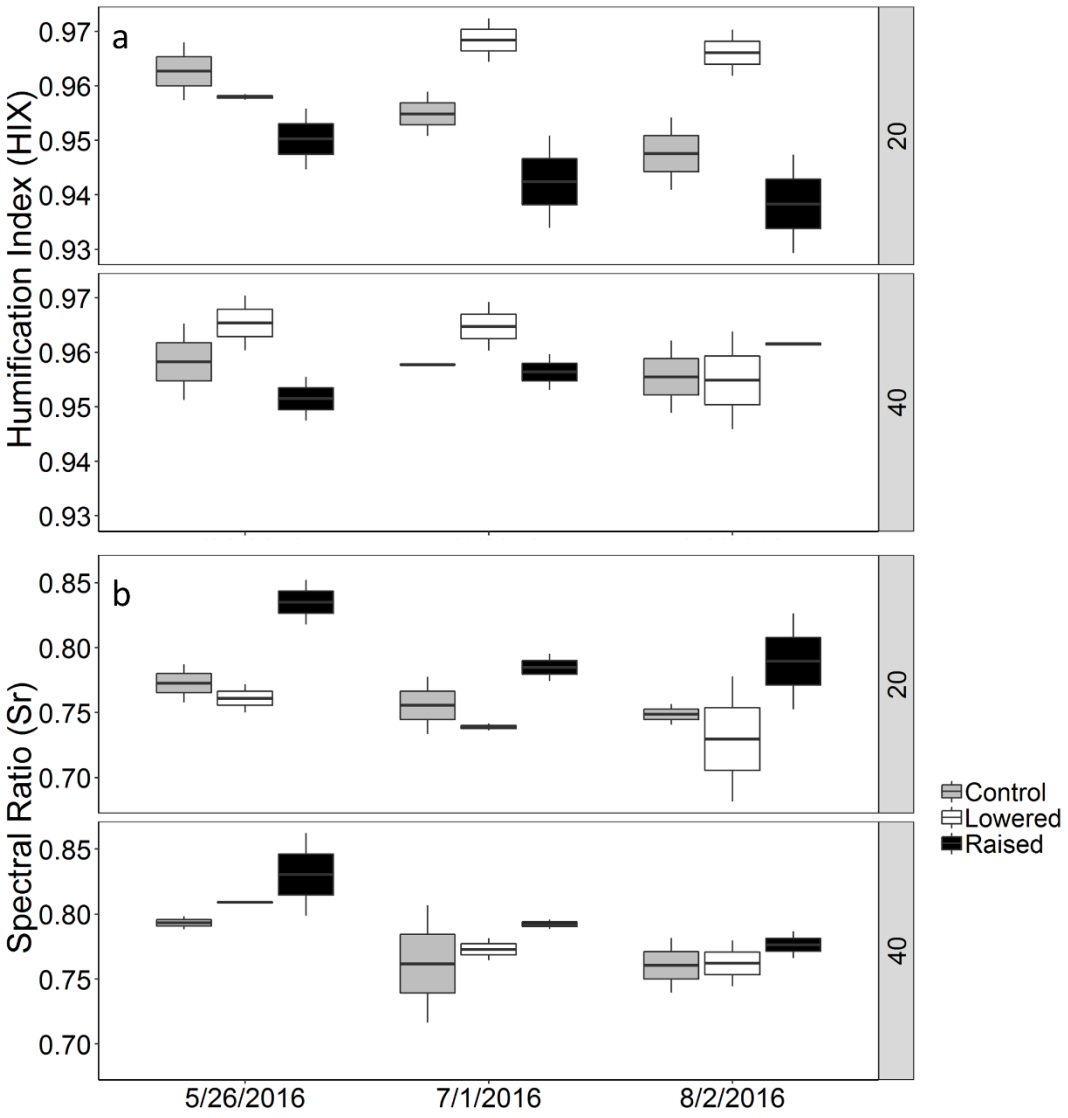

Fig. 4.5 Spectral characteristics of pore water from the water table treatments. a) The Humification Index (HIX), related to molecular complexity, was lower in the raised water table treatment (0.950 ± 0.010) vs. the lowered water table treatment (0.962 ± 0.007) at the 20 cm depth (p=0.003; F=8.09) but not the 40 cm depth b) Sr, inversely related to oxidation status and molecular weight, was higher in the raised plot (0.80 ± 0.04) than lowered (0.74 ± 0.03; p=0.008) and control (0.76 ± 0.02; p=0.047) plots at 20 but not 40 cm

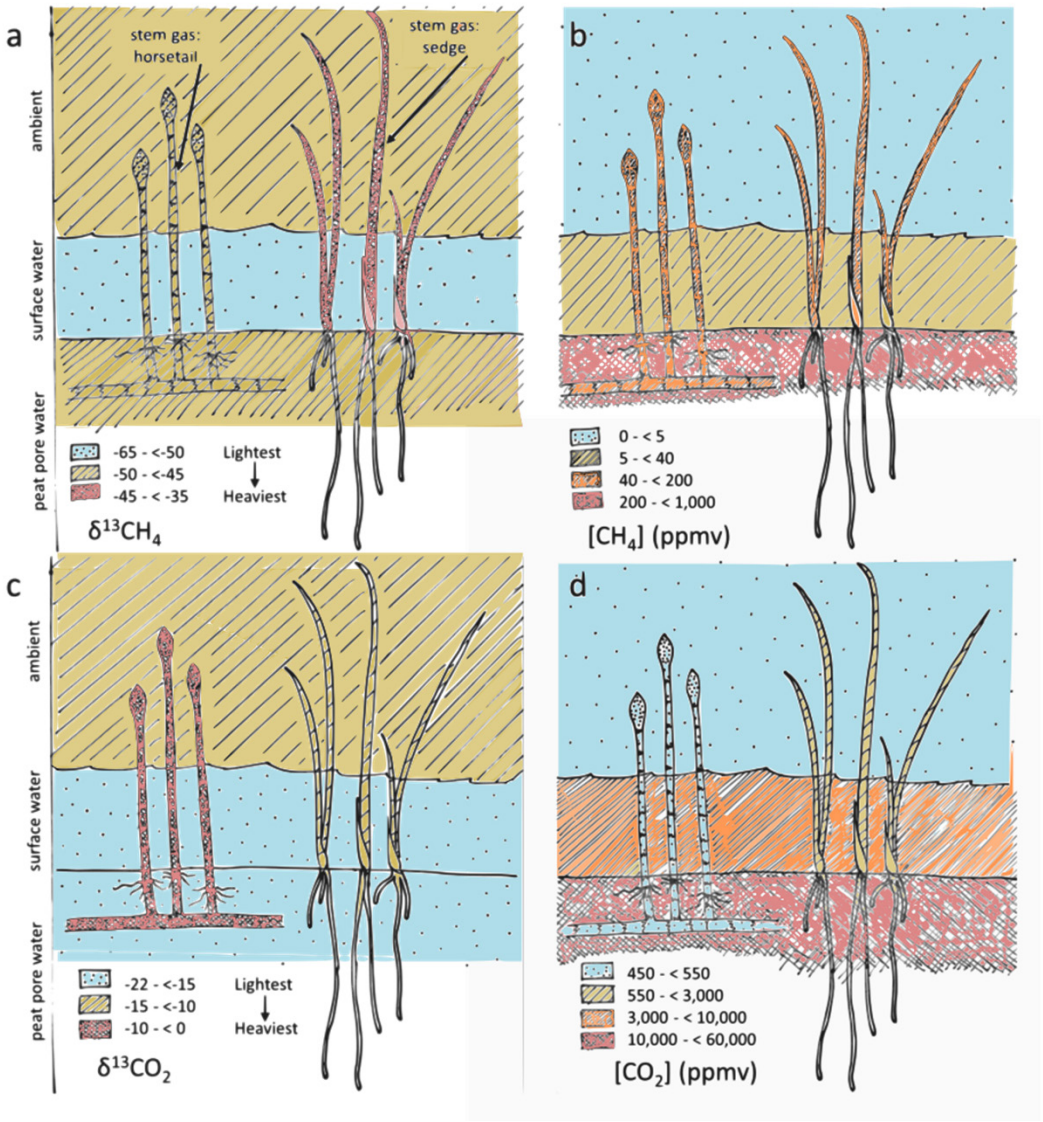

Fig. 4.6 Profile of a) $\delta^{13}CH_4$, b) $[CH_4]$, c) $\delta^{13}CO_2$, and d) $[CO_2]$ in pore water, surface water, inner cavities of horsetail (represented on the left) and sedge (represented on the right) stems, and (true) ambient gasses collected while the site was flooded in the 2017 growing season

Table 4.1. Summary of field season environmental variables from early June – late July, 2016. Mean values with an * are significantly different ($p<0.05$) from **bolded** values

	Control			Lowered			Raised		
Measure	Min	Mean	Max	Min	Mean	Max	Min	Mean	Max
NEE ($\mu M\ CO_2\ m^{-2}\ s^{-1}$)	-3.75	0.84	6.89	-4.00	0.48	6.51	-3.93	-0.90	4.91
CH_4 efflux ($\mu M\ m^{-2}\ m^{-1}$)	0.05	2.62*	7.66	0.38	2.97*	7.66	0.07	**5.62**	12.77
Eh7 at 20 cm (mV)	-68.1	27.2	387.1	-132.3	15.6	139.6	-48.4	12.9	111.4
Temperature (°C)	8.8	**13.3**	17.4	10.3	14.2*	18.0	9.0	14.5*	18.3
Peat to water table (cm)	-9.0	4.3	14.0	-33	-0.5*	13.0	-1.5	**4.0**	9.0

4.8 Appendix 4.A Supplemental Material

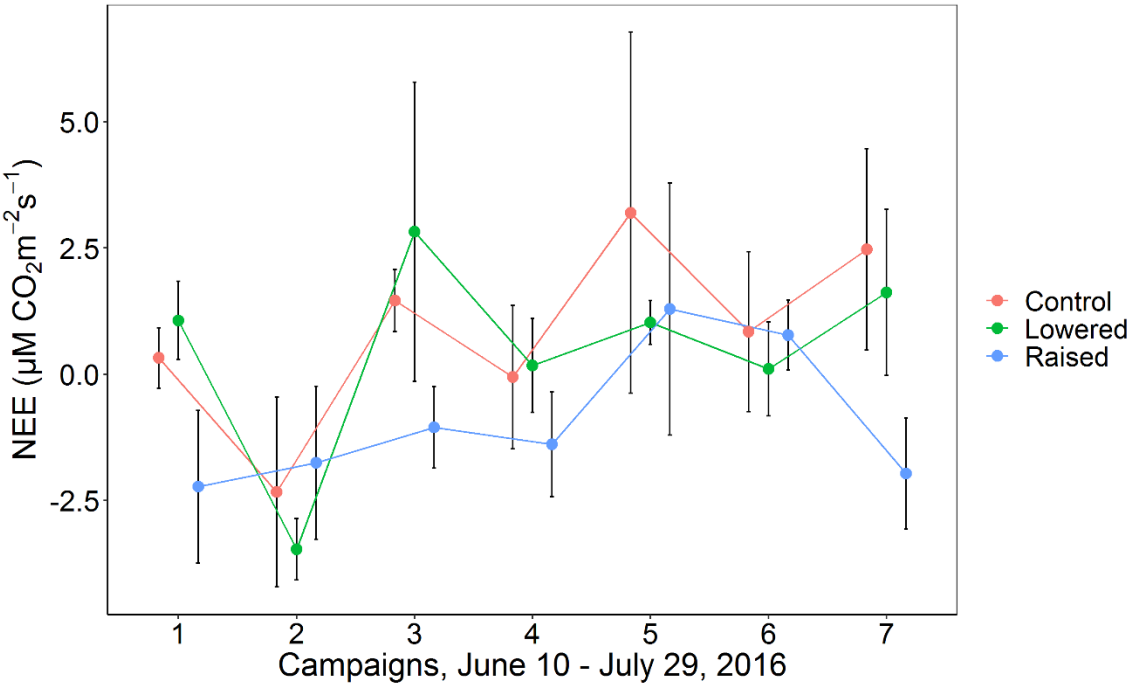

Fig. 4.A.1 Net ecosystem exchange (NEE) during the 2016 growing season in the water table treatment plots. NEE is significantly lower in the raised water table treatment plot than the lowered and control ($p=0.001$ vs. control and $p=0.014$ vs. lowered, $F=7.476$ for overall treatment differences). Negative values indicate net uptake, whereas positive

values indicate net efflux. Treatments were sampled on the same date for each campaign; data is offset for ease of interpretation

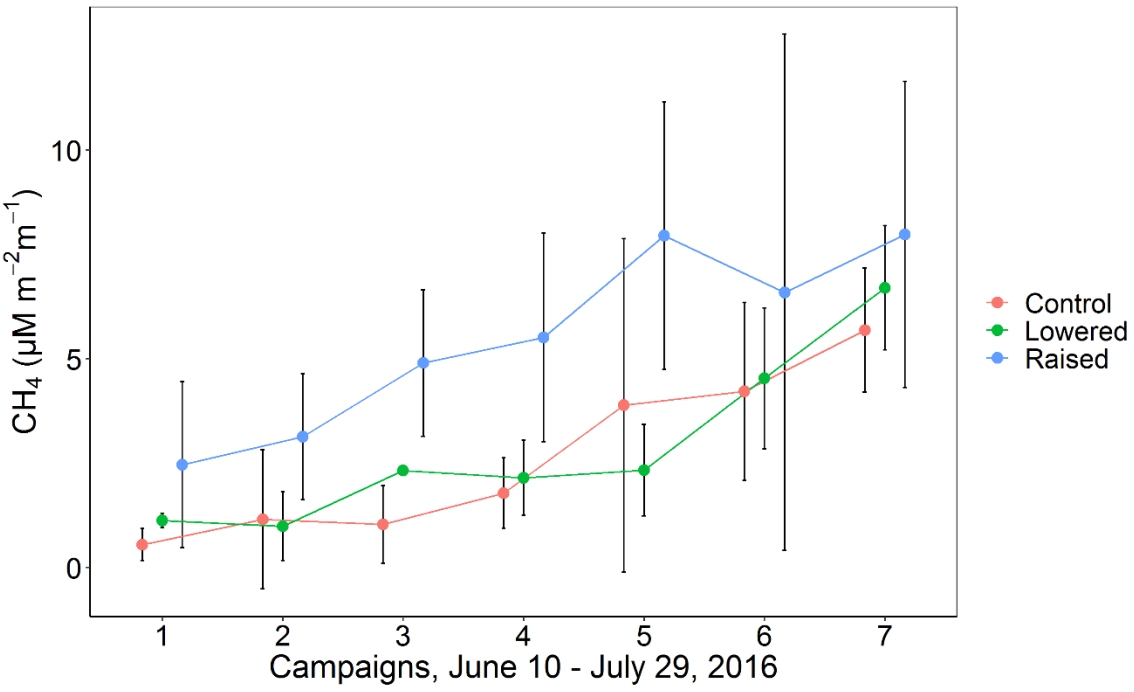

Fig. 4.A.2 CH_4 efflux from the water table treatments during the 2016 growing season. Methane efflux was significantly higher in the raised water table treatment than in the control and lowered plots ($p \leq 0.001$; $F=12.84$). Treatments were sampled on the same date for each campaign; data is offset for ease of interpretation

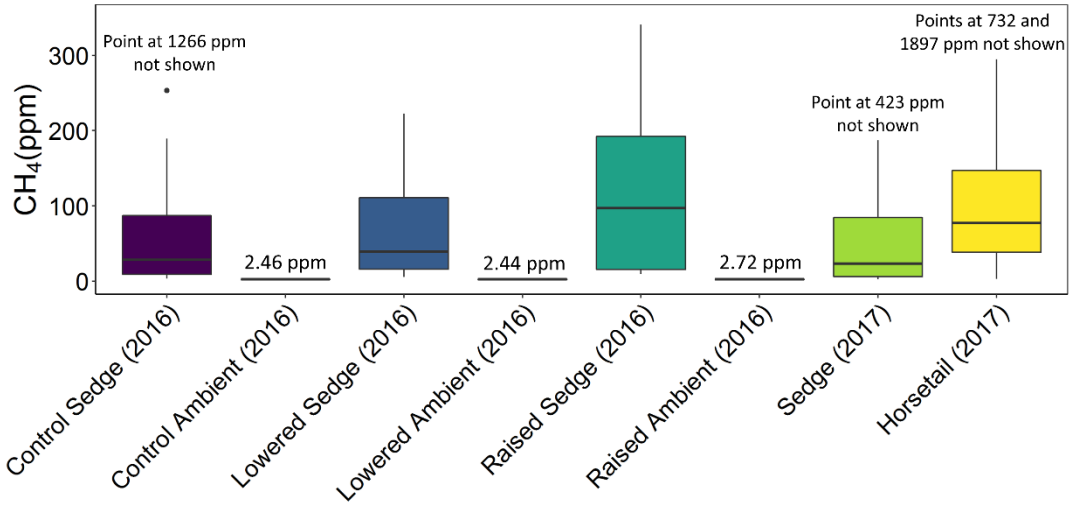

Fig. 4.A.3 Spread of [CH_4] from inner stem cavities of sedges from the control, lowered, and raised water table treatments during the 2016 growing season, and horsetail and sedges from the 2017 growing season, when the site was flooded. Ambient refers to true ambient conditions; not chamber efflux

Table 4.A.1 Properties of environment at time of 2017 profile collection, when the site was flooded

Site	**Eh7 (mV)**	**Eh7 (mV)**	**Temp (°C)**	**Temp (°C)**
	Surface water	*Pore water*	*Surface water*	*Pore water*
Site 1	-17.9	-60.3	27.5	23.3
Site 2	-45.2	-114.9	27.0	22.7
Site 3	-52.2	-114.6	27.4	23.3
Site 4	-29.1	-78.7	27.3	24.4
Site 5	**-53.2**	**-114.7**	**26.5**	**22.3**

5 Conclusion

As the arctic and boreal regions warm, the landscape will see conjoint changes in hydrology and vegetation, which will affect the global carbon cycle. Environmental shifts spurred by permafrost thaw and evolving precipitation regimes will likely lead, at least initially, toward a more fen-dominated landscape. The goal of this work was to specifically examine the effects of water table and plant functional groups on boreal fen ecosystems, and the consequences for carbon cycling. Taking into account microbial communities, plant traits, and geochemical variables, we present a biogeochemical perspective on Alaska's changing fens.

The findings in this dissertation, taken together, present several key observations and conclusions regarding fens in a changing climate. First, the fens investigated here, along with many northern peatlands, release a lower ratio of methane to carbon dioxide than would be expected regarding geochemical reactions. In northern fens, we suggest that this low ratio is in part due to methane oxidation within the rhizosphere. These conditions are supported by the oxidizing nature of many fen plant roots and their support of not only methanogenic (methane producing), but also methanotrophic (methane consuming) prokaryotes, per Chapter 2. Although wetter conditions in fens lead to higher rates of methane production due to favorable conditions for methanogenesis, geochemically and biologically (via plant root exudate – stimulation of methanogens), methane production is offset by the aforementioned processes.

Second, plants directly affect carbon above- and belowground. Belowground biomass (roots and rhizomes) is linked in Chapter 1 to carbon dioxide production. As demonstrated with dissolved organic carbon and dissolved pore water gas concentrations and isotopes, certain plants (e.g. sedges, horsetail, grasses) appear to remove dissolved organic carbon from the pore water while oxidizing the rhizosphere. Dissolved organic carbon in this fen is directly related to concentrations of pore water carbon dioxide concentrations, as shown in Chapter 3. Other wetland plants seem to have little effect on pore water carbon, but influence decomposition (e.g. cinquefoil) and/or exhibit signs of disease in the manipulated plots—consistent with findings linking disease outbreaks with environmental change (Anderson et al. 2004).

Several plant-related research questions are identified by the findings of this work. Warranted is an in-depth examination of plant-iron cycler-methanotroph-methanogen-endophyte interactions and how they affect CH_4:CO_2 gas ratios surfacing from the fen system. A laboratory experiment comparing plants with sterile roots, plants with only endophytes, and plants with different combinations of aforementioned prokaryotes could tease apart how each of these functional groups affects the final product of greenhouse gasses emerging from the peat. Each scenario should be tested with a raised and drawn down water table. The investigation of exactly how sedges affect pore water carbon is also a question of interest. Specifically, is the plant itself removing dissolved organic carbon from its surroundings, or is it the work of root endophytes and/or the surrounding microbial community?

Overall, it is apparent by all three studies presented in this dissertation that vegetation exerts a powerful control over carbon cycling, and is perhaps more important than other geochemical factors in agreement with a growing body of literature (Robroek et al. 2015; Ward et al. 2015; Dieleman et al. 2017). Not only do many plants act as conduits of gas between the surface and subsurface environment in wetlands, but they are also host, in the rhizosphere, to the very microorganisms that biologically produce and consume methane and carbon dioxide. That being said, hydrologic change is a direct cause for vegetation change (Minkkinen et al. 1999; Churchill et al. 2014; Pedrotti et al. 2014; Potvin et al. 2014; Dieleman et al. 2015). Therefore, hydrologic change is the vehicle by which change to the carbon cycle—in the form of vegetation change—takes place. The vegetation that moves to fill new niches cause by water table shifts then locally shapes its environment in close partnership with its microbiome.

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