Microbial Communities and Their Intermediary Ecosystem Metabolism Across Northern Peatlands

by

Analissa Flores Sarno

A Dissertation Presented in Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy

Approved November 2021 by the
Graduate Supervisory Committee:

Hinsby Cadillo-Quiroz, Chair
Ferran Garcia-Pichel
Rosa Krajmalnik-Brown
Daniel Childers

ARIZONA STATE UNIVERSITY

May 2022

ABSTRACT

Under current climate conditions northern peatlands mostly act as C sinks; however, changes in climate and environmental conditions, can change the soil carbon decomposition cascade, thus altering the sink status. Here I studied one of the most abundant northern peatland types, poor fen, situated along a climate gradient from tundra (Daring Lake, Canada) to boreal forest (Lutose, Canada) to temperate broadleaf and mixed forest (Bog Lake, MN and Chicago Bog, NY) biomes to assess patterns of microbial abundance across the climate gradient. Principal component regression analysis of the microbial community and environmental variables determined that mean annual temperature (MAT) (r^2=0.85), mean annual precipitation (MAP) (r^2=0.88), and soil temperature (r^2=0.77), were the top significant drivers of microbial community composition ($p < 0.001$). Niche breadth analysis revealed the relative abundance of *Intrasporangiaceae*, *Methanobacteriaceae* and Candidatus *Methanoflorentaceae* fam. nov. to increase when MAT and MAP decrease. The same analysis showed *Spirochaetaceae, Methanosaetaceae and Methanoregulaceae* to increase in relative abundance when MAP, soil temperature and MAT increased, respectively. These findings indicated that climate variables were the strongest predictors of microbial community composition and that certain taxa, especially methanogenic families demonstrate distinct patterns across the climate gradient.

To evaluate microbial production of methanogenic substrates, I carried out High Resolution-DNA-Stable Isotope Probing (HR-DNA-SIP) to evaluate the active portion of the community's intermediary ecosystem metabolic processes. HR-DNA-SIP revealed several challenges in efficiency of labelling and statistical identification of responders, however families like *Veillonellaceae*, *Magnetospirillaceae*, *Acidobacteriaceae* 1, were found ubiquitously active in glucose amended incubations. Differences in metabolic byproducts from glucose amendments show distinct patterns in acetate and propionate accumulation across sites. Families like *Spirochaetaceae and Sphingomonadaceae* were only found to be active in select sites of propionate amended incubations. By-product analysis from propionate incubations indicate that the northernmost sites were acetate-accumulating communities.

These results indicate that microbial communities found in poor fen northern peatlands are strongly influenced by climate variables predicted to change under current climate scenarios. I have identified patterns of relative abundance and activity of select microbial taxa, indicating the potential for climate variables to influence the metabolic pathway in which carbon moves through peatland systems.

DEDICATION

To Aaron, Carlos, Rosa, and Benjamin – mi familia

And to those who came before me, paving the way with opportunities.

ACKNOWLEDGMENTS

I would like to express my deepest gratitude to everyone who has helped me while on my Ph.D. journey. Hinsby, thank you for your patience, time, and guidance. I am grateful that you introduced me to peatlands and the important role they play in our planet, opening a whole new world of microbiology to me. Thank you to my committee members for your support, feedback, and insights on my research that was provided throughout the journey. I feel very grateful to have been able to gain an alternative perspective to enhance my thought processes and research. Thank you to my collaborators around the world, for contributing an imperative part to my projects, even if we haven't met in person.

A huge thank you to the current and former Cadillo lab members. For keeping me motivated and positive during long experiments and celebrating the victories along the way. To my fellow SOLS graduate students, thank you for the community and support throughout my time at ASU. To the SOLS Grad Office and Facilities staff, thank you for your patience, assistance, and guidance in all issues administrative. To my former teachers and mentors in grade school and at UCHS and CSUSM, thank you for taking the time to teach me the importance of gaining a strong education and being an inquisitive scholar.

To my mental health advisor for her constant support and encouragement to continue forging ahead. To my wellness trainer, for reminding me of my priorities and sticking with me through the tough times. To my friends near and far, for supporting me and bringing with you the good times to help me get through this journey.

To my extended family, for supporting my endeavors and always offering motivating words of encouragement. To my mom for teaching me the importance of sacrifice, hard work and dedication for the benefit of the greater good. To my dad for teaching me that "sometimes you just gotta be tough". To Ben for your camaraderie, keeping me on my toes, always ready for the next adventure. To Aaron for your sacrifice, unwavering support, constant encouragement, and technical skills to fix broken lab equipment, to help me achieve my goals.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

CHAPTER 1

DISSERTATION INTRODUCTION

Northern peatlands play an important role in global carbon (C) cycling. They cover less than 3% of Earth's continental surface but are estimated to contain 20-30% of all soil organic carbon (SOC) (Gorham 1991; Wu and Roulet 2014a). Northern peatlands are found mostly in boreal zones, between 40-70°N latitude (**Figure 1.1**) (Wuebbles and Hayhoe 2002; Abdalla *et al.* 2016).

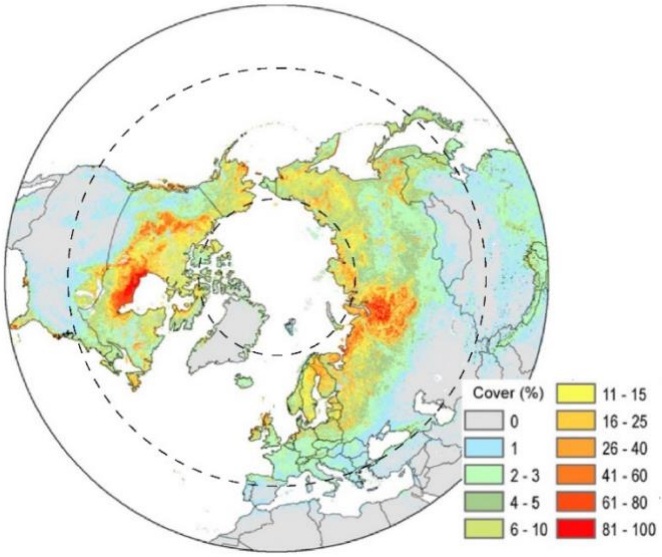


Figure 1.1 Taken from Hugelius *et al.* 2020, peatland coverage of Northern peatlands. Dashed lines were added and indicate 40 and 70°N latitude.

Peatlands are categorized based on their dominant water source which greatly affects the nutrient status; peatland types range from surface-water fed or minerotrophic (nutrient rich content) to rain-fed ombrotrophic (nutrient poor conditions), with poor fens being at intermediate nutrient status (Mitsch and Gosselink 2015). Peatland's large carbon stores are in the form of peat, a recalcitrant carbon pool estimated at 1.055 Giga tons of carbon, made up of partially decomposed plant matter (Nichols and Peteet 2019). Under the current climate conditions, pristine northern peatlands act as a net carbon sink, and are estimated to uptake 20-60 g CO_2-C meter^{-2} year^{-1} (Yu 2011; Wu and Roulet 2014b). Although a net carbon sink, northern peatlands

are also a main natural source of methane (CH_4), producing an estimated 36 Tera grams CH_4-C $year^{-1}$, just over 10% of total global methane emissions (Wuebbles and Hayhoe 2002; Fletcher *et al.* 2004; Abdalla *et al.* 2016).

Methane is a potent greenhouse gas and has 25 times more global warming potential when compared to carbon dioxide (CO_2) (Bridgham *et al.* 2013; IPCC Panel 2014). Methane is produced by a group of microorganisms called methanogens, which require specific conditions including anoxia, a reducing environment and low availability of alternative electron acceptors to allow them to outcompete metabolically more diverse counterparts (Conrad 2007; Strack *et al.* 2008a). The anaerobic carbon decomposition cascade of northern peatlands is a complex set of interdependent metabolic reactions resulting in the production and release of methane. There has been extensive research on environmental factors that influence methane production in peatlands (Blodau 2002; Lai 2009; Abdalla *et al.* 2016), however there has been little consideration for upstream processes that feed the pool of available methanogenic substrates (Schmidt *et al.* 2015).

In this dissertation, I investigated the microbial community composition and methane production of poor fen northern peatlands across a climate gradient while focusing on their primary and secondary fermentation metabolism. Primary and secondary fermentation are two stepwise reactions that degrade complex carbon rich molecules into less complex units that are transformed into the major compounds of the methanogenic substrate pool in peatlands. For the larger picture, it is important to further understand how a changing climate could alter the carbon decomposition cascade and potentially the sink/source status of norther peatlands due to their large carbon reservoir and ability to emit methane.

Below, I review the status of the field, introducing and defining northern peatlands, the role climate plays in the carbon flux of peatlands, spatial and temporal variability of peatlands and the microbial community of northern peatlands, focusing specifically on the anoxic reactions that feed methanogens known as intermediary ecosystem metabolism.

1. NORTHERN PEATLANDS AND CLIMATE CHANGE

1.1. NORTHERN PEATLANDS

Northern peatlands are found primarily in cool boreal zones where precipitation is greater than evapotranspiration and the persistence of water-logged soils perpetuates the accumulation of peat. Peatland classification type is determined by the major water source. Water source is largely influenced by the surrounding topography and bodies of water, greatly dictating the chemical composition and pH of the peat and porewater. Peatland types exist on a continuum where the extremes are minerotrophic fens and ombrotrophic bogs. Minerotrophic fens are primarily fed by groundwater which is enriched with nutrients from the surrounding mineral soils (Gorham 1991). Thus, minerotrophic fens have a relatively high calcium (Ca) and (Mg) ion concentration and a circumneutral pH of 6.5-7.0. Under these conditions, grasses, sedges and reeds commonly dominate minerotrophic peatland vegetation (Amon *et al.* 2002). Ombrotrophic bogs are fed exclusively through precipitation which contains minimal nutrients and a low buffering capacity (Gorham 1991; Mitsch and Gosselink 2015). This causes ombrotrophic bogs to have low Ca and Mg ion concentration and a pH of less than 4 (Ye *et al.* 2012). *Sphagnum* moss species dominate bogs, accompanied by other acidophilic shrubs, sedge and some trees (Mitsch and Gosselink 2015). Mesotrophic peatlands, also known as poor fens, are transitional peatlands because they receive decreased amounts of groundwater and rely on precipitation, while also containing a mixture of circumneutral and acidophilic vegetation. These peatlands will eventually become ombrotrophic and rely exclusively on precipitation for moisture and nutrient input, due to the accumulation and compaction of organic matter cutting off any ground water influx, essentially isolating the peatland from ground water. The variation in pH and dominant plant species across peatland types can greatly impact the rate at which carbon accumulates.

Decomposition is slow in northern peatlands due to environmental conditions such as low pH, cold temperatures, short growing seasons, and the persistence of frozen or water-logged, nutrient poor soils. These conditions allow for carbon fixation to exceed carbon decomposition, resulting in the accumulation of carbon in the form of peat; dead, partially decayed plant matter (Abdalla *et al.* 2016). Accumulation of carbon in northern peatland ecosystems is dependent on

the rate of decomposition, rather than the rate of carbon fixation. The dominant plant species and vegetation community structure of a peatland is influenced by the peatland type and nutrient status. This will in turn, affect the carbon quality of plant litter and rate of decomposition. Additional factors that affect the rate of decomposition in peatlands includes soil nitrogen and phosphorus availability, and soil carbon quality will vary drastically along with minerotrophic – ombrotrophic gradient (Ye *et al.* 2012). This set of environmental variables creates a unique ecosystem where only a specialized group of vegetation and microorganisms survive.

Peatlands are a major natural source of methane. Methane has a 25-30 times more potent heat retention potential than carbon dioxide (IPCC 2007), the other main greenhouse gas, making the understanding of methane dynamics a high priority for climate scientists. Methane emissions from peatlands are driven by factors such as water table level, climatic zone and temperature, plant community composition and soil pH. All these factors affect the rate of methane production by methanogens and methane oxidation by methanotrophs, ultimately affecting the amount of methane released into the atmosphere. Nutrient status of a peatland and the presence or absence of permafrost can also have a large effect on methane production (Ye *et al.* 2012; McCalley *et al.* 2014a). These environmental factors not only control the methane production and consumption but also influence the anaerobic carbon decomposition cascade. Specifically, nutrient status will influence the utilization of certain carbon decomposition pathways, changing the quantity and types of methanogenic substrates produced and thus methane flux rates themselves. Due to the complex microbial community and environmental conditions affecting the production and release of methane, it is difficult to predict how carbon decomposition and methane production in peatlands will respond to a changing climate.

1.2. CLIMATE CHANGE AND ITS EFFECT ON NORTHERN PEATLANDS

Under current climate conditions, northern peatlands act as a carbon sink due to their large capacity to store carbon in the form of peat. The change in carbon storage is calculated by subtracting the total carbon released by ecosystem respiration from the total carbon fixed by photosynthesis (Clymo 1984; Strack *et al.* 2008a). Future climate scenarios are predicting reduced precipitation and increased air temperature in areas where northern peatlands are found

(Serreze *et al.* 2000). These climatic changes could cause a decrease in water table level, permafrost thaw, and/or change in vegetation community structure. Even a slight change in any of these variables could alter the current carbon balance and change the carbon dynamics of northern peatlands.

The surface temperatures of biomes found in northern latitudes, including artic, subarctic, tundra and boreal forests have been warming faster than other ecosystem types subsequently causing increased soil respiration (Gorham 1991; Serreze *et al.* 2000; Commane *et al.* 2017). The effects of climate change in these biomes includes a longer growing seasons with less precipitation which could lower the water table level, increased risk of fire activity and degradation of permafrost. Studies have shown that increased temperature and decreased water table will increase decomposition rates due in part to increased enzyme activity as a result of increased soil temperature and to the drop in water table and the exposure of a larger peat profile for oxic decomposition (Dieleman *et al.* 2016). Permafrost zones are predicted to thaw and convert to net carbon emitting ecosystems under the current climate estimates, due to increased microbial respiration in thawed soils (Serreze *et al.* 2000; IPCC Panel 2014).

Under the influence of climate warming the carbon sink status of northern peatlands is in question due to potential changes in the balance of carbon dioxide fixation and organic matter decomposition, and methane production and oxidation. A change in the current carbon cycling regime of northern peatlands could further perpetuate the greenhouse gas positive feedback loop, due to the amount of carbon that peatlands could potentially release. To better understand how a changing climate will affect these systems, especially reactions affecting methane production, further research is needed on microbially mediated processes.

2. VARIABILITY OF NORTHERN PEATLANDS SITES

2.1. TEMPORAL VARIABILITY OF NORTHERN PEATLANDS

A peatland ecosystem is developed by an intricate set of interactions between topography, hydrology, chemistry, and vegetation succession, ultimately resulting in a positive water balance and the accumulation of peat. The amount of precipitation or external water input

to the ecosystem must be greater than evapotranspiration to have an accumulation of water. Generally, evapotranspiration of peatlands is 50 -70% of precipitation, allowing for the excess to accumulate as pore water (Mitsch and Gosselink 2015). With the presence of excess moisture, anoxic conditions dominate which decreases the rate of decomposition, ultimately contributing to the accumulation of peat. Although primary production of northern peatland ecosystems is low (estimated to be about 0.3 Pg C yr^{-1}) compared to other ecosystem types (warm wetlands and tundra are estimated to be 0.9-1.1 and 1.4-1.9 Pg C yr^{-1}, respectively) (Ito and Oikawa 2004), decomposition is even slower due to the cool temperature, anoxic conditions, and low nutrient status, resulting in the accumulation of peat (Clymo, Turunen and Tolonen 1998). Peatlands begin as minerotrophic ecosystems with a circumneutral pH and dominant wetland vegetation such as *Carex, Typha* and *Lythrum* (Mitsch and Gosselink 2015). Once a peatland is formed, the low pH and the water holding capacity of the peat perpetuate the development of conditions that are resistant to environmental changes or fluctuations (Belyea 2009).

There are two main mechanisms by which peatlands can develop in the presence of a positive water balance and organic carbon accumulation. The first mechanism is terrestrialization, where small shallow lakes are filled in with peatland plants and the second is paludification where a terrestrial ecosystem becomes blanketed by peatland vegetation, both of which perpetuate the further accumulation of peat (Mitsch and Gosselink 2015).

Regardless of the mechanism of formation, peatlands go through a succession progressing from a minerotrophic peatland to an ombrotrophic peatland, leading to changes in energetic state which causes shifts in the microbial and vegetation ecology (Chapman *et al.* 2016, 2017). The differential successional states a peatland can be in causes a wide range of peatland variability even within a small geographical area. The variation in successional states as well as the time scale on which peatland ecosystem level processes occur are important to consider when evaluating climate and peatland carbon feedback cycles or larger peatland complexes for carbon cycling or biogeochemical attributes (**Figure 1.2**).

2.2. SPATIAL VARIABILITY OF NORTHERN PEATLANDS

Northern peatlands are located across the globe between 40-70°N latitude, across different biomes and under different topographical circumstances. There are about 3.5 million km^2 of northern peatlands found globally (Mitsch and Gosselink 2015; Hugelius *et al.* 2020). Areas with a large percent of peatlands include Scandinavia, eastern Europe, west Siberia, Alaska, and Canada. The peatlands found in these areas can range in nutrient status and dominant vegetation, greatly influencing the microbial respiration and release of carbon dioxide and methane. This is especially true for methane flux, due to the variation in water table level and labile carbon input from vegetation across microtopographical features of peatlands. There are three main types of microtopographical features recognized in northern peatlands which include hummocks, hollows, and lawns. Hummocks are raised mounds of *Sphagnum* sp., which can be up to 50 cm higher than surrounding peat surface (Bubier *et al.* 1993). Hollows are depressions, filled with water and lawns are flat or slightly sloping areas that can cover larger areas than a hummock or a hollow. Differences in gas flux and microbial community composition have been documented across microtopographical features within the same peatland, further demonstrating intra-site variability (Bubier *et al.* 1993; Galand, Fritze and Yrjälä 2003; Wu *et al.* 2011). An example of the variability can be seen in **Figure 1.2**, where the differences in carbon between a hummock and a hollow are compared. In the *St. Charles-de-Bellechasse* poor fen hummocks are a source of carbon due to increased ecosystem respiration (Strack *et al.* 2006), likely due to the lower water table on the hummock and increased soil temperature allowing for higher levels of decomposition. The hollow has a higher water table, making a larger portion of the peat profile anoxic, which inhibits decomposition. Heterogeneity within and among peatland types makes it difficult to accurately extrapolate carbon flux of a peatland, because the aforementioned biotic and abiotic characteristics can have significant effects on microbial activity and carbon cycling (Waddington and Roulet 2000).

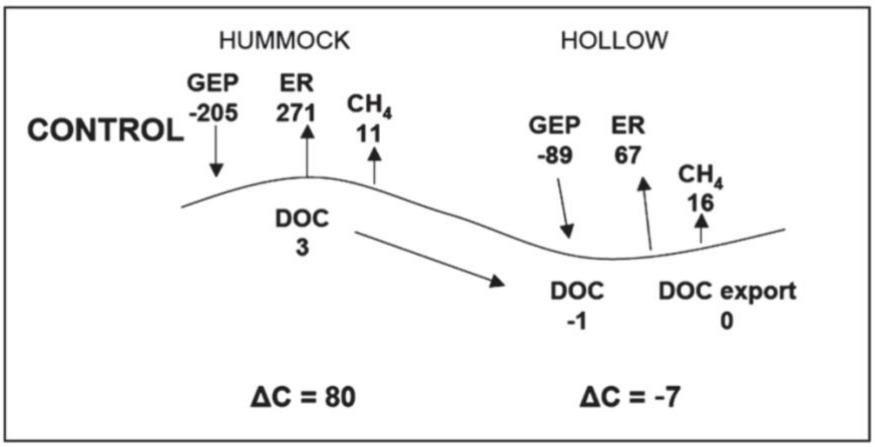


Figure 1.2 Taken from Strack *et al.* 2008, demonstrating differences in carbon exchange from *St. Charles-de-Bellechasse* poor fen microtopography during the growing season. DOC = dissolved organic carbon, GEP = gross ecosystem photosynthesis, ER= ecosystem respiration.

3. INTERMEDIARY ECOSYSTEM METABOLISM AND THE ROLE OF PRIMARY AND SECONDARY FERMENTATION IN NORTHERN PEATLAND CARBON CYCLING

3.1. MICROBIAL CONTRIBUTIONS TO CARBON CYCLING OF NORTHERN PEATLANDS

In anoxic environments such as northern peatlands, decomposition is limited by the availability of oxygen, alternative electron acceptors, organic nitrogen and phosphorus, temperature, pH, and carbon quality of available substrates. One of the main limiting factors is oxygen availability which decreases with peat depth due to waterlogged and anoxic conditions (Artz 2013). This lack of oxygen leads to the use of metabolic reactions with lower energetic potential and partial decomposition reactions. Under anoxic conditions decomposition does not fully cease and instead proceeds using fermentative processes or anaerobic respiration utilizing alternative electron acceptors such as Fe (III), SO_4^{-3}, NO^{-3} and CO_2 (Bridgham *et al.* 2013). The presence of alternative electron acceptors is greatly dependent on peatland water source. As mentioned previously, ombrotrophic peatlands which are fed exclusively by precipitation have a much lower concentration of alternative electron acceptors as compared to the minerotrophic peatlands which are recharged by ground water and overland flow.

The vertical profile of a peatland is composed of three major zones which are influenced by the water table and plant rooting zone differentially. The predominately oxic layer is called the

acrotelm, where O_2 is available and there are carbon inputs from plant root exudates and plant litter. Studies have found fungi and mycorrhizae to be dominant in this layer, while taxa such as *Proteobacteria, Acidobacteria,* were identified as abundant members of the actively growing *Sphagnum* associated microbiome (Myers *et al.* 2012; Kostka *et al.* 2016). Labile carbon substrates are quickly consumed while more recalcitrant structural compounds from plant litter rely on the extracellular hydrolytic enzymes from fungi and bacteria to become available.

The layer below that is the periodically oxic/anoxic zone called the mesotelm. This layer still has a large amount of substrate inputs from the plant root exudate but is greatly influenced by any shifts in the water table (Clymo and Bryant 2008). Both facultative and obligate anaerobes are found here due to the fluctuation of oxic/anoxic conditions. This is where fermentation reactions commonly occur, using monomers such as glucose produced from the exo-cellular enzymes (Bridgham *et al.* 2013) to produce low molecular weight fatty acids and alcohols.

The permanently anoxic zone, called the catotelm, has little input of plant root exudates. It is greatly constrained by lack of O_2 and, thus, relies exclusively on alternative electron acceptors for respiration reactions or must proceed with the energetically less favorable primary or secondary fermentation reaction. The microbial community is altered with depth due to O_2 limitation causing the community to become more metabolically specialized to utilize the intermediate substrates released by higher trophic levels. Due to the permanent anoxia in this layer, any recharge of terminal electron acceptors must occur via electron shuttling through the chemical reduction of organic matter (Lovley *et al.* 1998; Heitmann and Blodau 2006). Fermentation and methanogenesis become the main carbon transforming processes at this level.

3.2. MICROBIAL PHYSIOLOGY OF MICROBES PERFORMING PRIMARY AND SECONDARY FERMENTATION REACTIONS

Many microbial metabolic processes contribute to the complex processes of the carbon decomposition cascade found in northern peatlands. The trophic structure of terrestrial freshwater methanogenic communities' proceeds in a stepwise manner, as shown in **Figure 1.3**.

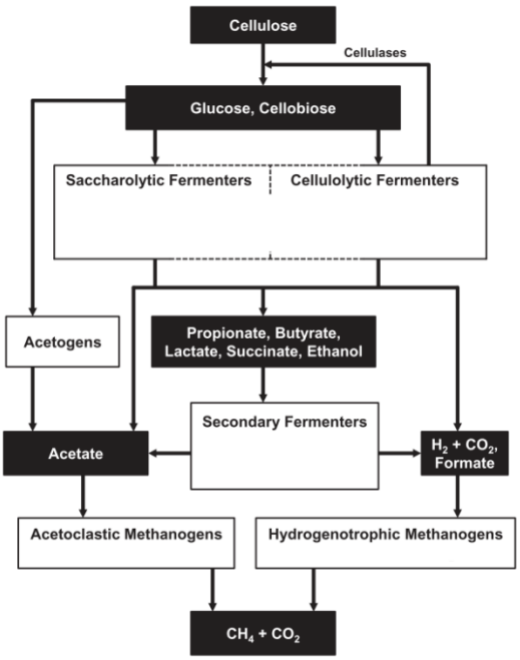


Figure 1.3. Taken from Schmidt *et al.* 2015, a hypothetical model of the step wise, intermediate ecosystem metabolism in Schlöppnerbrunnen fen.

Many of the microbial interactions are determined by thermodynamics predictions (Kotsyurbenko 2005). Due to thermodynamic constraints, the bacterial and archaeal members of the methanogenic community are metabolically reliant on one another and do not exist individually in peatlands. Trophic structures of these communities are also influenced by temperature, whereby changes in dominant members have shifted also causing a shift in the pathway of carbon flow (Metje and Frenzel 2005, 2007).

Under anoxic conditions the first step in the trophic cascade is the degradation of complex polymers such as cellulose and hemicellulose from decaying plant matter. These compounds are hydrolyzed by cellulolytic fermenters utilizing exoenzymes to produce soluble cellodextrins, cellobiose and/or glucose (**Figure 1.3**) (Wüst, Horn and Drake 2009; Schmidt *et al.* 2015). This process has been shown in peatlands to be the rate-limiting step. Saccharolytic and celluloytic fermenters compete for substrates, keeping the cellobiose and glucose levels low (Leschine 1995; Bayer, Shoham and Lamed 2006). In northern peatlands this process is commonly facilitated by primary fermenters from the *Pseudomonadaceae* and *Clostridiaceae*

families, but cellobiose and glucose can also be consumed by facultative anaerobes and acetogens (Leschine 1995). Primary fermentation reactions produce CO_2, hydrogen gas (H_2), formate, acetate and organic acids and alcohols (such as propionate, butyrate, ethanol, and methanol). Secondary fermenters such as *Syntrophobacteraceae* and *Syntrophaceae,* then ferment the organic acids and alcohols to acetate, formate, CO_2, and H_2. The two dominant types of methanogenesis that occur in peatlands are the reduction of CO_2 with H_2 (hydrogenotrophic methanogenesis) by families such as *Methanocella* and *Methanoregula,* and acetate fermentation (acetoclastic methanogenesis) by *Methanosarcina* and *Methanoseata* (Ye *et al.* 2012).

Environmental factors can affect the methanogenic carbon decomposition cascade. Changes in temperature can alter the carbon and electron flow, by favoring certain processes over others (Fey *et al.* 2000). Metje and Frenzel demonstrated that over a temperature gradient the consumption rate of secondary fermentation products and the rate and pathway of methane production varied (Metje and Frenzel 2005). Another example includes low pH (as low as pH 4.8), similar to ranges found in poor fen type peatlands has been shown to favor hydrogenotrophic methanogenesis (Kotsyurbenko *et al.* 2007). Although there are factors that have been shown to affect methanogenic activity in the laboratory such as temperature and pH, other more compounding factors such as carbon availability and electron flow are more difficult to test and are less studied *in situ* (Ye *et al.* 2012; Bridgham *et al.* 2013). This further points to the lack of detailed mechanistic understanding of primary and secondary fermentation reactions that feed the methanogenic substrate pool. There is a gap in knowledge on the effects of climate on microbial community composition in Northern peatlands and how these factors control carbon and energy movement through the decomposition cascade.

4. NORTHERN PEATLAND METHANE EMISSIONS AND THE MICROBIAL CONTRIBUTION TO ECOSYSTEM LEVEL FUNCTIONS

Although undisturbed peatlands contain large stores of carbon and are a carbon sink under the current climate regime, peatland-specific parameters are uncommonly represented in projections or broad-scale estimates of the global carbon budget (Schuur *et al.* 2015; Hugelius *et*

al. 2020). This includes greenhouse gas feedback from permafrost thaw and change in water table level. The carbon stored in peat is vulnerable to climate warming and changes in precipitation, affecting the overall water balance. *In situ* and laboratory incubations have demonstrated that increased temperatures and higher atmospheric carbon dioxide concentrations have potential to extend the length of the growing season and augment photosynthetic rates, ultimately stimulating CO_2 uptake (Cavicchioli *et al.* 2019). An increase in temperature is also predicted to cause an overall increase in decomposition and soil respiration due to an increase in evapotranspiration as well as extending the duration and depth of soil thaw. Under drier conditions, soil respiration is speculated to increase while observations have demonstrated that photosynthesis could increase or decrease, and that it likely is dependent on peatland type and dominant vegetation (Flanagan and Syed 2011).

Greenhouse gas flux, carbon feedbacks between peatlands and climate, and global climate models rarely include parameters for microbial community composition or microbial physiological characteristics (Riley *et al.* 2011). This includes abiotic and biotic factors that can influence methanogenesis, methane consumption and gas transport dynamics (Bridgham *et al.* 2013). As shown in **Figure 1.4**, little consideration is given to the microbial contribution to the ecosystem respiration or other accompanying processes.

Another source of uncertainty in flux models arises due to extrapolation of flux data to a large geographical scale from sites with high heterogeneity. It has been shown that processes such as peat initiation are greatly influenced by climate, but models have yet to effectively combine input values for climate and microbial activity into a single climate model that yields improved greenhouse gas flux estimates (Morris *et al.* 2018). Studies have shown that microbial community composition and activity regulate ecosystem level processes such as methane flux. Gaining a more detailed understanding of the community response to factors such as climate is an important step towards integrating these variables into greenhouse gas flux models (McCalley *et al.* 2014b).

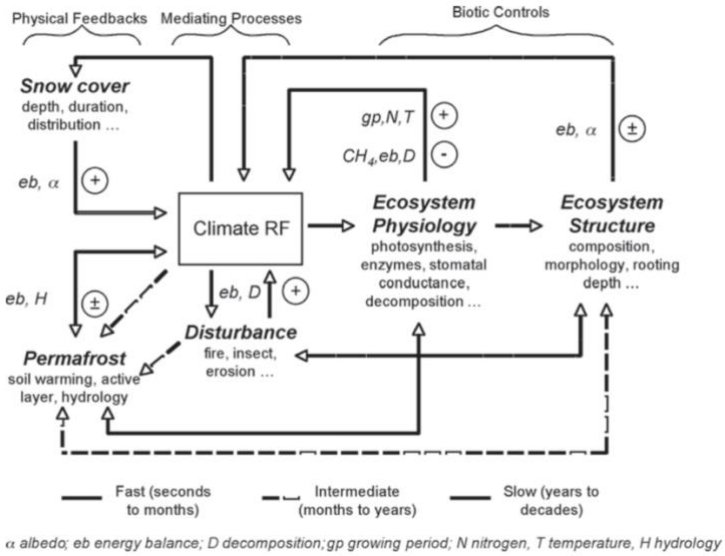


Figure 1.4. Taken from Strack *et al.* 2008, a peatlands and climate feedback model which considers different spatial and temporal scales, while lacking the microbial component of ecosystem respiration.

5. DISSERTATION RESEARCH OBJECTIVES

The overarching research objective for this dissertation was to evaluate the effect of climate differences (MAT and MAP) on microbial community structure, and composition, and methane production from poor fen northern peatlands to elucidate climate driven trends in metabolic functioning and phylogenetic distribution of the carbon decomposition cascade, focusing on processes that directly feed the methanogenic substrate pool.

6. EXPERIMENTAL APPROACH

I developed field surveys across a climate gradient, seeking to delineate possible climate driven trends in the microbial community composition of poor fen northern peatlands, because the poor fen type is one of the most abundant. I selected geochemically similar poor fen peatland field sites to reduce confounding variables, allowing the statical analyses to identify differences likely originating from the effects of the climate gradient. This natural gradient type experimental approach has been shown successful in peatlands when studying thaw, climate, latitude and warming gradients (Wieder and Yavitt 1994; Bragazza *et al.* 2013; McCalley *et al.* 2014b; Wilson *et al.* 2016a).

I chose to work in poor fen and bog peatlands because they are the most dominant type of northern peatland and harbor large amounts of organic carbon that has the potential to be released to the atmosphere due to changes in climate (Yu *et al.* 2003; Abdalla *et al.* 2016). For millennia northern peatlands have acted as methane sources and carbon dioxide sinks and, thus, a net carbon sink, accumulating more carbon year after year. With the threat of a changing climate, it is critical to better understand the factors affecting microbial community composition, the microbial trophic organization and methane production to be able to better model and anticipate the potential for change in carbon source/sink status.

From seven potential sites that were sampled, and a preliminary analysis performed, I focused this study on four peatland sites located across a climate gradient spanning permafrost, boreal, temperate, and transitional boreal/temperate conditions. The four sites were surveyed to detail their similarity in geochemistry, dominant plant species, methane production and microbial community composition. Statistical tests were performed to determine the degree of influence that geochemical and climate variables had on microbial community composition and methane production. Additional statistical tests were performed to identify the degree of inter- and intra-site variation. To further investigate the influence of climate on microbial community structure and function, DNA-Stable Isotope Probing (DNA-SIP) was used to identify active community members in the presence of glucose or propionate amendment. By monitoring microcosm methane and carbon dioxide production, substrate production and consumption and performing buoyant density separation of the microbial community DNA, I was able to determine methane and carbon dioxide ratios in response to glucose or propionate, quantify substrate production in response to a primary or secondary fermentation amendment and identify the active members of the community. This allowed me to compare the carbon decomposition cascade across sites and the climate gradient while also identifying key members in the methanogenic microbial community.

7. DISSERTATION STRUCTURE

This dissertation is divided into 4 chapters. The first chapter is an introduction which reviews the current literature and provides a background and framework for my dissertation. The second

and third chapters are experimental research chapters formatted as stand-alone publishable units. Lastly, the findings are summarized in a comprehensive conclusion chapter where future research directions are also suggested.

7.1. CHAPTER 1: DISSERTATION INTRODUCTION

This present chapter acts as a general introduction where I discuss the importance of northern peatlands, their propensity for storing carbon and the role that microorganisms play in carbon degradation, specifically in the context of a changing climate.

7.2. CHAPTER 2: ASSESSING MICROBIAL COMMUNITY COMPOSITION AND METHANE PRODUCTION OF NORTHERN PEATLANDS ACROSS A CLIMATE GRADIENT

Chapter 2 details a biological survey of microbial communities and biogeochemical characteristics such as pH, cation concentrations and dissolved methane from porewater of four northern peatland field sites, sampled across a climate gradient. In this study I sought to identify microbial community composition patterns associated with climate variables predicted to change under current climate scenarios. I identified several taxa who demonstrate niche preference across the climate gradient. These individuals included representatives from the following taxonomic groups, uncultured *Gemmatimonadetes, Fibrobacteraceae,* uncultured *Saccharimonadales*, *Methanobacteriaceae* and Candidatus '*Methanoflorentaceae'* fam. nov. associated with cooler sites receiving less precipitation. Individuals from *Magnetospirillaceae*, *Spirochaetaceae*, *Methanoregulaceae* and *Methanosaetaceae* were associated with warmer sites receiving more precipitation. Understanding niche preferences for members of the northern peatland community can assist us in better predicting microbial community structure and function in the face of a changing climate.

7.3. CHAPTER 3: USING DNA-SIP TO EVALUATE THE PREDOMINANCE OF PRIMARY VS SECONDARY FERMENTATION OF NORTHERN PEATLANDS ACROSS A CLIMATE GRADIENT

This chapter details a series of soil incubation experiments that investigate the active microbial community and primary fermentation *versus* secondary fermentation of northern peatland soils using DNA-Stable Isotope Probing. Soils from each field site studied in Chapter 2 are incubated in the presents of a glucose treatment as a proxy for primary fermentation or propionate as a proxy for secondary fermentation. Across sites, amended substrates were consumed and processed differentially based on site. Similarly, I also observed different community members consuming glucose or propionate across sites. Some of the active members that were identified mirrored their niche breadth preferences as characterized in Chapter 2. Revise main findings here.

7.4. DISSERTATION CONCLUSIONS:

The conclusion chapter compiles the major findings from each data chapter of my dissertation. I highlight the implications of my work to the field of northern peatland microbial ecology and consider future avenues for research.

CHAPTER 2

CLIMATE AND GEOCHEMICAL DRIVERS ORGANIZE MICROBIAL COMMUNITIES ACROSS POOR FEN NORTHERN PEATLANDS

In submission to the Microbiology Society's, Microbiology Journal for the special collection 'Implications of climate change for terrestrial microbiomes and global cycles'

Authors: Analissa F. Sarno[1], Mark C. Reynolds[1], Damien Finn[2], Joseph B. Yavitt[3], Elyn R. Humphreys[4], David Olefeldt[5], Liam Heffernan[6], D. Tyler Roman[7], Stephen D. Sebestyen[7], Randall K. Kolka[7] and Hinsby Cadillo-Quiroz[1,8]

Authors acknowledge the use of this work in my dissertation.

[1] School of Life Sciences, Arizona State University, Tempe, Arizona, USA

[2] Thünen Institut für Biodiversität, Johann Heinrich von Thünen Institut, Bundesallee 65 Braunschweig 38116, Germany

[3] Department of Natural Resources & the Environment, Cornell University, Ithaca, NY 14853, USA

[4] Department of Geography and Environmental Studies, Carleton University, Ottawa, ON K1S 5B6, Canada

[5]Department of Renewable Resources, University of Alberta, Edmonton, AB T6G 2H1, Canada.

[6]Evolutionary Biology Centre, Department of Ecology and Genetics/Limnology, Uppsala University, Norbyvägen 18D, 752 36, Uppsala, Sweden

[7]Northern Research Station, USDA Forest Service, Grand Rapids, MN, 55744, USA

[8]Biodesign Institute, Arizona State University, Tempe, Arizona, USA

Abstract:

Northern peatlands accumulate soil carbon because their anoxic waterlogged conditions slow organic matter decomposition, including microbial activity, being outpaced by plant growth and thus ecosystem carbon gain. However, how climate change may affect microbes participating in organic matter decomposition activities, their community composition and niche breadth in

northern peatlands is uncertain. Here we selected four peatlands situated along a climate and biome gradient from tundra (Daring Lake, Canada) to boreal forest (Lutose, Canada) to temperate broadleaf and mixed forest (Bog Lake, MN and Chicago Bog, NY) biomes to assess how the relative abundance of microbial taxa and microbial niche breadth might impact the decomposition of soil organic matter to methane (CH_4). The selected peatlands are poor fen type with similar hydrology and geochemistry. Microbial community composition derived from amplicon sequencing of the 16S rRNA gene, and geochemical and climate variables were analyzed with principal component regression analysis to determine major drivers of community variation. Niche breadth analyses allowed for the detection of putative patterns in microbial niche preference across the climate gradient. These results show that environmental factors predicted to change over time due to climate change, including mean annual temperature and precipitation, soil temperature and water table level, have the most significant impact on microbial community composition including the abundance of certain individuals from the families *Intrasporangiaceae*, *Prolixibacteraceae*, *Fibrobacteraceae*, *Methanobacteriaceae*, *Candidatus* '*Methanoflorentaceae*' fam. nov., *Magnetospirillaceae* and *Spirochaetaceae*. The observed patterns could be indicative of likely microbial community and functional shifts, resulting from the effects of climate change in poor fen northern peatlands.

1. INTRODUCTION:

Northern peatlands occur between 40° and 70°N latitude and are estimated to store about 30% of global soil organic carbon but make up only 3% of Earth's terrestrial surface (Yu 2012; Turetsky *et al.* 2015). Although these ecosystems have been a net carbon sink since their establishment, they also produce and emit 11% of atmospheric methane (CH_4) (Abdalla *et al.* 2016), a greenhouse gas 34 times more potent than carbon dioxide (Shindell *et al.* 2009). The carbon sequestration potential of northern peatlands may change under the influence of a warming climate (Gorham 1991; Keiser *et al.* 2019). For example, recent increases in air temperatures have increased CH_4 emissions from northern peatland soils, driven in part by changes in microbial community structure and trophic interactions (Tveit *et al.* 2015a; Zheng *et al.*

2018). If maintained, this will further perpetuate a positive feedback loop between climate change and greenhouse gas emissions (Chapin *et al.* 2005; IPCC 2007; Schuur *et al.* 2015). Our ability to predict effects of climate change on northern peatland ecosystems must take into account the diversity of peatland types including variation in nutrient status, heterogeneity of microtopographical features and their wide distribution across biome types (Strack *et al.* 2008; Qiu *et al.* 2020). Moreover, peatland-climate feedback models commonly lack specific parameters such as microbial respiration and small-scale processes such as oxic/anoxic zones across peat depth, which could be important regionally in carbon balancing (Dean *et al.* 2018; Hugelius *et al.* 2020).

Previous research has demonstrated that natural gradients can be utilized to investigate how ecosystems and their associated microbiomes could respond to a changing climate (Wieder and Yavitt 1994; Bragazza *et al.* 2013, 2015; Seward *et al.* 2020). Poor fen type northern peatlands in North America are frequently distributed between 40°- 70°N latitude, spanning multiple biomes including tundra, boreal, and temperate (Treat *et al.* 2015). The occurrence of poor fen peatlands across these regions facilitates the comparison of similar peatland type, hydrology, and geochemistry, and thus enabling the examination of climate-relate variables. However, peatlands also present diverse microtopographical features or local microhabitats at the site level with variability, causing some to be present or absent across sites and biomes (Strack *et al.* 2008a; Juottonen *et al.* 2015). For instance, “lawn” (*Sphagnum spp.* mat above the water table) or “hollow” (*Sphagnum spp.* mat at or below the water table facilitating the pooling of water) are common across poor fen and other peatlands in temperate and boreal regions, while “collapsed scar” (the result of permafrost degradation, an area of peat subsidence) or “tussock” (dominated by tussock forming graminoids above the water table) are frequent in permafrost affected sites in boreal and tundra but absent in temperate regions (Prater, Chanton and Whiting 2007; Lafleur and Humphreys 2008; Nijp *et al.* 2014). Differences in microbial and ecological measurements have demonstrated the variation across microtopographical features including microbial community composition, respiration, and CH_4 production (Juottonen *et al.* 2015). Thus,

microbiome evaluations of poor fen peatlands at distinct regions will need to test for the effects of inter-site (regional) and intra-site (microtopographical) derived variation.

In terms of CH_4 in peatlands as for all biological processes, rates of CH_4 production are temperature sensitive. However, a meta-analysis of ecosystem level studies have demonstrated the temperature dependence of CH_4 production is similar to the temperature dependence measurements from pure cultures (Yvon-Durocher *et al.* 2014). These metrics are equivalent to a 57-fold increase from 0°C to 30°C. Peatland soil incubations across a temperature range have demonstrated the CH_4 production rate to be 75% higher at 25°C than at 4°C (Tveit *et al.* 2015b). These dramatic changes were due to the temperature-dependence of rate-limiting steps during fermentation. Specifically, incubations below 7°C were limited by syntrophic propionate oxidation while above 7°C incubations were limited by polysaccharide hydrolysis. This shift was supported by a change in the microbial community structure and trophic cascade (Tveit *et al.* 2015b).

As demonstrated by different soil and wetland-based studies, MAT and MAP are important controllers of biological processes such as primary productivity, microbial respiration, and methanogenesis (Yvon-Durocher *et al.* 2014; Feng *et al.* 2020; Yang *et al.* 2022). In peatlands these processes then contribute to and affect ecosystem level outcomes such as total aquatic carbon export, which increases with greater precipitation, potentially affecting the overall carbon balance (Olefeldt *et al.* 2013). Similarly, peatland studies have demonstrated how an increase in temperature can expand the extent of ericaceous shrubs and how this change affects below ground labile carbon availability and affect the methanogenesis pathway and magnitude of production (Chasar *et al.* 2000; Bragazza *et al.* 2013).

This is especially relevant under predicted climate change scenarios where amount, frequency and duration of precipitation and intensity of warming events are unknown. Peatlands that are dominated by *Sphagnum spp.* are especially vulnerable to changes in precipitation due to their lack of stomata and reliance on capillary rise and retention to maintain water availability for photosynthesis (Ketcheson and Price 2014). Water availability can also affect the water table level and facilitate the diffusion of substrates and DOC throughout the pore space, in turn affecting microbial activity (Moore and Knowles 1989; Bergman, Klarqvist and Nilsson 2000).

In ecology, niche is used to define the environmental biotic and abiotic parameters (such as resources and interactions) that dictate the persistence of a species' abundance and metabolic activity (Zhou and Ning 2017). Only recently have microbiologists considered to quantitatively evaluate niche breadth, including environment-taxon and taxon-taxon interactions (Finn *et al.* 2020), although methods have been available for macro ecology systems for over 50 years (Levins 1968). The ability to quantitatively evaluate niche-based hypothesis allows microbiologists to further build upon the platform that next generation sequencing has established, especially in the context of a changing climate.

The objective of this study was to evaluate microbial community composition and niche breadth patterns of northern peatland soils influenced by climate variables. We used samples from northern peatlands situated across a climate and biome gradient, focusing on the regionally dominant poor fen type peatland. We hypothesized that climate variables such as MAT and MAP would be strong predictors of microbial community composition and key members would demonstrate climate-dependent distributions. We aim to test the influence of climate on microbial community composition across sites in a study set where the variability in other environmental and biogeochemical variables is limited by the selection of similar sites. We were able to evaluate the influence climate variables such as MAT and MAP have on microbial community composition and niche breadth patterns by utilizing poor fen type peatlands sampled across a climate gradient.

2. EXPERIMENTAL PROCEDURES

2.1. FIELD SITE CHARACTERISTICS

Four *Sphagnum*-dominated, poor fen type peatlands were selected across a natural climate gradient (**Figure 2.1**). The northernmost site, Daring Lake (DL), is a poor fen located in Northwest Territories, Canada found within the southern Arctic biome where permafrost is present. It is a treeless *Sphagnum* dominated tundra plain (64°52'N, 111°35'W) (Lafleur and Humphreys 2008). Lutose Bog (LT), a *Sphagnum* dominated collapse scar bog in Alberta, Canada (59°12'N,

117°12'W), is located in the northwest corner of Alberta within the boreal forest biome, surrounded by permafrost plateaus in a zone of discontinuous permafrost (Corbett *et al.* 2015). Bog Lake (BL), a poor fen in the Marcell Experimental Forest, Minnesota, USA (47°30'N, 93°27'W) is located in northern Minnesota near the Ontario border in the Laurentian Mixed Forest biome, a transitional zone between boreal and broadleaf deciduous forests (Sebestyen *et al.* 2011). The southernmost site, Chicago Bog (CB), is a *Sphagnum* dominated ombrotrophic bog, formed in a kettle-hole pond in upstate New York, USA (42°34'N, 76°14'W) located in the mixed broadleaf deciduous forest biome (Cadillo-Quiroz *et al.* 2006; Dettling, Yavitt and Zinder 2006).

Climate data for each site was collected from existing studies or national repositories. For Lutose climate data, MAT (Mean Annual Temperature) and MAP (Mean Annual Precipitation) values were estimated using the monthly average of the two closest weather stations including the Hay River weather station in Northwest Territories (Meterological Service of Canada 2021a) and High Level weather station in Alberta (Meterological Service of Canada 2021b). For Daring Lake climate data is based on the results from a previous study in this site (Lafleur and Humphreys 2008). For Bog Lake and Chicago Bog, monthly average temperature and precipitation data from Marcell, MN and Ithaca, NY were retrieved from the NOOA, U.S. Historical Climatology Network database (National Centers for Environmental Information 2007).

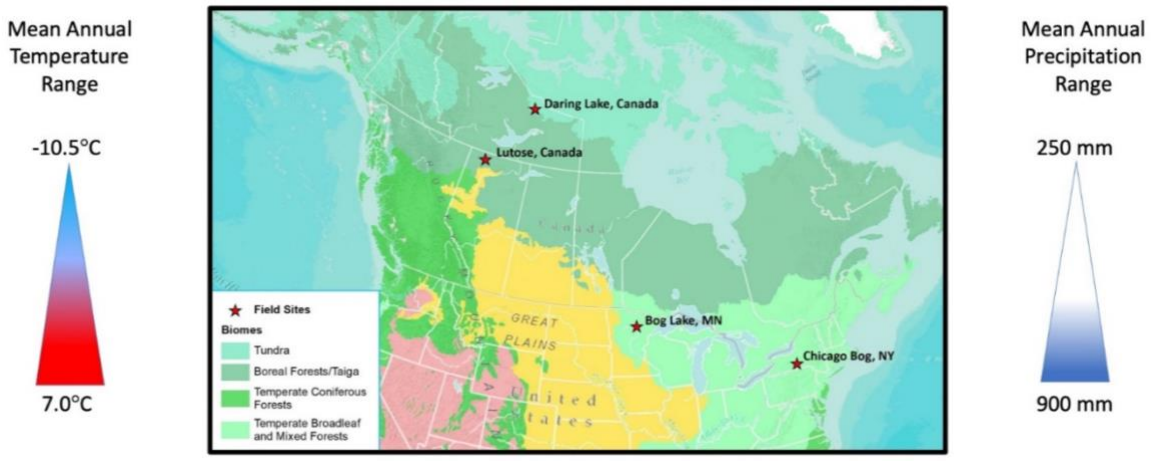


Figure 2.1: Four poor fen type northern peatlands sampled across a climate and biome gradient. Mean annual temperature (MAT) and mean annual precipitation (MAP) ranges are included on the left and right of the map respectively. Biome designations were retrieved from a publicly

available database (Bailey 1980; Wiken 1986; Olson and Dinerstein 2002; The Nature Conservency 2019).

2.2. SOIL AND POREWATER SAMPLING

The dominant microtopographical features at each site were identified and sampled (**Figure 2.2**, **Table 2.1C**). Six replicate soil and water samples were taken from each type of microtopographical feature along a 50 m transect through the middle of the peatland, for the purposes of comparing inter and intra-site variability. Water samples could not be taken of all soil sampling sites at Daring Lake field site due to the presence of permafrost. Therefore, their corresponding soil samples were not included in the microbial community analysis due to the absence of geochemical data. Soil temperature was recorded at time of sampling using an Ecosense, YSI probe (Dartmouth, NS). Soil for DNA analysis was collected in plastic zip-top bags during the summer of 2015, at the depth of the water table which ranged from +9 to -41 cm. If the water table was above the soil surface, soil was sampled at the soil surface. To preserve sample integrity, soil samples were stored on ice during transport to the lab, and then stored at -20°C until DNA extraction.

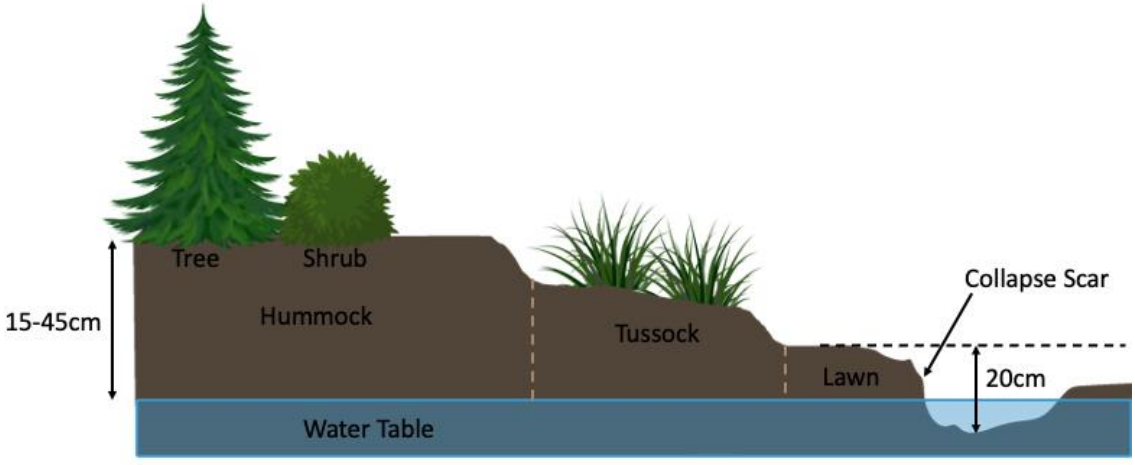


Figure 2.2: Graphical representation of the dominant microtopographical features identified and sampled at each of the four poor fen northern peatland sites.

2.3. GEOCHEMICAL MEASUREMENTS

We measured commonly used geochemical parameters to characterize northern peatlands including soil pH, water table depth, Mg^{2+} and Ca^{2+} ion concentration, moisture content of the peat soil, DOC and dissolved CH_4 of porewater. Water table level was measured from the peat surface, where below the peat surface was a negative water table value and above the water table was a positive water table value.

To analyze geochemical characteristics of each site, porewater was collected 5 cm below the water table with a porewater sampler made from a polyvinyl chloride capsule 5 cm long and 2.5 cm in diameter, with holes drilled on the sides, covered with litter-bag mesh to allow water to pass through while filtering large litter particles (2 mm mesh size). Water samples were drawn through PTFE tubing using a 30 mL syringe, passed through a PALL Acrodisc PF 32 mm Syringe filter with 0.8/0.2 µm Supor Membrane (Pall Corporation, Ann Arbor, MI), stored in sterile 15 ml sterile conical tubes, refrigerated for dissolved organic carbon (DOC) or frozen at -20C for ion chromatography (IC) analyses. IC samples were preserved with Thymol (ThermoFisher Scientific, Waltham, MA) at a final concentration of 500 mg/L (Cape *et al.* 2001).

To extract dissolved CH_4 from porewater, 15 mL of porewater was collected in a 30 mL syringe equipped with an air-tight valve. Fifteen mL of nitrogen gas (N_2) was added to the syringe from a serum bottle and the sample was shaken vigorously for 1 minute. Gas volume from the syringe was injected into an N_2-flushed, stoppered serum vial, and stored at 4°C until analysis. Soil pH was measured upon returning to the lab by adding 0.5 g peat soil to 4.5 mL of MilliQ water and was measured with a 3-point calibrated Orion 3 STAR bench top pH meter (Thermo Scientific, Waltham, MA). The gravimetric soil moisture of each sample was measured by comparing 10 g peat wet weight before and after 24 hours drying at 110°C or until there was no further mass loss.

Anions and cations were analyzed with IC using a Dionex ICS2000 series chromatograph with a 1 mL/min eluent flow rate. For anions, an AG18 pre-column, AS18 analytical column, and a 22 mM potassium hydroxide eluent were used. For cations, a CG12A pre-column, CS12A

analytical column, and an eluent of 35 mM methanesulfonic acid were used. DOC was analyzed with a TOC-V Total Organic Carbon Analyzer (Shimadzu Scientific Instruments, Koyoto, Japan). Dissolved CH_4 was measured using a gas-tight, N_2-flushed syringe (Hamilton, Reno, NV) and gas chromatograph (SRI Instruments, Torrance, CA) equipped with a flame-ionization detector (FID) fitted with a HayeSep column (Restek, Bellefonte, PA) with H_2 for combustion (supplied by an H_2 generator GCGS-7890, Parker Balston) and N_2 (UHP grade 99.999%, Praxair Inc) as carrier gas. Customized standard mixtures were used for CH_4 calibration (Scott Specialty Gases, accuracy $\pm$ 5%, Fremont, CA) over a range of 0.5-10,000 parts per million volume.

2.4. DNA EXTRACTION AND SEQUENCING

Total genomic DNA was extracted from 0.5 g of peat with the PowerSoil DNA Isolation Kit (MoBio, Carlsbad, CA). Minor modifications were made to the manufacturer's protocol to maximize DNA yield and minimize phenolic contamination. Samples were bead beat for 1 minute with solution C1 and centrifuged at 10,000 x g for 4 minutes to rupture cells and pellet the debris. All supernatant was transferred to a 2 mL microfuge tube, and solution C2 was added and incubated on ice for 15 mins to precipitate contamination. The tube was centrifuged at 10,000 x g for 2 minutes to pellet contamination. All supernatant was transferred to a 3 mL tube and 1.3 mL of solution C4 was added to bind DNA to the filter, tube was inverted to mix. Sample was then added to the spin filter and centrifuged per manufacturer's instructions. The spin filters that collected extra debris, indicated by a dark filter, were washed twice with solution C5 to remove excess phenolics. The sample was eluted with 85 µL of solution C6. Subsequently, all DNA samples were quantified with QuBit Broad Range dsDNA Assay Kit (ThermoFisher Scientific, Waltham, MA) and Nanodrop (ThermoFisher Scientific, Waltham, MA) per the manufacturer's instructions.

PCR amplification of the V4 region of the universal prokaryote 16S rRNA gene, targeting archaea and bacteria, was performed with 515F and 909R primers containing 6-10 nt unique barcodes for multiplexing, ordered from Integrated DNA Technologies (Tamaki *et al.* 2011; Herbold *et al.* 2015). Triplicate 25 µL reactions were performed using GoTaq Green Master Mix (Promega, Madison, WI), containing 0.3 mM of each primer and 0.2 mg/L Bovine serum albumin.

Polymerase chain reactions were carried out in an Eppendorff Mastercyler Pro with the following steps: Initial denaturation (95˚C, 5min), followed by 25 cycles of denaturation (94˚C, 30 sec), annealing (52˚C, 1 min), and extension (72˚C, 1 min), followed by final elongation (72˚C, 10 min) (Eppendorf, Hamburg, Germany).

The amplification of PCR products was verified visually with 0.8% w/v agarose gel electrophoresis with 0.5X Tris/Borate/EDTA (TBE) buffer. Reactions were cleaned and normalized with a SequalPrep Normalization Plate Kit (Invitrogen, Waltham, MA) following the manufacturer's instructions. Samples were pooled and submitted to DNASU core facility, for 300-bp paired-end Illumina MiSeq sequencing (Tempe, Az).

2.5. DNA SEQUENCE ANALYSIS

Forward and reverse reads were merged using Context-Aware Scheme for Paired-End Reads (CASPER) version 0.8.2 (Kwon, Lee and Yoon 2014), from high-throughput amplicon sequencing with the following parameters: Number of threads for parallel processing = 30, K-mer size = 19, threshold for difference of quality score = 19, threshold for mismatching ratio = 0.5, minimum length of overlap = 10, using Jellyfish = true. Sequences were demultiplexed with an in-house Python script (Reynolds *et al.* 2022). Amplicon sequence variants (ASVs) were assembled using Deblur's standalone tool using default parameters (Amir *et al.* 2017). Taxonomy was assigned to the ASVs using Naïve-Bayes taxonomic classifier trained with SILVA 132 database and the 16S 515F/909R primers with 355 nucleotide length using Qiime2 version 2020.2 (Tamaki *et al.* 2011; Bolyen *et al.* 2019). ASV tables were exported to R for further analysis.

2.6. STATISTICAL ANALYSIS

Analysis of Variance (ANOVA), T-test, or a non-parametric alternative was used to test for inter- and intra-site differences in geochemical, climatic, and other field-site characteristics (R Core Team 2013; R Studio Team 2020). Sites that demonstrated significant intra-site variation

across microtopographical features are indicated with an asterisk ($p < 0.05$). R (v. 4.0.1) and R studio (v. 1.3.959) were used for statistical analysis.

Geochemical and climate data was scaled to perform a Non-Metric Multidimensional Scaling (NMDS) and Analysis of Similarity (ANOSIM) of the Bray-Curtis dissimilarity index to evaluate differences in geochemical and climatic across sites (Oksanen *et al.* 2020). For the ANOSIM test, an R statistic near 1 implies a greater dissimilarity between groups, while a value near 0 implies an equal distribution of high and low ranks within and between groups. An R statistic less than 0 implies a greater dissimilarity within groups (Clarke 1993; Warton, Wright and Wang 2012; Buttigieg and Ramette 2014).

To further understand the contribution of each geochemical or climatic factor, a non-metric multi-dimensional scaling (NMDS) plot was used with linear regression of the variables was overlain and R^2 and p values.

The microbial community ASV table was exported from Qiime2, and Hellinger transformed in R, then redundancy analysis was performed at the family taxonomic level and plotted. The family taxonomic level was used for the redundancy analysis to minimize the presence of uncultured and unclassified ASVs. Then Permutational Multivariate Analysis of Variance (PERMANOVA) was performed in R to evaluate the potential differences across microbial communities.

The microbial community ASV table was rarefied to 6,000 per sample, exceptionally there were two samples that contained less than 6,000 reads included in the analysis. Any reads that could not be identified to at least the class level or were assigned chloroplast taxonomy were removed from subsequent analysis. The MicroNiche package (Finn *et al.* 2020) in R was used to analyze Levins' niche breadth to determine generalists *versus* specialists within the microbial community. Levins' niche breadth analysis of the 16S rRNA of the microbial communities allows us to determine if a taxon is a generalists or specialist when abundance is compared across the environmental gradient. Designation as a generalist is indicated by a niche breadth (B_N) value of 1, signifying the taxon can occupy a broad, non-discriminatory niche within the ecosystem.

Designation as a specialist is indicated by a B_N approaching 0, signifying that the taxon only occupies a narrow, discriminate niche within the ecosystem.

Hurlbert's niche breadth index was used to determine niche breadth of individual taxa across the climate gradient, testing the geochemical and climate variables that were found to be most significant in the linear regression analysis ($R^2 > 0.45$, p value < 0.05). Hurlbert's niche breadth analysis ranges between 0 and 1, where 0 implies an inverse relationship between the taxa and the environmental parameter and 1 implies a positive correlation between the taxa and the environmental parameter (Hurlbert 1978). Hurlbert's niche breadth index could not be used for the MAT data set due to negative data values. Alternatively, Spearman's coefficient was calculated for the specialists identified with Levins' niche breadth index to determine which taxa had had a significant relationship with MAT. Taxa that were found to have a significant relationship with MAT, MAP, soil temperature and water table level were graphed using the R "boxplot" function from the Graphics package (Murrell 2006).

3. RESULTS

3.1. EVALUATION OF INTRA- VERSUS INTER-SITE VARIATION OF CLIMATE AND GEOCHEMICAL PROPERTIES

The survey of the multisite (four), 50-meter transects inclusive of dominant microtopographical environments at each site resulted in 61 sampling points. The geochemical analyses and pairwise comparisons revealed the Ca^{2+} and Mg^{2+} levels across sites ranged from 1.6 - 3.3 mg/L and 2.0 - 3.9 mg/L respectively, characteristics of poor fens and that the intra-site variation for most measurements in these categories completed for this study were not statistically significant excepting pH at Lutose Bog (values 3.95 – 6.11) and Chicago Bog (values 3.95 - 5.40) and DOC at Daring Lake (16.0 – 87.0 mg/L) and at Bog Lake (29.7 – 59.6 mg/L) ($p < 0.05$). As shown in **Table 2.2** sites had a narrow range in geochemical conditions across microtopographical features per site.

A

Site	Latitude (°N)	MAT (°C)	MAP (mm)	Soil pH	Soil temp (°C)	Water table depth (cm)	DOC (mg/L)	Dissolved CH_4 (µmol/L)	Dry weight (g H2O/ g soil)
Daring lake	64.8	-10.5	250	4.9 ± 0.3	9.1 ± 4.9	-6.0 ± 13.7	41.6 ± 20.5*#	17.7 ± 15.3	0.09 ± 0.04
Lutose bog	59.2	-0.9	353	4.8 ± 0.8	7.3 ± 0.6	-35.7 ± 6.6	50.6 ± 5.2*	178.1 ± 88.7	0.08 ± 0.02
Bog lake	47.5	3.3	772	4.4 ± 0.1	16.0 ± 0.9	-22.1 ± 12.7	41.5 ± 8.1#	167.7 ± 66.0	0.06 ± 0.01
Chicago bog	42.5	7.9	890	4.5 ± 0.5	20.1 ± 1.8	-11.2 ± 4.5	54.5 ± 15.2*	115.1 ± 36.4*	0.05 ± 0.02

B

Site	Mg^{2+} ion (mg/L)	Ca^{2+} ion (mg/L)	Na^{+} ion (mg/L)	F^{-} ion (mg/L)	Cl^{-} ion (mg/L)	Br^{-} ion (mg/L)	SO_4^{-2} ion (mg/L)	PO_4^{-3} ion (mg/L)	NH_4^{+} ion (mg/L)	K^{+} ion (mg/L)
Daring lake	3.7 ± 1.3	1.6 ± 1.0	52.8 ± 5.5	0.4 ± 0.3	1.8 ± 0.8	0.5 ± 0.3	0.5 ± 0.3	0.4 ± 0.20	4.6 ± 4.1	4.5 ± 1.5
Lutose bog	3.8 ± 0.4	2.4 ± 0.4	7.7 ± 1.5	0.2 ± 0.1	1.9 ± 1.3	0.1 ± 0.02	0.1 ± 0.03	0.3 ± 0.2	15.3 ± 3.0	2.1 ± 1.8
Bog lake	3.9 ± 0.5	2.9 ± 0.6	45.1 ± 4.3	0.04 ± 0.03	2.6 ± 1.1	0.2 ± 0.1	0.1 ± 0.1	0.5 ± 0.1	0.1 ± 0.3	3.5 ± 1.2
Chicago bog	2.0 ± 2.3	3.3 ± 3.1	6.8 ± 2.9	0.2 ± 0.1	0.6 ± 0.4	0.3 ± 0.3	0.1 ± 0.04	0.3 ± 0.1	2.4 ± 3.0	2.6 ± 1.9

C

Site	Lawn	Hummock	Hummock with Shrub	Tree	Tussock	Collapse Scar
Daring lake	X				X[a]	X[b]
Lutose bog		X		X	X	
Bog lake	X		X			
Chicago bog	X	X	X			

Table 2.1: Summary of climate, geochemical and microtopography sampling data from each site. **A)** Climate and geochemical characteristics of each site. The mean ± standard deviation is reported. Pairwise statistical tests were performed to determine intra-site differences, indicated with an asterisk ($p < 0.05$). Soil temperature and water table depth were not statically tested because they are climate related variables. Pairwise statistical tests were performed to determine inter-site differences, indicated by #, where the two sites containing this symbol are statistically different ($p < 0.05$). **B)** Anion and cation concentrations for each site. The mean ± standard deviation is reported. **C)** Sampling scheme from each site is indicated by the presence of an X. Each microtopographical feature was sampled with an N of 6 unless otherwise noted. [a] Only 5 replicates were sampled. [b] Only 4 replicates were sampled. The following abbreviations are used: Mean Annual Temperature (MAT), Mean Annual Precipitation (MAP), Dissolved Organic Carbon (DOC).

A non-metric multi-dimensional scaling (NMDS) analysis of all environmental variables showed the relative distribution of the climatic and geochemical characteristics (**Figure 2.3**), which was complemented with a linear regression analysis of each climate or geochemical variable overlain to demonstrate the individual variable's contribution to the total environmental variation across sites. The most important contributors to the environmental variation across sites are MAP, MAT, soil temperature, dissolved CH_4, water table depth and Na^+ ion concentration ($p < 0.001$) with R^2 scores from 0.99 to 0.45 as shown in **Table 2.2**. Analysis of Similarity (ANOSIM) test, a rank-based analysis was used to determine the inter-site *versus* intra-site geochemical variation. ANOSIM test of climatic and geochemical variables resulted in an R statistic of 0.91, p < 0.001, indicating inter-site variation with low intra-site variation.

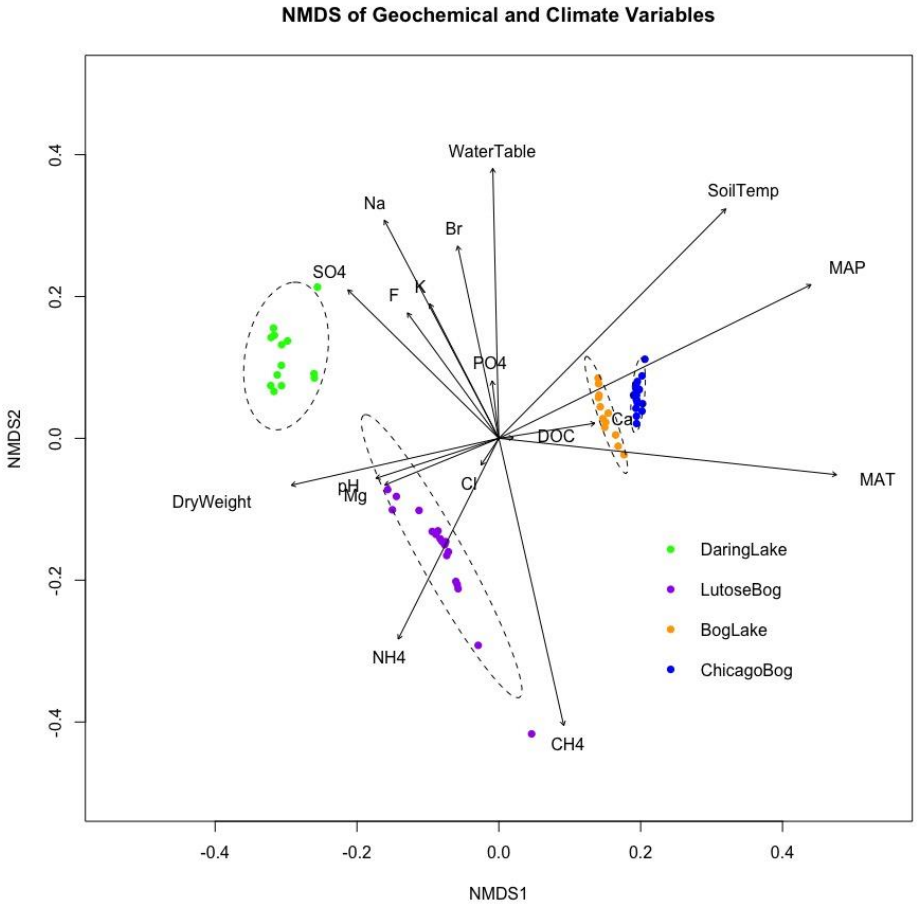


Figure 2.3: Non-metric multi-dimensional scaling plot of geochemical and climate variables with the linear regression of each variable overlain to account for each variable's contribution.

Variable	r^2	*P* value ≤
MAP (mm)	0.9886	0.001
MAT (°C)	0.9424	0.001
Soil temp (°C)	0.8503	0.001
Dissolved CH_4 µmol/L	0.7080	0.001
Water table (cm)	0.5956	0.001
Na^+ (mg/L)	0.4954	0.001
NH_4^+ (mg/L)	0.4117	0.001
Dry weight (g H_2O/g soil)	0.3696	0.001
SO_4^{-2} (mg/L)	0.3661	0.001
Br^- (mg/L)	0.3159	0.001
F^- (mg/L)	0.1968	0.008
K^+ (mg/L)	0.1868	0.005
Soil pH	0.1363	0.010
Mg^{+2} (mg/L)	0.1239	0.017
Ca^{+2} (mg/L)	0.0773	0.093
PO_4^{-3} (mg/L)	0.0273	0.486
Cl^- (mg/L)	0.0084	0.830
DOC (mg/L)	0.0018	0.956

Table 2.2: Results from the linear regression of climatic and geochemical variables overlain on the NMDS of the geochemical variables, to account for each variable's contribution to the variation. MAT= Mean Annual Temperature, MAP = Mean Annual Precipitation, DOC = Dissolved Organic Carbon.

3.2. MICROBIAL COMMUNITY COMPOSITION ACROSS A CLIMATE GRADIENT

Principal component analysis (PCA) of the 16S rRNA indicated that some intra-site variation occurred (see points per site dispersal), however a larger portion of the variation occurred across sites as illustrated by tight clustering within the 95% confidence interval (**Figure 2.4**).

Daring Lake and Lutose each showed only one replicate that falls outside of the 95% confidence interval. Importantly, the complementary PERMANOVA analysis of the microbial community across sites rejected the null hypothesis indicating that the centroids of each group are different significantly ($R^2 = 0.49$, $p < 0.001$). Linear regression of the climatic and geochemical variables was overlain on the PCA plot to demonstrate the contribution of each variable to the microbial community distribution (**Figure 2.4**). The climatic and geochemical variables most strongly contributing to the microbial community dispersion as indicated by the strong R^2 values include MAP, MAT, soil temperature, Na^+ ion concentration and water table depth ($R^2 > 0.45$, $p < 0.001$) as shown in **Table 2.3**.

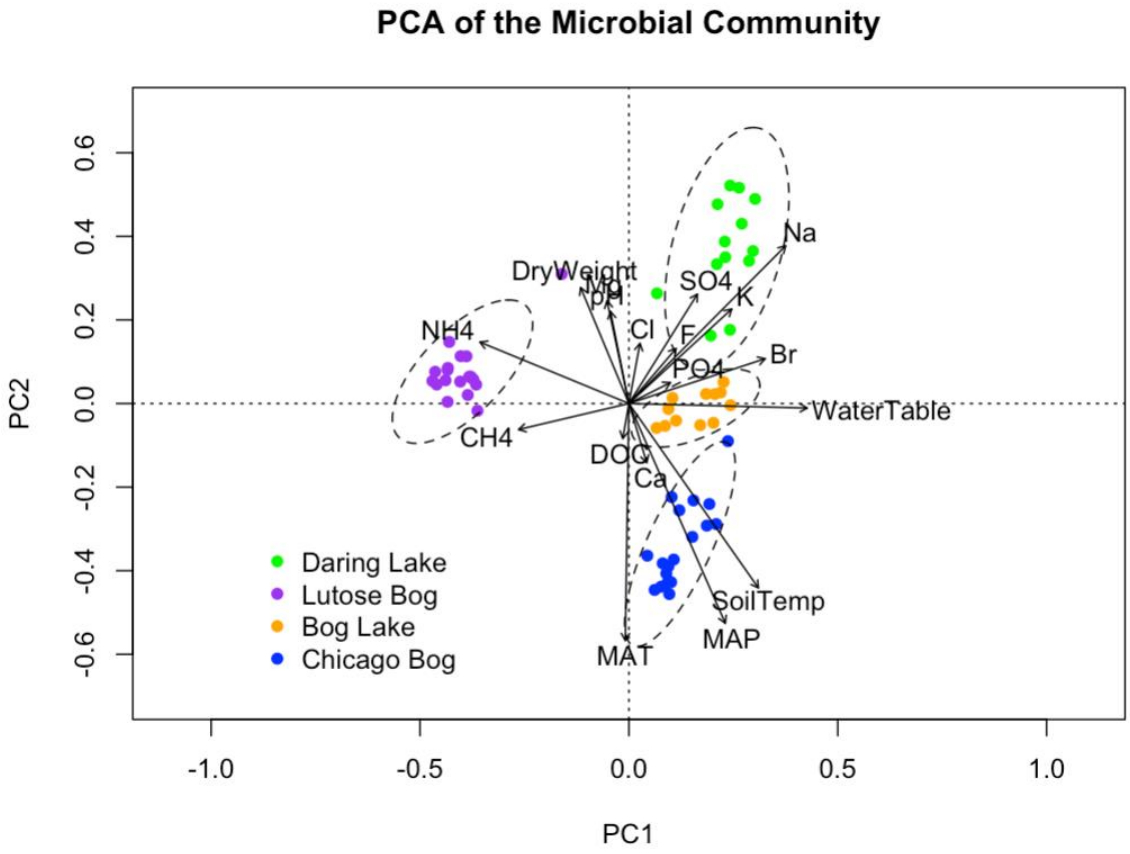


Figure 2.4: Principal component analysis of the microbial community at the family level with a 95% confidence interval indicated by the dashed circles. Linear regression of the geochemical and climate variables overlain to visualize each variable's contribution to the variation within the community.

Variable	**r^2**	***P* value ≤**
MAP (mm)	0.8759	0.001
MAT (°C)	0.8539	0.001
Soil temp (°C)	0.7717	0.001
Na^+ (mg/L)	0.7521	0.001
Water table (cm)	0.4840	0.001
NH_4^+ (mg/L)	0.395	0.001
Br^- (mg/L)	0.3132	0.001
K^+ (mg/L)	0.2968	0.001
SO_4^{-3} (mg/L)	0.2534	0.001
Dry weight (g H_2O/g soil)	0.2397	0.001
Dissolved CH_4 µmol/L	0.1953	0.002
Mg^{+2} (mg/L)	0.1674	0.003
Soil pH	0.1340	0.014
Ca^{+2} (mg/L)	0.0799	0.055

Variable	r^2	*P* value ≤
F^- (mg/L)	0.0579	0.193
PO_4^{-3} (mg/L)	0.0568	0.186
Cl^- (mg/L)	0.0328	0.376
DOC (mg/L)	0.0191	0.588

Table 2.3: Linear regression of climatic and geochemical variables to the microbial community at the family level ranked by r^2 value from greatest to least. MAT= Mean Annual Temperature, MAP = Mean Annual Precipitation, DOC = Dissolved Organic Carbon.

3.3. EVALUATION OF NICHE BREADTH USING LEVINS' B_N OF 16S COMMUNITY RRNA AMPLICON SEQUENCING

Levins' niche breadth analysis was performed on the rarefied dataset of about 174,000 sequences assigned to the family level to maximize phylogenetic identification and minimizing unidentified assignment. The Levins' niche breadth analysis identified 119 bacterial and 18 archaeal specialist taxa, only 71 of which could be resolved to the family level ($B_N < 0.52$, $p < 0.05$) (**Table 2.4**). Of the archaeal specialists, individuals from Crenarchaeota and Euryarchaeota made up the largest percentages (33%) each, followed by Thaumarchaeota (22%), with Diapherotrites and Nanoarchaeaeota making up the smallest percentage (6%) each. Of the archaeal specialists that could be resolved to the family level, there were individuals from the *Methanobacteriaceae*, *Methanoregulaceae*, *Candidatus* 'Methanoflorentaceae' fam. nov., and *Methanosaetaceae* families. Unresolved archaeal taxa included representatives from the classes Batharchaeia, Micrarchaeia, Thermoplasmata, Woesearchaeia and Thaumarchaeota Group 1.1c.

Of the bacterial specialists, the top five phyla represented were Proteobacteria (34%), Bacteroidetes (14%), Chloroflexi (11%), Actinobacteria (8%) and Acidobacteria (7%). Specialists from the phylum Proteobacteria included taxa from the Alpha-, Delta- and Gamma-classes of Proteobacteria. Specifically, there were representatives from the following families within the Alphaproteobacteria class; *Elsteraceae*, *Reyranellaceae*, *Hyphomicrobiaceae* and *Rickettsiaceae*. Within the Deltaproteobacteria class, specialists were identified from the families *Bdellovibrionaceae*, *Desulfovibrionaceae*, *Archangiaceae*, *Syntrophaceae* and *Syntrophobacteraceae.* Within the Gammaproteobacteria class, specialists were identified from the families *Aeromonadaceae*, *Neisseriaceae*, *Nitrosomonadaceae*, *Rhodocyclaceae*,

Methylococcaceae, *Methylomonaceae*, *Steroidobacteraceae*, *Rhodanobacteraceae*, and *Xanthomonadaceae.* From Bacteroidetes there were five taxa identified to the family level which included *Paludibacteraceae*, *Prolixibacteraceae*, *Chitinophagaceae*, *Microscillaceae*, and *Sphingobacteriaceae.* Out of 13 taxa from the Chloroflexi phylum, there was only taxon that could be identified to the family level which was *Ktedonobacteraceae.* From the Actinobacteria phylum, individuals from *Mycobacteriaceae*, *Acidothermaceae*, *Frankiaceae*, *Kineosporiaceae*, *Intrasporangiaceae* and *Microbacteriaceae* families were identified as specialists. From the Acidobacteria phylum, specialists included taxa from uncultured Acidobacteria subgroups 2, 7, 18 and 6. The Levins' niche breadth analysis identified no archaeal and 1 bacterial generalist, from the family *Beijerinckiaceae* ($B_N > 0.97$, $p < 0.05$) (**Table 2.4**).

3.4. 16S RRNA HURLBERT'S NICHE BREADTH

To better understand the effects of the strongest environmental predictors on taxa abundance across the gradient, we performed Hurlbert's niche breadth analysis of the microbial community. Hurlbert's niche breadth analysis of the microbial community and MAP data identified 93 bacterial and 4 archaeal taxa that are in high abundance when MAP is low ($B_N \leq 0.30$, $p < 0.005$) (**Table 2.4**). Only 67 of the taxa could be resolved to the family level.

Of the archaeal taxa that were significant when MAP was low, there were two unidentified Bathyarchaeia and one representative from each *Methanobacteriaceae* and *Candidatus* 'Methanoflorentaceae' fam. nov..

Of the bacterial taxa that were at high abundance under low MAP conditions, 65 could be resolved to the family level. Taxa from Proteobacteria, Bacteroidetes, Chloroflexi, Actinobacteria and Acidobacteria made up the greatest portion of the families identified to be in high abundance when MAP was low. Proteobacteria included representatives from the Alpha-, Delta- and Gamma-classes of Proteobacteria. Individuals from the Alphaproteobacteria class that could be resolved to the family level included *Micropepsaceae*, *Reyranellaceae*, *Hyphomicrobiaceae*, *Xanthobacteraceae.* Individuals from the Deltaproteobacteria class included representatives from *Bdellovibrionaceae*, *Haliangiaceae*, *Syntrophacea* and *Syntrophobacteraceae.* Individuals from

the Gammaproteobacteria class include *Nitrosomonadaceae*, *Enterobacteriaceae*, *Methylococcaceae* and *Steroidobacteraceae.* Families represented from the Chloroflexi phylum included *Anaerolineaceae* and *Ktedonobacteraceae.* Families represented from the Verrucomicrobia phylum include *Xiphinematobacteraceae* and *Terrimicrobiaceae*, *Chthoniobacteraceae.* Other families represented include *Fibrobacteraceae*, *Clostridiaceae* 1, *Thermoanaerobacteraceae* and *Nitrospiraceae.*

Hurlbert's niche breadth analysis of the microbial community and MAP identified an unclassified *Alphaproteobacteria*, *Magnetospirillaceae* and *Spirochaetaceae* to be in high abundance when MAP is high ($B_N > 0.89$, $p < 0.001$) (**Table 2.4**). No archaeal taxa were identified to have a significant response when MAP is high.

Hurlbert's niche breadth analysis of the microbial community and soil temperature identified 97 bacterial and 8 archaeal taxon that are in high abundance when soil temperature is low ($B_N < 0.37$, $p < 0.005$). Taxa from Proteobacteria, Bacteroidetes, Chloroflexi, Actinobacteria and Acidobacteria made up the greatest portion of the families identified to be in high abundance when soil temperature was low. The Proteobacteria phylum included representatives from the Alpha-, Delta- and Gamma-classes of Proteobacteria. Individuals from the Alphaproteobacteria class that could be resolved to the family level included *Reyranellaceae* and *Hyphomicrobiaceae.* Individuals from the Deltaproteobacteria class included representatives from *Bdellovibrionaceae*, *Syntrophacea* and *Syntrophobacteraceae*. Individuals from the Gammaproteobacteria class include *Aeromonadaceae*, *Nitrosomonadaceae*, *Methylococcaceae*, *Methylomonaceae*, *Steroidobacteraceae* and *Xanthomonadaceae.*

The archaeal taxon whose abundance had an inverse relationship with soil temperature included an uncultured *Bathyarchaeia* and *Thaumarchaeota Group 11c*, one representative from each *Methanobacteriaceae* and *Candidatus* 'Methanoflorentaceae' fam. nov.

Hurlbert's niche breadth analysis of the microbial community and soil temperature identified 10 bacterial and no archaeal taxon that are in high abundance when soil temperature was high ($B_N > 0.87$, $p < 0.05$). Proteobacteria made up the largest portion of families identified to be in high abundance when temperature is high, which included representatives from the Alpha-

and Delta-classes of Proteobacteria. These included individuals from the families *Beijerinckiaceae*, *Magnetospirillaceae* and *Geobacteraceae*. Individuals from other families identified included *Nostocaceae* and *Spirochaetaceae.*

Hurlbert's niche breadth analysis of the microbial community and water table depth identified 91 bacterial and 5 archaeal taxa with a high abundance when water table depth was low (below the peat surface) ($B_N < 0.24$, $p < 0.01$) (**Table 2.4**). Of the archaeal taxa identified, only 1 could be resolved to the family level. The taxa identified included individuals from the Crenarchaeota, Diapherotrites, Euryarchaeota, and Nanoarchaeaeota phyla. Specifically, an individual from the *Methanobacteriaceae* family was identified to have a high abundance while water table depth was low.

Hurlbert's niche breadth analysis of the microbial community and water table depth identified 7 bacterial and no archaeal taxa that were in high abundance when water table level was high ($B_N > 0.86$, $p < 0.001$) (**Table 2.4**). These taxa included representatives from the *Coxiellaceae* and *Syntrophorhabdaceae* families, all others could not be resolved to the family level.

Hulbert's niche breadth analysis of the microbial community and Na^+ ion concentration identified 70 bacterial and 12 archaeal taxa to be in high abundance when Na^+ ion concentration is low ($B_N < 0.2$, $p < 0.01$). Of the archaeal taxa identified to be in high abundance when Na^+ ion concentration is low, included individuals from the phyla Asgardaeota, Crenarchaeota, Diapherotrites, Euryarchaeota, Nanoarchaeaeota and Thaumarchaeota, of which only *Nitrosopumilaceae* could be resolved to the family level.

Of the 70 bacterial taxa identified to be in high abundance under low Na^+ ion concentrations, only 18 individuals could be resolved to the family level. From the Firmicutes phyla this included individuals from the *Staphylococcaceae*, *Lactobacillaceae*, *Gracilibacteraceae*, *Peptococcaceae*, *Limnochordaceae*, *Acidaminococcaceae* families. Proteobacteria included representatives from the Alpha-, Delta- and Gamma-classes of Proteobacteria. Individuals from the Alphaproteobacteria class that could be resolved to the family level included *Hyphomonadaceae* and *Dongiaceae*. Individuals from the

Deltaproteobacteria class included representatives from *Desulfobacteraceae*, *Desulfobulbaceae* and *Sandaracinaceae.* Individuals from the Gammaproteobacteria class include *Methylophilaceae*, *Leptospiraceae* and *Synergistaceae.* Families from the Actinobacteria phylum included *Actinospicaceae*, *Micromonosporaceae*. Individuals from the phylum Bacteroidetes and Chloroflexi included *Blattabacteriaceae* and *Roseiflexaceae* respectively.

Hurlbert's niche breadth of the microbial community and Na^+ ion concentration identified 4 bacterial and 1 archaeal taxa to be in high abundance when Na^+ ion concentration is high. This included taxa from Thermoplasmata, Elusimicrobia Lineage IIa, Phycisphaerae, Delta- and Gamma- proteobacteria. An individual from the *Methanomassiliicoccaceae* family was the only one representative able to be resolved to the family level.

Microbial members identified as specialists were subsequently analyzed using Spearman's correlation coefficient with MAT data to determine taxa with the strongest relationships to MAT. This analysis identified 19 representatives, 11 archaeal and 7 bacterial taxa with moderate to very strong (r_s = 0.51 to 0.83, $p < 0.001$) (**Table 2.4**) strength of correlation, indicating a positive association of ranks with MAT. Archaeal individuals were from the Crenarchaeota, Diapherotrites, Euryarchaeota, Nanoarchaeaeota, Thaumarchaeota phyla, but only *Methanosaetaceae*, *Methanoregulaceae*, *Nitrosotaleaceae* could be resolved to the family level. Bacterial individuals were from the Bacteroidetes, Chloroflexi, Elusimicrobia, Proteobacteria and Spirochaetes phyla, but only an individual from *Brevinemataceae* could be resolved to the family level.

Spearman's correlation coefficients of microbial members identified as specialists and MAT data identified 8 taxa with a moderate to strong (r_s = -0.63 to -0.50, $p < 0.001$) (**Table 2.4**) strength of correlation, indicating a negative association of ranks with MAT. This included individuals from the phyla Actinobacteria, Bacteroidetes, Chloroflexi, Fibrobacteres and Patescibacteria. Of these phyla, only *Intrasporangiaceae*, *Prolixibacteraceae*, and *Fibrobacteraceae* could be resolved to the family level.

A summary of the niche breadth analyses can be found in **Table 2.4**. Eleven taxa were identified to have a high abundance when the strongest environmental predictors were low

including MAP, MAT, soil temperature and water table. This indicates that these taxa should have a higher abundance in the northern most sites and a lower abundance in the southernmost sites, as evidenced in **Figure 2.5**.

Table 2.4: Summary of taxa identified to be a generalist or a specialist and/or to have a significant relationship with MAT, MAP, soil temperature or water table level. The count column is the sum of all the number of variables under which the taxon was found to be significant. Taxon that could not be identified to the class level were removed. High/low indicates that the taxon has a high abundance when the environmental variable is high/low. Any empty cells indicates that the taxon was not statistically significant for that variable. N or P under putative function indicates nitrogen (N) or phosphorus (P) cycling. [1](Kim *et al.* 2017), [2](Sizova *et al.* 2007), [3](Hunger, Gößner and Drake 2015), [4](Schmidt *et al.* 2016), [5](Fortin, Song and Anderson 2021), [6](Mason *et al.* 2020; Wang *et al.* 2022), [7](Xia *et al.* 2016), [8](Mondav *et al.* 2014), [9](Oren 2014)

ASV Identification	Generalist (G) Specialist (S)	MAP	Soil Temp.	MAT	Water Table	Putative Function
Bacteria, Actinobacteria, Actinobacteria, Frankiales	S	Low	Low	Low	Low	
Bacteria, Actinobacteria, Actinobacteria, Micrococcales, *Intrasporangiaceae*	S	Low	Low	Low	Low	Cellulose hydrolysis, N[1]
Bacteria, Actinobacteria, Coriobacteriia, OPB41	S	Low	Low	Low	Low	
Bacteria, Bacteroidetes, Bacteroidia, Bacteroidales, *Prolixibacteraceae*	S	Low	Low	Low	Low	N[5]
Bacteria, Chloroflexi, Anaerolineae, SBR1031, Uncultured Gemmatimonadetes bacterium	S	Low	Low	Low	Low	Carbohydrate fermentation[7]
Bacteria, Fibrobacteres, Fibrobacteria, Fibrobacterales, *Fibrobacteraceae*	S	Low	Low	Low	Low	Secondary fermentation[4]
Bacteria, Patescibacteria, Saccharimonadia, Saccharimonadales, Uncultured bacterium	S	Low	Low	Low	Low	P[6]
Bacteria, Patescibacteria, Saccharimonadia, Saccharimonadales, Uncultured soil bacterium	S	Low	Low	Low	Low	P[6]
Archaea, Crenarchaeota, Bathyarchaeia, Unidentified archaeon	S	Low	Low		Low	
Archaea, Euryarchaeota, Methanobacteria, Methanobacteriales, *Methanobacteriaceae*	S	Low	Low		Low	Hydrogenotrophic methanogenesis[9]
Bacteria, Acidobacteria, Holophagae, Subgroup 7	S	Low	Low		Low	
Bacteria, Acidobacteria, Holophagae, Subgroup 7, Freshwater sediment metagenome	S	Low	Low		Low	
Bacteria, Acidobacteria, Holophagae, Subgroup 7, Uncultured Thermaerobacter sp.	S	Low	Low		Low	
Bacteria, Acidobacteria, Subgroup 6	S	Low	Low		Low	
Bacteria, Actinobacteria, Actinobacteria, Frankiales, *Acidothermaceae*	S	Low	Low		Low	
Bacteria, Actinobacteria, Actinobacteria, Frankiales, *Frankiaceae*	S	Low	Low		Low	
Bacteria, Actinobacteria, Actinobacteria, Kineosporiales, *Kineosporiaceae*	S	Low	Low		Low	

ASV Identification	Generalist (G) Specialist (S)	MAP	Soil Temp.	MAT	Water Table	Putative Function
Bacteria, Actinobacteria, Actinobacteria, Micrococcales, *Microbacteriaceae*	S	Low	Low		Low	
Bacteria, Bacteroidetes, Bacteroidia, Bacteroidales, Bacteroidetes vadinHA17	S	Low	Low		Low	
Bacteria, Bacteroidetes, Bacteroidia, Bacteroidales, *Paludibacteraceae*	S	Low	Low		Low	
Bacteria, Bacteroidetes, Bacteroidia, Bacteroidales, SB 5	S	Low	Low		Low	
Bacteria, Bacteroidetes, Bacteroidia, Chitinophagales, *Chitinophagaceae*	S	Low	Low		Low	
Bacteria, Bacteroidetes, Bacteroidia, Cytophagales, *Microscillaceae*	S	Low	Low		Low	
Bacteria, Bacteroidetes, Bacteroidia, Sphingobacteriales, S15 21	S	Low	Low		Low	
Bacteria, Bacteroidetes, Bacteroidia, Sphingobacteriales, *Sphingobacteriaceae*	S	Low	Low		Low	
Bacteria, Bacteroidetes, Ignavibacteria, Ignavibacteriales, SR FBR L83	S	Low	Low		Low	
Bacteria, Bacteroidetes, Ignavibacteria, OPB56	S	Low	Low		Low	
Bacteria, Bacteroidetes, Ignavibacteria, OPB56, Uncultured bacterium	S	Low	Low		Low	
Bacteria, Bacteroidetes, Ignavibacteria, OPB56, Uncultured bacterium KF JG30 B11	S	Low	Low		Low	
Bacteria, Chloroflexi, Anaerolineae, SBR1031	S	Low	Low		Low	
Bacteria, Chloroflexi, Anaerolineae, SBR1031, Uncultured bacterium	S	Low	Low		Low	
Bacteria, Chloroflexi, KD4 96	S	Low	Low		Low	
Bacteria, Chloroflexi, KD4 96, Uncultured *Anaerolineaceae* bacterium	S	Low	Low		Low	
Bacteria, Chloroflexi, KD4 96, Uncultured Chloroflexi bacterium	S	Low	Low		Low	
Bacteria, Chloroflexi, Ktedonobacteria, B12 WMSP1, Uncultured Chloroflexi bacterium	S	Low	Low		Low	
Bacteria, Chloroflexi, Ktedonobacteria, Ktedonobacterales, JG30 KF AS9	S	Low	Low		Low	
Bacteria, Chloroflexi, Ktedonobacteria, Ktedonobacterales, *Ktedonobacteraceae*	S	Low	Low		Low	
Bacteria, Elusimicrobia, Lineage IIa, Uncultured soil bacterium	S	Low	Low		Low	
Bacteria, Patescibacteria, Microgenomatia, Candidatus Woesebacteria, Uncultured Microgenomates group bacterium	S	Low	Low		Low	
Bacteria, Planctomycetes, Phycisphaerae, Tepidisphaerales, Cpla 3 termite group	S	Low	Low		Low	
Bacteria, Planctomycetes, Planctomycetacia, Planctomycetales	S	Low	Low		Low	
Bacteria, Planctomycetes, Planctomycetacia, Planctomycetales, Uncultured bacterium	S	Low	Low		Low	
Bacteria, Proteobacteria, Alphaproteobacteria, Elsterales, URHD0088	S	Low	Low		Low	
Bacteria, Proteobacteria, Alphaproteobacteria, Reyranellales, *Reyranellaceae*	S	Low	Low		Low	

ASV Identification	Generalist (G) Specialist (S)	MAP	Soil Temp.	MAT	Water Table	Putative Function
Bacteria, Proteobacteria, Alphaproteobacteria, Rhizobiales, Rhizobiales Incertae Sedis	S	Low	Low		Low	
Bacteria, Proteobacteria, Deltaproteobacteria, Bdellovibrionales, *Bdellovibrionaceae*	S	Low	Low		Low	
Bacteria, Proteobacteria, Deltaproteobacteria, Myxococcales	S	Low	Low		Low	
Bacteria, Proteobacteria, Deltaproteobacteria, Myxococcales, KD3 10	S	Low	Low		Low	
Bacteria, Proteobacteria, Deltaproteobacteria, Myxococcales, mle1 27	S	Low	Low		Low	
Bacteria, Proteobacteria, Deltaproteobacteria, Myxococcales, P3OB 42	S	Low	Low		Low	
Bacteria, Proteobacteria, Gammaproteobacteria, Betaproteobacteriales, A21b	S	Low	Low		Low	
Bacteria, Proteobacteria, Gammaproteobacteria, Betaproteobacteriales, *Nitrosomonadaceae*	S	Low	Low		Low	
Bacteria, Proteobacteria, Gammaproteobacteria, Betaproteobacteriales, SC I 84	S	Low	Low		Low	
Bacteria, Proteobacteria, Gammaproteobacteria, JG36.TzT.191, Uncultured Proteobacterium	S	Low	Low		Low	
Bacteria, Verrucomicrobia, Verrucomicrobiae, Chthoniobacterales, *Xiphinematobacteraceae*	S	Low	Low		Low	
Bacteria, Verrucomicrobia, Verrucomicrobiae, SBQ257 soil group	S	Low	Low		Low	
Bacteria, Planctomycetes, Pla4.lineage, Uncultured Planctomycete	S		Low		High	
Bacteria, Actinobacteria, Actinobacteria, Corynebacteriales, *Mycobacteriaceae*	S		Low		Low	
Bacteria, Proteobacteria, Alphaproteobacteria, Rhizobiales, *Hyphomicrobiaceae*	S		Low		Low	
Bacteria, Proteobacteria, Alphaproteobacteria, Rhizobiales, KFJG30B3	S		Low		Low	
Bacteria, Proteobacteria, Alphaproteobacteria, Uncultured bacterium	S		Low		Low	
Bacteria, Proteobacteria, Gammaproteobacteria, Betaproteobacteriales, Uncultured bacterium	S		Low		Low	
Bacteria, Proteobacteria, Gammaproteobacteria, Steroidobacterales, *Steroidobacteraceae*	S		Low		Low	
Bacteria, Nitrospirae, Nitrospira, Nitrospirales, *Nitrospiraceae*	S		Low		Low	
Bacteria, Proteobacteria, Deltaproteobacteria, MBNT15	S		Low		Low	
Archaea, Diapherotrites, Micrarchaeia, Uncultured archaeon	S			High	Low	

ASV Identification	Generalist (G) Specialist (S)	MAP	Soil Temp.	MAT	Water Table	Putative Function
Archaea, Euryarchaeota, Thermoplasmata, Uncultured	S			High	Low	
Archaea, Nanoarchaeaeota, Woesearchaeia	S			High	Low	
Bacteria, Chloroflexi, Anaerolineae, SJA15	S			High	Low	
Bacteria, Proteobacteria, Deltaproteobacteria, SAR324 clade Marine group B, Uncultured bacterium zdt33i5	S			High	Low	
Bacteria, Spirochaetes, Spirochaetia, Brevinematales, *Brevinemataceae*	S			High	Low	
Archaea, Euryarchaeota, Methanomicrobia, Methanomicrobiales, Candidatus *Methanoflorentaceae* fam. nov.	S	Low	Low			Hydrogenotrophic methanogenesis[8]
Bacteria, Bacteroidetes, Ignavibacteria, Ignavibacteriales	S	Low	Low			
Bacteria, Chloroflexi, AD3, Uncultured bacterium	S	Low	Low			
Bacteria, Chloroflexi, Dehalococcoidia, GIF9, Uncultured bacterium	S	Low	Low			
Bacteria, Firmicutes, Clostridia, Thermoanaerobacterales, *Thermoanaerobacteraceae*	S	Low	Low			
Bacteria, Planctomycetes, Phycisphaerae, MSBL9, SG8.4	S	Low	Low			
Archaea, Crenarchaeota, Bathyarchaeia	S		Low	High		
Archaea, Thaumarchaeota, Group.1.1c, Uncultured archaeon	S		Low	High		
Bacteria, Actinobacteria, Acidimicrobiia, IMCC26256, Bacterium enrichment culture clone auto734W	S		Low		Low	
Bacteria, Actinobacteria, Thermoleophilia, Solirubrobacterales, *Solirubrobacteraceae*	S		Low		Low	
Bacteria, Chloroflexi, Anaerolineae, Anaerolineales, *Anaerolineaceae*	S		Low		Low	
Bacteria, Chloroflexi, Chloroflexia, Elev1554, Uncultured bacterium	S		Low		Low	
Bacteria, Elusimicrobia, Lineage IIa	S		Low		Low	
Bacteria, Firmicutes, Clostridia, Clostridiales, *Clostridiaceae* 1	S		Low		Low	
Bacteria, Bacteroidetes, Bacteroidia, *Sphingobacteriales*	S		Low		High	
Bacteria, Proteobacteria, Alphaproteobacteria	S	High	High			
Bacteria, Proteobacteria, Alphaproteobacteria, Rhodospirillales, *Magnetospirillaceae*	S	High	High			N^2
Bacteria, Spirochaetes, Spirochaetia, Spirochaetales, *Spirochaetaceae*	S	High	High			Saccharolytic fermenter, Acetogenesis[3]
Bacteria, Proteobacteria, Gammaproteobacteria, Methylococcales, *Methylomonaceae*	S		Low			
Bacteria, Bacteroidetes, Bacteroidia, Sphingobacteriales, env OPS 17	S				Low	
Bacteria, Bacteroidetes, Bacteroidia, Sphingobacteriales, KD3 93	S				Low	

ASV Identification	Generalist (G) Specialist (S)	MAP	Soil Temp.	MAT	Water Table	Putative Function
Bacteria, Proteobacteria, Alphaproteobacteria, uncultured, Bacterium Ellin6089	S				Low	
Archaea, Crenarchaeota, Bathyarchaeia, Archaeon RBG.16.50.20,	S		Low			
Archaea, Crenarchaeota, Bathyarchaeia, Uncultured crenarchaeote,	S		Low			
Archaea, Crenarchaeota, Bathyarchaeia, Uncultured methanogenic archaeon	S		Low			
Bacteria, Acidobacteria, Acidobacteriia, Subgroup 2	S		Low			
Bacteria, Acidobacteria, Acidobacteriia, Subgroup.2, Uncultured Acidobacteria bacterium	S		Low			
Bacteria, Bacteroidetes, Bacteroidia, Sphingobacteriales, Uncultured bacterium	S		Low			
Bacteria, Elusimicrobia, Elusimicrobia, Lineage IV	S		Low			
Bacteria, Elusimicrobia, Lineage.IIa, Uncultured bacterium	S		Low			
Bacteria, Firmicutes, Bacilli, Bacillales	S		Low			
Bacteria, Patescibacteria, CPR2, Uncultured eubacterium WCHB1.60	S		Low			
Bacteria, Proteobacteria, Deltaproteobacteria, Sva0485, Uncultured Deltaproteobacterium	S		Low			
Bacteria, Proteobacteria, Deltaproteobacteria, Syntrophobacterales, *Syntrophaceae*	S		Low			
Bacteria, Proteobacteria, Deltaproteobacteria, Syntrophobacterales, *Syntrophobacteraceae*	S		Low			
Bacteria, Proteobacteria, Gammaproteobacteria, Aeromonadales, *Aeromonadaceae*	S		Low			
Bacteria, Proteobacteria, Gammaproteobacteria, Methylococcales, *Methylococcaceae*	S		Low			
Bacteria, Proteobacteria, Gammaproteobacteria, Xanthomonadales, *Xanthomonadaceae*	S		Low			
Bacteria, Rokubacteria, NC10, Methylomirabilales, *Methylomirabilaceae*	S		Low			
Bacteria, Verrucomicrobia, Verrucomicrobiae, Chthoniobacterales, *Terrimicrobiaceae*	S		Low			
Archaea, Euryarchaeota, Methanomicrobia, Methanomicrobiales, *Methanoregulaceae*	S			High		Hydrogenotrophic methanogenesis[9]
Archaea, Euryarchaeota, Methanomicrobia, Methanosarcinales, *Methanosaetaceae*	S			High		Hydrogenotrophic / acetoclastic methanogenesis[9]
Archaea, Euryarchaeota, Thermoplasmata, Uncultured, Uncultured archaeon	S			High		

ASV Identification	Generalist (G) Specialist (S)	MAP	Soil Temp.	MAT	Water Table	Putative Function
Archaea, Thaumarchaeota, Group.1.1c, Uncultured Thaumarchaeote	S			High		
Archaea, Thaumarchaeota, Nitrososphaeria, Nitrosotaleales, *Nitrosotaleaceae*	S			High		
Bacteria, Bacteroidetes, Ignavibacteria, Ignavibacteriales, Uncultured bacterium	S			High		
Bacteria, Elusimicrobia, Uncultured Acidobacteria bacterium	S			High		
Bacteria, Proteobacteria, Deltaproteobacteria, Sva0485	S			High		
Bacteria, Proteobacteria, Gammaproteobacteria, Betaproteobacteriales	S			High		
Bacteria, Proteobacteria, Alphaproteobacteria, Rhizobiales, *Beijerinckiaceae*	G		High			
Bacteria, Acidobacteria, Acidobacteriia, Subgroup 13, Uncultured bacterium	S				High	
Bacteria, Chlamydiae, Chlamydiae, Chlamydiales, cvE6	S				High	
Bacteria, Dependentiae, Babeliae, Babeliales, Uncultured bacterium	S				High	
Bacteria, Proteobacteria, Deltaproteobacteria, Deltaproteobacteria Incertae Sedis, *Syntrophorhabdaceae*	S				High	
Bacteria, Acidobacteria, Holophagae, Holophagales, *Holophagaceae*	S				Low	
Bacteria, Actinobacteria, Acidimicrobiia, Uncultured	S				Low	
Bacteria, Actinobacteria, Acidimicrobiia, Uncultured, Uncultured Forest soil bacterium	S				Low	
Bacteria, Bacteroidetes, Bacteroidia, Bacteroidales	S				Low	
Bacteria, Bacteroidetes, Bacteroidia, Sphingobacteriales, AKYH767	S				Low	
Bacteria, Firmicutes, Negativicutes, Selenomonadales, Veillonellaceae	S				Low	
Bacteria, Patescibacteria, Parcubacteria, Uncultured bacterium	S				Low	
Bacteria, Proteobacteria, Alphaproteobacteria, Rhizobiales, *Xanthobacteraceae*	S				Low	
Bacteria, Proteobacteria, Deltaproteobacteria, Myxococcales, *Haliangiaceae*	S				Low	
Bacteria, Proteobacteria, Gammaproteobacteria, Betaproteobacteriales, TRA3.20	S				Low	
Bacteria, Proteobacteria, Gammaproteobacteria, Enterobacteriales, *Enterobacteriaceae*	S				Low	
Bacteria, Verrucomicrobia, Verrucomicrobiae, Chthoniobacterales, *Chthoniobacteraceae*	S				Low	
Bacteria, Elusimicrobia, Elusimicrobia, Lineage IV, Uncultured Termite group 1 bacterium	S				Low	

ASV Identification	Generalist (G) Specialist (S)	MAP	Soil Temp.	MAT	Water Table	Putative Function
Bacteria, Proteobacteria, Alphaproteobacteria, Micropepsales, *Micropepsaceae*	S				Low	
Bacteria, Proteobacteria, Gammaproteobacteria, Coxiellales, *Coxiellaceae*	S				High	
Bacteria, Chloroflexi, AD3, uncultured Chloroflexi bacterium,	S		Low			
Bacteria, Acidobacteria, Acidobacteriia, Acidobacteriales	S		High			
Bacteria, Chloroflexi, Dehalococcoidia, RBG.13.46.9, Uncultured bacterium	S		High			
Bacteria, Cyanobacteria, Oxyphotobacteria, Nostocales, *Nostocaceae*	S		High			
Bacteria, Elusimicrobia, Elusimicrobia, Lineage.IV, Uncultured bacterium	S		High			
Bacteria, FCPU426, Uncultured bacterium	S		High			
Bacteria, Fibrobacteres, Fibrobacteria, Fibrobacterales, B122	S		High			
Bacteria, Proteobacteria, Deltaproteobacteria, Desulfuromonadales, *Geobacteraceae*	S		High			
Bacteria, Dependentiae, Babeliae, Babeliales, *Vermiphilaceae*	S	High				

These ASVs include individuals from the phyla Actinobacteria, Bacteriodetes, Chloroflexi, Fibrobacteres, Patescibacteria. Of these phyla, only three ASVs could be resolved to the family level, which included *Intrasporangiaceae, Prolixibacteraceae, Fibrobacteraceae. Methanobacteriaceae,* Candidatus '*Methanoflorentaceae*' fam. nov. and an unclassified Bathyarchaeia were also found to be in high abundance when differing environmental conditions were low (**Figure 2.5**).

Six ASVs were identified to be in high abundance when different environmental conditions were high indicating these taxa should be in high abundance in the southernmost sites (**Figure 2.6**). These include individuals from the Proteobacteria, Spirochaetes and Euryarchaeota phyla. Specifically, individuals from the families *Magnetospirillaceae, Spirochaeraceae, Methanoregula and Methanosaetaea* could be taxonomically resolved.

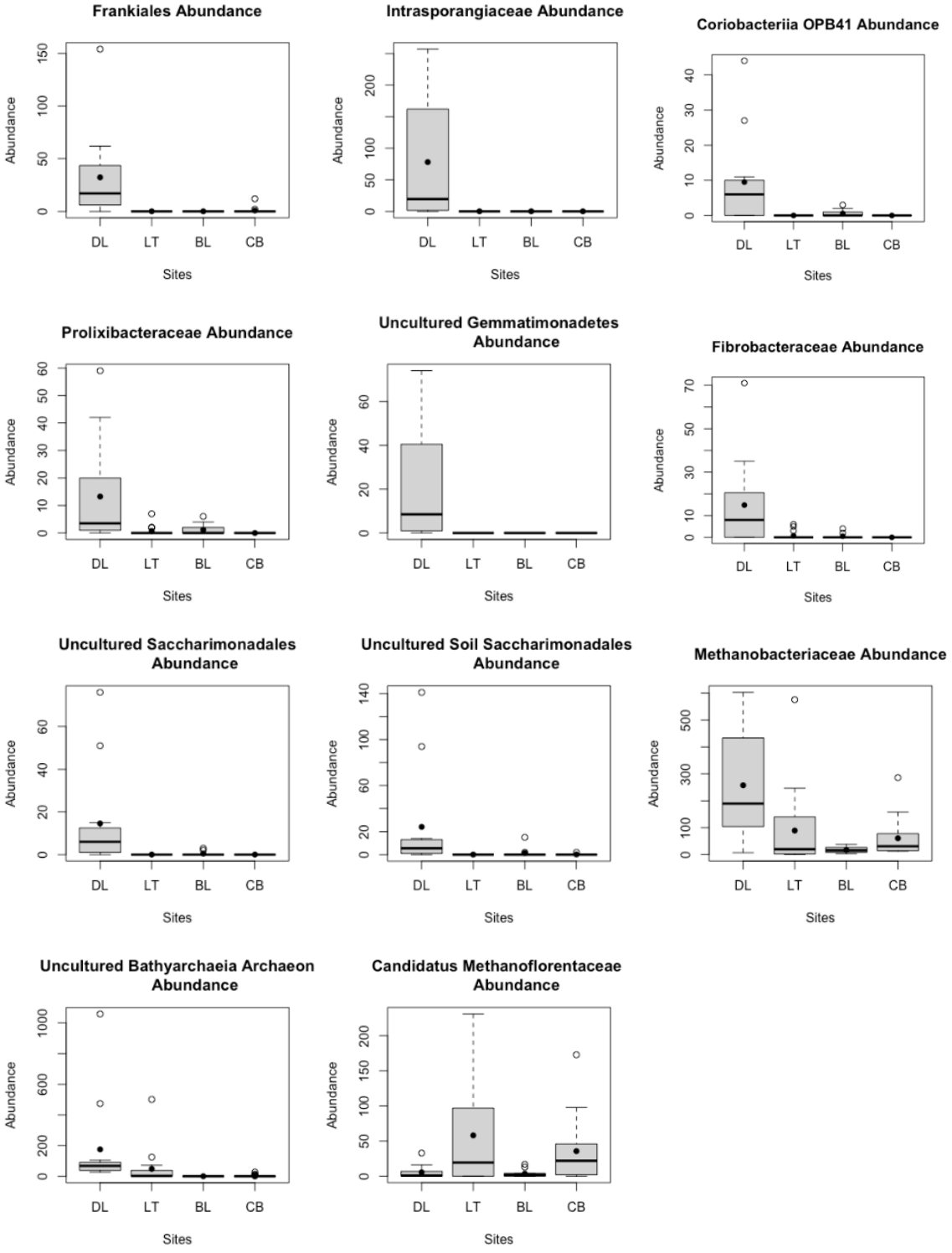


Figure 2.5: Box and whisker plots of the ASV taxa distribution that were identified to have a significant relationship with multiple environmental variables using niche breadth analyses when environmental variables are low. The box spans the interquartile range, and the whiskers marks the highest and lowest points. The mean is indicated by the closed circle, outliers are indicated by the open circles and the horizontal bar within the box indicates the median. DL= Daring Lake, LT= Lutose Bog, BL= Bog Lake, CB= Chicago Bog.

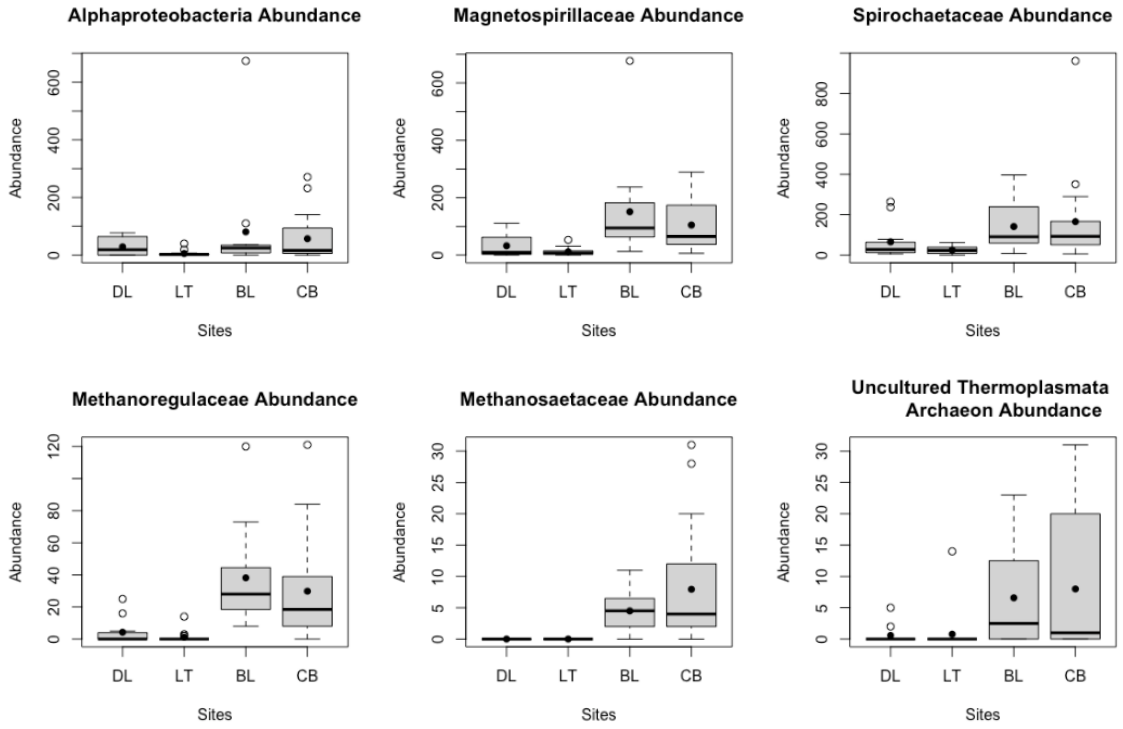


Figure 2.6: Box and whisker plot of ASV abundance that were identified to have a significant relationship with one or more environmental variables using niche breadth analyses when environmental variables are high. Reference **Table 2.4** and for more niche breadth details. These taxa demonstrate high abundance at the colder and drier end of the climate gradient. The box spans the interquartile range, and the whiskers marks the highest and lowest points. The mean is indicated by the closed circle, outliers are indicated by the open circles and the horizontal bar within the box indicates the median. DL= Daring Lake, LT= Lutose Bog, BL= Bog Lake, CB= Chicago Bog.

4. DISCUSSION

Based on previous studies of these poor fen sites, we expected low geochemical variability in comparison to other peatland types (Dettling *et al.* 2007; Prater, Chanton and Whiting 2007; Lafleur and Humphreys 2008; Feng *et al.* 2020). We also expected a greater portion of the variability attributed to climate related variables across sites. This was due to the natural climate and biome gradient on which the sites are located. This expectation was met by most geochemical parameters across sites, although there were slight differences in some sites including a pH range of 4.5 - 4.9, Mg^{+2} ion concentration range of 2.0 – 3.9 mg/L and DOC 41.5 - 54.5 mg/L (**Table 2.2**). The ranges of geochemical values are comparable to those found in

studies using poor fen sites situated across a natural gradient (Wieder and Yavitt 1994; Myers *et al.* 2012; Zalman *et al.* 2018; Seward *et al.* 2020), supporting that our site selection was representative of poor fens.

As predicted, there were differences in microtopographic feature types within a site (**Table 2.2**), likely due to the influence that climate and site specific biotic factors can have on the development of different features (Mitsch and Gosselink 2015). Microtopographic features have been shown to play an important role in biogeochemical processes and C balance within a peatland complex, contributing a critical component to the ecosystem's functioning (Moore *et al.* 2011; Graham *et al.* 2020). Importantly, the observed geochemical and microbial variation within and among sites was small, as demonstrated by the clustering by site analysis (**Figure 2.3** and **2.4**), indicating that the dataset was amenable to analyze climate related variables.

Analysis of the geochemical and climatic values measured across the sites indicated that MAP, MAT, soil temperature, dissolved CH_4 and water table level were the greatest contributors to overall variation across sites (**Figure 2.3** and **Table 2.3**). Mean annual precipitation, MAT, soil temperature and water table were among the top contributors to microbial community variation across sites (**Figure 2.3** and **Table 2.2**) further demonstrating the importance of the climate and climate-related variables.

The literature demonstrates the influence environmental conditions can have on the microbial community structure including an increase in diversity indices as conditions become more circumneutral and nutrient rich, a change in trophic structure as temperature decreases and increase in microbial respiration and methanogenesis with permafrost thaw (Galand *et al.* 2005; McCalley *et al.* 2014b; Schmidt *et al.* 2015).

4.1. MICROBIAL TAXA DEMONSTRATE NICHE PATTERS ACROSS A CLIMATE GRADIENT.

The microbial communities sampled across the climate and biome gradient were dominated by bacterial phyla common to poor fen type peatlands including Acidobacteria, Proteobacteria, Chloroflexi, Bacteroidetes, and Actinobacteria (Mondav *et al.* 2017; Seward *et al.* 2020). Although the sites shared several members, the PCA and PERMANOVA analyses

demonstrated significant differences among sites as depicted by the tight clustering by site in **Figure 2.4**. This is contradictory to the results from Seward *et al.* 2020, where microbial community structure investigation and beta-diversity dissimilarity ordination of fens from the southern Appalachian Mountain resembled those characterized from more northern latitudes, demonstrating little to no difference across the climate gradient. There could be confounding factors of this comparison because the taxonomic resolution from Seward *et. al.*, was at the phylum level, while this study focused on the family level to better associate traits from cultured individuals while minimizing unidentified assignments (Seward *et al.* 2020).

Previous studies have predicted the role climate variables such as MAT, MAP and soil temperature can have on microbial community composition and carbon cycling dynamics. These include a shift in community composition and functional guilds with an increase in temperature (Tveit *et al.* 2015a), or changes in methanogenic community composition, with an increase in *Methanocella* abundance when incubated at warmer temperatures, and an increase in *Methanoregula* and *Methanosaeta* abundance when incubated at cooler temperatures (Schmidt *et al.* 2015). The latter is the opposite methanogen abundance pattern of what we see from our soil samples, indicating that there may be different factors regulating community composition in a microcosm-based soil incubation *versus* abundance analysis from an *in-situ* soil sample. These observations may also be impacted by the difference in sampling a community and altering the incubation temperature, compared to sampling soils from an intact community at different temperatures.

A soil study based in an artic-alpine ecosystem evaluating the effect of microtopography and climate variables on microbial community composition study found that temperature and precipitation were the strongest drivers of community composition. Specifically, there were significant differences in abundance reported for *Spirochaetaceae*, *Syntrophobacterales* and *Methylococcales* in soils with higher soil moisture content. We see similar patterns of microbial abundance response to moisture related parameters such as MAP of individuals from the same taxonomic groups. This could be due to the interdependence of some *Syntrophobacterales* and *Methylococcales* individuals on methanogens and associated anaerobic conditions.

Importantly, this study has identified several taxa including individuals from *Intrasporangiaceae* and Anaerolineae SBR1031 taxonomic groups that have previously been identified in peatlands, whose presence or abundance has an inverse relationship with the climate variables MAT, MAP, soil temperature and water table depth. These taxa were found to be more abundant in the northern most, colder, and drier sites along the climate gradient. This includes individuals from the *Oryzihumus* genus that were initially identified as abundant in the Zoige wetland located on the Tibet plateau where the mean annual temperature is 1.1°C (Gu *et al.* 2018). Similarly, an uncultured representative from the order *Saccharimonadales* was identified as an indicator species of native undisturbed wetlands in China where the mean annual temperature is 2.5°C (Song *et al.* 2009; Wei *et al.* 2019). An uncultured individual found to have the same inverse relationship with the environmental gradient from the SBR1031 order of *Anaerolineae*. Only a few genomes have been characterized in this order but all have contained key genes for acetogenic dehydrogenation which could facilitate the use of ethanol as a carbon source (Xia *et al.* 2016). Comparative genomics revealed genes facilitating a highly versatile carbohydrate-based fermentative metabolism, including genes for cellulose hydrolysis that have been found in other *Anaerolinea* phylotypes, and perhaps this metabolic flexibility allows them to remain successful in cold environments. Previous wetland studies have identified this type of metabolic versatility to be a product of the challenging environmental conditions, heterogeneity of wetland ecosystems and niche differentiation within the community (Dalcin Martins *et al.* 2018).

Other taxa that were identified to have a high abundance in the northern-most, colder sites with less precipitation include individuals from *Methanobacteriaceae* and Candidatus *'Methanoflorentaceae'* fam. nov., of which are a characterized methanogen family or are putatively assigned methanogenic capabilities based on metagenome and proteomic analysis (Mondav *et al.* 2014; Oren 2014). The majority of the members of the family *Methanobacteriaceae* utilize hydrogenotrophic methanogenesis for energy conservation and many isolates have come from cold environments, some of which even demonstrate activity at low temperatures in pure culture (0-10°C) (Cadillo-Quiroz *et al.* 2014; Oren 2014; Schirmack *et al.* 2014). Specifically, the abundance of Candidatus *'Methanoflorentaceae'* fam. nov., dominant in a

partially thawed permafrost peatland, was a key predictor of the transition from a hydrogenotrophic to partly acetoclastic methanogenic community as demonstrated by the shift in δ^{13} signature (McCalley *et al.* 2014b; Mondav *et al.* 2014). The high abundance at Lutose Bog, may be due to the semi-continuous permafrost characteristics of this site, with an expected decrease in abundance at Chicago bog, a temperate ombrotrophic bog with no permafrost. Gaining a better understanding of the environmental-dependent factors that influence microbial community composition and distribution could improve our understanding of the potential community shifts climate change could cause. Across the climate gradient we identified individuals in high abundance under high MAT, soil temperature and MAP conditions. Since each environmental variable was analyzed separately, we were able to determine the taxonomic groups that best fit the climatic gradient with significance from the most variables. These included individuals from the families *Intrasporangiaceae*, *Prolixibacteraceae*, *Fibrobacteraceae*, *Methanobacteriaceae* and *Candidatus* '*Methanoflorentaceae*' fam. nov..

At the other end of the climate gradient were sites with warmer temperatures receiving more annual precipitation and thus selecting for a different niche type. This included individuals from the families *Magnetospirillaceae* and *Spirochaetaceae, Methanosaetaceae* and *Methanoregulaceae*. The pattern and differences in microbial community abundance and composition across the gradient could indicate a pattern of similar processes taking place, with contributions from different taxa depending on the environmental conditions as previously described by Hunger *et. al.* 2015.

A subset of the families that were significant for climate related variables were compared to the literature in more detail, allowing for a comparison of putative functions within the northernmost cooler sites and the southernmost warmer sites. Individuals from the families *Magnetospirillaceae* and *Spirochaetaceae*, *Methanoregulaceae*, *Methanosaetaceae* were identified to have a higher abundance under warmer conditions with higher precipitation. The literature supports a wide variety of metabolic functions from these families including nitrogen fixation, saccharolytic fermentation, hydrogenotrophic and acetoclastic methanogenesis

respectively. These functions represent important steps in the intermediary metabolic ecosystem of northern peatlands, demonstrating the potential to predict these decomposition cascades.

Similarly, individuals from the taxonomic groups *Fibrobacteraceae*, *Intrasporangiaceae*, *Prolixibacteraceae*, *Saccharimonadales*, Anaerolineae SBR1031, Candidatus *Methanoflorentaceae* fam. nov. and *Methanobacteriaceae* have demonstrated functional potential that mirrors the established intermediary ecosystem metabolic processes that have been previously hypothetically modeled by Schmidt and colleagues. This includes secondary fermentation, cellulose hydrolysis, nitrogen and phosphorus cycling, carbohydrate, and alcohol fermentation, and hydrogenotrophic methanogenesis respectively. This subset of taxa and their putative functional identification supports the potential for similar processes occurring by different taxa to support the intermediary ecosystem metabolism under different climatic regimes. Similar results were found from a survey of mire soils sampled across a geochemical gradient, where distinct taxa were identified with the similar capabilities to support these processes across the gradient. The similarity in processes could indicate that although communities are predicted to change with changing climate, that processes, or ecosystem level outcomes may not. Further research quantifying these processes and validating taxonomic functional traits will be required to resolve these patterns.

5. CONCLUSIONS

We identified climate variables such as MAT and MAP to be the strongest predictors of microbial community composition of the variables measured (**Figure 2.4**, **Table 2.3**). We also identified microbial taxa potentially demonstrating a climate influenced niche selection pattern across a climate gradient. Taxonomic ranks such as *Saccharimonadales, Anaerolinea,* and *Methanobacteriaceae* were identified to have a significant relationship with colder sites with less precipitation, while *Magnetospirillaceae, Methanoregulaceae* and *Methanosaetaceae* demonstrated higher abundance in warmer sites with higher precipitation, respectively (**Figure 2.5** and **2.6**).

Complex ecosystems such as peatlands are commonly treated as black boxes with minimal predetermined parameters when put into a model. To be able to predict the future of

these ecosystems more accurately under a changing climate, priorities should be placed on characterizing components that greatly contribute to ecosystem level processes, such as CH_4 production from peatlands. Further elucidation of *in-situ* parameters affecting decomposition and CH_4 production will be imperative to enhancing the ability to predict the fate of store carbon more accurately in environments like peatlands. The environmental drivers and associated taxa that we have identified as most vulnerable to differences in climate regime can further inform parameters used to evaluate potential shifts more accurately in microbial community activity and trophic structure when influences by a changing climate.

Acknowledgments

Funding for this project was provided in part by the NSF-GRFP awarded to Analissa Flores Sarno and the IMSD Institutional training grant to Dr. S. Newfeld at Arizona State University (R25). I would also like to thank the field and lab team members who assisted in sample collection and analysis, Benjamin G. Sarno, Electra Skaruup and Aaron Thompson.

CHAPTER 3

EVALUATING PRIMARY *VERSUS* SECONDARY FERMENTATION OF NORTHERN PEATLAND SOILS SAMPLED ACROSS A CLIMATE GRADIENT[1]

Abstract:

The influence of primary versus secondary fermentation on methanogenesis of poor fen Northern peatland soil incubations was investigated. To assess the relationship to climate of primary and secondary fermentation and its effect on methane production, I study four poor fens with similar hydrology and geochemistry along a climate gradient in four biomes: tundra (Daring Lake, NWT), boreal forest (Lutose, AB) and temperate broadleaf and mixed forest (Bog Lake, MN and Chicago Bog, NY). Anaerobically sampled soils from each site were assayed by High Resolution-DNA-Stable Isotope Probing (HR-DNA-SIP) amendments with: 13C-Glucose as a biotracer for primary fermentation or 13C-Propionate as a biotracer for secondary fermentation. Clear differences in production of metabolic byproducts from glucose separate the southernmost sites from northernmost as propionate-accumulating. By-product analysis from propionate incubations indicate that the southernmost sites are acetate-accumulating communities.

The extent of climates' influence on the metabolic processes these taxa perform is unknown, but it was identified that there were significant relationships with microbial abundance across sites as well as amendment incorporation. Gaining a better understanding of the relationship of climate and microbial community composition and function will allow a more precise prediction of how microbial processes could be affected by a changing climate.

1. INTRODUCTION

Under the current climate regime northern peatlands are a methane source, although they are a net carbon sink (Limpens *et al.* 2008; Bragazza *et al.* 2013; Hanson *et al.* 2020). This is because carbon fixation exceeds decomposition due to the cold climate, water-logged soil, and

[1] This work is in preparation for submission to Applied and Environmental Microbiology, 2022. Authors acknowledge the use of this work in my dissertation. Authors: Analissa F. Sarno, Michaela Hayer, Egbert Schwartz, Samuel E. Barnett, Joseph B. Yavitt, Elyn R. Humphreys, David Olefeldt, Liam Heffernan, D. Tyler Roman, Stephen D. Sebestyen, Randall K. Kolka and Hinsby Cadillo-Quiroz.

low concentration of alternative electron acceptors. Although peatlands only account for 3% of terrestrial cover they are estimated to contain 30% of all soil organic carbon. Studies have shown that changes in a regions climate regime could cause the release of more carbon from peatlands due to drawn down water tables and increased mean annual temperatures (Gorham 1991; Keiser *et al.* 2019). The climate has been changing more rapidly in northern, boreal and tundra ecosystems than any other ecosystem type, and for this reason northern peatland carbon stores have a large potential to be released (Serreze *et al.* 2000; Commane *et al.* 2017).

Microcosm incubation experiments have demonstrated the change in microbial community with change in climate parameters such as temperature (Metje and Frenzel 2007). In mildly acidic permafrost soil, the dominant methanogenic processes was shown to change from hydrogenotrophic dominated at 4°C to acetoclastic dominated at 26°C (Metje and Frenzel 2007). Intermediary ecosystem metabolism has only recently begun to be untangled in northern peatlands, and further resolution is needed to better predict the effect of a changing climate.

Due to the limited substrate range of methanogens, they rely on the production of substrates from the 'intermediary ecosystem metabolism' to drive methanogenesis (Drake, Horn and Wüst 2009). The intermediary ecosystem metabolism of northern peatlands is a group of intricately involved microbial metabolisms working together to degrade complex plant biomass to produce methanogenic substrates such as formate, acetate, ethanol and H_2. These partnerships are commonly made up of methanogens and a syntrophic partner which facilitates conditions for each of the partners to survive at the energetic limit of life (Schink 1997).These intermediate reactions have recently become a topic of interest due to their potential to enhance methane production from peatlands, but many of the reactions have yet to be fully resolved.

Another factor that further complicates the system is the metabolic diversity that can be found in peatlands, and the lack of phylogenetic cohesiveness of metabolic abilities (Godin *et al.* 2012). For example, acetogens are not monophyletic and are able to ferment and respire a range of electron acceptors (Drake, Horn and Wüst 2009).

In this study we evaluated whether carbon compounds will move through the microbial decomposition cascade differentially in soils from sites under different climate, influenced by

niche selection. This was quantified by monitoring community respiration and substrate production and consumption of glucose or propionate amended incubations in conjunction with DNA-Stable Isotope Probing (DNA-SIP). Soil was sampled from a suite of four previously characterized field sites situated across a climate gradient. Previous work has established the geochemical similarity across sites, minimal inter and intra-site variation along with establishing the influence climate plays in microbial community structure across the gradient (Chapter 2).

2. METHODS

2.1. SOIL SAMPLE COLLECTION

Four poor fen-type northern peatlands located across a climate and biome gradient were sampled for this study and have previously been characterized (**Figure 3.1,** see Chapter 2).

Figure 3.1: Four poor fen type northern peatlands sampled across a climate and biome gradient. Mean annual temperature (MAT) and mean annual precipitation (MAP) ranges are included on the left and right of the map respectively. Biome designations were retrieved from a publicly available database (Bailey 1980; Wiken 1986; Olson and Dinerstein 2002; The Nature Conservency 2019).

Daring Lake is the northern most site and is in central Northwest Territories, Canada. It is a poor fen located in the southern artic biome, a treeless tundra environment (Lafleur and Humphreys 2008). Lutose is a collapse scar bog located in the discontinuous permafrost zone of Northern Alberta, Canada in the boreal forest biome (Prater, Chanton and Whiting 2007). Bog lake is a poor fen located in the Laurentian Mixed Forests of Minnesota, a transitional biome between boreal forests and broadleaf deciduous forests (Wieder and Yavitt 1994; Sebestyen *et*

al. 2011). Chicago Bog is an ombrotrophic bog located in upstate New York in the deciduous and broadleaf forest biome (Cadillo-Quiroz *et al.* 2006). Soil was sampled anaerobically from the water table level at each site during June - July of 2018 and stored on ice while in the field and stored at 4°C upon arrival to the lab.

2.2. INCUBATION SET UP AND MONITORING:

Anoxic soil slurries were prepared in an anaerobic glove box (Coy Laboratory Products, Grass Lake, MI), by blending soil using a sterilized Magic Bullet Mini Blender and sterile anaerobic de-ionized water in a 1:10 ratio. Soil slurry headspace was flushed with N_2 for 10 min upon crimping to remove any residual H_2 from the glovebox. Soils were pre-incubated in three, 120 ml serum vials with blue stoppers on their sides in the dark at 22°C for 40 days or until methane production began to level off, to exhaust any alternative electron acceptors and labile carbon sources. The pre-incubations were pooled into a sterile Erlenmeyer flask in the glove box and swirled to mix. The preincubated soil slurry was then split into 15 mL aliquots and transferred into 30 mL sterile serum bottles. Amendment was added anaerobically, and the headspace was flushed with N_2/CO_2 for 5 min. Glucose amendments were made in two increments of 1.5 mM, to minimize acetate accumulation (U-13C6 D-Glucose, Cambridge Isotope Laboratories, Tewksbury, MA). Only data from the first addition will be discussed herein. Propionate amendments were made in one addition of 1.5 mM (13C3 Sodium Propionate, Cambridge Isotope Laboratories, Tewksbury, MA). Incubations were inverted to homogenize, then sampled anaerobically every 2-4 days for CH_4, CO_2, and every 4-6 days for organic acids and alcohols. CH_4 and CO_2 were measured using a gas tight, N_2 flushed syringe (Hamilton, Reno, NV) gas chromatograph (SRI Instruments, Torrance, CA) equipped with a flame-ionization detector (FID). The FID was fitted with a HayeSep column (Restek, Bellefonte, PA) with N_2 as a carrier gas (UHP grade 99.999%, Praxair Inc) and H_2 for combustion (supplied by an H_2 generator GCGS-7890, Parker Balston). Calibration standards were made from customized standard mixtures, (Scott Specialty Gases, accuracy $\pm$ 5%, Fremont, CA) over a range of 0.5-10,000 ppmv. Organic acids and alcohols were measured with a 200 µL injection volume using a Shimadzu HPLC, 0.6 ml flow

rate, 65°C, 5 mM H_2SO_4 eluent, through two Aminex HPX-87H columns 300 x 7.8 mm (BioRad, Hercules, Ca). Signals were quantified using the UV-VIS detector at 210 nm for succinate, formate, acetate, propionate, butyrate, and ethanol. Refractive index detector was used to quantify glucose and lactate. A multi-point calibration standard was used for identification and quantification of compounds.

2.3. NUCLEIC ACID EXTRACTION

Soil incubations were transferred to a 15 mL sterile conical tube and centrifuged for 10 min to separate soil from liquid phase. The liquid phase was removed and discarded. Soil pellets were stored at -20°C until DNA extraction. Total genomic DNA was extracted from 0.350 g of peat with the NuceloSpin Soil kit, (Macherey-Nagel, Düren, Germany). Manufacturer's protocol was followed as written with minor modifications as described below to maximize DNA yield from this soil type and minimize phenolic contamination. Lysis buffer SL1 was used, and DNA was resuspended in 40 µL of SE buffer. To increase DNA recovery, multiple extractions were pooled. Samples were quantified using Qubit dsDNA Broad Range Assay Kit as instructed by the manufacturer (ThermoFisher Scientific, Waltham, MA). The DNA quality was confirmed with a 1% agarose gel electrophoresis using 0.5X Tris-Borate-EDTA (TBE) buffer. DNA samples were stored at -20°C until isopycnic ultracentrifugation.

2.4. ISOPYCNIC CENTRIFUGATION AND FRACTIONATION

DNA was fractionated on CsCl density gradients for one timepoint from each treatment. Glucose incubations were destructively sampled for DNA extraction after 5-8 uM of C-glucose were consumed, which coincided with 7 days post addition. Propionate incubations were destructively sampled for DNA extraction after 360 - 410 uM of C-propionate was consumed which on average was day 23.5 post addition. Saturated CsCl solution was prepared to a final density of 1.9 g/mL in sterile water and filter sterilized. Gradient buffer was prepared and autoclaved as follows, 200 mM Tris-HCl pH 8.0, 200 mM KCl, 2 mM EDTA (Schwartz *et al.* 2019). Each density gradient contained 6.90 g of the saturated CsCl solution, 300 µL of gradient buffer,

2 µg DNA was added into a 4.7 mL OptiSeal Tube 13x48 mm (Beckman Coulter, Palo Alto, CA). Gradients were ultracentrifuge in an Optima Max-TL using a TLA-110 rotor for approximately 70 hours at 50,000 rpm at 18°C. Gradient tubes were fractionated immediately following ultracentrifugation and separated into 200 µL fractions using a Beckman Coulter fraction recovery system (Beckman Coulter Inc, Palo Alto, CA). The fractions were measured using a Reichert AR200 digital refractometer (Reichert Analytical 161 Instruments, Depew, NY, USA). DNA from each fraction was precipitated by adding 20 µg linear polyacrylamide, molecular biology grade (Fisher Scientific), 500 µL of Polyethylene glycol and inverting to mix. Incubate for 2 hours at room temperature. Samples were centrifuged at 13,000 x G for 30 minutes at 4°C and the supernatant was discarded. Pellet was washed with 500 µL of 70% molecular grade ethanol and centrifuged for 10 minutes at 13,000 x G at 4°C. Supernatant was discarded and pellet was air dried in the molecular hood for 15 minutes. Pellet was then resuspended in 50 µL molecular grade water. Resulting DNA was quantified using the Qubit dsDNA Broad Range Assay Kit.

2.5. PCR AMPLIFICATION

Samples from the glucose addition experiment were processed as follows: Polymerase chain reaction (PCR) was performed using 515F/909R primers (Tamaki *et al.* 2011) with a unique 6-10 nucleotide barcode on the forward and reverse primer (Integrated DNA Technologies, Coralville, IA). Samples were run in an Eppendorff Mastercyler Pro (Eppendorf, Hamburg, Germany) in triplicate with the following steps: Initial denaturation (95˚C, 5 min), followed by 25 cycles of denaturation (30 sec, 94˚C), annealing (1 min, 52˚C), and extension (1 min, 72˚C), followed by final elongation (10 min, 72˚C). PCR products from both experiments were verified with 0.8% w/v agarose gel electrophoresis with 0.5X Tris-Borate-EDTA (TBE) buffer.

Samples from the propionate addition experiment were processed as follows: Polymerase chain reaction was performed in two PCRs, the first of which is performed with 515F/909R primers (Tamaki *et al.* 2011) containing a 16 nucleotide linker sequence (Herbold *et al.* 2015) using the same thermocycling conditions reported above. PCR products were confirmed on a 0.8% agarose gel run in 0.5X TBE. PCR samples were cleaned with QIAquick PCR

Purification Kit per the manufacturer's protocol (Qiagen, Hilden, Germany) and the cleaned product was used as template for a second PCR using a forward and reverse pair of primers containing the nucleotide linker sequence and a unique barcode using the following conditions: Initial denaturation (95˚C, 3 min), followed by 5 cycles of denaturation (30 sec, 95˚C), annealing (45 sec, 46˚C), and extension (1 min, 72˚C) (Integrated DNA Technologies, Coralville, IA) (Herbold *et al.* 2015). PCR products were confirmed on a 2% agarose gel run in 0.5X TBE.

SequalPrep Normalization Plate Kit (Invitrogen, Carlsbad, CA) were used to clean up and normalize PCR products from both experiments. Amplicons were sequenced using MiSeq 2x300 bp, V3 chemistry at DNASU Sequencing Core Facility, Biodesign Institute at Arizona State University in Tempe, Az.

2.6. DNA SEQUENCE ANALYSIS

DNA sequences were merged using CASPER version 0.8.2 (Kwon, Lee and Yoon 2014) with the following parameters: Number of threads for parallel processing = 30, K-mer size = 19, Threshold for difference of quality score = 19, threshold for mismatching ratio = 0.5, minimum length of overlap = 10, using Jellyfish = true. Sequences were demultiplexed with an in-house Python script and trimmed to 355 nucleotides in length. Sequences were analyzed using the Qiime2 platform with the following workflow: Dereplication using Vsearch, Clustering using Vsearch Closed Reference Cluster Features, Chimera Check with Vsearch Uchime-denovo and Aligned using Mafft. Taxonomy was assigned using Naïve-Bayes taxonomic classifier trained with SILVA 132 99% consensus database and the 16S 515F/909R primers with 355 nucleotide length using Qiime2 version 2020.2 (Tamaki *et al.* 2011; Bolyen *et al.* 2019). Family-level taxonomic rank was used for downstream analysis to minimize uncultured/unidentified taxonomic assignments. Operational taxonomic unit (OTU) tables were exported to R for further analysis.

2.7. STATISTICAL ANALYSIS

I utilized High Throughput Sequencing of Stable Isotope Probing Data Analysis (*HTSSIP*, R package) (Youngblut, Barnett and Buckley 2018a) a high throughput sequencing analysis

framework for evaluating potential incorporators based on their increase in buoyant density in 13C treatments. Sample outputs from Qiime2 were imported to *HTSSIP* as a Phyloseq object and then analyzed using High Resolution-Stable Isotope Probing (HR-SIP) workflow (Github, https://github.com/buckleylab/HTSSIP). Briefly, 12C samples were paired with their 13C counter parts and the Bray-Curtis dissimilarities were compared along with the distribution of the fractions across the buoyant density windows. Individual fractions were rarefied to account for uneven sequencing depth. For HR-SIP analysis, sparsity threshold = 3, window set minimum = 1.7, window set maximum = 1.75, 1.77 and p value cutoff set = 0.1. To demonstrate 13C incorporation or to be identified as an incorporator/responder, the relative abundance of an OTU must increase in high density fractions compared to its 12C control indicating its increase in buoyant density due to 13C incorporation. Figures from the resulting analysis were produced in R using *ggplot2* (Wickham, Navarro and Pedersen 2016).

Methane, CO_2, organic acid and alcohol production and consumption data were collected in Excel then exported and analyzed in R using *Stats* and *Car* packages. Gas and substrate production and consumption rates were calculated based on the linear portion of the curve.

3. RESULTS

3.1. METHANE AND CARBON DIOXIDE PRODUCTION FROM GLUCOSE AMENDED PEAT INCUBATIONS

Methane was produced in all glucose amended soils, with Daring Lake producing the most methane at 8.5 $\mu M\ g^{-1}\ soil_{dw}$ per day and Bog Lake producing the least amount of methane at 0.37 $\mu M\ g^{-1}\ soil_{dw}$ per day as shown in **Figure 3.2**. Carbon dioxide was also produced in all glucose amended soils with Lutose producing the most amount of CO_2 at 117.35 $\mu M\ g^{-1}\ soil_{dw}$ per day and Daring Lake producing the least at 16.40 $\mu M\ g^{-1}\ soil_{dw}$ per day.

The CO_2:CH_4 ratios of the glucose amended incubations are shown in **Table 3.1**. The lowest CO_2:CH_4 ratio was from Daring Lake at 1.10 and 1.04 for 12C-glucose and 13C-glucose amended incubations respectively. The highest CO_2:CH_4 ratio was from the Bog Lake at 97.02 and 136.92 for 12C-glucose and 13C-glucose amended incubations respectively.

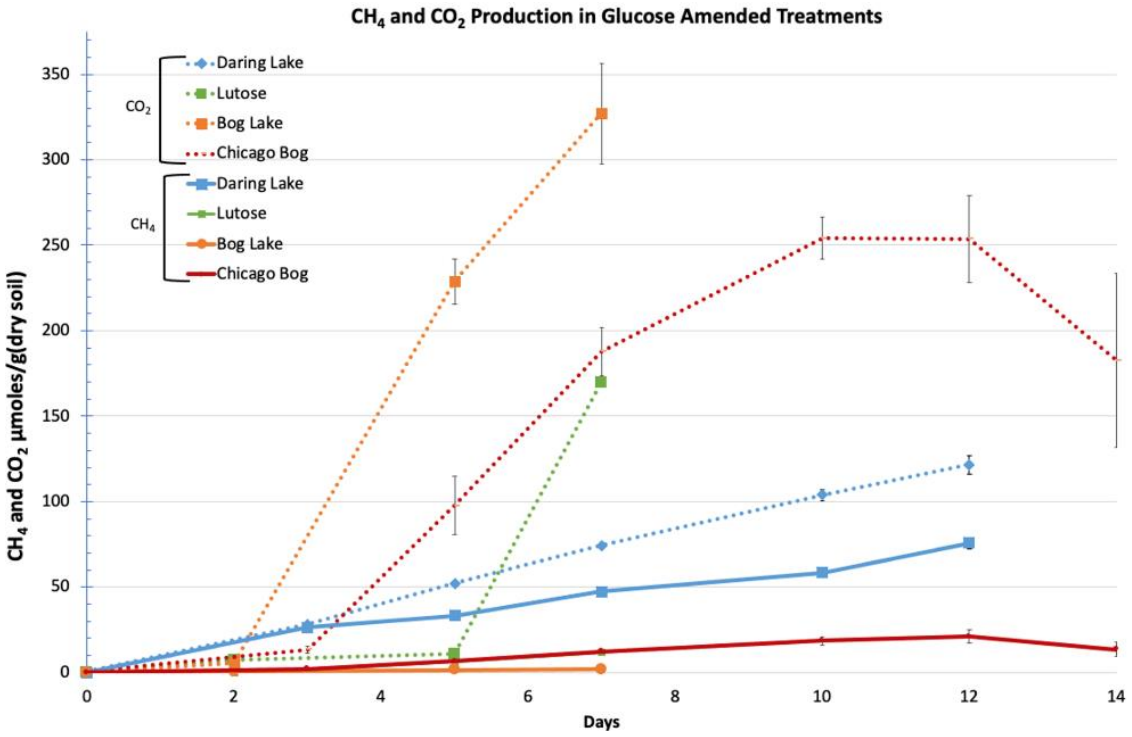


Figure 3.2: Methane and CO_2 production from 13C-glucose amended incubations for the duration of the first glucose addition. Reported is the average of six replicates ± the standard deviation starting at day 0, whereby samples were destructively sampled throughout the experiment.

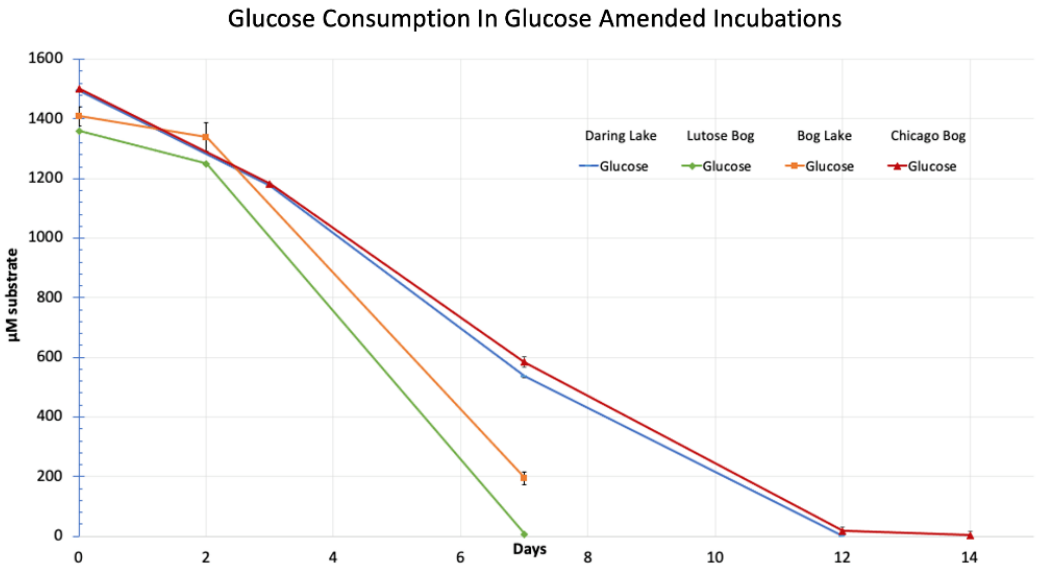
Table 3.1: Ratio of CO_2 to CH_4 accumulated gases in 12C and 13C-glucose amended incubations. The average of three samples, taken on day 7 before destructive sampling for DNA-SIP.

Sites	CO_2:CH_4 12C-Glucose Incubations	CO_2:CH_4 13C-Glucose Incubations
Daring Lake	1.10	1.04
Lutose Bog	9.62	9.65
Bog Lake	97.02	136.92
Chicago Bog	10.31	10.29

3.2. SUBSTRATE CONSUMPTION AND ENDOGENOUS SUBSTRATE PRODUCTION FROM GLUCOSE AMENDED PEAT INCUBATIONS

The addition of glucose stimulated community respiration in soils of all sites but was consumed at different rates for each site (**Figure 3.3**).

A)



B)

Site	12C-glucose consumption mM g^{-1} $soil_{dw}$ per day	13C-glucose consumption mM g^{-1} $soil_{dw}$ per day
Daring Lake	2.25 ± 0.13	1.73 ± 0.09
Lutose Bog	3.46 ± 0.41	3.47 ± 0.38
Bog Lake	4.65 ± 0.75	4.96 ± 0.42
Chicago Bog	3.84 ± 0.20	3.50 ± 0.13

Figure 3.3: **A**) Glucose consumption of 13C-glucose amended incubations. 12C-glucose amended incubations showed very similar glucose consumption patterns (data not shown). Reported is the average of 3 replicates and the error bars represent the standard deviation **B**) Calculated rate of glucose consumption derived from the linear portion of the curve. Reported is the average of 3 replicates ± standard deviation.

Bog Lake showed the greatest rate of glucose consumption at 4.65 mM g^{-1} $soil_{dw}$ per day and Daring Lake had the slowest rate of glucose consumption at 2.25 mM g^{-1} $soil_{dw}$ per day.

Endogenous substrate production in response to glucose amendments were measured. To compare substrate production across sites, percent carbon recovery from glucose and reductant recovered from glucose were calculated, which are shown in **Table 3.2**. Of the 13C-glucose incubations, Lutose Bog had the greatest carbon and reductant recovered from glucose at 103.72% and 118.74% respectively. Bog Lake had the lowest carbon and reductant recovered from glucose for both the 12 and 13C-glucose incubations, with a range of 17.42 - 39.94%. Of the 12C-glucose incubations, Daring Lake had the greatest carbon and reductant recovered from glucose at 77.81% and 85.11% respectively.

Table 3.2: Carbon and reductant recoveries from glucose amended incubations. Due to the nature of destructive sampling and this experimental design, the 0 time point calculations includes 6 gas replicates for each treatment (control, 12C-glucose, and 13C-glucose), and 3 liquid substrate measurements per treatment. For the final timepoint, the calculation includes 3 gas replicates per treatment and 3 liquid substrate measurements per treatment.

	Daring Lake		**Lutose Bog**		**Bog Lake**		**Chicago Bog**	
	Carbon recovered from glucose (%)							
	Addition 1							
	12C incubation	13C incubation	12C incubation	13C incubation	12C incubation	13C incubation	12C incubation	13C incubation
Acetate	33.01	34.20	7.65	15.59	7.02	13.80	1.98	7.40
Formate	0.21	0.22	0.74	0.05	2.06	3.37	0.90	0.95
Propionate	43.08	40.29	40.49	82.89	3.41	5.34	14.95	31.38
Succinate	0.25	0.19	1.00	1.05	0.44	0.94	0.07	0.12
Lactate	0.06	0.00	0.16	0.10	0.13	0.12	0.06	0.00
Butyrate	0.91	2.07	0.00	1.73	0.00	1.63	2.89	5.21
Ethanol	0.13	0.00	0.81	2.15	4.14	10.16	0.66	2.44
CO_2	0.10	0.09	0.13	0.17	0.23	0.24	0.04	0.03
CH_4	0.06	0.06	0.01	0.01	0.00	0.00	0.00	0.00
sum	**77.81**	**77.12**	**51.00**	**103.72**	**17.42**	**35.60**	**21.56**	**47.54**
	Reductant recovered from glucose (%)							
	Addition 1							
	12C incubation	13C incubation	12C incubation	13C incubation	12C incubation	13C incubation	12C incubation	13C incubation
Acetate	33.01	34.20	7.65	15.59	7.02	13.80	1.98	7.40
Formate	0.10	0.11	0.37	0.03	1.03	1.68	0.45	0.47
Propionate	50.26	47.01	47.23	96.70	3.98	6.23	17.45	36.61
Succinate	0.22	0.17	0.88	0.91	0.38	0.83	0.07	0.10
Lactate	0.06	0.00	0.16	0.10	0.13	0.12	0.06	0.00
Butyrate	1.14	2.59	0.00	2.16	0.00	2.03	3.61	6.51
Ethanol	0.19	0.00	1.22	3.22	6.21	15.24	0.99	3.66
CO_2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CH_4	0.13	0.12	0.02	0.02	0.00	0.00	0.01	0.01
sum	**85.11**	**84.19**	**57.54**	**118.74**	**18.75**	**39.94**	**24.61**	**54.77**

3.3. QUANTIFYING THE DNA BUOYANT DENSITY SHIFT ACROSS GLUCOSE AMENDED PEAT INCUBATIONS

The shift in buoyant density of DNA from each treatment can be used to qualitatively estimate the difference in 12C and 13C-glucose DNA peaks (**Figure 3.4**). It is difficult to compare

across sites due to differences in G+C% and/or efficacy of incorporation, but this can be a useful metric to complement measurement of DNA atom%.

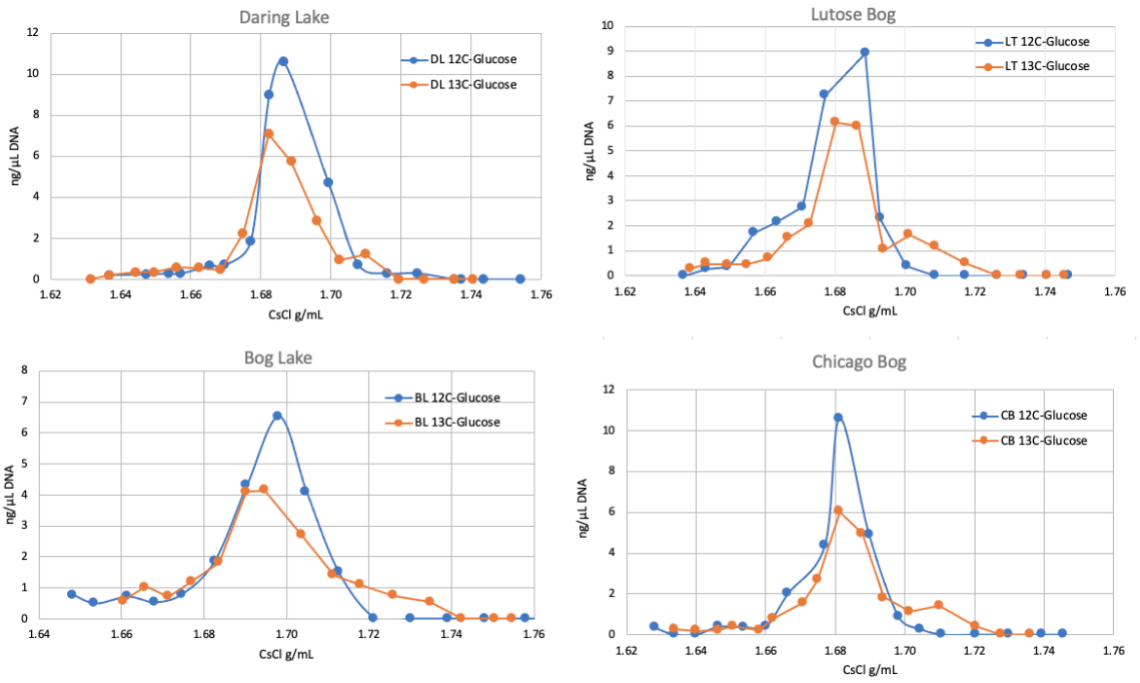


Figure 3.4. The buoyant density of each fraction from 12C-glucose and 13C-glucose of each site are overlayed to demonstrate the shift to the right that occurs in the 13C-treatment due to the increase in buoyant density.

3.4. INCORPORATORS FROM GLUCOSE AMENDED PEAT INCUBATIONS

Sixteen families were identified to be incorporators in response to glucose amendment, over half of which were identified in chapter 2 to have a significant niche breadth relationship with climate related variables (**Table 3.3**). At the Daring Lake site, 24 distinct OTUs were identified as incorporators across 11 families, only 16 of which could be identified to the genus level. Lutose had 12 incorporators identified, only 7 of which could be identified to the genus level. The analysis of Bog Lake yielded the most incorporators at 56, only 13 of which could be identified to the genus level. The high number of incorporators was expected due to the significant shift in the Bog Lake buoyant density of the 13C-glucose fractions. Chicago Bog had 12 identified incorporators, only 7 of which could be identified to the genus level. The high number of unidentified taxa at the genus level is why I choose to present the results at the family taxonomic rank.

Table 3.3. Glucose responders identified from 13C-glucose amended incubations. Closed squares indicate presence of the incorporator and open squares indicate absence of the incorporator at that site. Niche breadth analysis results are included from Chapter 2. High/low indicates that the taxon has a high abundance when the environmental variable is high/low. S indicates specialist. If the cell was left open under Specialist, MAP, Soil Temp, MAT or Water Table indicates the taxon was not statistically significant for the niche breadth analysis for that environmental variable. Taxon unassigned at the class level were removed.

Taxa	Specialist (S)	MAP	Soil Temperature	MAT	Water Table	DL	LT	BL	CB
Chromobacteriaceae							■	■	■
Veillonellaceae	S				Low	■	■	■	■
Magnetospirillaceae	S	High	High			■	■	■	■
Acidobacteriaceae (Subgroup 1)						■	■	■	■
Holophagaceae	S				Low	■		■	
Spirochaetaceae	S	High	High			■		■	
Micropepsaceae	S				Low	■			
Paludibacteraceae	S	Low	Low		Low	■			
Pedosphaeraceae						■			
Chthoniobacteraceae	S				Low	■			
Armatimonadales, uncultured bacterium						■			
Acetobacteraceae						■			
Enterobacteriaceae	S				Low		■		
Clostridiaceae 1	S		Low		Low			■	
Aeromonadaceae	S		Low					■	
Lentimicrobiaceae								■	

3.5. METHANE AND CARBON DIOXIDE PRODUCTION FROM PROPIONATE AMENDED PEAT INCUBATIONS

Methane and CO_2 were produced in all propionate amended soils with Daring Lake producing the most CH_4 at a rate of 1.3 $\mu M\ g^{-1}\ soil_{dw}$ per day and Bog Lake producing the least amount at a rate of 0.03 $\mu M\ g^{-1}\ soil_{dw}$ per day as shown in **Figure 3.5**.

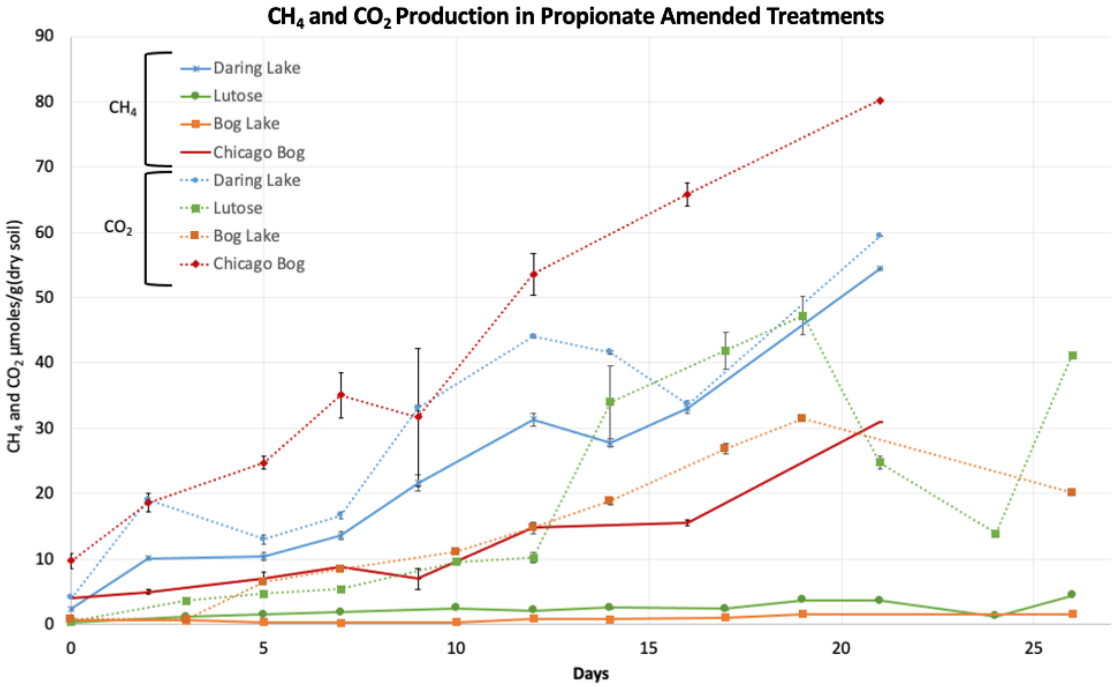


Figure 3.5: Methane and CO_2 production of 13C-propionate amended incubations. Reported is the average of five replicates ± the standard deviation starting at day 0, whereby samples were destructively sampled throughout the experiment.

The CO_2:CH_4 ratios of the propionate amended incubations are shown in **Table 3.4**. The lowest CO_2:CH_4 ratio was from Daring Lake at 0.81 and 0.81 for 12C and 13C amended incubations respectively. The highest CO_2:CH_4 ratio was from the Bog Lake at 8.90 and 8.70 for 12C and 13C amended incubations respectively.

Table 3.4: Ratio of CO_2 to CH_4 of accumulated gases in 12C and 13C-propionate amended incubations taken on day 26 for BL and LT and day 21 for DL and CB before destructive sampling for DNA-SIP. One replicate per sample.

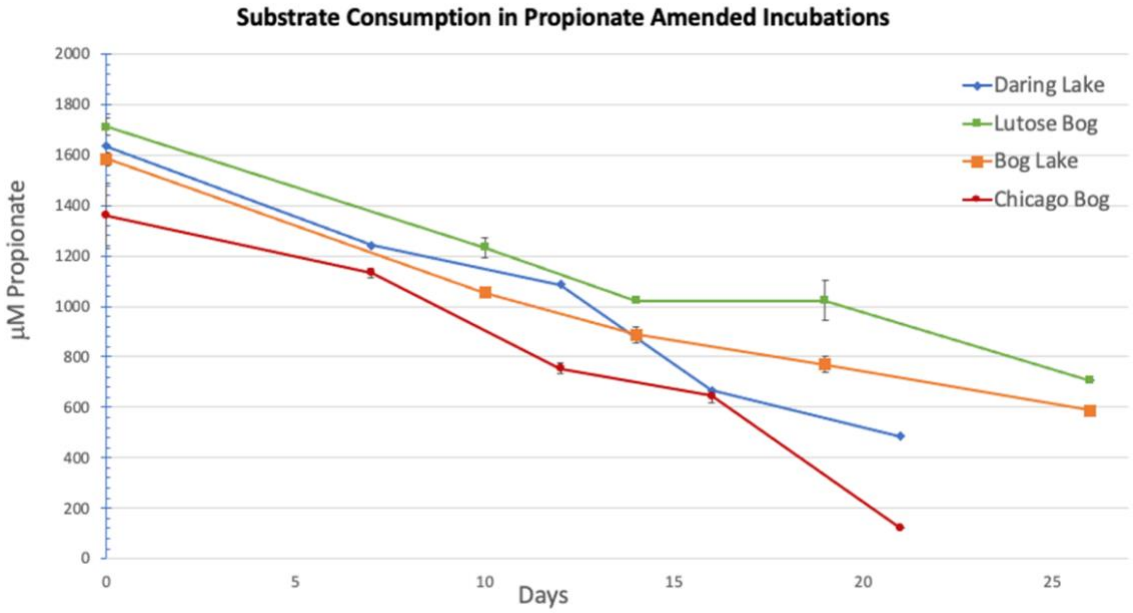
Sites	CO_2:CH_4 12C-Propionate Incubations	CO_2:CH_4 13C-Propionate Incubations
Daring Lake	0.81	0.81
Lutose Bog	5.25	6.29
Bog Lake	8.90	8.70
Chicago Bog	1.54	1.47

3.6. SUBSTRATE CONSUMPTION AND ENDOGENOUS SUBSTRATE PRODUCTION IN PROPIONATE AMENDED PEAT INCUBATIONS

Propionate was consumed in all propionate amended incubations but not at the same rate and was not necessarily indicated by the amount of CH_4 or CO_2 produced (**Figure 3.5** and **3.6**). Although the consumption rates were slightly different across incubations, the range was very small, 1.2 – 1.6 g^{-1} $soil_{dw}$ per day across both 12 and 13-C incubations.

Lutose Bog 12-C incubation showed the greatest rate of propionate consumption at 1.6 g^{-1} $soil_{dw}$ per day and Bog Lake 12-C incubation showed the lowest rate of propionate consumption at 1.2 g^{-1} $soil_{dw}$ per day. Chicago Bog 13-C incubation showed the greatest rate of propionate consumption at 1.5 g^{-1} $soil_{dw}$ per day and Daring Lake 13-C showed the least rate of incubation at 1.2 g^{-1} $soil_{dw}$ per day.

Endogenous substrate production in response to propionate amendments were measured. To compare substrate production across sites, percent carbon recovery from propionate and reductant recovered from propionate were calculated (**Table 3.5**). Chicago Bog had the greatest recovery of carbon and reductant from propionate for both the 12 and 13-C incubations which ranged between 98.63 and 119.82%. Lutose Bog 12 and 13-C incubations had the lowest recovered carbon and reductant from propionate with a range from 21.42 - 27.72%.



Site	12C-propionate consumption µM g^{-1} $soil_{dw}$ per day	13C-propionate consumption µM g^{-1} $soil_{dw}$ per day
Daring Lake	1.4 ± 1.0	1.2 ± 0.4
Lutose Bog	1.6 ± 0.3	1.3 ± 0.1
Bog Lake	1.2 ± 0.1	1.4 ± 0.2
Chicago Bog	1.4 ± 0.1	1.5 ± 0.3

Figure 3.6: A) Propionate consumption of 13C-propionate amended incubations. Reported is the average of 3 replicates and the error bars represent the standard deviation starting at day 0, whereby samples were destructively sampled throughout the experiment. **B)** Calculated rate of propionate consumption. Reported is the average of 3 replicates ± standard deviation.

Table 3.5: Carbon and reductant recoveries calculated for propionate amended incubations. Due to the nature of destructive sampling and this experimental design, the 0 time point calculations includes 5 gas replicates for each treatment (control, 12C-propionate, and 13C-propionate), and 3 liquid substrate measurements per treatment. For the final timepoint, the calculation includes 1 gas replicates per treatment and 1 liquid substrate measurements per treatment.

	Daring Lake		**Lutose Bog**		**Bog Lake**		**Chicago Bog**	
	Carbon recovered from propionate (%)							
	Addition 1							
	12C incubation	13C incubation	12C incubation	13C incubation	12C incubation	13C incubation	12C incubation	13C incubation
Acetate	4.74	10.95	17.22	15.43	26.21	36.62	29.94	5.76
Formate	9.56	8.14	1.55	5.53	14.25	4.19	23.23	28.15
Propionate	0.00	0.00	0.23	0.11	1.64	2.09	0.13	0.11
Succinate	0.00	0.00	0.91	0.37	24.92	1.00	0.59	0.44
Lactate	9.62	6.77	0.00	0.00	0.00	0.00	25.15	0.31
Butyrate	46.77	1.78	6.59	1.73	0.00	0.00	40.71	75.49
Ethanol	0.00	0.00	0.00	2.79	0.00	0.00	0.00	0.00
CO_2	0.04	0.04	0.01	0.03	0.01	0.01	0.01	0.03
CH_4	0.05	0.04	0.00	0.00	0.00	0.00	0.05	0.03
sum	**70.79**	**27.72**	**26.52**	**26.00**	**67.04**	**43.91**	**119.82**	**110.32**
	Reductant recovered from propionate (%)							
	Addition 1							
	12C incubation	13C incubation	12C incubation	13C incubation	12C incubation	13C incubation	12C incubation	13C incubation
Acetate	4.07	9.39	14.76	13.23	22.47	31.39	25.66	4.94
Formate	4.10	3.49	0.67	2.37	6.11	1.80	9.96	12.06
Propionate	0.00	0.00	0.20	0.09	1.41	1.79	0.11	0.09
Succinate	0.00	0.00	0.69	0.28	18.69	0.75	0.44	0.33
Lactate	8.25	5.80	0.00	0.00	0.00	0.00	21.55	0.27
Butyrate	50.11	1.91	7.06	1.86	0.00	0.00	43.62	80.89
Ethanol	0.00	0.00	0.00	3.59	0.00	0.00	0.00	0.00
CO_2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
CH_4	0.09	0.06	0.00	0.00	0.00	0.00	0.08	0.05
sum	**66.61**	**20.65**	**23.37**	**21.42**	**48.68**	**35.73**	**101.43**	**98.63**

3.7. QUANTIFYING THE DNA BUOYANT DENSITY SHIFT ACROSS PROPIONATE AMENDED INCUBATIONS

The shift in buoyant density of DNA from fractions of the propionate incubations can be seen observed in **Figure 3.7.**

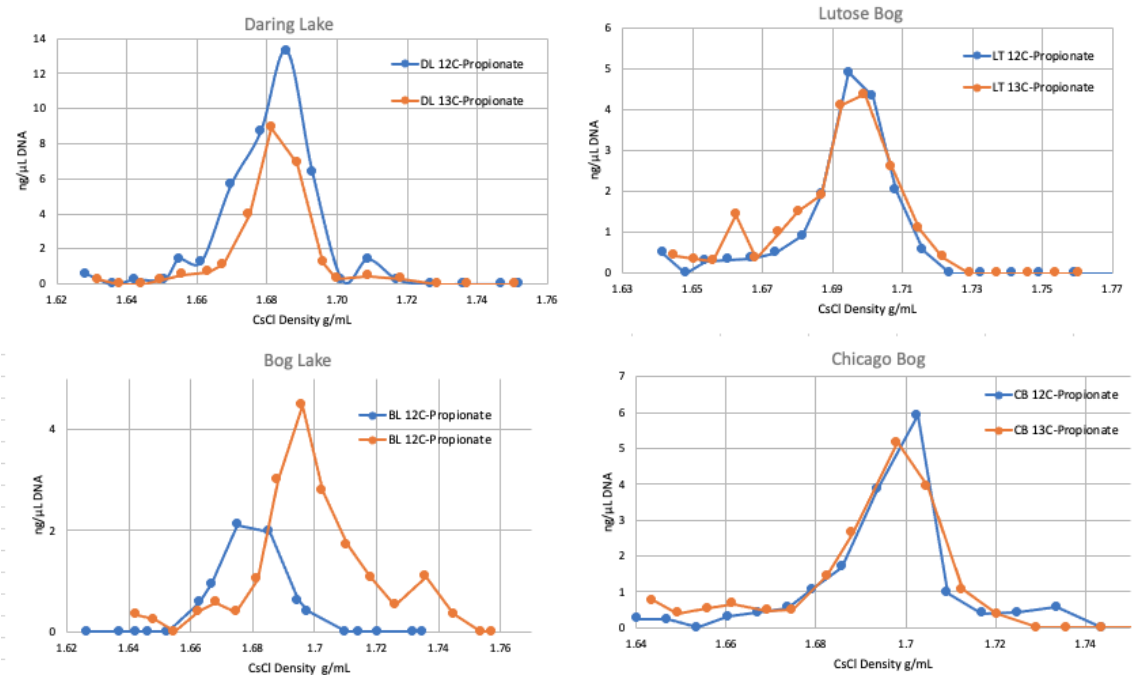


Figure 3.7. The buoyant density of each fraction from 12C-propionate and 13C-propionate of each site are overlayed to demonstrate the shift to the right that occurs in the 13C-treatment due to the increase in buoyant density.

3.8. INCORPORATORS FROM PROPIONATE AMENDED SOIL INCUBATIONS

Eight families were identified to be incorporators across at least two sites, in response to propionate amendment (**Table 3.6**). An additional 65 families were identified as incorporators from Bog Lake that were not shared across other sites (**Table 3.6)**. Only three of the eight families that had incorporators found in at least two sites have statistically significant niche breadth results from chapter 2. At the Daring Lake site, five distinct OTUs were identified as incorporators across four families, only four of which could be identified to the genus level. Lutose

had three incorporators identified, all of which could be identified to the genus level. The analysis of Bog Lake yielded the most incorporators at 258. The high number of incorporators was expected due to the significant shift in the Bog Lake buoyant density of the 13C-propioinate fractions (**Figure 3.7**). Chicago Bog had 3 identified incorporators, only one of which could be identified to the genus level.

Table 3.6. Incorporators identified from 13C-propionate amended incubations. Closed squares indicate presence of the incorporator and open squares indicate absence of the incorporator at that site. Niche breadth analysis results are included from Chapter 2. High/low indicates that the taxon has a high abundance when the environmental variable is high/low. S indicates specialist and G indicates generalist. If the cell was left open under Generalist/Specialist, MAP, Soil Temp, MAT or Water Table indicates the taxon was not statistically significant for the niche breadth analysis for that environmental variable. Taxon unassigned at the class level were removed. *Likely to be taxa falsely identified as propionate consumers and instead be a product of cross-feeding.

Taxa	Specialist (S)	MAP	Soil Temperature	MAT	Water Table	DL	LT	BL	CB
Bacillaceae						■		■	
Chromobacteriaceae						■		■	
Pseudomonadaceae						■		■	
Veillonellaceae	S				Low	■		■	
Sphingomonadaceae							■	■	
Acidobacteriia, Subgroup 13, uncultured bacterium	S				High			■	■
Acidobacteriaceae (Subgroup 1)								■	■
Spirochaetaceae	S	High	High					■	■
Thermodesulfovibrionia, uncultured								■	
Acidobacteria, Subgroup 18, uncultured bacterium								■	
Acidobacteria, Subgroup 6, unidentified	S	Low	Low		Low			■	
Chloroflexi, KD4-96, unidentified	S	Low	Low		Low			■	
Crenarchaeota, Bathyarchaeia, unidentified	S	Low	Low		Low			■	

Taxa	Specialist (S)	MAP	Soil Temperature	MAT	Water Table	DL	LT	BL	CB
Thaumarchaeota, Group 1.1c	S		Low	High					
Acidimicrobiia, uncultured	S				Low				
Acidobacteriia, Subgroup 2	S		Low						
Alphaproteobacteria, uncultured	S		Low		Low				
Dehalococcoidia, GIF9	S	Low	Low						
Deltaproteobacteria, MBNT15	S		Low		Low				
Deltaproteobacteria, RCP2-54									
Deltaproteobacteria, Sva0485	S		Low						
Gammaproteobacteria, JG36-TzT-191	S	Low	Low		Low				
Gammaproteobacteria, WD260									
Microgenomatia, Candidatus Gottesmanbacteria									
Thermoplasmata, uncultured	S			High					
Acidobacteriales, uncultured	S		High						
Elsterales, uncultured									
Gaiellales, uncultured									
Planctomycetales, uncultured	S	Low	Low		Low				
Verrucomicrobiae, S-BQ2-57, soil group	S	Low	Low		Low				
Solirubrobacterales, Ambiguous taxa	S		Low		Low				
Acetobacteraceae									
Aeromonadaceae	S		Low						
Anaerolineaceae	S		Low		Low				
Azospirillaceae									
Bacteroidetes, vadinHA17	S	Low	Low		Low				
Beijerinckiaceae	G		High						
Burkholderiaceae									

Taxa	Specialist (S)	MAP	Soil Temperature	MAT	Water Table	DL	LT	BL	CB
Chitinophagaceae	S	Low	Low		Low				
Clostridiaceae 1	S		Low		Low				
Corynebacteriaceae									
Dysgonomonadaceae									
Enterobacteriaceae	S				Low				
Fibrobacteraceae	S	Low	Low	Low	Low				
Gemmatimonadaceae									
Geobacteraceae	S		High						
Haliangiaceae	S				Low				
Heliobacteriaceae									
Holophagaceae	S				Low				
Isosphaeraceae									
Koribacteraceae									
Lentimicrobiaceae									
Magnetospirillaceae	S	High	High						
Marinobacteraceae									
Candidatus Methanomethyliaceae*									
Methanoregulaceae*	S			High					
Methanosarcinaceae*									
Micropepsaceae	S				Low				
Opitutaceae									
Paludibacteraceae	S	Low	Low		Low				
Peptostreptococcaceae									
Pirellulaceae									
Propionibacteriaceae									
Candidatus Methanoflorentaceae*	S	Low	Low						
Solibacteraceae (Subgroup 3)									
Solirubrobacteraceae	S		Low		Low				
Spirochaetaceae	S	High	High						
Staphylococcaceae									
Streptococcaceae									
Syntrophaceae	S		Low						

Taxa	Specialist (S)	MAP	Soil Temperature	MAT	Water Table	DL	LT	BL	CB
Syntrophobacteraceae	S		Low						
Thermoanaerobaculaceae									
Vermiphilaceae	S	High							
Xanthobacteraceae	S				Low				

4. DISCUSSION

4.1. MICROBIAL COMMUNITY RESPIRATION AND SUBSTRATE PRODUCTION IN RESPONSE TO GLUCOSE CONSUMPTION

Methane and CO_2 are products of terminal decomposition reactions in peatlands and other anaerobic systems, resulting from the degradation of complex plant polymers such as cellulose (Drake, Horn and Wüst 2009). All the glucose amended incubations produced CH_4 and CO_2, although in different proportions and both Lutose and Bog Lake had a 4-day delay to achieve detectable methane levels. Chicago Bog and Bog Lake incubations, the southernmost sites, accumulated the most CO_2 and produced the least amount of CH_4. Understanding the CO_2:CH_4 ratios of each site can give us insight into the proportion of methanogenesis *versus* other terminal CO_2 producing reactions of each incubation. Due to the ombrotrophic nature of these peatlands, there are very low levels of alternative electron acceptors (data not shown). Furthermore, any low levels of alternative electron acceptors that were present would have been consumed during the preincubation period and should not be contributing to the CO_2:CH_4 ratios greater than one (Schmidt *et al.* 2016). Although sources of organic electron acceptors such as humic substances, which are found in high concentrations in peat, could be contributing to the increased level of carbon dioxide production (Keller, Weisenhorn and Megonigal 2009; Lipson *et al.* 2013). The CO_2:CH_4 ratios of all the glucose amended incubations were greater than 1 indicating that methanogenesis was not the only terminal decomposition process occurring in these incubations (Schmidt *et al.* 2016) (Schmidt *et al.* 2016). The calculation for this conclusion assumes that the original substrate from which CO_2 and CH_4 are derived is at the same oxidation state as glucose. The CO_2:CH_4 ratios of Daring Lake were only slightly higher than one, indicating that the majority

of the terminal reactions occurring in these incubations were methanogenesis. Bog Lake shows a very high CO_2:CH_4 ratio, about ten times greater than the next greatest site. The high CO_2:CH_4 ratio from Bog Lake indicates that the terminal decomposition processes were dominated by fermentation or anaerobic respiration reactions. Previous studies have shown incubations of Bog Lake soil to demonstrate a similar trend with a CO_2:CH_4 ratio greater than 100 (Wieder and Yavitt 1994).

Insight into the reactions occurring at each site can also be gleaned from the consumption of glucose and the non-gas compounds released by the community such as acetate and propionate. Glucose was consumed at different rates by each site and different compounds were produced in response to glucose consumption as shown in **Table 3.2**. The northernmost sites demonstrated slower glucose consumption than the warmer southernmost sites. Acetate and propionate were the main accumulated products of the Daring Lake and Lutose incubations, the northernmost sites. The dominant accumulated products at Bog Lake were acetate and ethanol. The southernmost site, Chicago Bog was dominated by the accumulation of acetate, propionate, and butyrate. The differences in substrate accumulation across sites could be indicative of functional differences in the active microbial community and their associated substrate preference based on many variables including presence/absence of syntrophic or cooperative partners, or environmental conditions.

4.2. TAXA AFFILIATED WITH ANAEROBIC CONSUMPTION OF GLUCOSE

Individuals from the families *Veillonellaceae*, *Magnetospirillaceae*, and *Acidobacteriaceae (Subgroup 1)* were identified as glucose incorporators across all sites. Individuals from these taxa have been identified in peatlands previously and have previously been implicated in degradation of cellulose derived carbon compounds (Schmidt *et al.* 2015, 2016; Johnston *et al.* 2016; Kaupper *et al.* 2021). At this point it is difficult to know more about the metabolic role these putative glucose consumers play due to the large portion of uncultured assignments at the genus and species level. This is mainly due to the lack of cultured representatives from peatlands soils in the

database, which has been previously discussed (Dedysh 2011; Hunger, Gößner and Drake 2015; Seward *et al.* 2020).

One of the individuals that did have a cultured representative assignment, *Propionispira arboris* with a confidence of 93% was found in the northernmost sites, Daring Lake and Lutose Bog. This individual was isolated from wetwoods, a Cottonwood tree trunk disease site and characterized as obligately anaerobic, able to fix nitrogen gas, consume lactate and other saccharides, and mainly produced propionate, acetate and CO_2 as by product (Schink, Thompson and Zeikus 1982). Similarities can be seen in the environmental conditions of the sample origin, including carbon and/or carbohydrate rich, anoxic conditions. The carbon and reductant recovered from glucose **Table 3.2** indicates that of all the sites, glucose was mainly converted to propionate and acetate, main products produced by *Propionispira arboris.*

Certain taxa identified to have significant niche breadth results with climate related variables are also identified as incorporators from glucose amended incubations. This includes the families *Paludibacteraceae* and *Spirochaetaceae,* both of which have previously identified members from peat soils with the potential to consume glucose. Previously individuals from the family *Spirochaetaceae* have demonstrated incorporation from 13C-cellulose, likely indicating that they are scavengers of cellobiose or glucose that are common cellulose degradation components in peat soils. This is likely why they are identified glucose incorporators at Daring Lake and Bog Lake (Schmidt *et al.* 2015).

4.3. MICROBIAL COMMUNITY RESPIRATION AND SUBSTRATE PRODUCTION IN RESPONSE TO PROPIONATE CONSUMPTION

Methane and CO_2 were produced in all propionate amended incubations, although amounts were less than the amount produced in response to glucose. The balance between CH_4 and CO_2 production is influenced by many factors including the presence of humic substances acting as terminal electron acceptors, the accumulation of fermentation products, carbon quality and/or nutrient status (Blodau 2002; Vile, Bridgham and Kelman Wieder 2003; Yavitt and Seidman-Zager 2006). The CO_2:CH_4 ratios of propionate amended incubations ranged from 0.81

at Daring Lake to 8.90 at Bog Lake, indicating a large difference across sites in the proportions of terminal reactions producing CO_2:CH_4. The theoretical ratio of propionate converted to CO_2 and CH_4, is 1 propionate: 1.25 CO_2: 1.75 CH_4. Incubations from other studies of northern peatland soil amended with propionate have shown similar CO_2:CH_4 ratios of greater than 1 (Schmidt *et al.* 2016). Although there was a range of CO_2:CH_4 ratios across sites, they were not in agreement with the rate of propionate consumption or, carbon or reductant recovered from propionate. The main products accumulating across sites varied indicating that different processes were taking place within the incubations.

4.4. TAXA AFFILIATED WITH ANAEROBIC CONSUMPTION OF PROPIONATE

Propionate amended incubations also showed differential incorporators across sites (**Table 3.6**). Bog lake had the greatest number of propionate incorporators, which likely was due to an increased rate of incorporation into DNA, rather than rate of propionate consumption because the rates were similar across sites. It is very difficult to anticipate the rate of incorporation and cannot be easily calculated from the rate of propionate consumption but can be qualitatively evaluated from the change in buoyant density of DNA across fractions in the 12C and 13C-propionate treatments (**Figure 3.7**). The optimal method of quantifying this is by performing DNA-SIP incubations trials with 13-C substrate and amplicon sequencing, which can become very expensive. Many of the same families identified as glucose incorporators were also identified as propionate incorporators including, *Chromobacteriaceae, Veillonellaceae, Sphingomonadaceae*, *Acidobacteriaceae* Subgroup 1 and 13. Although there were fewer families with significant niche breadth relationships with climate variables within the group of propionate incorporators there were still some patterns to be observed. *Acidobacteriaceae* Subgroup 13 showed propionate incorporation in sites with mid – high water table levels and *Spirochaetaceae* demonstrated incorporation in the two sites with greatest soil temperature and mean annual precipitation, both of which are further supported by the niche breadth relationships identified in Chapter 2.

4.5. CONSIDERATIONS FOR DNA-STABLE ISOTOPE PROBING OF PEATLAND SOIL

DNA-SIP is a culture independent, molecular based method of identifying the active portion of a microbial community using stable isotope abundance as a tracer or biomarker (Radajewski *et al.* 2000). Although this technique can be a very robust and informative, it is also a very fastidious technique requiring meticulous attention to detail and honing of the technique, to achieve reproducible results. One of the most difficult obstacles to overcome with this technique, especially in a lab newly applying this technique is funding. Previously published DNA-SIP resources and studies have cited the importance of isotope trials and the financial obstacles that can be associated with performing DNA-SIP (Schmidt *et al.* 2016; Youngblut, Barnett and Buckley 2018b). Other challenges include efficacy of incorporation, incubation length and temperature, cross-feeding and optimizing concentration of amendment.

Length of incubation is an important attribute that can be determined through using isotope labeled substrate trials. Specifically, optimizing the incubation length can maximize incorporation by primary substrate consumers and minimize the potential for cross-feeding (Uhlík *et al.* 2009). Cross-feeding is when an organism is identified as a responder, that likely consumed a labeled metabolic product from the primary consumer or its dead biomass. The Bog Lake propionate responders indicates the potential of cross-feeding. This likely occurred through acetate or formate, a common product of anaerobic propionate oxidation and a known substrate for methanogenesis (Costa and Leigh 2014; Schmidt *et al.* 2016; Enzmann *et al.* 2018). Individuals from the methanogenic families *Methanoregulaceae, Methanoflorentaceae* and *Methanosarcinaceae* were identified as incorporators. At the time of writing this, to my knowledge no propionate consuming methanogens have been identified, making it very likely that 13C-carbon was incorporated into the DNA via formate or acetate cross-feeding (Costa and Leigh 2014; Schmidt *et al.* 2016; Enzmann *et al.* 2018). Before sequencing, the difference in buoyant density (ΔBD) of DNA can be compared across the 12C-control and the 13C-treatment as a qualitative metric for amount of incorporation. As can be seen in **Figure 3.7,** Bog Lake 13C-propionate has the largest shift to the right, indicating the greatest difference between the

buoyant density of the 12C-control and the 13C-treatment. This change can be used as a proxy for 13C-substrate incorporation. Although this shift will change for different microbial communities and substrates, the minimum incorporation recommended for 13C-carbon compounds is 5-500 µmol per gram of sample (Dunford and Neufeld 2010).

Incubation temperature is another important factor that requires test incubations. Initial trials of this experiment attempted to incubate the soil at a lower temperature, such as 15°C. Incubation at this temperature, using this experimental set up caused very slow consumption of substrate and low community respiration levels. With slow substrate consumption, proper incorporation can be difficult to achieve while minimizing cross-feeding. For these reasons we altered the experimental design to have the soil slurries incubate at 22°C.

Other experimental design considerations include the data analysis pipeline. The *HTSSIP* package has many different options for data analysis, depending on your sample set and hypothesis. These include the option for high resolution stable isotope probing, multi-window high-resolution stable isotope probing, quantitative stable isotope probing and ∆BD. Although the *HTSSIP* analysis presented herein was high resolution stable isotope probing, we tried other variations to ensure the best fit for the data. For example, if multiple window SIP is used, there must be at a minimum of three fractions per window for the analysis to be meaningful. Although multiple window SIP can produce more robust results, our sample set did not meet the three-fraction requirement, so these results were discarded.

With all these considerations in mind and of course many others, I attempted to use DNA-SIP to identify the active portion of the microbial population consuming glucose and propionate in northern peatland soil incubations, from sites sampled across a climate gradient as demonstrated in the hypothetical trophic cascade in **Figure 3.8** and **Table 3.7**.

4.6. PROPOSED TROPHIC CASCADE: INTEGRATING GLUCOSE AND PROPIONATE RESPONDERS

We have combined the incorporator results from glucose and propionate to assemble a hypothetical anoxic carbon decomposition cascade of the four northern peatland sites (**Figure 3.8**

and Table 3.7)**.** This diagram highlights the importance of the intermediary ecosystem metabolism in northern peatlands and the dependence on syntrophic relationships for methane production. **Figure 3.9** displays the number of taxa that were identified at incorporators with each amendment and those that were incorporators under both treatments. This further demonstrates the complexity of the peatland carbon decomposition cascade, highlighting that an individual organism could consume different substrates under similar environmental conditions but different substrate availability conditions. This complexity is what makes predicting the effects of a changing climate on northern peatlands so difficult. The results from this study suggest that across the climate gradient, primary and secondary fermentation substrates are processed differently, indicated by the differential accumulation of fermentation products such as CH_4 and CO_2 as well as substrate accumulation like acetate and butyrate. This is further supported by the distinct active glucose or propionate consuming community across each site. When these results are combined with the niche breadth analysis from chapter 2, we see a replicated pattern of certain individuals and their affinity for warmer sites with higher precipitation or cooler sites with lower precipitation. Continuing to elucidate main taxonomic groups and their associated metabolic processes across ecosystems will continue to improve the ability to predict more accurately ecosystems that are most vulnerable to a changing climate.

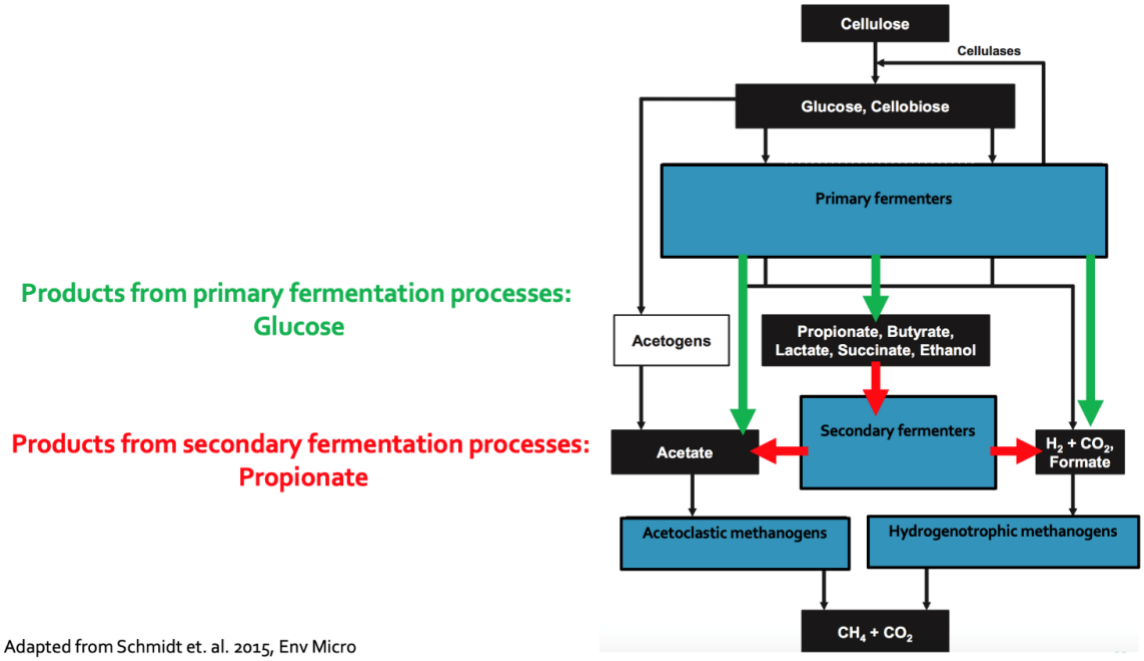


Figure 3.8: Based on the incorporators identified from glucose and propionate incubations, a hypothetical anaerobic decomposition cascade of the four poor fen sites investigated was assembled.

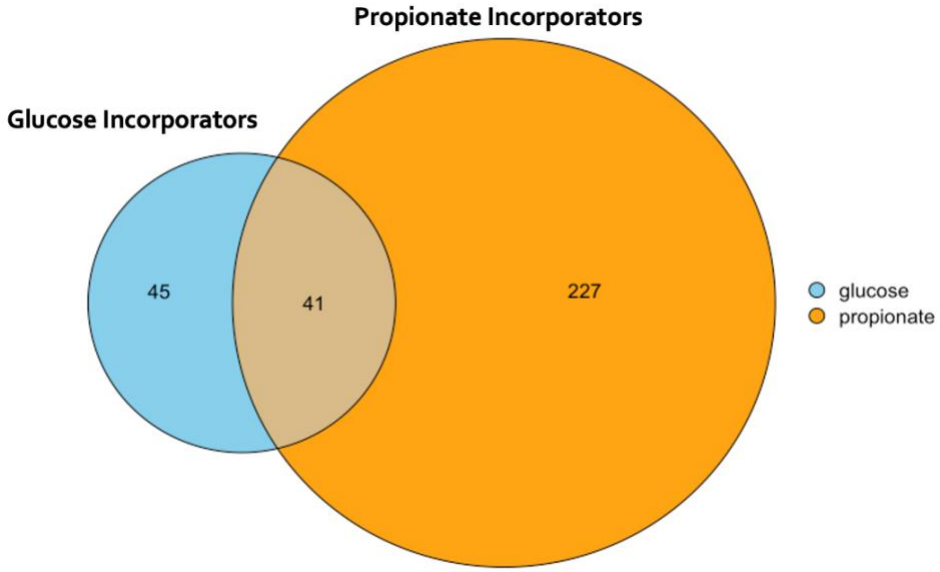


Figure 3.9: Venn diagram indicating the number of ASVs that were identified as glucose incorporators, propionate incorporators and those identified as both.

Table 3.7: Table indicating incorporating taxa at their putatively assigned step in the decomposition process. A taxon can be present at multiple stages in the decomposition processes.

Daring Lake	Lutose Bog	Bog Lake	Chicago Bog
Primary Fermenters			
Veillonellaceae *Magnetospirillaceae* *Acidobacteriaceae* (Subgroup 1) *Spirochaetaceae* *Holophagaceae* *Spirochaetaceae* *Micropepsaceae* *Paludibacteraceae* *Pedosphaeraceae* *Chthoniobacteraceae* Armatimonadales, uncultured bacterium *Acetobacteraceae*	*Veillonellaceae* *Magnetospirillaceae* *Acidobacteriaceae* (Subgroup 1) *Chromobacteriaceae* *Enterobacteriaceae*	*Veillonellaceae* *Magnetospirillaceae* *Acidobacteriaceae* (Subgroup 1) *Chromobacteriaceae* *Holophagaceae* *Spirochaetaceae* *Clostridiaceae* 1 *Aeromonadaceae* *Lentimicrobiaceae*	*Veillonellaceae* *Magnetospirillaceae* *Acidobacteriaceae* (Subgroup 1) *Chromobacteriaceae*
Secondary Fermenters			
Bacillaceae *Chromobacteriaceae* *Pseudomonadaceae* *Veillonellaceae*	*Sphingomonadaceae*	*Bacillaceae* *Chromobacteriaceae* *Pseudomonadaceae* *Veillonellaceae* *Sphingomonadaceae* Acidobacteriia, Subgroup 13, uncultured bacterium *Acidobacteriaceae* (Subgroup 1) *Spirochaetaceae*	Acidobacteriia, Subgroup 13, uncultured bacterium *Acidobacteriaceae* (Subgroup 1) *Spirochaetaceae*

Acknowledgments:

Funding for this project was provided in part by an NSF-GRFP to Analissa Flores Sarno and IMSD Institutional training grant to Dr. S. Newfeld at Arizona State University (R25).

CHAPTER 4

CONCLUSIONS

5. MAIN FINDINGS FROM EACH CHAPTER AND DISSERTATION CONTRIBUTIONS AND IMPLICATIONS

Northern peatlands have a multi-faceted function in global carbon cycling, including carbon storage via peat formation and sequestration, carbon sink via photosynthetic fixation and carbon source via DOC release to surrounding aquatic systems and CH_4 and CO_2 gas flux (Bridgham *et al.* 2013). Covering less than 3% of Earth's terrestrial surface, they are estimated to contain 20-30% of all soil organic carbon (Gorham 1991; Wu and Roulet 2014b). Found most commonly in boreal zones, between 40-70°N latitude (Wuebbles and Hayhoe 2002; Abdalla *et al.* 2016), Northern peatlands are being exposed to the most rapidly climate warming conditions (Serreze *et al.* 2000; Commane *et al.* 2017). Field and incubation-based studies have shown a change in ecosystem level properties, influenced by microbial processes in response to changing climate parameters (Wilson *et al.* 2016b). Understanding the microbial community composition and their interactions that provide input to the methanogenic substrate pool is an important next step into understanding how peatland microbiology contributes to global methane flux. This dissertation investigates the microbial community composition, microbial community respiration and microbial pathways to methane production in poor fen northern peatlands sampled across a climate gradient to further elucidate the potential effects of a changing climate on microbial niche breadth patterns, differential microbial community carbon uptake and metabolic processing to methane. In this chapter, I summarize the main findings or each chapter, present a list of newly identified microbial taxa implicated in intermediary ecosystem metabolism and synthesize the major contributions of my research to the field.

In chapter 2, I resolved putative patterns of microbial niche breadth influenced by climate related variables and identified the individuals demonstrating the greatest change in abundance at the climate extremes. To do this, I conducted a multi-site survey of poor fen northern peatlands and the associated microbial communities, geochemistry, and climate data to identify field sites that could be used for a climate-variable based research inquiry. Attention was paid during site

selection to minimize geochemical and biological inter- and intra-site variation to facilitate robust statistical results. 16S rRNA amplicon sequencing was coupled with geochemical and climate data to evaluate environmental-dependent microbial distributions across the gradient. Then taxon abundance was further investigated with the variables found to be most significant drivers of community composition, including MAT, MAP, soil temperature, water table level and Na^+ ion concentration. These results demonstrate that climate change associated variables such as mean annual temperature and precipitation have a significant effect on microbial community composition of Northern peatland soils. Specifically, I demonstrated an increase in abundance of individuals from *Nitrosotaleaceae, Magnetospirillaceae and Spirochaetaceae Methanoregulaceae, Methanosaetaceae,* under warm, high precipitation environments, while the abundance of individuals from *Intrasporangiaceae*, *Prolixibacteraceae*, *Fibrobacteraceae*, *Methanobacteriaceae*, *Candidatus* '*Methanoflorentaceae*' fam. nov., increased under cold, low precipitation environments. As Mondav and colleagues have done for permafrost thaw ecosystems, and Seward and colleagues have done for northern peatlands across a range of peatland types, I have identified microbial taxa of poor fens demonstrating significant change in abundance when compared across climate change-relevant environmental conditions (Mondav *et al.* 2017; Seward *et al.* 2020). This further contributes to the elucidation of northern peatland microbial ecology dynamics and how microbial taxa could respond to a changing climate.

In chapter 3, I utilized DNA-Stable Isotope Probing, a molecular based tracer technique on soil incubations supplemented with glucose or propionate to resolve the primary and secondary fermentation (intermediary ecosystem metabolic) processes and active members of the microbial community. Implementing soil samples from sites studied in Chapter 2, I demonstrated that communities consume and utilize glucose and propionate differentially to produce methane and other terminal decomposition products. Although each site exhibited differences in glucose consumption and substrate production; a general trend emerged of the northernmost sites, characterized by cold temperatures and low precipitation, consumed glucose more slowly than the southernmost sites characterized by warmer temperatures and more

precipitation. Acetate was an accumulating product in response to glucose addition across all sites, but the northern most sites also accumulated propionate, while the southernmost sites accumulated propionate, ethanol, and butyrate. Individuals from the families *Veillonellaceae*, *Magnetospirillaceae*, *Acidobacteriaceae* (Subgroup 1) were active consumers of glucose across all four sites, indicating population similarities in active across sites. Conversely, *Clostridiaceae* 1, *Enterobacteriaceae*, *Acetobacteraceae*, *Aeromonadaceae* and *Lentimicrobiaceae* were identified as active members of only a single community, potentially contributing to the differences in metabolic product accumulation. Similar to the patterns identified in glucose supplemented incubations, propionate was consumed and utilized differently across all four sites. Although rates of consumption were similar across sites, metabolic product accumulation patters were distinct. These differences were further demonstrated by the differences in active members of each population in response to propionate addition. Individuals from the families *Bacillaceae*, *Chromobacteriaceae*, *Pseudomonadaceae*, *Veillonellaceae*, *Sphingomonadaceae*, Acidobacteriia subgroup 13 and 1, and *Spirochaetaceae* were identified at two or more sites, but not with any discernable pattern in relation to the climate gradient. Pairing putative taxa and demonstrating their affiliated metabolic functions with climate related patterns could assist in more accurate predictions of community shifts caused by a changing climate. These results suggest that although similar processes are occurring across sites, they are likely being performed by different distinct taxa. Similar to the findings of Hunger and colleagues, I contribute to a revised system-level taxonomic and metabolic information of syntrophic and methanogenic communities of northern peatlands (Hunger, Größner and Drake 2015). In contrast to the Hunger *et al.* study, my sampling scheme represented the same type of peatlands under different biome and climate conditions, compared to different peatland types, thus shedding light on the functional redundancy in carbon cycling processes but by distinct members across climates.

Previous studies have only begun to identify taxa playing a significant role in microbial metabolism and contributing to the pool of methanogenic substrates in northern peatlands. Although much focus has been on environmental conditions affecting methanogenesis, little focus

has been put on upstream reactions “feeding” the methanogenic substrate pool. My dissertation contributes to the elucidation of northern peatland microbial ecology, specifically niche breadth patterns in relation to climate change-related variables and intermediary ecosystem metabolic processes impacting the production of methane. Specifically, that poor fen microbial communities are distinct across a climate and biome gradient and that certain taxa show putative patterns relating to climate change-related variables. The information in my dissertation also contributes to the growing body of molecular microbiology resolving the intermediary ecosystem metabolism that has been deemed a ‘black box’, made up of primary and secondary fermentation processes and their successive production and transformation of metabolic products (Drake, Horn and Wüst 2009). Specifically, I have demonstrated differences in primary and secondary fermentation processes across sites and identified distinct active members performing these processes.

As a body of work, my dissertation contributes to the further resolution of microbial patterns and processes contributing to the production of methane in poor fen type northern peatlands. These findings can inform specific components of the equation to estimate methane flux from poor fen northern peatlands. With the added component of climate variables, these results could assist in further contributing to the understanding of how a shift in climate could affect methanogenesis and other reactions releasing carbon that has been sequestered for millennia.

Identifying and evaluating the contribution of climate variables to the variation in microbial community composition and elucidating patters of microbial abundance and processes across a climate gradient can inform predictions of ecosystem level processes. This includes the ability to re-evaluate parameters used in models and prediction systems that represent microbial processes and bring new knowledge to the model. The simplistic approach used in **Figure 1.4** and discussed by Schuur and colleagues to represent microbial processes in evaluation of ecosystem level processes and future climate models need to be reevaluated (Strack *et al.* 2008b; Schuur and Mack 2018). My findings can assist in informing updated approaches,

allowing for a more robust parameter to represent the microbial component of the complex methane producing systems in northern peatlands.

REFERENCES

Abdalla M, Hastings A, Truu J *et al.* Emissions of methane from northern peatlands: a review of management impacts and implications for future management options. *Ecol Evol* 2016;**6**:7080–102.

Amir A, McDonald D, Navas-Molina JA *et al.* Deblur Rapidly Resolves Single-Nucleotide Community Sequence Patterns. *mSystems* 2017;**2**:1–7.

Amon JP, Thompson CA, Carpenter QJ *et al.* Temperate zone fens of the glaciated Midwestern USA. *Wetlands* 2002;**22**:301–17.

Artz RRE. Microbial Community Structure and Carbon Substrate use in Northern Peatlands. *Carbon Cycling in Northern Peatlands*. American Geophysical Union, 2013, 111–29.

Bailey R. *Description of the Ecoregions of the United States*. Ogden, Utah: U.S. Department of Agriculture, 1980.

Bayer EA, Shoham Y, Lamed R. Cellulose-decomposing bacteria and their enzyme systems. In: Dworkin M, Falkow S, Rosenberg E, et al. (eds.). *The Prokaryotes*. New York: Springer, 2006, 578–617.

Belyea L. Nonlinear dyanmics of peatlands and potential feedback on the climate system. *Carbon Cycling in Northern Peatlands*. 2009, 5–18.

Bergman I, Klarqvist M, Nilsson M. Seasonal variation in rates of methane production from peat of various botanical origins: Effects of temperature and substrate quality. *FEMS Microbiol Ecol* 2000;**33**:181–9.

Blodau C. Carbon cycling in peatlands-A review of processes and controls. *Environ Rev* 2002;**10**:111–34.

Bolyen E, Ram Rideout J, Dillon MR *et al.* Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat Biotechnol* 2019;**37**:848–57.

Bragazza L, Bardgett RD, Mitchell EAD *et al.* Linking soil microbial communities to vascular plant abundance along a climate gradient. *New Phytol* 2015;**205**:1175–82.

Bragazza L, Parisod J, Buttler A *et al.* Biogeochemical plant–soil microbe feedback in response to climate warming in peatlands. *Nat Clim Chang* 2013;**3**:273–7.

Bridgham SD, Cadillo-Quiroz H, Keller JK *et al.* Methane emissions from wetlands: Biogeochemical, microbial, and modeling perspectives from local to global scales. *Glob Chang Biol* 2013;**19**:1325–46.

Bubier J, Costello A, Moore TR *et al.* Microtopography and methane flux in boreal peatlands, in northern Ontario Canada. *Can J Bot* 1993;**71**:1056–63.

Buttigieg PL, Ramette A. A guide to statistical analysis in microbial ecology: A community-focused, living review of multivariate data analyses. *FEMS Microbiol Ecol* 2014;**90**:543–50.

Cadillo-Quiroz H, Bräuer S, Yashiro E *et al.* Vertical profiles of methanogenesis and methanogens in two contrasting acidic peatlands in central New York State, USA. *Environ*

Microbiol 2006;**8**:1428–40.

Cadillo-Quiroz H, Brauer SL, Goodson N *et al.* Methanobacterium paludis sp. nov. and a novel strain of Methanobacterium lacus isolated from northern peatlands. *Int J Syst Evol Microbiol* 2014;**64**:1473–80.

Cape JN, Kirika A, Rowland AP *et al.* Organic nitrogen in precipitation: real problem or sampling artefact? *Sci World* 2001;**1 Suppl 2**:230–7.

Cavicchioli R, Ripple WJ, Timmis KN *et al.* Scientists' warning to humanity: microorganisms and climate change. *Nat Rev Microbiol* 2019;**17**:569–86.

Chapin FS, Sturm M, Serreze MC *et al.* Role of land-surface changes in arctic summer warming. *Science* 2005;**310**:657–60.

Chapman EJ, Cadillo-quiroz H, Childers DL *et al.* Soil microbial community composition is correlated to soil carbon processing along a boreal wetland formation gradient. *Eur J Soil Biol* 2017;**82**:17–26.

Chapman EJ, Childers DL, Shock EL *et al.* A Thermodynamic Analysis of Soil Ecosystem Development in Northern Wetlands. *Wetlands* 2016;**36**:1143–53.

Chasar LS, Chanton JP, Glaser PH *et al.* Methane concentration and stable isotope distribution as evidence of rhizospheric processes: Comparison of a fen and bog in the Glacial Lake Agassiz Peatland complex. *Ann Bot* 2000;**86**:655–63.

Clarke KR. Non-parametric multivariate analyses of changes in community structure. *Aust J Ecol* 1993;**18**:117–43.

Clymo RS. The limits to peat bog growth. *Trans R Soc Lond B* 1984;**303**:605.

Clymo RS, Bryant CL. Diffusion and mass flow of dissolved carbon dioxide, methane, and dissolved organic carbon in a 7-m deep raised peat bog. *Geochemica Cosmochim Acta* 2008;**72**:2048–66.

Clymo RS, Turunen ; J, Tolonen ; K. *Carbon Accumulation in Peatland*., 1998.

Commane R, Lindaas J, Benmergui J *et al.* Carbon dioxide sources from Alaska driven by increasing early winter respiration from Arctic tundra. *Proc Natl Acad Sci U S A* 2017;**114**:5361–6.

Conrad R. Microbial Ecology of Methanogens and Methanotrophs. In: Sparks DL (ed.). *Advances in Agronomy*. 96th ed. San Diego: Elsevier, 2007, 1–63.

Corbett JE, Tfaily MM, Burdige DJ *et al.* The relative importance of methanogenesis in the decomposition of organic matter in northern peatlands. *J Geophys Res Biogeosciences* 2015;**120**:280–93.

Costa KC, Leigh JA. Metabolic versatility in methanogens This review comes from a themed issue on Cell and pathway engineering. *Curr Opin Biotechnol* 2014;**29**:70–5.

Dalcin Martins P, Danczak RE, Roux S *et al.* Viral and metabolic controls on high rates of microbial sulfur and carbon cycling in wetland ecosystems. *Microbiome* 2018;**6**, DOI:

10.1186/s40168-018-0522-4.

Dean JF, Middelburg JJ, Röckmann T *et al.* Methane Feedbacks to the Global Climate System in a Warmer World. *Rev Geophys* 2018;**56**:207–50.

Dedysh SN. Cultivating Uncultured Bacteria from Northern Wetlands: Knowledge Gained and Remaining Gaps. *Front Microbiol* 2011;**2**:1–15.

Dettling MD, Yavitt JB, Cadillo-Quiroz H *et al.* Soil–Methanogen Interactions in Two Peatlands (Bog, Fen) in Central New York State. *Geomicrobiol J* 2007;**24**:247–59.

Dettling MD, Yavitt JB, Zinder SH. Control of organic carbon mineralization by alternative electron acceptors in four peatlands, Central New York State, USA. *Wetlands* 2006;**26**:917–27.

Dieleman CM, Lindo Z, McLaughlin JW *et al.* Climate change effects on peatland decomposition and porewater dissolved organic carbon biogeochemistry. *Biogeochemistry* 2016:1–12.

Drake HL, Horn M a., Wüst PK. Intermediary ecosystem metabolism as a main driver of methanogenesis in acidic wetland soil. *Environ Microbiol Rep* 2009;**1**:307–18.

Dunford E a., Neufeld JD. DNA Stable-Isotope Probing (DNA-SIP). *J Vis Exp* 2010:1–6.

Enzmann F, Mayer F, Rother M *et al.* Methanogens: biochemical background and biotechnological applications. *AMB Express* 2018;**8**:1.

Feng X, Deventer MJ, Lonchar R *et al.* Climate Sensitivity of Peatland Methane Emissions Mediated by Seasonal Hydrologic Dynamics. *Geophys Res Lett* 2020;**47**:1–9.

Fey A, Conrad R, Fey A *et al.* Effect of Temperature on Carbon and Electron Flow and on the Archaeal Community in Methanogenic Rice Field Soil Effect of Temperature on Carbon and Electron Flow and on the Archaeal Community in Methanogenic Rice Field Soil. *Appl Environ Microbiol* 2000;**66**:4790–7.

Finn DR, Yu J, Ilhan ZE *et al.* MicroNiche: an R package for assessing microbial niche breadth and overlap from amplicon sequencing data. *FEMS Microbiol Ecol* 2020;**96**:131.

Flanagan LB, Syed KH. Stimulation of both photosynthesis and respiration in response to warmer and drier conditions in a boreal peatland ecosystem. *Glob Chang Biol* 2011;**17**:2271–87.

Fletcher SE, Tans PP, Bruhwiler LM *et al.* CH4 sources estimated from atmospheric observations of CH4 and its 13C/ 12C isotopic ratios: 2. Inverse modeling of CH4 fluxes from geographical regions. *Glob Biogeochem Cycles* 2004;**18**, DOI: 10.1029/2004GB002224.

Fortin SG, Song B, Anderson IC. Microbially mediated nitrogen removal and retention in the York River Estuary One sentence summary: Determining geochemical and microbial controls on benthic nitrogen cycling processes in the York River Estuary. *FEMS Microbiol Ecol* 2021;**97**:118.

Galand PE, Fritze H, Conrad R *et al.* Pathways for methanogenesis and diversity of methanogenic archaea in three boreal peatland ecosystems. *Appl Environ Microbiol* 2005;**71**:2195–8.

Galand PE, Fritze H, Yrjälä K. Microsite-dependent changes in methanogenic populations in a

boreal oligotrophic fen. *Environ Microbiol* 2003;**5**:1133–43.

Godin A, McLaughlin JW, Webster KL *et al.* Methane and methanogen community dynamics across a boreal peatland nutrient gradient. *Soil Biol Biochem* 2012;**48**:96–105.

Gorham E. Northern Peatlands: Role in the carbon cycle and probable responses to climatic warming. *Ecol Appl* 1991;**1**:182–95.

Graham JD, Glenn NF, Spaete LP *et al.* Characterizing Peatland Microtopography Using Gradient and Microform-Based Approaches. *Ecosystems* 2020, DOI: 10.1007/s10021-020-00481-z.

Griffiths NA, Hanson PJ, Ricciuto DM *et al.* Temporal and Spatial Variation in Peatland Carbon Cycling and Implications for Interpreting Responses of an Ecosystem-Scale Warming Experiment. *Soil Sci Soc Am J* 2017;**81**:1668–88.

Gu Y, Bai Y, Xiang Q *et al.* Degradation shaped bacterial and archaeal communities with predictable taxa and their association patterns in Zoige wetland at Tibet plateau. *Sci Rep* 2018;**8**.

Hanson PJ, Griffiths NA, Iversen CM *et al.* Rapid Net Carbon Loss From a Whole-Ecosystem Warmed Peatland. *AGU Adv* 2020;**1**, DOI: 10.1029/2020AV000163.

Heitmann T, Blodau C. Oxidation and incorporation of hydrogen sulfide by dissolved organic matter. *Chem Geol* 2006;**235**:12–20.

Herbold CW, Pelikan C, Kuzyk O *et al.* A flexible and economical barcoding approach for highly multiplexed amplicon sequencing of diverse target genes. *Front Microbiol* 2015;**6**:1–8.

Hugelius G, Loisel J, Chadburn S *et al.* Large stocks of peatland carbon and nitrogen are vulnerable to permafrost thaw. *Proc Natl Acad Sci U S A* 2020;**117**:20438–46.

Hunger S, Gößner AS, Drake HL. Anaerobic trophic interactions of contrasting methane-emitting mire soils: Processes versus taxa. *FEMS Microbiol Ecol* 2015;**91**:1–14.

Hurlbert SH. The Measurement of Niche Overlap and Some Relatives. *Ecology* 1978;**59**:67–77.

IPCC. *Climate Change 2007: Impacts, Adaptation and Vulnerability: Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel*. Parry L, Canziani OF, Palutikof JP, et al. (eds.). United Kindgom: Cambridge University Press, 2007.

IPCC Panel. *IPCC, 2014: Climate Change 2014: Synthesis Report*. Geneva, Switzerland, 2014.

Ito A, Oikawa T. Global Mapping of Terrestrial Primary Productivity and Light-Use Efficiency with a Process-Based Model. In: Shiyomi M, Kawahata H, Koizumi H, et al. (eds.). *Global Environmental Change in the Ocean and on Land*. Tokyo: Terrapub, 2004, 343–58.

Johnston ER, Rodriguez-R LM, Luo C *et al.* Metagenomics reveals pervasive bacterial populations and reduced community diversity across the Alaska tundra ecosystem. *Front Microbiol* 2016;**7**, DOI: 10.3389/fmicb.2016.00579.

Juottonen H, Kotiaho M, Robinson D *et al.* Microform-related community patterns of methane-cycling microbes in boreal Sphagnum bogs are site specific. *FEMS Microbiol Ecol*

2015;**91**:1–13.

Kaupper T, Mendes LW, Harnisz M *et al.* Recovery of Methanotrophic Activity Is Not Reflected in the Methane-Driven Interaction Network after Peat Mining MICROBIAL ECOLOGY. 2021, DOI: 10.1128/AEM.02355-20.

Keiser AD, Smith M, Bell S *et al.* Peatland microbial community response to altered climate tempered by nutrient availability. *Soil Biol Biochem* 2019;**137**.

Keller JK, Weisenhorn PB, Megonigal JP. Humic acids as electron acceptors in wetland decomposition. *Soil Biol Biochem* 2009;**41**:1518–22.

Ketcheson SJ, Price JS. Characterization of the fluxes and stores of water within newly formed Sphagnum moss cushions and their environment. *Ecohydrology* 2014;**7**:771–82.

Kim DU, Kim SG, Lee H *et al.* Oryzihumus soli sp. Nov., isolated from soil and emended description of the genus oryzihumus. *Int J Syst Evol Microbiol* 2017;**67**:3960–4.

Kostka JE, Weston DJ, Glass JB *et al.* The Sphagnum microbiome: New insights from an ancient plant lineage. *New Phytol* 2016;**211**:57–64.

Kotsyurbenko OR, Friedrich MW, Simankova M V. *et al.* Shift from acetoclastic to H2-dependent methanogenesis in a West Siberian peat bog at low pH values and isolation of an acidophilic Methanobacterium strain. *Appl Environ Microbiol* 2007;**73**:2344–8.

Kotsyurbenko ORR. Trophic interactions in the methanogenic microbial community of low-temperature terrestrial ecosystems. *FEMS Microbiol Ecol* 2005;**53**:3–13.

Kwon S, Lee B, Yoon S. CASPER : context-aware scheme for paired-end reads from high-throughput amplicon sequencing. *BMC Bioinformatics* 2014;**15**:1–11.

Lafleur PM, Humphreys ER. Spring warming and carbon dioxide exchange over low Arctic tundra in central Canada. *Glob Chang Biol* 2008;**14**:740–56.

Lai DYF. Methane Dynamics in Northern Peatlands: A Review. *Pedosphere* 2009;**19**:409–21.

Leschine SB. Cellulose Degradation in Anaerobic Environments. *Annu Rev Microbiol* 1995;**49**:399–426.

Levins R. *Evolution in Changing Environments*. Princeton: Princeton University Press, 1968.

Limpens J, Berendse F, Blodau C *et al.* Peatlands and the carbon cycle: from local processes to global implications-a synthesis. *Biogeosciences* 2008;**5**:1475–91.

Lipson DA, Haggerty JM, Srinivas A *et al.* Metagenomic Insights into Anaerobic Metabolism along an Arctic Peat Soil Profile. *PLoS One* 2013;**8**, DOI: 10.1371/journal.pone.0064659.

Lovley DR, Fraga JL, Blunt-Harris EL *et al.* Humic Substances as a Mediator for Microbially Catalyzed Metal Reduction. *Acta Hydrochim hydrobiol* 1998;**26**:152–7.

Mason L, Eagar A, Patel P *et al.* Potential microbial bioindicators of phosphorus mining in a temperate deciduous forest. 2020, DOI: 10.1111/jam.14761.

McCalley CK, Woodcroft BJ, Hodgkins SB *et al.* Methane dynamics regulated by microbial community response to permafrost thaw. *Nature* 2014a;**514**:478–81.

McCalley CK, Woodcroft BJ, Hodgkins SB *et al.* Methane dynamics regulated by microbial community response to permafrost thaw. *Nature* 2014b;**514**:478–81.

Meterological Service of Canada. Hay River Climate, Northwest Territories. 2021a.

Meterological Service of Canada. High Level A, Alberta. 2021b.

Metje M, Frenzel P. Effect of temperature on anaerobic ethanol oxidation and methanogenesis in acidic peat from a Northern Wetland. *Appl Environ Microbiol* 2005;**71**:8191–200.

Metje M, Frenzel P. Methanogenesis and methanogenic pathways in a peat from subarctic permafrost. *Environ Microbiol* 2007;**9**:954–64.

Mitsch WJ, Gosselink JG. Peatlands. *Wetlands*. 5th ed. Hoboken: John Wiley & Sons, Inc., 2015, 413–51.

Mondav R, McCalley CK, Hodgkins SB *et al.* Microbial network, phylogenetic diversity and community membership in the active layer across a permafrost thaw gradient. *Environ Microbiol* 2017;**19**:3201–18.

Mondav R, Woodcroft BJ, Kim E-H *et al.* Discovery of a novel methanogen prevalent in thawing permafrost. *Nat Commun* 2014;**5**:3212.

Moore TR, Knowles R. The Influence of Water Table Levels on Methane and Carbon Dioxide Emissions From Peatland Soils. *Can J Soil Sci* 1989;**69**:33–8.

Moore TR, De Young A, Bubier JL *et al.* A Multi-Year Record of Methane Flux at the Mer Bleue Bog, Southern Canada. *Ecosystems* 2011;**14**:646–57.

Morris PJ, Swindles GT, Valdes PJ *et al.* Global peatland initiation driven by regionally asynchronous warming. *Proc Natl Acad Sci* 2018;**115**:4851–6.

Murrell P. *R Graphics*. New York: Chapman & Hall/CRC, 2006.

Myers B, Webster KL, Mclaughlin JW *et al.* Microbial activity across a boreal peatland nutrient gradient: The role of fungi and bacteria. *Wetl Ecol Manag* 2012;**20**:77–88.

National Centers for Environmental Information NO and AA. U.S. Historical Climatology Network. 2007.

Nichols JE, Peteet DM. Rapid expansion of northern peatlands and doubled estimate of carbon storage. *Nat Geosci* 2019;**12**:917–21.

Nijp JJ, Limpens J, Metselaar K *et al.* Can frequent precipitation moderate the impact of drought on peatmoss carbon uptake in northern peatlands? *New Phytol* 2014;**203**:70–80.

Oksanen J, Guillaume Blanchet F, Friendly M *et al.* Vegan: Community Ecology Package. 2020.

Olefeldt D, Roulet N, Giesler R *et al.* Total waterborne carbon export and DOC composition from ten nested subarctic peatland catchments—importance of peatland cover, groundwater

influence, and inter-annual variability of precipitation patterns. *Hydrol Process* 2013;**27**:2280–94.

Olson DM, Dinerstein E. The Global 200: Priority Ecoregions for Global Conservation. *Ann Missouri Bot Gard* 2002;**89**:199–224.

Oren A. The Prokaryotes: Other Major Lineages of Bacteria and the Archaea. In: Rosenberg E, DeLong E, Lory S, et al. (eds.). Fourth. Berlin: Springer, 2014, 165–93.

Prater JL, Chanton JP, Whiting GJ. Variation in methane production pathways associated with permafrost decomposition in collapse scar bogs of Alberta , Canada. 2007;**21**:1–10.

R Core Team. R: A language and environment for statistical computing. 2013.

R Studio Team. RStudio: Integrated Development Environment for R. 2020.

Radajewski S, Ineson P, Parekh NR *et al. Stable-Isotope Probing as a Tool in Microbial Ecology*. Nature Publishing Group, 2000.

Reynolds MC, Finn D, Sarno AF *et al.* Delineating the Drivers and Functionality of Methanogenic Niches within an Arid Landfill. Villanueva L (ed.). *Appl Environ Microbiol* 2022, DOI: 10.1128/AEM.02438-21.

Riley WJ, Subin ZM, Lawrence DM *et al.* Barriers to predicting changes in global terrestrial methane fluxes: Analyses using CLM4Me, a methane biogeochemistry model integrated in CESM. *Biogeosciences* 2011;**8**:1925–53.

Schink B. Energetics of syntrophic cooperation in methanogenic degradation. *Microbiol Mol Biol Rev* 1997;**61**:262–80.

Schink B, Thompson TE, Zeikus JG. Characterization of Propionispira arboris gen. nov. sp. nov., a Nitrogen-fixing Anaerobe Common to Wetwoods of Living Trees. *Microbiology* 1982;**128**:2771–9.

Schirmack J, Mangelsdorf K, Ganzert L *et al.* Methanobacterium movilense sp. nov., a hydrogenotrophic, secondary-alcohol-utilizing methanogen from the anoxic sediment of a subsurface lake. *Int J Syst Evol Microbiol* 2014;**64**:522–7.

Schmidt O, Hink L, Horn MA *et al.* Peat: home to novel syntrophic species that feed acetate- and hydrogen-scavenging methanogens. *ISME J* 2016;**10**:1–13.

Schmidt O, Horn MA, Kolb S *et al.* Temperature impacts differentially on the methanogenic food web of cellulose-supplemented peatland soil. *Environ Microbiol* 2015;**17**:720–34.

Schuur EAG, Mack MC. Ecological Response to Permafrost Thaw and Consequences for Local and Global Ecosystem Services. 2018, DOI: 10.1146/annurev-ecolsys-121415.

Schuur EAG, McGuire AD, Schädel C *et al.* Climate change and the permafrost carbon feedback. *Nature* 2015;**520**:171–9.

Schwartz E, Hayer M, Hungate BA *et al.* Stable Isotope Probing of Microorganisms in Environmental Samples with H218O. In: Dumont MG and MHG (ed.). *Stable Isotope Probing: Methods and Protocols, Methods in Molecular Biology*. Vol 2046. Springer

Science+Business Media, LLC, part of Springer Nature 2019, 2019, 129–36.

Sebestyen SD, Dorrance C, Olson DM *et al.* Long-Term Monitoring Sites and Trends at the Marcell Experimental Forest. In: Kolka, Randall K.; Sebestyen, Stephen D.; Verry, Elon S.; Brooks KN (ed.). *Peatland Biogeochemistry and Watershed Hydrology*. Boca Raton, FL: Taylor and Francis Group, LLC, 2011, 15–71.

Serreze MC, Walsh JE, Chapin FS *et al.* Observational evidence of recent change in the northern high-latitude environment. *Clim Change* 2000;**46**:159–207.

Seward J, Carson MA, Lamit LJ *et al.* Peatland Microbial Community Composition Is Driven by a Natural Climate Gradient. *Microb Ecol* 2020;**80**:593–602.

Shindell DT, Faluvegi G, Koch DM *et al.* Improved attribution of climate forcing to emissions. *Science* 2009;**326**:716–8.

Sizova M V., Panikov NS, Spiridonova EM *et al.* Novel facultative anaerobic acidotolerant Telmatospirillum siberiense gen. nov. sp. nov. isolated from mesotrophic fen. *Syst Appl Microbiol* 2007;**30**:213–20.

Song C, Xu X, Tian H *et al.* Ecosystem-atmosphere exchange of CH4 and N2 O and ecosystem respiration in wetlands in the Sanjiang Plain, Northeastern China. *Glob Chang Biol* 2009;**15**:692–705.

Strack M, Waddington JM, Rochefort L *et al.* Response of vegetation and net ecosystem carbon dioxide exchange at different peatland microforms following water table drawdown. *J Geophys Res Biogeosciences* 2006;**111**:2006.

Strack M, Waddington JM, Turetsky M *et al. Northern Peatlands, Greenhouse Gas Exchange and Climate Change*., 2008a.

Strack M, Waddington JM, Turetsky MR *et al.* Northern peatlands, greenhouse gas exchange and climate change. In: Strack M (ed.). *Peatlands and Climate Change*. 2008b, 44–69.

Tamaki H, Wright CL, Li X *et al.* Analysis of 16S rRNA amplicon sequencing options on the roche/454 next-generation titanium sequencing platform. *PLoS One* 2011;**6**:1–6.

The Nature Conservency. Terrestrial Ecoregions. *Geospatial Conserv Atlas* 2019.

Treat CC, Natali SM, Ernakovich J *et al.* A pan-Arctic synthesis of CH4 and CO2 production from anoxic soil incubations. *Glob Chang Biol* 2015;**21**:2787–803.

Turetsky MR, Benscoter B, Page S *et al.* Global vulnerability of peatlands to fire and carbon loss. 2015, DOI: 10.1038/NGEO2325.

Tveit AT, Urich T, Frenzel P *et al.* Metabolic and trophic interactions modulate methane production by Arctic peat microbiota in response to warming. *Proc Natl Acad Sci* 2015a;**112**:E2507–16.

Tveit AT, Urich T, Frenzel P *et al.* Metabolic and trophic interactions modulate methane production by Arctic peat microbiota in response to warming. *Proc Natl Acad Sci U S A* 2015b;**112**:E2507-16.

Uhlík O, Jecná K, Leigh MB *et al.* DNA-based stable isotope probing: a link between community structure and function. *Sci Total Environ* 2009;**407**:3611–9.

Vile MA, Bridgham SD, Kelman Wieder R. RESPONSE OF ANAEROBIC CARBON MINERALIZATION RATES TO SULFATE AMENDMENTS IN A BOREAL PEATLAND. *Ecol Appl* 2003;**13**:720–34.

Waddington JM, Roulet NT. Carbon balance of a Boreal patterned peatland. *Glob Chang Biol* 2000;**6**:87–97.

Wang G, Jin Z, Wang X *et al.* 104274 Available online. *Appl Soil Ecol* 2022;**170**:929–1393.

Warton DI, Wright ST, Wang Y. Distance-based multivariate analyses confound location and dispersion effects. *Methods Ecol Evol* 2012;**3**:89–101.

Wei J, Gao J, Wang N *et al.* Differences in soil microbial response to anthropogenic disturbances in Sanjiang and Momoge Wetlands, China. *FEMS Microbiol Ecol* 2019;**95**:110.

Wickham H, Navarro D, Pedersen TL. *Ggplot2: Elegant Graphics for Data Analysis*. 3rd ed. New York: Springer Verlang New York, 2016.

Wieder RK, Yavitt JB. Peatlands and global climate change: Insights from comparative studies of sites situated along a latitudinal gradient. *Wetlands* 1994;**14**:229–38.

Wiken E. *Terrestrial Ecozones of Canada*., 1986.

Wilson RM, Hopple AM, Tfaily MM *et al.* Stability of peatland carbon to rising temperatures. *Nat Commun* 2016a;**7**, DOI: 10.1038/ncomms13723.

Wilson RM, Hopple AM, Tfaily MM *et al.* Stability of peatland carbon to rising temperatures. *Nat Commun* 2016b;**7**:13723.

Wu J, Roulet NT. Climate change reduces the capacity of northern peatlands to absorb the atmospheric carbon dioxide: The different responses of bogs and fens. *Global Biogeochem Cycles* 2014a;**27**:1005–24.

Wu J, Roulet NT. Global Biogeochemical Cycles. 2014b:1005–24.

Wu J, Roulet NT, Moore TR *et al.* Dealing with microtopography of an ombrotrophic bog for simulating ecosystem-level CO2exchanges. *Ecol Modell* 2011;**222**:1038–47.

Wuebbles DJ, Hayhoe K. Atmospheric methane and global change. *Earth-Science Rev* 2002;**57**:177–210.

Wüst PK, Horn MA, Drake HL. Trophic links between fermenters and methanogens in a moderately acidic fen soil. *Environ Microbiol* 2009;**11**:1395–409.

Xia Y, Wang Y, Wang Y *et al.* Cellular adhesiveness and cellulolytic capacity in Anaerolineae revealed by omics-based genome interpretation. *Biotechnol Biofuels* 2016;**9**:111.

Yang J, Jia X, Ma H *et al.* Effects of warming and precipitation changes on soil GHG fluxes: A meta-analysis. *Sci Total Environ* 2022;**827**:154351.

Yavitt JB, Seidman-Zager M. Methanogenic Conditions in Northern Peat Soils. *Geomicrobiol J* 2006;**23**:119–27.

Ye R, Jin Q, Bohannan B *et al.* pH controls over anaerobic carbon mineralization, the efficiency of methane production, and methanogenic pathways in peatlands across an ombrotrophic-minerotrophic gradient. *Soil Biol Biochem* 2012;**54**:36–47.

Youngblut ND, Barnett SE, Buckley DH. HTSSIP: An r package for analysis of high throughput sequencing data from nucleic acid stable isotope probing (sip) experiments. *PLoS One* 2018a;**13**, DOI: 10.1371/journal.pone.0189616.

Youngblut ND, Barnett SE, Buckley DH. SIPSim: A Modeling Toolkit to Predict Accuracy and Aid Design of DNA-SIP Experiments. *Front Microbiol* 2018b;**9**:1–16.

Yu Z. Holocene carbon flux histories of the world's peatlands: Global carbon-cycle implications. *Holocene* 2011;**21**:761–74.

Yu Z, Vitt DH, Campbell ID *et al.* Understanding Holocene peat accumulation pattern of continental fens in western Canada. *Can J Bot* 2003;**81**:267–82.

Yu ZC. Northern peatland carbon stocks and dynamics: A review. *Biogeosciences* 2012;**9**:4071–85.

Yvon-Durocher G, Allen AP, Bastviken D *et al.* Methane fluxes show consistent temperature dependence across microbial to ecosystem scales. *Nature* 2014;**507**:488–91.

Zalman C, Keller JK, Tfaily M *et al.* Small differences in ombrotrophy control regional-scale variation in methane cycling among Sphagnum-dominated peatlands. *Biogeochemistry* 2018;**139**:155–77.

Zheng J, RoyChowdhury T, Yang Z *et al.* Impacts of temperature and soil characteristics on methane production and oxidation in Arctic tundra. *Biogeosciences* 2018;**15**:6621–35.

Zhou J, Ning D. Stochastic Community Assembly: Does It Matter in Microbial Ecology? *Microbiol Mol Biol Rev* 2017;**81**, DOI: 10.1128/MMBR.00002-17.