

Theoretical and Empirical Investigations of
Ecosystem Development in Boreal Wetlands

by

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ABSTRACT

Despite the breadth of studies investigating ecosystem development, an underlying theory guiding this process remains elusive. Several principles have been proposed to explain ecosystem development, though few have garnered broad support in the literature. I used boreal wetland soils as a study system to test a notable goal oriented principle: The Maximum Power Principle (MPP). The MPP posits that ecosystems, and in fact all energy systems, develop to maximize power production or the rate of energy production. I conducted theoretical and empirical investigations to test the MPP in northern wetlands.

Permafrost degradation is leading to rapid wetland formation in northern peatland ecosystems, altering the role of these ecosystems in the global carbon cycle. I reviewed the literature on the history of the MPP theory, including tracing its origins to The Second Law of Thermodynamics. To empirically test the MPP, I collected soils along a gradient of ecosystem development and: 1) quantified the rate of adenosine triphosphate (ATP) production—literally cellular energy—to test the MPP; 2) quantified greenhouse gas production (CO_2 , CH_4 , and N_2O) and microbial genes that produce enzymes catalyzing greenhouse gas production, and; 3) sequenced the 16s rRNA gene from soil microbes to investigate microbial community composition across the chronosequence of wetland development. My results suggested that the MPP and other related theoretical constructs have strong potential to further inform our understanding of ecosystem development. Soil system power (ATP) decreased temporarily as the ecosystem reorganized after disturbance to rates of power production that approached pre-disturbance levels. Rates of CH_4 and N_2O production were higher at the newly formed bog and microbial genes

involved with greenhouse gas production were strongly related to the amount of greenhouse gas produced. DNA sequencing results showed that across the chronosequence of development, the two relatively mature ecosystems—the peatland forest ecosystem prior to permafrost degradation and the oldest bog—were more similar to one another than to the intermediate, less mature bog. Collectively, my results suggest that ecosystem age, rather than ecosystem state, was a more important driver for ecosystem structure and function.

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CHAPTER 1

INTRODUCTION

General Context: Ecosystem ecology and ecosystem development

Ecosystem ecology is the study of the interactions among abiotic and biotic system components. Although there were early studies in physics, geology, and chemistry in the 18th and 19th centuries that later influenced the development of ecosystem ecology, it was not until the early part of the 20th century that a unified conceptual approach to ecosystem ecology began to emerge. Work by Elton (1927) and Vernadsky (1926) established the ideas of energy flow through trophic structures in ecosystems and the importance of biogeochemistry, respectively. But it was not until Tansley (1935) that the term “ecosystem” even appeared in the literature. Tansley coined the term to highlight the importance of material and energy exchanges among living and nonliving system components. Work by Lindeman (1942) and Hutchinson (1961) reinforced an energetic perspective to studying ecosystems. The attention to, and interest in, energy flows in ecosystem ecology demonstrated the importance of physical concepts, such as thermodynamics, into the discipline.

Many early ecosystem studies focused on understanding how ecosystems respond to disturbance. Clements (1916) argued that following disturbance, ecosystems develop in a predictable manner and ultimately reach stable climax communities. Gleason (1926) disagreed, arguing that ecosystem development was not as deterministic as Clements posited. Though there were other important contributions from ecologists studying disturbance following Gleason (1926), it was not until 1969 when Odum (1969) outlined

two-dozen expected trends during ecosystem development with regards to nutrient cycling, community structure, and ecosystem energetics. For example, Odum stated that in a young, developing ecosystem more energy is used for ecosystem growth and production, whereas in older, more mature ecosystems more energy is relegated through respiration to maintaining ecosystem structure. Odum (1969) inspired a generation of scientists to test the mechanistic hypotheses he discussed in this seminal work (e.g. Connell and Slatyer, 1977; Fisher and Likens, 1973; Vitousek and Reiners, 1975).

Despite the breadth of work investigating ecosystem development, an underlying theory of this process remains elusive. The explicit consideration of energy flows in ecosystem ecology makes The Laws of Thermodynamics—specifically The Second Law of Thermodynamics, a construct from physics,—an appropriate place to start. The Second Law of Thermodynamics states that the entropy of an isolated system does not decrease. In other words, the spontaneous dispersal of energy, or entropy tends to increase in isolated systems. Despite the ties to physical concepts such as energy and the inextricable importance of The Second Law of Thermodynamics to ecosystem ecology, there are surprisingly few studies that explicitly consider The Second Law of Thermodynamics.

One approach that incorporates a thermodynamic perspective to investigate energy flow and material cycling within ecosystems involves quantifying the redox environment. Redox potential (oxidation-redox potential) is a measure of the availability of electrons in a system and has long been used in ecosystems to quantify the tendency of an environment to oxidize or reduce substances (Becking et al. 1960). For example, when oxygen is present and redox potentials are high (> 250 mv) oxygen is used as a terminal

electron acceptor to oxidize carbon compounds during cellular respiration. In oxygen-poor systems such as wetland soils, however, terminal electron acceptors other than oxygen are used for cellular respiration (Mitch and Gosselink 2007). In decreasing order of redox potential, NO_3^- (250 mV), Mn^{4+} (225 mV), Fe^{3+} (+100 to -100 mV), SO_4^{2-} (-100 to -200 mV), and CO_2 (< -200 mV) are used as terminal electron acceptors for cellular respiration. Using oxygen as a terminal electron acceptor yields the highest energy gain during cellular respiration. However, when oxygen is absent microbes are able to derive lower amounts of energy through cellular respiration by using these alternative electron acceptors. Using a terminal electron acceptor other than oxygen is inherently less efficient and results in less energy generation during cellular respiration. In fact, along this gradient of redox potential there is almost a ten-fold reduction in energy gain when using oxygen versus CO_2 . Redox potential is a powerful tool to relate energetics to efficiency in soils and thus is one important approach to linking ecosystem ecology with thermodynamic principles.

Another approach in the ecosystem ecology literature with explicit links to The Second Law of Thermodynamics is the Maximum Power Principle (MPP), developed by Lotka (1922) and later built upon by Odum and Pinkerton (1955) where power, traditionally measured in J s^{-1} in physics, is a measurement of energy per unit time (energy flow). Lotka (1922) argued that in a system with sufficient available energy, those organisms able to capture this untapped energy source would have a competitive advantage. Odum and Pinkerton (1955) expanded the MPP concept and argued that entire ecosystems, not just organisms, organize to maximize power production. They also

framed power production in terms of system efficiency and suggested that maximum power occurs at an intermediate system efficiency, where efficiency is a dimensionless ratio of output over input (Figure 1). Given that more mature ecosystems relegate more energy to ecosystem maintenance, per Odum (1969), it follows that these systems would maximize energy flow or power through the ecosystem and operate at an intermediate efficiency. The MPP is thus one way to unite theories of ecosystem development and ecosystem ecology with The Second Law of Thermodynamics.

Research Context

My research sought to test a generalizable theory for how systems develop, using boreal peat wetlands as my experimental ecosystem. My research was conducted in collaboration with scientists at the University of Guelph and the United States Geological Survey in Alaskan wetlands outside of Fairbanks (Figure 2). These boreal ecosystems are particularly important because of their importance to the global carbon cycle (Bridgman et al. 2008; Tarnocai et al. 2009). Because long-term production is generally greater than decomposition in these northern ecosystems, northern Alaskan wetlands store large amounts of carbon. For example, northern boreal ecosystems underlain by permafrost account for less than 1/5 of the global land area, but contain roughly 50% of the global soil carbon (Tarnocai et al. 2009). Additionally, climate change is leading to extensive permafrost degradation in these northern ecosystems (Davidson and Janssens 2006; Schuur et al. 2008, IPCC 2007). Permafrost degradation is leading to the rapid mineralization of these large carbon pools, and release of that carbon to the atmosphere

(Grosse et al. 2011; Jorgenson et al. 2010; Schuur et al. 2009).

Depending on the resulting ecosystem state following permafrost degradation, mineralized soil carbon is released to the atmosphere as CO₂ and/or CH₄. For example, the degradation of permafrost in low-lying areas of central Alaska commonly leads to peat wetland formation when soils collapse into the relic ice space previously occupied by permafrost (Jorgenson et al. 2001). In the resulting anaerobic and waterlogged conditions following permafrost degradation, CH₄ emission may be enhanced (Christensen et al. 2004; Turetsky et al. 2002; Wagner et al. 2007). In fact, Karhu et al. (2014) recently compared the temperature sensitivity of soil respiration rates from soils collected across global ecosystems and found that high latitude soil carbon stores could be more vulnerable to climate change than previously thought. The outcome of permafrost degradation may thus have large impacts on the type and magnitude of resulting greenhouse gas emissions from these systems.

Microbial metabolic pathways in soils largely drive carbon emissions from high latitude ecosystems. For example, CO₂ that is fixed by plants from the atmosphere and turned into organic compounds during photosynthesis is later degraded by microbes and released back to the atmosphere soil organic matter (SOM) if it is not stored. Microbes derive energy from heterotrophic respiration and release CO₂, CH₄, and N₂O as by-products. Microbial processes operating on micro spatial scales drive a macro biogeochemical ecosystem process. Soil microbial systems are integral players in soil biogeochemistry and thus regulate many of the earth's biogeochemical cycles (Falkowski et al. 2008). For this reason, I used the microbial soil system as my "model ecosystem."

Goals of Dissertation

The overall goals of my dissertation research were: 1) to provide a historical review of the role The Second Law of Thermodynamics has played in ecosystem ecology; 2) to empirically and explicitly test the MPP using Alaskan peat soils as a model system; 3) to quantify greenhouse gas emissions from these peat soils, and; 4) to assess functional and structural microbial responses during ecosystem development using molecular biology tools. The overarching question guiding my dissertation work was: **How can a thermodynamic perspective better inform how ecosystems develop and organize?**

Dissertation Research Questions, Approach, and Organization

While much work has been done on understanding changes in nutrient cycling and energy flow during ecosystem development, from a mechanistic or process perspective, less work has focused on developing generalizable theories for how ecosystems develop and organize. Additionally, despite ecosystem ecology's inextricable ties to thermodynamics, surprisingly few ecosystem ecologists have attempted to develop a general theory for ecosystem development that is grounded in thermodynamics. One notable exception, Brown et al. (2004), proposed using metabolism as a general theory for ecology across all scales. While the metabolic theory of ecology has received considerable attention and application in organismal ecology, its potential to be applied to community and ecosystem ecology has not been realized (Tilman et al. 2004). To assess

the extent to which a thermodynamic perspective can better inform Alaskan wetland ecosystem development, using the soil system as a model, I ask the following questions:

Question 1: How has The Second Law of Thermodynamics informed ecosystem ecology through its history?

In CHAPTER 2 I reviewed the literature using Web of Science and examined the role that The Second Law of Thermodynamics has played in shaping the field of ecosystem ecology. Throughout the history of ecosystem ecology there have been many attempts to develop a general theory governing how systems develop and organize. Within the literature there are a multitude of these “goal-oriented principles,” many of which are complementary (e.g. Fath et al. 2001). In this chapter I examined the historical developments that led to formation of several prominent goal-oriented principles that are grounded in the Second Law of Thermodynamics. To address the research question for this chapter, I conducted a meta-analysis of papers from the ecosystem science literature that use and tested either the MPP or the Maximum Entropy Production Principle (MEPP). This chapter provides a theoretical construct for the empirical work I present in the remaining dissertation chapters.

Question 2: Do boreal wetland soil ecosystems develop to maximize power or to maximize efficiency?

In CHAPTER 3 I explicitly and empirically tested the MPP along a gradient of wetland ecosystem development using a space-for-time substitution. I used peat soils as

my model system, and because soil systems are microbially dominated, I used adenosine triphosphate (ATP)—literally the chemical energy that does cellular work—as a proxy for soil system power. I conducted aerobic and anaerobic soil incubation experiments to quantify prominent microbial metabolic pathways for energy production. The work of this chapter builds on a small body of literature that has empirically tested the MPP (Cai et al. 2006; DeLong, 2008).

Question 3: How does the emission of greenhouse gases (CO₂, CH₄, and N₂O) change over a multi-decadal time scale along a gradient of wetland development, and how does this gas production relate to microbial gene abundances?

In CHAPTER 4 I examined the temporal dynamics of greenhouse gas emissions across the same chronosequence gradient of ecosystem development using soil incubation experiments in the laboratory. I related the production of CO₂, CH₄, and N₂O to the abundance of microbial functional genes responsible for the production of CH₄ (*mcrA*) using quantitative polymerase chain reaction (qPCR). This approach allows me to link greenhouse gas emissions with microbial functional genes a predisturbance permafrost forested bog, in newly developed wetlands, and older more mature wetlands.

Question 4: How do microbial soil communities change—structurally and functionally—to ecosystem development during wetland formation?

In CHAPTER 5 I investigated the structural and functional changes in the microbial soil system after a state change and during subsequent ecosystem development.

One approach to studying these microbial responses uses next-generation sequencing technologies, such as the Illumina MiSeq 2000 platform. In this chapter, I targeted a specific gene (16s rRNA) to assess microbial structural changes during wetland ecosystem development using PCR. I also compared sequence results with reference databases to investigate Phylum-, Class-, and Order-level taxonomic shifts in the microbial community associated with carbon and nitrogen cycling.

In addition to the research questions and the corresponding dissertation chapters discussed above, in the current section—CHAPTER 1—I provide theoretical context as well as context for using northern boreal wetlands for this dissertation work. I highlight early work in ecosystem ecology and ecosystem development and the importance of northern ecosystems in the global carbon cycle. This introductory chapter also emphasizes the role that The Laws of Thermodynamics has implicitly had on the discipline of ecosystem ecology. In CHAPTER 6, I conclude with a summary of each dissertation chapter, a short discussion on the application of The Laws of Thermodynamics within ecosystem ecology studies, and highlight novel contributions of this work to the field of systems ecology.

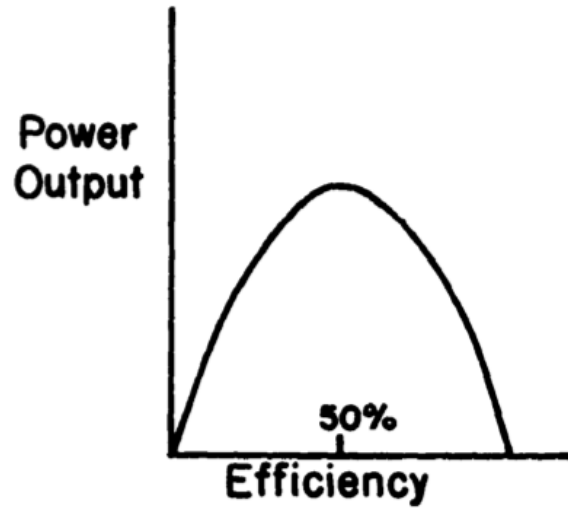


Figure 1. Conceptual diagram of the Maximum Power Principle. Maximum power occurs at some intermediate efficiency, not maximum efficiency (From Odum and Pinkerton 1955).

CHAPTER 2

A REVIEW OF HOW THE SECOND LAW OF THERMODYNAMICS HAS INFORMED ECOSYSTEM ECOLOGY THROUGH ITS HISTORY

Abstract

Many attempts have been made to develop a general principle governing how systems develop and organize in ecology. I reviewed the historical developments that led to conceptualization of several goal-oriented principles in ecosystem ecology. I focused this review on two prominent principles—the Maximum Power Principle and the Maximum Entropy Production Principle—and the literature that applies to both. While these principles have conceptual overlap, I found considerable differences in their historical development, the disciplines that apply these principles, and their adoption in the literature. These principles were more similar than dissimilar and maximization of power in ecosystems occurs with maximum entropy production. These principles have great potential to explain how systems develop, organize, and function, but there are no widely agreed upon theoretical derivations for the MEPP and MPP, hindering their broader use in ecological research. I end with recommendations for how ecosystems-level studies may better use these principles.

I. Introduction

Many theoretical frameworks have been proposed to unify fundamental concepts of systems organization and development. For example, there are hypotheses that systems organization involves maximizing power (Lotka 1922), maximizing entropy production

(Paltridge 1975), minimizing entropy production (Prigogine and Nicolis 1971), enhancing ascendancy (Ulanowicz 1986), and maximizing embodied energy, or emergy (Odum 1988). There have been many frameworks grounded in non-equilibrium thermodynamics that seek to explain how systems develop, but a consensus remains elusive. Martyushev and Seleznev (2006) noted that many of these principles have been independently proposed. They argue that various researchers, unaware of the other studies in different subjects, have proposed these principles under different names, leading to considerable delays in theoretical advancements. Because these principles aim to provide a mechanism for why systems develop, these principles are sometimes referred to as “goal” functions. To add more complexity, Fath et al. (2001) argued that there is considerable overlap and that many of these approaches are complementary. Here, I focused on two of these goal principles that have received considerable attention in the ecosystem ecology literature: The Maximum Power Principle (MPP) and the maximum entropy production principle (MEPP) and explored the links between these principles as well as possible reasons that they have gained surprisingly little traction with ecosystem ecologists writ large.

Originally presented by Alfred Lotka in the 1920s, the MPP states that systems develop to increase the total flow of energy or power through the system. Lotka framed the MPP in the context of natural selection, arguing that organisms that most efficiently harness available energy would be preserved (Lotka 1922). Lotka even proposed that the MPP should be considered the 4th Law of Thermodynamics. Odum and Pinkerton (1955) reformulated the original principle and argued that systems develop to an efficiency level

that maximizes power production. The reformulation of the MPP by Odum and Pinkerton (1955) explicitly addressed ecosystems level thinking while Lotka focused on organisms as systems. Though the MPP was formulated nearly 100 years ago, there is still considerable interest in applying the principle to ecological studies (e.g. Cai et al. 2006; DeLong 2008), yet surprisingly few ecosystem ecologists use the construct in their research.

The MEPP is an extension of the Second Law of Thermodynamics in non-equilibrium systems. It is argued that an open system far from equilibrium will maximize the production of entropy while relaxing to equilibrium over time. Similar to the use of the MPP in many fields, the MEPP has been applied to a variety of studies including physics, astronomy, mathematics, and computer science. Several researchers throughout the 20th century have independently proposed the MEPP. For example, Ziegler (1963) is credited with a derivation of the MEPP from a statistical mechanics framework, while Jaynes (1957) derived the MEPP in information theory (Martyushev and Seleznev 2006). The MPP and the MEPP are relatively young and their importance and applicability in ecosystems ecology remains contested and unresolved (Sciubba 2011, Mansson and McGlade 1993).

As with these non-equilibrium thermodynamic principles, the concepts of ecosystem development and succession have their roots in the early 20th century. Clements (1916) and Gleason (1926) laid the foundation for ecological succession and development. Odum (1969) argued that ecosystems in different states of system development share particular traits. His seminal work inspired many of these so-called

contemporary “goal function” principles as researchers sought to provide a theoretical basis for the traits that Odum (1969) described. For example, one of the traits of a maturing ecosystem was the ecosystem production (P) to respiration (R) ratio. As systems age, Odum (1969) argued, P:R approaches 1. One of the goal functions that was developed to address this was the principle of maximum energy dissipation (Fath et al. 2001). The idea behind the principle of maximum dissipation is that through the creation of complex but ordered structure, at least in biological systems far from equilibrium, the rate of entropy production or energy dissipation is actually accelerated relative to simpler, non-ordered systems. The MPP and the MEPP are principles that provide a mechanistic explanation for how systems develop and organize in the context of energy inputs (e.g. power) and energy use for system maintenance and growth (e.g. entropy).

While there has been some advance and evolution in the theoretical aspects of these principles, there have been fewer empirical ecosystem studies that have tested the MPP and to a lesser extent, the MEPP. The reason for the latter is that the MEPP appeared in the literature considerably more recently than the MPP. In this review I asked: Has the MEPP theoretically evolved from the MPP, and if so, how? By examining the theoretical underpinnings and the applied empirical studies of both of these principles, I explored the similarities and differences between the MPP and the MEPP.

My objectives of this review are to (1) characterize the development of these principles in ecosystem ecology in the historical context of The Second Law of Thermodynamics, (2) examine the current state of the MEPP and the MPP literature, (3)

elucidate common themes and challenges of applications of these principles, and (4) explore possible reasons that neither has received more traction in ecosystem science.

II. Methods

To explore the links, similarities, and differences between these principles, I conducted targeted keyword searches using Web of Science. I started by examining articles that were important in the theoretical development of the MEPP and the MPP. To examine the disciplines that have drawn from these principles, I examined a total of 520 papers that cited two papers that first proposed the MPP, albeit at different levels of ecological organization: 1) Alfred Lotka's 1922a paper entitled *Contribution to the energetics of evolution* paper, and; Odum and Pinkerton's 1955 ecosystems-level paper. This exploration of the theoretical development of the MEPP involved searches for papers citing Jaynes (1957) and Ziegler (1983), which produced a total of 5324 records.

For a closer examination of how these principles have been used in the literature, I performed additional keyword searches using “maximum power principle” and “maximum entropy production.” From the 520 papers that cited Lotka (1922a) and Odum and Pinkerton (1955) and the 246 papers that cited “maximum entropy production” I limited this meta-analysis to 32 papers by focusing on articles with a strong emphasis on ecological research, although not all were at the ecosystems scale. For the sake of simplicity, papers that used concepts such as empower and exergy were considered to be complementary with maximum power and were included in this analysis.

Although Ziegler (1963) is often credited with some of the first theoretical

research concerning the MEPP, I used Ziegler (1983) as a substitute for Ziegler (1963). This earlier paper was not in the Web of Science database. By analyzing papers that cited these pioneers in the theoretical development of the MPP and the MEPP, I was able to account for studies that did not explicitly state an application of these principles. For example, Kemp and Boynton (2004) argued that Odum's early work examining the trophic structure of Silver Springs (Odum 1957) was one of the first empirical studies of the MPP, but this was not explicitly stated in his 1957 paper. In Web of Science it is possible for papers to be listed across multiple research areas. Thus, papers listed in more than one research area were counted more than one time in my examination of these principles and the research disciplines that use them.

III. Results and Discussion

The following section is organized in the following subsections: history, current trends, interdisciplinarity and the MEPP, and interdisciplinarity and the MPP. I start my examination of the MEPP, the MPP, and their role in ecosystems ecology within a historical context. I review the origin of The Second Law of Thermodynamics and how The Second Law of Thermodynamics gave rise to the MPP and the MEPP. Next, I focus on the current use of the MEPP and MPP within the broader literature as well as within the ecological literature. I conclude this section with an exploration of the disciplines that use the MEPP and the MPP.

A. History

Non-equilibrium theoretical principles such as the MPP and the MEPP are grounded in the classical equilibrium Laws of Thermodynamics, specifically the Second Law of Thermodynamics. Here, I briefly discuss the history of The Second Law of Thermodynamics to put non-equilibrium thermodynamic principles into a historical context. I discuss the early importance of the Carnot Engine, The Second Law of Thermodynamics, work by Boltzmann, and [most importantly] the development of the concept of entropy for the development of the MPP and the MEPP. For a more comprehensive review of the history of the Second Law of Thermodynamics see Ozawa (2003) and Kondepudi and Prigogine (1998).

The Second Law of Thermodynamics has its roots in the early part of the 19th century (Figure 1). Carnot (1824) made large theoretical strides with his only published work, a book originally published in French titled *Reflections on the Motive Power of Fire and on Machines Fitted to Develop that Power*. His work on heat engines would become the foundation and inspiration for the development of the Second Law of Thermodynamics.

Although Carnot (1824) laid the theoretical framework for what was to become the Second Law of Thermodynamics, German mathematician and physicist Rudolf Clausius first articulated the Second Law of Thermodynamics in the 1850s. In *The Mechanical Theory of Heat*, translated into English in 1879, Clausius (1879) refined Carnot's work to develop The Second Law of Thermodynamics and introduced the concept of entropy. Clausius (1879) defined entropy as the energy that is dissipated when

internal work is done within a system. The Second Law of Thermodynamics states that in isolated systems, entropy must increase. His famous 1865 proclamation “the entropy of the universe tends to maximum” is still one of the most common ways to state and teach The Second Law of Thermodynamics.

Following Clausius, Boltzmann (1886) developed the field of statistical mechanics and refined the Clausius definition of entropy using a statistical framework (Figure 1). The Boltzmann Formula fundamentally linked entropy with the spatial arrangement of atoms and molecules within a thermodynamic system. In addition to the importance of early theoretical developments in classical thermodynamics, the formulation of non-equilibrium thermodynamics was equally important to the development of the MPP and the MEPP. It was not until the 1930s that considerable theoretical development in non-equilibrium thermodynamics occurred. Onsager (1931) provided the first deduction of non-equilibrium thermodynamics. Fundamentally, advancements in non-equilibrium thermodynamics provided a theoretical bridge that allowed Odum and Pinkerton (1955) to make their ecosystem-scale step from the evolutionary/organismal scale by Lotka (1922a). I argue that one of the reasons that so little of ecosystem ecology touches on MPP and the MEPP is because few ecosystem ecologists know of Onsager’s work or this bridge to non-equilibrium systems.

The early development of thermodynamics was entirely and explicitly using physical systems. It was not until Lotka (1922a) that biological systems were viewed with a thermodynamic construct. Lotka (1922a,b) linked the early thermodynamic concepts of statistical mechanics, pioneered by Boltzmann and Gibbs in physical systems, with

evolution and natural selection to develop the MPP at the organismal level (Figure 1). In fact, Lotka's work (1922a) preceded most of the empirical and theoretical work that led to the modern discipline of ecosystem ecology, including: 1) early seminal work by Elton (1927) on food chains; 2) interactions among soil, minerals, and plants by Hutchinson (numerous publications); 3) introduction of the term “ecosystem” by Tansley (1935), and; 4) the concept of energy flow through an ecosystem by Lindeman (1942). It was Lotka's merging of evolution and natural selection with Boltzmann's approach to entropy, statistical mechanics, and the Second Law of Thermodynamics that first gave rise to the MPP.

The Second Law of Thermodynamics and the concept of entropy inspired many new disciplines. In addition to the statistical mechanics framework of Boltzmann and Gibbs, information theory gave rise to a new approach to studying systems, led by the work of Shannon (1948; Figure 1). Using information theory, Shannon (1948) defined information entropy in his paper *A Mathematical Theory of Communication*. Shannon argued that information entropy (units of bits in information theory) is a function of probability. In other words, information entropy is a measure of unpredictability. The Shannon entropy approach inspired Jaynes (1957) to explore the link between statistical mechanics and information theory. Jaynes (1957) is often credited as starting the Maximum Entropy (MaxEnt) approach to thermodynamics—not to be confused with the MEPP (Figure 1). Martyushev and Seleznev (2006) noted that Dewar (2003) attempted to theoretically ground the MEPP using the Jaynes (1957) formalism. Although Martyushev and Seleznev (2006) were critical of Dewar (2003), the usefulness of his approach is still

debated; in fact, many of the papers I reviewed cite Dewar (2003) as the sole source of theoretical foundations for the studies.

It is difficult to attribute the theoretical origin of the MEPP to a single researcher. This is in contrast to the MPP, which has a clear origin in Lotka (1922a) at the organismal level and Odum and Pinkerton (1955) at the ecosystem level. Martyushev and Seleznev (2006) credited Ziegler (1963) with the introduction of the MEPP. Martyushev and Seleznev (2006) also discussed the importance of the work done by Prigogine from 1945-1947 on his Minimum Entropy Production. It is important to note that while the principle of minimum entropy may seem contradictory to the MEPP, several researchers have argued that this is not the case (Martyushev and Seleznev 2006). Different principles have been developed for different scales or domains of application. Although Martyushev and Seleznev (2006) acknowledged the importance of Prigogine's contributions to the development of the MEPP, they credited Ziegler with the derivation of the MEPP because of its wider applicability. They acknowledged that several researchers before and after Ziegler developed the idea of the MEPP independently, including a slightly different minimum entropy production principle by Prigogine, but they also argued that Ziegler's formulation was the "most evident and simplest." Although the historical origin of the MEPP is convoluted, the derivation of the MEPP by Ziegler is favored for its grounding in non-equilibrium thermodynamic theory.

B. Current trends

Despite the origins of the MPP and the MEPP being almost 100 and almost 70

years ago, respectively, I found fewer examples of these principles being applied in current ecosystems ecological literature than I expected. That said, both of these principles are being tested more than they have been before. I argue that the MPP and the MEPP may be undergoing a renaissance within the general literature as well as within ecosystems research (Figures 2 and 3). To visualize this current trend within the MEPP and the MPP literature, I analyzed the cumulative number of published articles from the general literature that used “maximum entropy production” and “maximum power principle” as keywords (Figure 2). Not only were studies investigating either the MEPP or the MPP increasing at an increasing rate, but this acceleration seems to have begun as recently as the early 2000s (Figure 2). I found this same trend of increasing interest in ecological publications that cite either the MEPP or the MPP in the last 10-20 years (Figure 3).

C. Interdisciplinarity and the MEPP

In the following two sections I expand the historical foundational work of the MEPP and the MPP (Figure 1) by explicitly tying them to a range of research disciplines. Since the introduction of The Second Law of Thermodynamics by Clausius, countless studies from a range of research disciplines, from psychology to ecosystem ecology, have drawn on his formulation of the Second Law. I used the keywords “maximum power principle” or “maximum entropy production” or citations of Lotka (1922a) or Odum and Pinkerton (1955) to determine the disciplines that are using the MEPP and the MPP approach. I also searched for papers that cited Ziegler (1983) or Jaynes (1957) as key

foundational papers. Additionally, I used the keyword “The Second Law of Thermodynamics” to determine the extent of overlap across disciplines among the other keyword searches. I assessed to what extent disciplines that used the MPP or the MEPP overlapped with disciplines that frequently cite The Second Law of Thermodynamics.

A keyword search using “Second Law of Thermodynamics” produced 1768 papers cross-listed among more than 50 Web of Science research areas for a total number of 2704 records. Sixty-one percent of the papers I found were published in three disciplines: physics and mechanics (51%), and engineering (10%; Table 1). This finding was not surprising given that these are disciplines frequently associated with thermodynamic constructs. Despite its inextricable link to and foundation in The Second Law of Thermodynamics, ecologically related studies accounted for only 54 of the 2704 records, or 2% of the total studies. It was particularly surprising that ecosystem ecology was not well represented in this keyword search. I suggest that this may be because ecosystem-scale research either only implicitly considers The Second Law of Thermodynamics or that a sizable population of ecosystem ecologists feel no intrinsic connection to or importance of The Second Law. I suggest that while the former is understandable, the latter is troubling.

To explore the extent of disciplinary overlap between The Second Law of Thermodynamics and the MEPP, I focused on research areas that most often cited the MEPP and I found considerable overlap. Papers that cited pioneers in the MEPP framework (Ziegler 1983, Jaynes 1957) showed a strong connection with disciplines that traditionally study The Second Law of Thermodynamics research: physics, mechanics,

engineering, mathematics, and materials science (Table 1). Papers that cited Ziegler (1983) were also most likely to identify with disciplines that frequently refer to the Second Law of Thermodynamics: mechanics and engineering accounted for 11% and 10% of papers referring to “the Second Law of Thermodynamics” and 27% and 18% of those that cited Ziegler (1983), respectively.

Although Jaynes (1957) and Ziegler (1983) have both been acknowledged as foundational in the development of the MEPP, papers that cited Jaynes (1957) came from a wider range of disciplines than those that cited Ziegler (1983; Table 1). This is likely because Jaynes’ maximum entropy (MaxEnt) derivation was a much broader theory, of which entropy can be thought of as one case. For example, papers from more than 90 disciplines across more than 5000 citation records cited Jaynes (1957). In other words, Jaynes (1957) reached a broad range of disciplines, but 52% of all articles citing this paper were published in physics, engineering, mathematics, and computer science.

Interestingly, environmental sciences ecology was the 6th most common discipline to cite Jaynes (1957), for 3% of the total articles. Although 3% of the total articles does not sound like much, Jaynes (1957) is cited by over 5000 articles. This suggested that the derivation of the MEPP by Jaynes (1957) has reached a broad audience in the ecological community.

Although Martyushev and Seleznev (2006) credited Ziegler with the first use of the MEPP concept, based on his comprehensive non-thermodynamic theoretical approach, Ziegler (1983) did not explicitly state his theoretical influences. For this reason, Martyushev and Seleznev (2006) could only infer that his derivation of the MEPP

followed the theoretical developments in statistical physics from Boltzmann (Figure 1). The Ziegler approach to MEPP was derived independently from other research on the MEPP, and this may explain why I found considerable overlap among disciplines citing his work and those that referred to “the Second Law of Thermodynamics” (Table 1). Relative to Jaynes (1957), Ziegler (1983) was cited by far fewer research disciplines—mechanics, engineering, materials science, and physics papers accounted for 69% of all Ziegler (1983) citations (Table 1). Notably, I was unable to find a single ecosystems ecology study, or even ecological study, that cited Ziegler’s derivation of the MEPP. I thus concluded that the Ziegler (1983) derivation of the MEPP has not received traction by ecologists, or even by ecosystems ecologists.

The final keyword search relating to the MEPP was for papers that have referred to “maximum entropy production” (Table 1). This search generated 307 total records that spanned 28 different research areas. As with papers that cited Ziegler (1983) and Jaynes (1957), most of these were physics or atmospheric sciences papers. Studies from the ecological sciences were less common, but much more prevalent than papers that cited Ziegler (1983) or Jaynes (1957). While the environmental sciences ecology research area accounted for 6% of the total studies that referred to “maximum entropy production,” in papers published since 2010 this proportion increased to 10%. This appears to be driven by 15 articles in a special issue in *Philosophical Transactions of the Royal Society Biological Sciences*, published in 2010, which focused on the MEPP in biological systems.

D. Interdisciplinarity and the MPP

My focus on the MPP in the literature was based on the papers of Lotka (1922a), Odum and Pinkerton (1955), and use of the term “maximum power principle.” A total of 275 papers have cited Lotka (1922a) representing 55 different research areas for a total number of 424 records. One-third of papers that cited Lotka (1922a) were from the environmental sciences ecology research area (Table 1). Additionally, papers on some form of ecology accounted for 43% of the total records. Given the strong influence that Darwin had on Lotka, and the interest Lotka had in biological systems, these results are not surprising from an organismal perspective.

Papers that cited Odum and Pinkerton (1955) showed a similar research area breakdown. Of the 364 papers I found, spread across 39 research areas, 211 or 58% had research that at least overlapped with ecosystems science (Table 1). This is not particularly surprising given that most of Odum’s work was firmly entrenched in ecosystem ecology. I also found that the keyword “maximum power production” had substantial representation in the ecological literature (Table 1)—of the 25 records, 60% had a focus in ecological sciences. My analyses of the MEPP and the MPP papers suggested that there were clear disciplinary distinctions in the use of these principles, and that ecosystem ecologists and ecologists in general have, by and large, focused their work on the MPP on a percentage basis. That is, 60% of the papers that refer to the MPP are within ecological sciences, but only 8% of the MEPP papers are. However, in terms of a total number of records, there are more ecology studies within the MEPP than the MPP.

Additionally, many disciplines have used the MEPP over time, but the use of the MPP has mainly been by ecologists.

The pattern of an increasing rate of articles being published that apply the MEPP (Figure 3) was also observed with the number of articles that cited the foundational MPP papers (Figure 4). The number of articles published per year that cited either of Lotka's 1922 publications or Odum and Pinkerton (1955) increased substantially in the 2000s. I found close parallels in the pattern of citations of these three papers (Figure 4), suggesting that authors commonly cited them all in the same paper. This common co-citation of Lotka (1922a) and Odum and Pinkerton (1955) suggests broad recognition of the theoretical link between them, but does not acknowledge that Lotka focused his MPP work mainly at the organismal level while Odum's focus was on ecosystems. Confusion about this distinction may be one reason that these Second Law principles have not gained greater traction in ecosystem ecology.

IV. Maximum Power Principle with a Focus on Ecology and Ecosystems

A. The Early work

As I noted above, Lotka (1922a) was the first formulation of the MPP. While referencing Boltzmann's contribution that energy is the basis of life struggle, Lotka argued that systems—more specifically organisms—that best capture energy to ensure future survival would have an evolutionary advantage. He further stated that if there are untapped energy sources within the system and there are mechanisms to use that energy, the total energy flux through the system might be increased.

Although Lotka pioneered the conceptual context for the MPP, Odum and Pinkerton (1955) provided a much stronger theoretical framework and applied it directly to ecosystems. For example, their paper framed system power output as a function of system efficiency and hypothesized a general power-efficiency relationship (Figure 5). They also discussed several ecosystems that operate at maximum power. Since Lotka (1922a) and Odum and Pinkerton (1955), there have been about a dozen studies that have explicitly tested the MPP. Odum's early papers, from the 1950s, did not always explicitly state that testing the MPP was an objective; thus, accounting for all of the early implicitly tested studies of the MPP by Odum or others was somewhat difficult. That is, studies that did not explicitly use the term MPP or cite key papers within the framework were difficult to study. For this reason it is likely that the number of studies that have tested the MPP are underreported in the literature. One of the earliest applications of the MPP was Odum (1957; Kemp and Boynton, 2004). In this study of Silver Springs, he used a modified diel approach to measure ecosystem production (P) and respiration (R) in an attempt to characterize the trophic structure, productivity, and total energy flow through this aquatic ecosystem. Even though Odum (1957) did not explicitly state that this study was a test of the MPP, through studying trophic structure and energy flow through Silver Springs, it seems reasonable to assume that this was his intent. By examining early MPP empirical studies and the theoretical underpinnings of the MPP, a clearer picture emerges of what some of the early pioneers in MPP literature intended from these types of ecosystem studies.

B. Types of Studies and Ecological Scales

I found 11 studies in the literature that tested the MPP. Within these 11 papers I reviewed that use the MPP approach to studying ecosystems, there is not one particular type of study or method that is more prevalent (Table 1). I found a broad range in the types of studies that have used the MPP, and they were more than a collection of studies that used P/R ratios to study energy flow in ecosystems, as Odum did early in his career. I found many studies using models to apply or test the MPP and similar principles (exergy and MePP) in a variety of contexts. For example, the MPP has been applied to studies that: 1) used models to optimize exergy relative to the effects on planktonic and zooplankton body size in aquatic ecosystems (Ray et al. 2001); 2) used the MPP to constrain hydrological conceptual models (Westhoff and Zehe 2013); modeled the role of exotic species in ecosystem self-organization (Campbell et al. 2009), and; 4) simulated the role of the MPP in self-organizing forest plantation ecosystems (Li et al. 2013). The MPP has also been used to predict outcomes of competitive exclusion experiments (DeLong 2008), as an indicator for benthic ecosystem recovery following a disturbance (Libralato et al. 2006), and in examinations of microcosm ecosystem self-regulation using pH to control photoperiod (Cai et al. 2006). It has also been used to model ecosystem organization of energy flows and storage (Fath et al. 2004) and in simulations of ecosystem responses to resource pulses (Lee 2014). It is clear that the MPP has guided systems-based ecological studies at all levels of organization—not just with ecosystems—for decades.

C. Common Themes

I found that half of the papers (6) cited both of the well-recognized theoretical foundational MPP papers. All of them cited (Lotka 1922a), except for one (i.e. Westhoff and Zehe 2013), and that study discussed the theoretical background of the MEPP, citing work by Dewar, but designed their model to test the MPP. In the studies that did not cite Lotka (1922a) and Odum and Pinkerton (1955), I found that the authors typically cited other Odum papers (e.g. Ray et al. 2001, Lee 2014, and Campbell et al. 2009). One paper cited foundational papers from both the MPP and the MEPP literature (Fath et al. 2004).

While the MPP has guided organismal and community-scale ecological research, it has most often been tested at the ecosystem scale. The difficulty of designing experiments and models to test the MPP translated to a limited literature of empirical MPP studies. Given this, I found it surprising that a large percentage of the studies attempted to draw results from large and relatively complex ecosystems. This may be a direct reflection of the legacy and influence of H.T. Odum and his ecosystem approach. In addition to testing the MPP at the ecosystem scale, a number of the studies I reviewed used the principle in the context of ecosystem development and system organization (per Odum 1969; e.g. Fath et al. 2004, Lee 2014, Campbell et al. 2009, and Li et al. 2013).

V. Is the Maximum Entropy Production Principle an Evolving Theory?

A. Early Work

Paltridge (1975) is commonly cited as the first known application of the MEPP in his attempt to model global atmospheric circulation patterns (Vallino, 2010; Virgo, 2010;

Ozawa, 2003). If the global transport of heat is assumed to operate at maximum entropy, he argued, then meridional energy flux, cloud cover, and meridional temperature distributions should all be predictable. Interestingly, while Paltridge is widely credited as the first empirical application of the MEPP, he did not cite any of theoretical foundational MEPP papers (Figure 1, Table 3). Paltridge originally framed the global atmospheric circulation patterns in the context of minimum entropy production; it was only later that others recognized his approach as the MEPP. This lack of an explicit link between his approach and the MEPP is surprising, given the large impact that his study has had on the MEPP field. For example, the Paltridge approach has also been applied to the atmosphere of Titan and Mars (Lorenz et al. 2001).

B. Types of Studies and Ecological Scales

Studies using the MEPP in an ecological context constituted a smaller percentage of the MEPP literature compared to the ecological fraction of the MPP literature, but overall I found more ecological MEPP studies. I reviewed 22 MEPP articles for this study, compared with the 11 I found for the MPP. I found a similar variety in the types of studies and the scale of application, and again a large fraction of them were modeling studies. The latter included models of ATP synthase enzyme design (Dewar et al. 2006), enzyme kinetics (Dobovišek et al. 2011), chemical replicators (Martin and Horvath 2013), systems with multiple equilibria (Herbvert et al. 2011b), detrital-based ecosystems (Meysman and Bruers 2007), ecosystem biogeochemistry (Vallino 2010), food webs (Meysman and Bruers 2010), watershed development (Kleidon et al. 2013), ecological

succession (Skene 2013), and glacial maximum climates (Herbert et al. 2011a). Other studies include using eddy-flux data to calculate entropy in developing ecosystems (Holdaway et al. 2010) and several studies describing community organization and physiology in the context of MEPP (Jia et al. 2012, Lin et al. 2009, Volkov et al. 2009, Dewar 2010, Schymanski et al. 2010). Among the studies applying the MEPP, I found a diverse range of scales being tested and a heavy emphasis on using modeling.

C. Common Themes

To a larger extent than MPP studies, I found a high prevalence of MEPP studies relied on modeling approaches. This is not surprising giving the difficulty and complexity of the types of systems ecosystems scientists study. I also found evidence of a highly fragmented and disjointed historical development of the MEPP, and thus of its impact on MEPP studies in ecosystem ecology and ecology in general. For example, five of the 21 studies did not cite any of the recognized theoretical foundations within the MEPP approach. All of these independently proposed variations of the MEPP have contributed to the strength of this approach, however there seems to be two main disconnects within the theoretical development: (1) some use entropy in the thermodynamic context, while others use it in an information theory context, and (2) the appropriate scale to apply the MEPP. For instance, it is a strength of the principle that it has been developed many times independently; however, this fragmented history hinders critical feedback and theoretical development. The theoretical disconnect across these studies suggests that while the MEPP is a general principle, some confusion remains about how it is currently

being applied to ecological systems. This is in contrast to the MPP, which is less used, but more uniformly accepted and cited.

VI. Complementarity and Challenges

By examining the historical theoretical development of the MPP and MEPP and the studies that have applied these principles, I concluded that the MPP and the MEPP actually guide complementary, not discrete or competing, approaches to studying ecosystems (Fath et al. 2001). Despite their parallel historical developments (Figure 1) and relatively few crossover citations in the literature between these two approaches (Table 2, Table 3), MPP and MEPP studies both tested fundamental thermodynamic concepts that are deeply linked. One way to consider the complementarity of these approaches is through the concept of thermodynamic maximization in each principle. For instance, in the MPP, power is thermodynamic work being done over time (power is measured in J s^{-1}), and it takes energy to do work. In other words, power is the flow of energy through some system per unit time. The MEPP argues that entropy production is maximized in energy systems, and entropy is the thermodynamic measure of energy dissipation. When work is done in a system, free energy is degraded and ultimately lost as heat, which is the Gibbs definition of entropy. If a system is operating at maximum power and degrading free energy to do work, this system will also be maximizing entropy production. And thus the MPP and the MEPP are simultaneously operating in this energy system provided system boundaries are carefully drawn, but with different units of measure—energy throughput versus entropy output.

My review of the MPP and the MEPP literature also suggested some difficulties with these approaches. One of the challenges with the MPP, and to a lesser extent the MEPP, was a lack of empirical studies testing the principle(s). While I found that interest in both has recently been increasing (Figure 2, Figure 3), there are still relatively few MPP or MEPP studies in the literature. Another difficulty is designing and implementing studies that explicitly test concepts in the theoretical foundations of the principles. For example, many of the MPP papers that I reviewed cited Lotka (1922a) and Odum and Pinkerton (1955). However, there are also many MPP papers that only cite Lotka (1922a) (Table 1). While Lotka originally conceptualized the MPP for organismal systems, it was Odum and Pinkerton (1955) that added theoretical rigor and an ecosystems level focus. Additionally, within those papers that cite Odum and Pinkerton (1955) I found none that explicitly used power output as a function of system efficiency. I suggest that future studies strive to test the original concept demonstrated in Odum and Pinkerton (1955) Figure 2 (Figure 5). The strength of this revolutionary idea is framing system power output as a function of system efficiency.

One of the main challenges to the MEPP approach is the lack of agreement about the scale over which it applies, and to a lesser extent the confusion regarding entropy itself. While Prigogine's minimum entropy production can be shown to be a special case of MEPP (Kleidon and Lorenz 2005), others have found that subparts of a system operate at minimum entropy production, but the system as a whole operates under MEPP. For instance, river networks organize to minimize drag (i.e., minimum entropy), but this maximizes the dissipation rate of gravitational potential (Kleidon et al. 2013). Likewise,

do individual organisms follow MEPP, or only ecosystems? As to entropy, there still seems to be some confusion between thermodynamic entropy, and information, where order is important for the latter, but not the former (Morrison 1964). These issues of scale and definition of entropy must be resolved before a mature MEPP can effectively advance ecosystem science.

VII. Conclusion

The MPP and the MEPP have been applied to ecosystem ecology in complementary ways and have often been focused on ecosystem development and organization. It is important to note that these are still young and developing principles, and their application is clearly not completely settled. However, both the MEPP and the MPP have strong potential to further inform our understanding of ecosystem structure and function. My historical review of The Second Law of Thermodynamics literature revealed that although the MEPP is generating more interest among ecologists than the MPP, the MEPP did not evolve from the MPP. Instead, both of these approaches were developed independently and are complementary to one another. The MPP is more widely used by ecosystem ecologists while there are more ecological studies that refer to the MEPP.

Through this study of the historical development of the MPP and the MEPP from The Second Law of Thermodynamics I sought to clarify theoretical developments and misconceptions within the principles. Despite their shortcomings, my examination of MPP and MEPP studies clearly demonstrates the general applicability of the MEPP and

the MPP across scales and their promise to better inform how ecosystems structure and function.

Besides a recent renewed interest in testing the MEPP and the MPP in ecosystems research, I was encouraged to see a wide variety in the ecological scales at which these principles are being applied. We need to test the MEPP at the ecosystem scale and across multiple systems to assess validity not at one particular scale. Because the MPP is clearly an ecosystem-scale construct, I suggest that the MPP has the most potential at the ecosystem scale. I end with a couple of suggestions for future studies within the MEPP and the MPP approaches: (1) I encourage broader collaboration across the multitude of disciplines that test these principles, (2) focus on theoretical development and an agreeable theoretical derivation of the MEPP, (3) increase the number of experiments to test the MPP and the MEPP, and (4) those studies that test the MPP should strive to frame the experimental design in terms of the approach outlined in Odum and Pinkerton (1955).

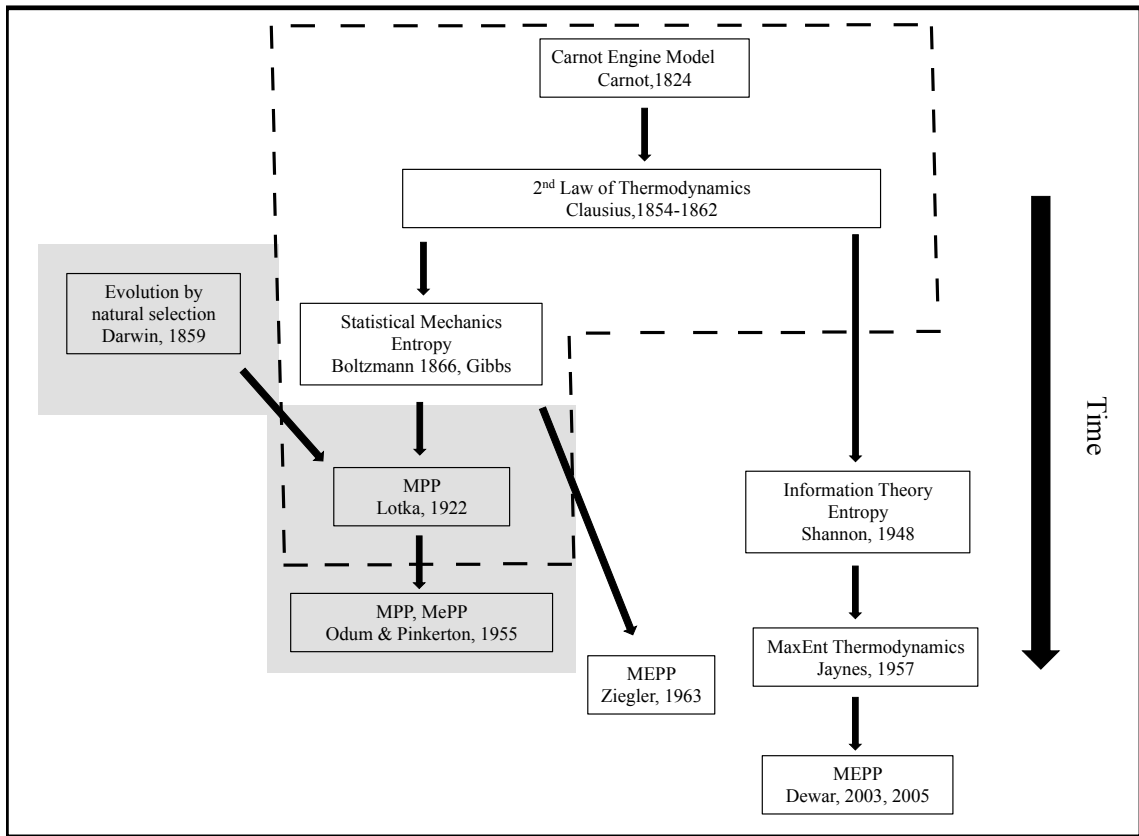


Figure 1. Conceptual map of the evolution and origin of several optimality principles in biology, physics, and chemistry. The MPP and the MEPP both evolved from the Second Law of Thermodynamics. The dashed line represents theoretical developments prior to the introduction of non-equilibrium thermodynamics approach by Onsager (1931). The gray box represents explicit theoretical development in biological sciences. MPP: Maximum Power Principle; MePP: Maximum emPower Principle; MaxEnt: Maximum Entropy; MEPP: Maximum Entropy Production Principle. Reviewed studies did not widely cite derivations of MEPP from Swenson (1989) or Prigogine and Nicolis (1971) and were omitted from this map.

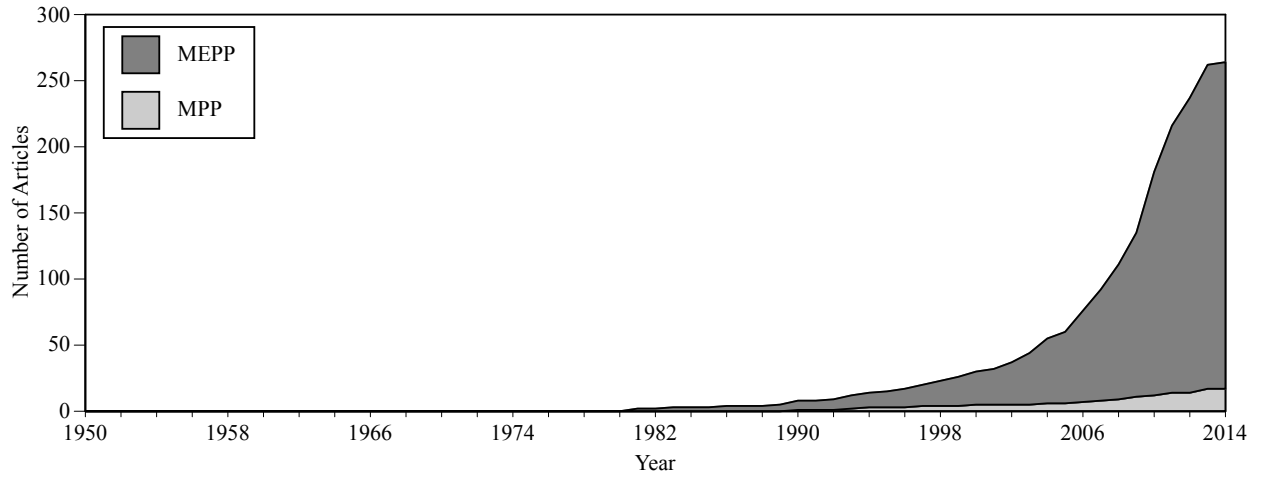


Figure 2. Maximum Entropy Production Principle and Maximum Power Production cumulative number of published papers. Since the early 2000s there has been an exponential increase in the number of articles that discuss the MEPP and to a lesser extent the MPP.

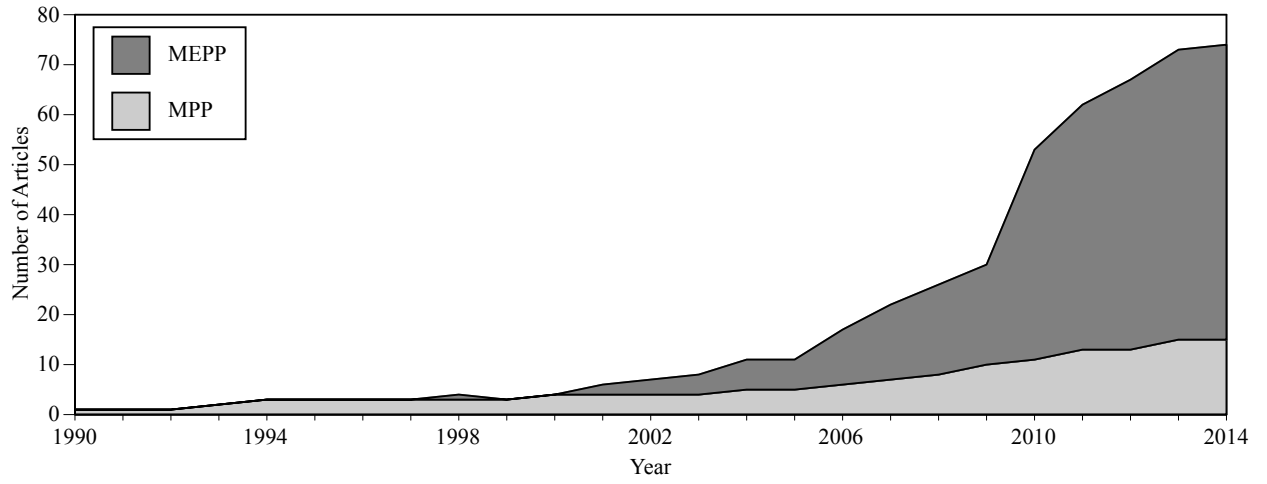


Figure 3. Maximum Entropy Production Principle and Maximum Power Production cumulative number of ecological studies published papers by year.

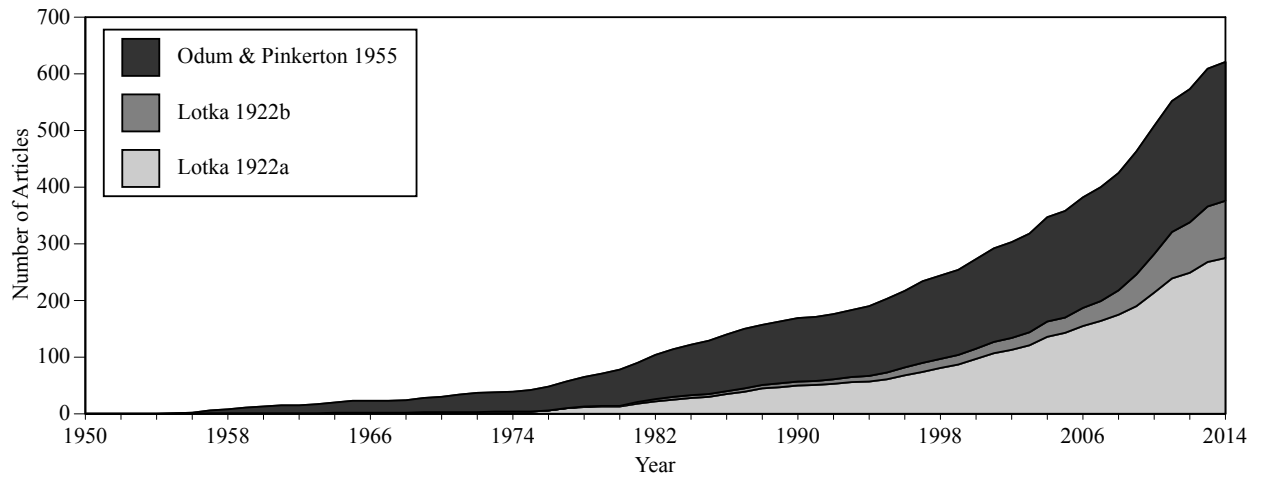


Figure 4. Cumulative number of publications that cite foundational MPP publications. From the 1950s to the 1970s these papers were largely ignored, however, since the mid 1980s all three of these papers have become increasingly recognized. These papers all follow similar temporal patterns, suggesting co-citations.

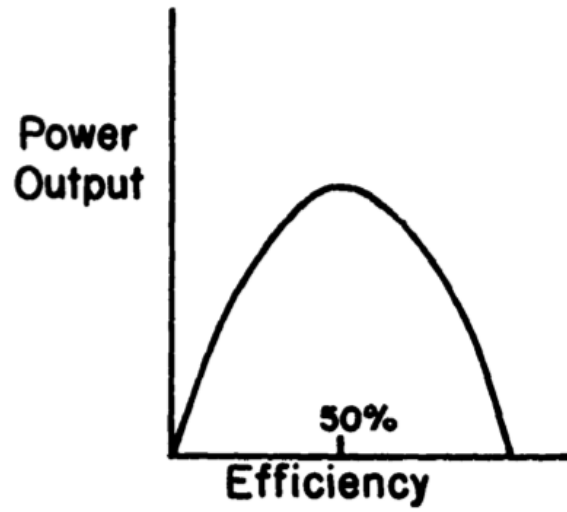


Figure 5. Power output as a function of efficiency. Maximum power output is observed at an intermediate frequency (from Odum and Pinkerton (1955), Figure 2.).

Percent of each discipline across different keyword searches

	“second law of thermodynamics”	Jaynes 1957	Ziegler 1983	“maximum entropy production”	Lotka 1922	Odum and Pinkerton 1955	“maximum power principle”
Physics and mechanics	51	40	45	26	4	0	0
Engineering	10	9	18	3	5	0	8
Thermodynamics	8	0	3	0	0	0	0
Mathematics	4	8	6	0	0	0	0
Materials science	4	0	14	0	0	0	0
Environmental science ecology	0	3	0	6	33	41	48
Biology miscellaneous	0	0	0	20	19	28	36
Geology	0	0	6	8	0	0	4
Other	23	40	9	37	39	31	4

Table 1. Keyword searches by percentage of each discipline. Web of Science keyword searches are in columns and disciplines are in rows. Numbers represent percentages.

List of reviewed MPP studies

Type	Scale	Principle	Theoretical Foundations	Source
Exergy as indicator of recovery	Ecosystem	(MPP)	Lotka	Libralato et al. 2006
Planktonic body size and exergy	Organism	(MPP)	Lotka, Odum	Ray et al. 2001
Exergy and ecosystem development model	Ecosystem	(MPP)	Lotka, Odum and Pinkerton, Prigogine	Fath et al. 2004
Constraining hydrological models	Watersheds	MPP	Dewar	Westhoff and Zehe 2013
Competition exclusion experiments	Community	MPP	Lotka, Odum and Pinkerton	DeLong 2008
Photoperiod and power acquisition microcosm	Ecosystem	MPP	Lotka, Odum and Pinkerton	Cai 2006
Review of Odum Silver Springs study (1957)	Ecosystem	MPP	Lotka, Odum and Pinkerton	Kemp and Boynton 2004
Behavioral thermal regulation in fish model	Organism	MPP	Lotka, Odum and Pinkerton	Bryan et al. 1990
Ecosystem based power model	Ecosystem	MePP	Lotka, Odum	Lee 2014
Invasion/ introduction of exotic species model	Ecosystem	MePP	Lotka, Odum	Campbell et al. 2009
Mechanistic model for plantation empower	Ecosystem	MePP	Lotka, Odum and Pinkerton	Li et al. 2013

Table 2. Reviewed studies that use the Maximum Power Principle. The types, scales, and theoretical foundations of these papers varied greatly. (MPP) refers to studies that use exergy as an indicator or principle. MePP refers to Maximum emPower Principle studies. Studies that cited Odum, but not Odum and Pinkerton (1955) have “Odum” listed as theoretical foundations.

List of reviewed MEPP studies

Type	Scale	Principle	Theoretical Foundations	Source
Plant physiology and maximum entropy	Organism	MaxEP	None	Jia et al. 2012
Self organization of plant communities	Community	Maximum dissipation	Lotka, Odum, Prigogine	Lin et al. 2009
Energetics of succession model	Ecosystem	MEPP	Boltzmann, Lotka, Odum and Pinkerton	Skene 2013
Species interaction models in tropical forest	Community	MEPP	Boltzmann, Shannon, Jaynes, Dewar	Volkov et al. 2009
Carbon assimilation and ecosystem spatial organization	Ecosystem	MEPP	Dewar	Jesus et al. 2012
Semiarid system heterogeneity models	Ecosystem	MEPP	Dewar	Schymanski et al. 2010
Chemical replicator model	Organism	MEPP	Dewar, Prigogine	Martin and Howarth 2013
Glacial maximum climates	Global	MEPP	Jaynes, Dewar	Herbert et al. 2011
Multiple equilibria models	Global	MEPP	Jaynes, Dewar	Herbert et al. 2011
Bacterial chemotaxis and maximum entropy	Bacterial	MEPP	Jaynes, Prigogine, Swenson, Dewar	Zupanovic et al. 2010
Amazon ecosystem development	Ecosystem	MEPP	Lotka, Odum and Pinkerton	Holdaway et al. 2010
Ecosystem biogeochemistry model	Ecosystem	MEPP	Lotka, Odum and Pinkerton, Prigogine, Dewar, Swenson	Vallino 2010
Organization of watersheds models	Watersheds	MEPP	Lotka, Odum, Dewar	Kleidon et al. 2013
Metabolic networks	Molecular, Bacterial	MEPP	None	Unrean and Srienc 2011
Soil hydrological processes model	Ecosystem	MEPP	None	Porada et al. 2011
Plant optimization theories and	Multiple	MEPP	Dewar	Dewar 2010
Climate system dynamics	Global	MEPP	None	Paltridge 1975
Detrital based ecosystems model	Ecosystem	MEPP	Odum	Meysman and Bruers 2007
Food webs models	Ecosystem	MEPP	Prigogine	Meysman and Bruers 2010
Enzyme kinetics and maximum entropy	Molecular	MEPP	Swenson, Dewar, Prigogine, Ziegler	Doboviseik et al. 2011
ATP synthase enzyme design	Molecular	MEPP	Jaynes, Dewar	Dewar et al. 2006

Table 3. Reviewed papers that use the Maximum Entropy Production Principle and similar principles. The MEPP was used for ecosystem succession models to bacterial chemotaxis studies. The scale of the study ranged from bacterial to ecosystem and global level. There were two studies within this literature that were considered complementary to the MEPP: maximum dissipation and MaxEP (Maximum Entropy Production).

CHAPTER 3

A THERMODYNAMIC ANALYSIS OF ECOSYSTEM DEVELOPMENT IN NORTHERN WETLANDS

Abstract

Higher temperatures associated with climate change are preferentially impacting high latitude ecosystems underlain by permafrost. To a large extent, permafrost governs the availability of soil organic carbon to be mineralized ultimately to the release of carbon dioxide (CO₂) and methane (CH₄) from these soils. Higher temperatures are leading to extensive areas of permafrost degradation, with the subsequent formation of wetlands in some cases. In this study, I tested the Maximum Power Principle (MPP)—a theoretical construct with its foundation in the Second Law of Thermodynamics—to characterize ecosystem development in wetlands in central Alaska, USA. The MPP argues that systems develop to maximize energy throughput, or power. I used adenosine triphosphate (ATP) as a proxy for power in high latitude wetland soil ecosystems to test the MPP along a gradient of wetlands that have developed following permafrost degradation: a bog with permafrost (forested bog, or FB) and a young bog and older bog that have formed since their permafrost thawed (young collapse scar, or YCS and old collapse scar, or OCS, respectively). To do this, I conducted soil incubation experiments and measured production rates of CO₂, CH₄, nitrous oxide (N₂O), and ATP. I also measured a suite of organic acid ions associated with fermentation reactions (i.e. citrate, formate, lactate, acetate, propanoate) during soil incubation experiments. Rates of potential ATP production were significantly lower ($p < 0.05$) at YCS compared to FB;

OCS rates were not statistically different from either site. I found significantly higher rates of CH₄ and N₂O flux from YCS compared to the other sites. These results suggested that system power decreased temporarily and that the system reorganized to rates of power production that approached pre-disturbance levels, but in a markedly different state. This approach allowed me to characterize the outcome of permafrost thaw disturbance on subsequent changes in boreal wetland soil ecosystem structure and function in an explicitly thermodynamic construct using the MPP.

I. Introduction

The response of northern ecosystems to climate change has been the subject of numerous recent studies, although scientists have long studied these ecosystems and speculated about their importance to the global climate (Gorham 1991). In more recent studies, there has been a concerted effort to understand the effects of a changing climate on ecosystem structure and function. For example, studies have examined: 1) the vulnerability of soil organic carbon in permafrost to mineralization (Schuur et al. 2008); 2) the northward expansion of arctic shrubs and its effect on surface albedo (Chapin et al. 2005; Myers-Smith et al. 2011; Tape et al. 2006); 3) the ecological changes associated with permafrost degradation (Jorgenson et al. 2001; Shur and Jorgenson 2007); 4) the role of a changing fire regime on ecosystem processes, particularly in Alaska (Johnstone et al. 2010; Kasischke et al. 2010; Waldrop and Harden 2008), and; 5) the effects of water table position on greenhouse gas emissions (Turetsky et al. 2008). Here, I investigated wetland ecosystem development following a permafrost thaw disturbance using a thermodynamic construct.

Direct and indirect effects of climate change are leading to extensive areas of permafrost degradation in interior Alaska, resulting in conditions that can lead to wetland formation (Jorgenson et al. 2001). Degradation of permafrost often results in ground subsidence that forms collapse pits called thermokarst. The formation of thermokarst occurs because ice occupies more volume than liquid water, causing the soil and vegetation above to collapse into relic ice space when permafrost thaws. Thermokarst formation often leads to poorly drained conditions and thus the formation of wetlands (Camill et al. 2001; Jorgenson et al. 2001).

Peatlands in high latitude ecosystems are of particular importance because of their role in the global carbon cycle. Thousands of years of peat accumulation, from carbon fixation rates that exceeded rates of carbon mineralization, have led to large stores of soil organic carbon. For example, peatlands cover roughly 3% of the terrestrial land surface of the globe, but account for up to 30% of the global soil carbon pool (Gorham, 1991). Additionally, Alaskan wetlands within northern peatland ecosystems are estimated to store ~42 Pg of carbon, or roughly 10% of the 529 Pg stored in wetlands globally (Bridgham et al. 2006). However, high latitude ecosystems are important sources of methane (CH₄). It is estimated that these ecosystems release 36 Tg CH₄ y⁻¹, mostly from boreal wetlands (Zhuang et al. 2006). Despite peatlands and boreal wetlands storing carbon over the long-term, increasing CH₄ emissions may negatively impact their carbon sequestering qualities (Whiting and Chanton, 2001).

The Second Law of Thermodynamics has been instrumental to the field of ecosystem ecology. Despite this, surprisingly few ecosystem-scale studies have explicitly

considered The Second Law of Thermodynamics (CHAPTER 2). One approach, with explicit ties to The Second Law of Thermodynamics, is the Maximum Power Principle (MPP). The MPP, originally developed in the 1920s by Lotka (1922) and refined by H.T. Odum and Richard Pinkerton in the 1950s, argues that ecosystems develop to maximize energy throughput (Odum and Pinkerton, 1955). Those processes that enhance the flow of energy through a system will be reinforced. Odum and Pinkerton (1955) argued that maximum system power output occurs at some intermediate efficiency, not at maximum efficiency (Figure. 1). While the MPP is nearly 100 years old, only a few empirical studies have directly tested it (e.g. Cai et al. 2006; DeLong, 2008). In his foundational paper on ecosystem development, Odum (1969) stressed the importance of energetics and links to The Second Law of Thermodynamics. The MPP is a promising and testable linkage between ecosystem ecology and The Second Law of Thermodynamics. In this paper I use the MPP as a guiding framework to test concepts in ecosystem ecology.

The goal of this study was to apply a thermodynamic approach (specifically the MPP) to Alaskan thermokarst wetlands that are at differing stages of ecosystem development, using their peat soils as a model ecosystem. I asked: How can a thermodynamic perspective better inform our understanding of the ecosystem development process? More specifically, do boreal wetland soil systems develop to maximize efficiency or to maximize power output? Because soil systems are microbially dominated, I used adenosine triphosphate (ATP) — literally the chemical energy used to do cellular work — as a surrogate for system power. I defined system efficiency, in terms

of power production, as the dimensionless ratio of power output (ATP production) to input (total potential power within the system, based on Gibbs energies; Equation 1):

$$Efficiency = \frac{output}{input} \quad (1)$$

The objectives of this study were: 1) to explicitly and empirically test the MPP using rates of ATP production from soil incubation experiments to measure power output; 2) to quantify the potential energy (input in Equation 1) in the soil system under aerobic conditions using standard Gibbs energies (ΔG°); 3) to measure the rates of soil respiration and organic acid production to assess the prevalence of metabolic fermentation pathways for energy production during anaerobic soil incubations in order to assess the prominent anaerobic pathways for energy production, and; 4) to measure the potential energy (and input in Equation 1) in the soil system under anaerobic conditions using the following equation:

$$\Delta G = 2.303*RT\log(Q/K).$$

where R= gas constant ($8.314 \text{ J deg}^{-1} \text{ mol}^{-1}$), T= temperature ($^\circ\text{K}$), Q= reaction quotient, and K= the equilibrium constant. I used Objectives 2 and 3 to compute the potential energy in the soil system (input in Equation 1) and Objective 1 to quantify the actual energy produced (output in Equation 1). By plotting power output as a function of efficiency, I explicitly tested the MPP (Figure 1).

II. Methods

Study Site

My study used established research sites located within the Tanana River floodplain in interior Alaska. The Tanana Flats are located approximately 35 km southwest of Fairbanks, AK USA and adjacent to the experimental forest of the Bonanza Creek Long Term Ecological Research Program (BNZ LTER; Figure 2). Additionally, the Alaska Peatland Experiment (APEX), a long-term peatland manipulation study, is also situated adjacent to the Tanana Flats. The Tanana Flats is a low-lying area in the discontinuous permafrost region characterized by an assemblage of peatlands that are of differing ages because of permafrost degradation. Regions within the Tanana Flats have undergone extensive permafrost degradation and permafrost is now 4 to 5 m below the surface in many places (Osterkamp et al. 2000). Within the Tanana Flats, the common thermokarst wetlands include ombrotrophic bogs, minerotrophic fens, and thermokarst pits.

Using space as a substitution for time, I identified three different bogs within the Tanana Flats that represented an age gradient of thermokarst wetlands. All of my sites were located within 100 m of one another. One of these sites was a forested bog (FB) characterized by intact permafrost, a stand of black spruce (*Picea mariana*), brown mosses, and *Sphagnum*. In the gradient of wetland development FB was considered pre-disturbance. Along the gradient of ecosystem development my next site was a young collapse scar site (YCS). Following a permafrost thaw disturbance and subsequent collapse of the vegetation into relic ice space, YCS was formed roughly 100 years ago.

The old collapse scar (OCS) site formed in the same way, but roughly 400-500 years passed since the permafrost thaw disturbance. Despite their similar plant community compositions, OCS had a larger presence of bog shrubs compared to YCS. Since more time had passed since permafrost thaw disturbance, OCS had accumulated more peat than YCS. Subsequently, the vegetation was further above the water table at OCS compared to YCS.

Sampling

In June 2012 I sampled FB, YCS, and OCS by taking five soil cores from each site. The cores were haphazardly located to ensure that I obtained representative samples. I used a serrated sharpened steel tube (i.d. 5.4 cm) to extract the soil cores by gently spinning the steel tube into the peat, minimizing soil compaction. I measured the length of the core and the depth of the hole left behind to assess degree of soil compaction, then removed the soil from the barrel of the corer with a PVC plunger. Cores were 20-30 cm in length. While in the field, I subsectioned the soil cores into surface (top) and deep (bottom) sections by dividing the cores at the position of the water table. Because water table position governs the availability of oxygen in the field, the deep sections were stored and transported submerged in bog water to maintain anaerobicity. On the day of sampling, the active layer, a seasonally thawed layer of soil, ranged in depth from 20-30 cm at FB and > 1 m at YCS and OCS. I measured a suite of variables to assess how permafrost degradation and subsequent thermokarst wetland formation impacted conditions. For example, I recorded pH, redox potential (Eh), soil depth to the water table, depth to ice, and temperature (Table 1). Samples were shipped on ice to Arizona

State University for processing and to conduct the soil incubation studies. Soil samples were stored at 4°C until the incubation experiments were conducted.

Incubation Experiment

I conducted peat incubations by placing fresh peat samples (~3 g) in 100 mL Wheaton vials that were fitted with rubber septa and sealed with aluminum crimp rings. Upon removing the stored samples at 4°C, soils were pre-incubated for three days to allow microbial activity to stabilize (Dilly and Nannipieri, 2001). I homogenized peat soils manually by hand and incubated subsections (surface or deep) of the cores in separate vials. To explore the effects of oxygen status on soil system ATP production and to model the oxygen status of the soils in the field, I conducted both aerobic and anaerobic incubation experiments. The position of the water table in these wetlands is dynamic. To experimentally simulate water tables higher and lower than at the time I extracted my cores, I incubated surface soils aerobically and anaerobically and deep soils aerobically and anaerobically. Aerobic incubations were conducted by placing a piece of parafilm to allow for free exchange of O₂ over the incubation jar until the headspace gas was sampled. I placed rubber septa on the aerobic incubation vials for one hour prior to collecting headspace gas. Anaerobic incubations were conducted by flushing the peat sample and Wheaton vial with N₂ for 3 minutes. These vials were immediately capped with rubber butyl stoppers and sealed with aluminum crimp rings. All vials were incubated in the dark at 25°C. Replicate soil samples were sacrificed at 1, 24, 48, 72, and 192 h over the course of the 8-day (192 h) incubation to quantify concentrations of CO₂,

CH₄, and N₂O, as well as ATP content and organic ion content. When soil samples were sacrificed, they were split between destructive sampling for ATP extraction and organic ion content analyzes.

ATP and Soil Ecosystem Power

ATP has long been called the energy currency of the cell (Fillingame, 1999; Itoh et al. 2004; Stock et al. 1999). As such, soil microbes use energy gained from mineralizing soil organic matter to produce ATP. Because microbes play this central role in soil ecosystems by metabolizing C and N to produce energy, I used rates of ATP production as a microbially specific metric of soil system power production. When ATP is hydrolyzed, the high-energy bond between phosphate groups is cleaved. Upon cleaving, ATP is converted to adenosine diphosphate (ADP) and subsequently releases ~32 kJ of energy mol⁻¹ ATP. By measuring the rate of potential ATP production, I was able to quantify the flow of energy through the soil system across the permafrost thaw disturbance gradient of bogs.

Soil ATP content and rates of ATP production were determined by extracting ATP with a trichloroacetic acid (TCA)-based extractant and a luciferin/ luciferase-based assay kit using a 96-well plate luminometer. The ATP extractant solutions were prepared following methods discussed in Redmile-Gordon et al. (2011), which used a modified and updated approach to Jenkinson and Oades (1979). ATP was extracted with a solution of 1.07 M TCA, 0.25 M PO₄³⁻, and 0.6 M Imidazole. To determine the extraction efficiency of the ATP extractant solution, two different ATP extraction solutions were

made: one extractant that contained a known amount of ATP, and a second extractant without ATP. Thus, for every sample, I used two different extractants on subsamples.

After a soil sample had been sacrificed, I added 5 mL of each extractant to ~1 g peat in separate vials. The peat-extractant mixture was sonicated using a Branson 450 Sonifier with a microtip adapter. Each sample was sonicated for 1 minute at 30% power output. The samples were placed on ice for at least 5 minutes and ATP extracts were filtered using Whatman 44 filter paper. Extracts were stored at -20°C until all samples had been collected and extracted. To quantify extracted ATP, I added 50 uL of the ATP extracts to 5 mL of an arsenate-based buffer (Jenkinson and Oades 1979; Redmile-Gordon et al. 2011). I measured ATP using a luciferin-luciferase assay (Molecular Probes, Grand Island, NY). Standard curves were generated and ATP was quantified using a 96-well microplate GloMax luminometer (Promega, Madison, WI). When luciferin and firefly luciferase from the kit were added in the presence of ATP from the soil samples, the reaction produced light of intensities that related to the concentration of ATP in the sample.

Measuring Power Input in Aerobic Incubations: CO₂ Production

I measured the power input (Equation 1) for my aerobic soil incubations by using standard Gibbs energies (ΔG°). To calculate the energy associated with the aerobic oxidation of glucose I used Equation 2. In the aerobic oxidation of glucose the $RT\ln Q$ term is negligible compared to the ΔG° term. That is, the change in Gibbs energies

associated with the oxidation of glucose to CO₂ was so large that the standard Gibbs energy captured the majority of the energy associated with this reaction.

$$\Delta G = \Delta G^\circ + RT \ln Q \quad (2)$$

Under aerobic conditions, glucose is oxidized to CO₂ in the following reaction: C₆H₁₂O₆ + 6O₂ → 6CO₂ + 6H₂O. I measured CO₂ production in my aerobic incubations to determine the potential energy (power input) of the entire surface soil system (denominator of Equation 1). By quantifying the net change in CO₂ concentration through the incubation experiment, I used a stoichiometric mass balance approach to calculate how much glucose would have been used to produce the measured change in CO₂. For example, the standard Gibbs energy produced when 1 mol of glucose is oxidized to CO₂ is -2870 kJ. Theoretically if 6 mols of CO₂ were produced, 1 mol of C₆H₁₂O₆ was oxidized. This would yield -2870 kJ of energy. I used this stoichiometric mass balance approach to calculate the total potential energy within the surface soil system during my aerobic incubations. When I divided the amount of energy in the ATP generated during the incubation experiment from the soil system by the total potential energy within the surface soil system I was able to calculate soil system efficiency (Equation 1).

Measuring Power Input in Anaerobic Incubations: Gas and Organic Acid Measurements

The approach for quantifying anaerobic power input differed from quantifying

aerobic system power input. In the aerobic incubation experiment I relied on standard Gibbs energies (ΔG°) to calculate soil system power output. I used a combination of approaches to determine the power input for the anaerobic soil incubation experiment (Equation 1). In order to assess the prominent metabolic pathways used during the anaerobic incubations I measured CO_2 , CH_4 , and N_2O production. I also measured organic acid production throughout the experiment to quantify to what extent fermentation pathways contributed to the production of power during the incubations. A combination of approaches was necessary to assess the anaerobic soil metabolic pathways, given the complexity of energy producing processes in an anaerobic environment. Once I determined the prominent metabolic pathways that the microbial system used to produce power, I used Equation 3 (below) to calculate the potential energy associated with those particular pathways. Dividing the energy contained in the ATP that was produced by this maximum amount of potential energy in the system yielded the power producing efficiency of the system (Equation 1). More specifically, instead of using standard Gibbs energies (ΔG°) alone to calculate the total potential energy in the system, I used Equation 3 and collected data on CH_4 , CO_2 , and acetate to calculate a reaction specific Gibbs energy that I observed.

$$\Delta G = 2.303RT * \text{Log}(Q/K) \quad (3)$$

where ΔG is Gibbs Energy, R is the gas constant ($8.314 \text{ J mol}^{-1} \text{ K}$), T is temperature ($^\circ\text{K}$), Q is the reaction quotient of the measured reaction (measured from the production of

CH₄, CO₂, and acetate during the anaerobic incubations), and K is the equilibrium constant (calculated value from SUPCRT92). When I divided the total energy in the ATP generated by this number, I calculated system efficiency.

Gas samples were stored in 20 mL vials that were sealed with rubber butyl stoppers and aluminum crimp rings until the incubation study was complete, and that were evacuated and flushed with N₂ prior to sampling. I analyzed the gas samples on a Varian CP-3800 gas chromatograph fitted with an autosampler. Gas fluxes were calculated as the change of the gas concentration between subsequent samplings (e.g. Hall et al. 2008).

Different anaerobic metabolic processes produce different amounts of potential power. Organic acids were quantified in my anaerobic incubation experiments to assess to what extent the soil system utilized fermentation pathways for energy production during the anaerobic incubation experiment. From these data, I disentangled the metabolic pathways that the soil system used to produce power in my incubation experiments. This allowed me to present my results as power output as a function of efficiency, per from Odum and Pinkerton (1955; Figure 7).

Organic acids were extracted at the beginning, middle, and end of the incubations (1, 48, 192 h). I extracted organic acids following methods described by Kane et al. (2013). Briefly, for each extraction, ~1 g of soil was mixed with 10 mL of deionized water and inverted 10 times. After an hour-long equilibration period I filtered the samples using ashed Whatman GF/C filters. Samples were frozen immediately at -20°C. Extracted organic acids were quantified on an ICS 1500 ion chromatograph (Dionex, Bannockburn,

IL). Standard curves were generated using ion chromatograph-grade standards of citrate, formate, lactate, acetate, and propanoate. Deionized water blanks were run approximately every 5 samples.

Statistical Analysis

I used R to perform an Analysis of Variance (ANOVA) on the production rates of ATP across site, soil section, and oxygen status of the incubation (aerobic or anaerobic) among the different sites. I also performed ANOVA tests on the rates of gas production (CO₂, CH₄, N₂O) across site, soil section, and oxygen status of the incubation. In all of my analyses, I conducted multiple post hoc analyses using Tukey's HSD test to compare means that were significantly different. I tested assumptions of equal variance and normality assumptions for the ANOVA models in R by using residuals vs. fitted and normal Q-Q plots, respectively. Data that did not meet the assumptions of the ANOVA were log transformed.

III. Results and Discussion

In the following subsections I discuss: 1) ATP results from my incubation study; 2) aerobic CO₂ production from my incubation; 3) anaerobic CO₂, CH₄, and acetate production results from my incubation study. In the final section, I explicitly tie the results of my incubation experiment, where I measured system power output and system efficiency, to the Odum and Pinkerton (1955) definition of power output as a function of efficiency (Figure 1).

Soil System Power Output

ATP Production Rates Independent of Ecosystem Development Gradient

The rates of ATP production were higher in the surface soil system sections compared to the deep sections regardless of whether or not the incubation experiment was aerobic or anaerobic (Figure 3). This suggested that the surface section soil system was dominated by metabolic pathways that produce more ATP than the deep soil sections (Figure 3). Many studies that measure soil ATP in the literature use ATP as a proxy for bacterial biomass. In their analysis of ATP content as a function of soil depth in forest and arable ecosystems, Vinther et al. (1999) found decreasing ATP content with increasing depth, despite finding higher counts of bacteria at depth. They attributed this finding to a larger population of fungi in surface soils. Additionally, I found significantly higher rates of ATP production ($p < 0.05$) in aerobic incubations compared to anaerobic incubations (Figure 3). Given that aerobic metabolic pathways yield higher levels of ATP production, these results were not surprising. Using oxygen as a terminal electron acceptor yields the most cellular energy. In paddy soil incubations, Inubushi et al. (1989) found that when aerobic soils were incubated anaerobically, soil ATP content dropped more than 90%. Conversely, when anaerobic soils were incubated aerobically they found that ATP content rapidly increased within minutes. The combination of these results suggests that soil systems are strongly influenced by environmental conditions and can quickly shift to the most thermodynamically favorable pathway for the conditions to produce energy to do cellular work.

ATP Production Rates Across the Ecosystem Development Gradient

Across the gradient of ecosystem development, in the aerobic surface soil incubations I found the highest rates of ATP production from the FB site with intact permafrost (Figure 4). I measured the lowest rates of ATP production in YCS, the youngest wetland site that formed following the degradation of permafrost at the FB site. In the aerobic surface soil at the OCS site I found an intermediate rate of ATP production, although this rate of ATP production was not statistically different from the rate of ATP production at the FB site. These results suggest that prior to the permafrost thaw disturbance, the soil system was organized to maximize the flow of energy through the soil system. Following the permafrost degradation event, I saw a marked decrease in power output at YCS compared to FB. Over time, the OCS system appeared to reorganize to a level of ATP production that was statistically the same as the FB system, albeit in a different ecosystem state without permafrost. Fioretto (2009) found similar results in their study of microbial activity along a developmental gradient of Mediterranean ecosystems. They measured higher soil ATP content in ecosystems at later developmental stages and argued that following disturbance, there was an increase microbial activity and soil function. In a study using a seasonal scale to investigate soil microbial response to differing pesticide regimes, Ahtiainen (2003) observed successional patterns in soil ATP content during a growing season. They used ATP as a measure for microbial biomass. They observed low soil ATP content early and late in the growing season and peak ATP content in the summer months. These results suggest that following during the growing season, soil systems organize to maximize energy throughput as measured by ATP.

I observed a similar pattern of ATP production rates across the gradient of ecosystem development in each of the different soil incubations. For example, in the deep soil sections that were incubated aerobically, I observed the highest rates of ATP production at the FB site, lowest rates at YCS, and intermediate rates at OCS. I also observed this pattern in the anaerobic deep sections as well as the surface soil sections that were incubated anaerobically. However, this pattern of ATP production was not as evident in all other incubation conditions compared to the aerobic surface soil incubations. Although these differences were not statistically significant in some cases, this consistent pattern suggested that [regardless of the conditions at each site] rates of power output were highest at the undisturbed site (FB) compared to the system formed immediately following permafrost thaw (YCS). The OCS site soils had higher rates of ATP production compared to YCS, but lower rates than at the FB site, although FB and OCS were statistically similar. This suggested that following the degradation of permafrost, the OCS site had recovered in terms of the rate of power production compared to FB.

When I incubated soils under conditions that simulated different water table depths (i.e. surface soil sections in anaerobic conditions and deep soil sections in aerobic conditions), I observed large changes in ATP production rates. For example, although surface aerobic sections produced the highest rates of ATP production during my incubations, when I incubated those soils anaerobically, the rates of ATP were more comparable to the anaerobic deep soils. The rates of ATP production were statistically similar when soils were incubated anaerobically regardless of their position relative to the

water table. This suggested that regardless of the age of the ecosystem, anaerobic conditions had lower rates of power output and anaerobic conditions negatively impacted the rate of power output across the gradient of ecosystems. When I incubated deep soils that were below the water table aerobically, I saw higher rates of ATP production compared to all anaerobic incubations. This indicated that the soil system was capable of quickly switching to aerobic metabolic pathways to produce higher rates of ATP, given the presence of oxygen.

Aerobic System Efficiency: Aerobic CO₂ Production

The cumulative aerobic production of CO₂ did not differ across the wetland ecosystem development gradient (Figure 5). These results suggest that the corresponding amount of glucose oxidized during the incubation was not different across the wetland ecosystem development gradient. In terms of aerobic surface soil system efficiency, each of the three sites along the chronosequence of wetland development had similar denominators in Equation 1. That is, the total potential energy in the aerobic surface soils was similar across each site in the chronosequence. Across the chronosequence of wetland development, the soil systems appeared to mineralize similar amounts of organic matter to produce energy.

Anaerobic System Efficiency: Anaerobic CO₂, CH₄, and Acetate Production

Across the gradient of wetland development I found evidence for distinct metabolic pathways during my soil incubation experiments. At the FB and OCS sites, the

rates of CO₂ and acetate production were not statistically different from one another (Figure 6). At sites where I observed any CH₄ production (YCS, OCS), the rate of CH₄ production was not statistically different to the rate of CO₂ production (Figure 6). I did not see any CH₄ production from the FB soils. Using these gas and acetate data, I developed theoretical stoichiometric models for the major metabolic pathways the soil system used to produce energy at each site. At YCS, for example, for every 1 mol of CO₂ produced, there was 1 mol of CH₄ produced. The concentration of acetate (CH₃COOH) did not change during the incubation at this site. To summarize the metabolic activity at YCS I used the following equations: C₆H₁₂O₆ → 3CH₃COOH and 3CH₃COOH → 3CH₄ + 3CO₂ for an overall equation of C₆H₁₂O₆ → 3CH₄ + 3CO₂. Because I saw a net increase in acetate concentration at OCS that I did not see at YCS, the following equations summarized the data I observed from the OCS soils: 2C₆H₁₂O₆ → 6CH₃COOH and 3CH₃COOH → 3CH₄ + 3CO₂ for an overall equation of 2C₆H₁₂O₆ → 3CH₃COOH + 3CH₄ + 3CO₂. At the FB site, where I saw acetate and CO₂ production, but not CH₄ production, I developed the following parsimonious model: 2C₆H₁₂O₆ → 6CH₃COOH and 3C₆H₁₂O₆ → 6C₂H₅OH + 6CO₂. To produce CO₂ anaerobically without producing CH₄, different terminal electron acceptors other than CO₂ must be used. Other common terminal electron acceptors include NO₃⁻, SO₄²⁻, Fe³⁺ and Mn⁴⁺. Because my study sites were ombrotrophic and rely on precipitation as the only source of inputs of these electron acceptors, I assumed that concentrations of these terminal electron acceptors were too low to account for more than a small fraction of the observed anaerobic CO₂ production at the FB site. In their incubation study to explore the source of anaerobic CO₂ production

in Alaskan peat, Duddleston et al. (2002) found that nitrate, sulfate, and iron reduction could not account for more than 15% of the observed anaerobic CO₂ production. They attributed the bulk of the anaerobic CO₂ production to fermentation processes. In another anaerobic peat incubation study, Metje and Frenzel (2005) observed very high concentrations of ethanol production. They argued that this intermediate pool of ethanol was slowly converted to acetate throughout their month-long study. By determining the metabolic pathways that these systems were using to produce power, I was able to calculate the theoretical maximum amount of energy produced by each pathway (input, Equation 1) and compare that to the amount of energy captured in ATP production (output, Equation 1) to calculate system efficiency. Depending on the pathway used to produce energy, power-producing efficiencies change. For example, the conversion of glucose to acetate yields 207 kJ mol⁻¹ glucose, CO₂ and CH₄ production from acetate yields 31 kJ mol⁻¹ acetate, and the production of ethanol and CO₂ from glucose yields 235 kJ mol⁻¹ glucose. In other words, different likely metabolic pathways produced different energy yields that influenced my calculations of system efficiency.

Soil Systems And the Maximum Power Principle

To summarize the relationship between power output and efficiency, I plotted power output as a function of system efficiency from my incubation experiments as per Odum and Pinkerton (1955; Figure 7, based on Figure 1). I found very low power-production efficiencies; that is, the amount of energy in the ATP that was generated during the incubation experiment was very low relative to the total potential energy of the

metabolic pathways that the soil systems used. Efficiencies ranged from 0.069% in the anaerobic deep soil incubation at YCS to 2.34% in the aerobic surface soil incubation at FB. In other words, the FB soil system converted 2.34% of the whole-system potential energy into actual usable cellular energy to do work. While soil systems are complex, soils are not complete ecosystems. Soil systems in ombrotrophic bogs rely on C inputs from macrophytic photosynthesis and precipitation for various elements. The low efficiencies I observed relative to what Odum and Pinkerton (1955) proposed may be attributed to the fact that my soil systems did not include the whole-system energy transformations at the ecosystem level. It is possible that using the heterotrophic soil system as a model system limited the potential power and efficiency of my soil system study. However, multiple ecosystem studies have observed varying efficiencies across ecosystem type and efficiency metric. Using the ratio of respiration to total input of organic carbon (a variation of output/input, Equation 1), Fisher and Likens (1972) calculated an ecosystem efficiency of 34% for a stream. In another study of carbon use efficiency (respiration/ NPP), Chambers et al. (2004) measured ecosystem efficiency of a tropical forest of 30%. While neither of these ecosystem-level studies measured an optimum efficiency for maximum power production, they both calculated much higher levels of system efficiency than I did in my soil system study, suggesting that when the ecosystem scale is used higher system efficiencies are observed.

Besides observing very low soil system efficiencies, I saw a distinct separation between the surface aerobic soils and the deep anaerobic soils when the power output was plotted against efficiency (Figure 7). Aerobic surface soil systems had both a higher

efficiency and a higher power output. Given that metabolic processes that use O₂ as a terminal electron acceptor to produce energy are inherently more efficient than anaerobic processes, this was not surprising. Higher power output from aerobic processes was also expected. Reactions that use oxygen as a terminal electron acceptor yield the maximum possible cellular energy. This is evident in the oxidation of glucose (-2870 kJ mol⁻¹ glucose) when compared to the conversion of glucose to acetate anaerobically (-207 kJ mol⁻¹ glucose). I also found greater variability in power production and system efficiency among the aerobic soil incubations when compared with the anaerobic soil incubations. Under anaerobic conditions there was much less variability in power output and power-producing efficiency across the gradient of ecosystem development (Figure 7).

Using the MPP and plotting power output as a function of system efficiency I quantified the effects of permafrost degradation on the flow of energy through these soil systems. Across the gradient of wetland ecosystem development in the surface soil sections, the site that had not experienced permafrost degradation disturbance (FB), showed the highest power output and efficiency. Following the permafrost degradation disturbance, the youngest wetland soil (YCS) showed the lowest efficiency and lowest power output. As the wetland ecosystem aged and developed it produced similar levels of power production (Figure 4) across the ecosystem gradient. That is, the OCS site appeared to have developed and organized to produce rates of power production that were similar to the pre-disturbance ecosystem (FB). Although I measured similar rates of ATP production at OCS compared to FB, it was in a markedly different ecosystem state.

Plotting power output as a function of system efficiency along the gradient of

wetland development allowed me to explicitly link the MPP with ecosystem development (Odum, 1969; Figure 7). More specifically, this figure shows the trajectory of power output and efficiency along a gradient of ecosystem development in aerobic surface soil and anaerobic deep soil following permafrost degradation. In his classic ecosystem development model, Odum (1969) argued that as systems develop, local system entropy decreases while total system entropy must increase. Furthermore, Odum and Pinkerton (1955) argued that as systems develop, more power (or work per unit time) must be relegated to fight total system entropy to maintain low local system entropy.

IV. Conclusions

In this study I used the MPP as a guiding framework to test the extent to which developing ecosystems follow the thermodynamic principles outlined by Odum and Pinkerton (1955). I found that shortly after the permafrost degradation disturbance, wetland soil ecosystems produced significantly less power and became less efficient. Along the gradient of ecosystem development I found that over time the soil system power output recovers, albeit in a different ecosystem state. That is, following disturbance and the subsequent change in ecosystem state, the wetland soil system developed to maximize power production. In addition to a marked reduction in system power output following disturbance, soil systems experienced a reduction in power production efficiency. This suggests that during ecosystem development, systems reorganize to enhance power output as well as system efficiency. In this study I found no evidence of an intermediate optimum efficiency because I observed power producing

efficiencies much lower than the 50% that Odum and Pinkerton (1955) argued would be optimal. I posit that the MPP can be a powerful tool to help explain how ecosystems develop following a permafrost degradation disturbance in northern wetlands, but the entire ecosystem should be studied when doing so.

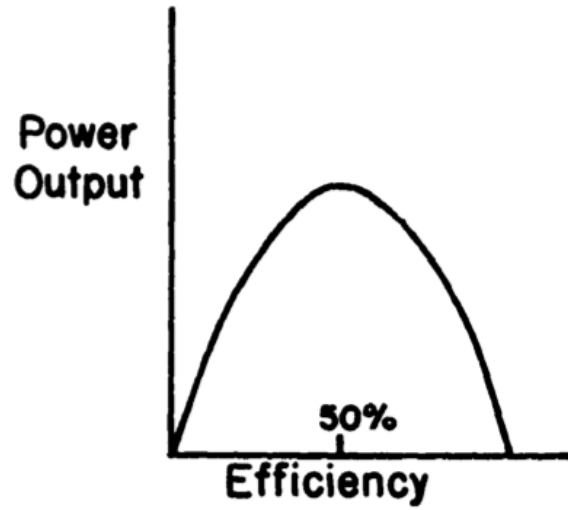


Figure 1. System power output as a function of efficiency. Maximum power production occurs at some intermediate efficiency, not maximum efficiency (From Odum and Pinkerton, 1955).

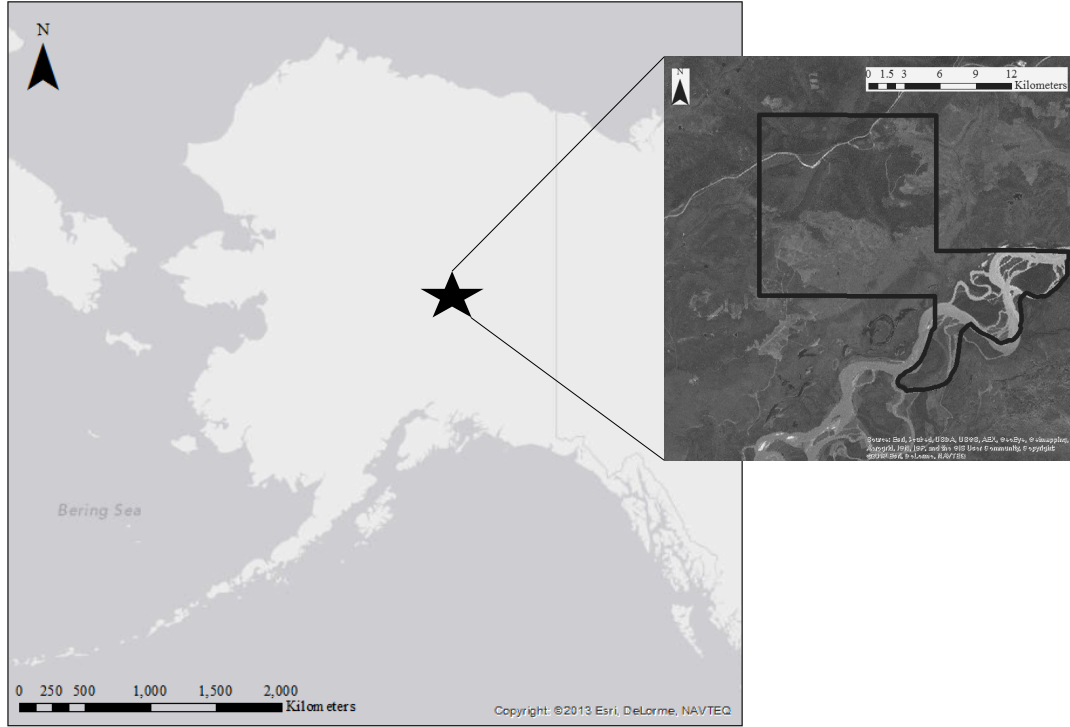


Figure 2. Map of the study site outside of the Bonanza Creek Long Term Ecological Research (BNZ LTER) station. The location of the star indicates Fairbanks, Alaska. The inset image shows the boundary of BNZ LTER station 35 km southeast of Fairbanks. I sampled just outside of the BNZ LTER in the Tanana River Flats.

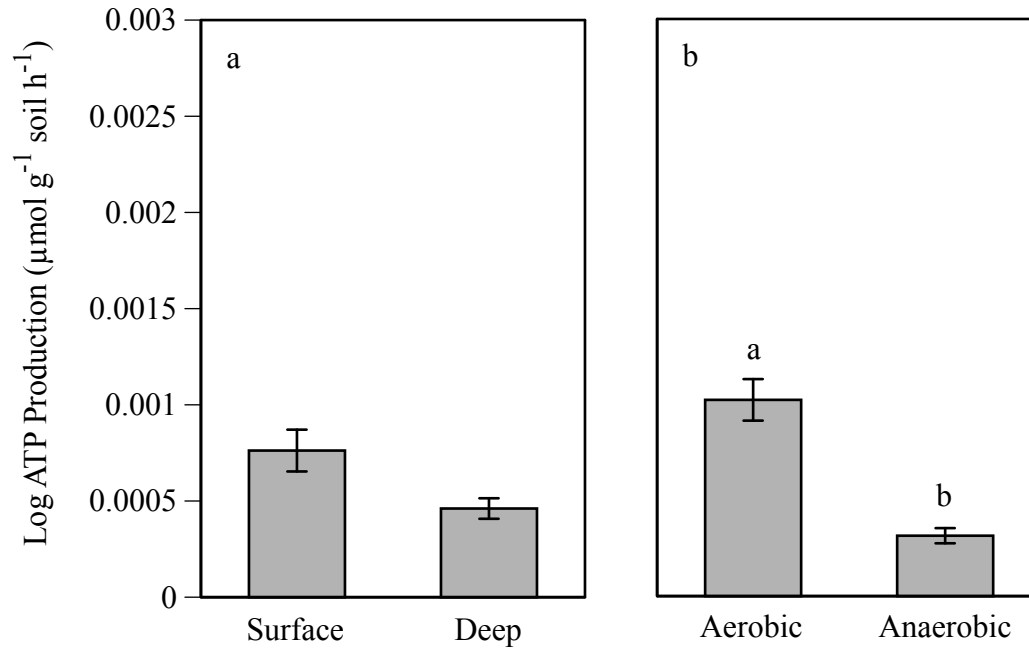


Figure 3. (a) Rate of ATP production from soils from surface and deep soil sections regardless of oxygen status of incubation. Soils that originated from the surface section had higher rates of ATP production. (b) Rate of ATP production in aerobic and anaerobic incubations regardless of section of soil. Aerobic incubations had significantly higher rates of ATP production relative to the anaerobic incubations.

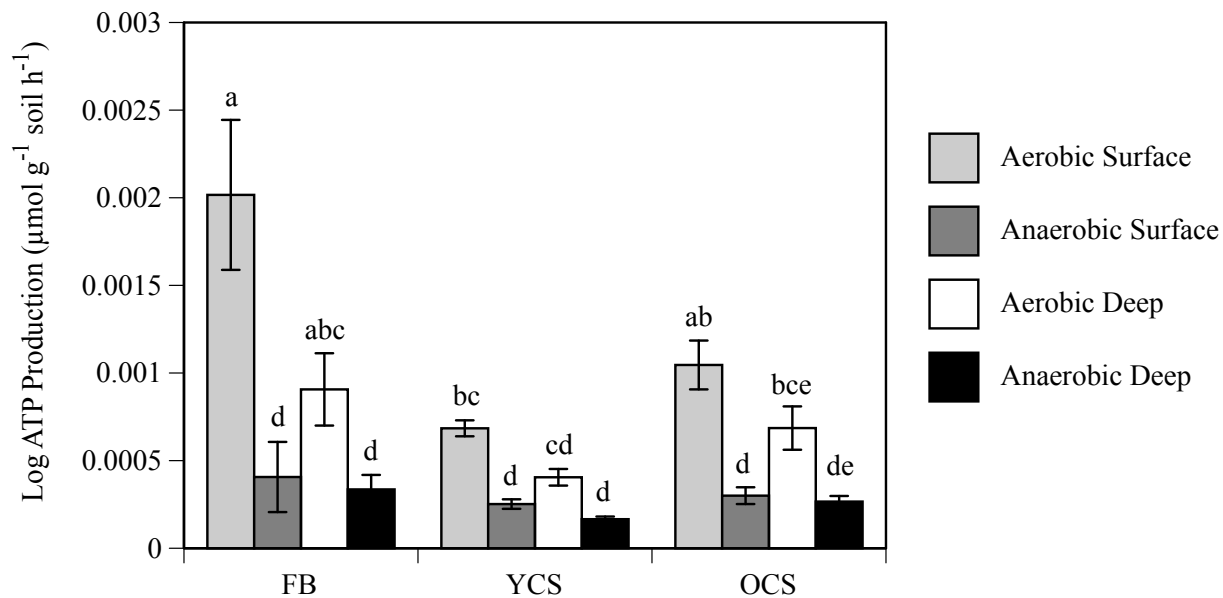


Figure 4. Rates of ATP production from the surface peat section (above water table) and deep peat section (below water table) during the aerobic and anaerobic incubation experiments from each site. FB had the highest rates of ATP production followed by OCS, and YCS. Different letters depict statistically different ($p < 0.05$) results from Tukey's post hoc comparison.

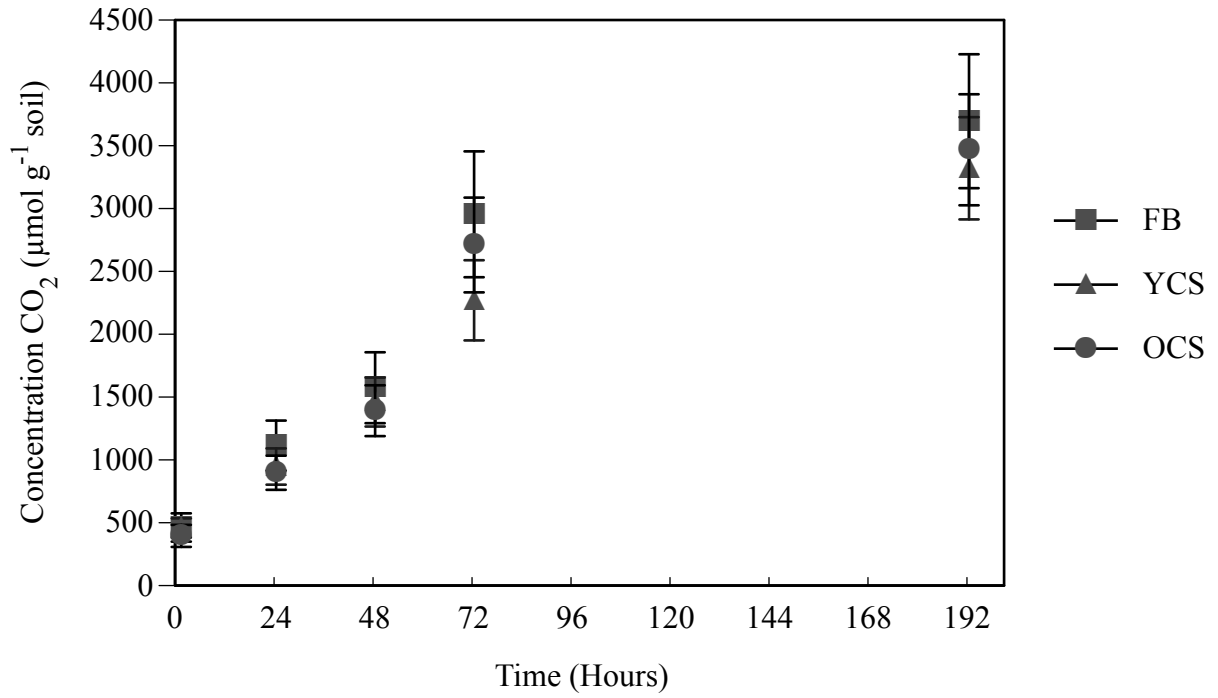


Figure 5. CO₂ production during the aerobic incubation experiment in the surface soil sections. All three sites produced similar amounts of CO₂.

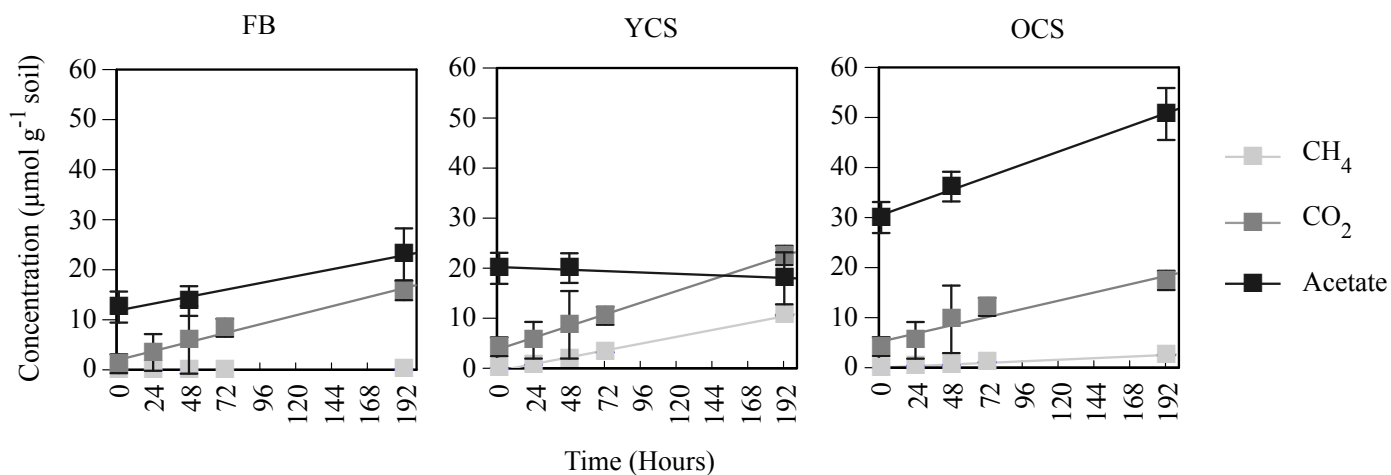


Figure 6. Concentration of CO_2 , CH_4 , and acetate during the anaerobic incubation experiment in the FB (left panel), YCS (center panel), and OCS (right panel). I used multiple t-tests to test whether or not these slopes were significantly different from one another. With the exception of acetate in the YCS panel, all of the other slopes were not statistically different from one another.

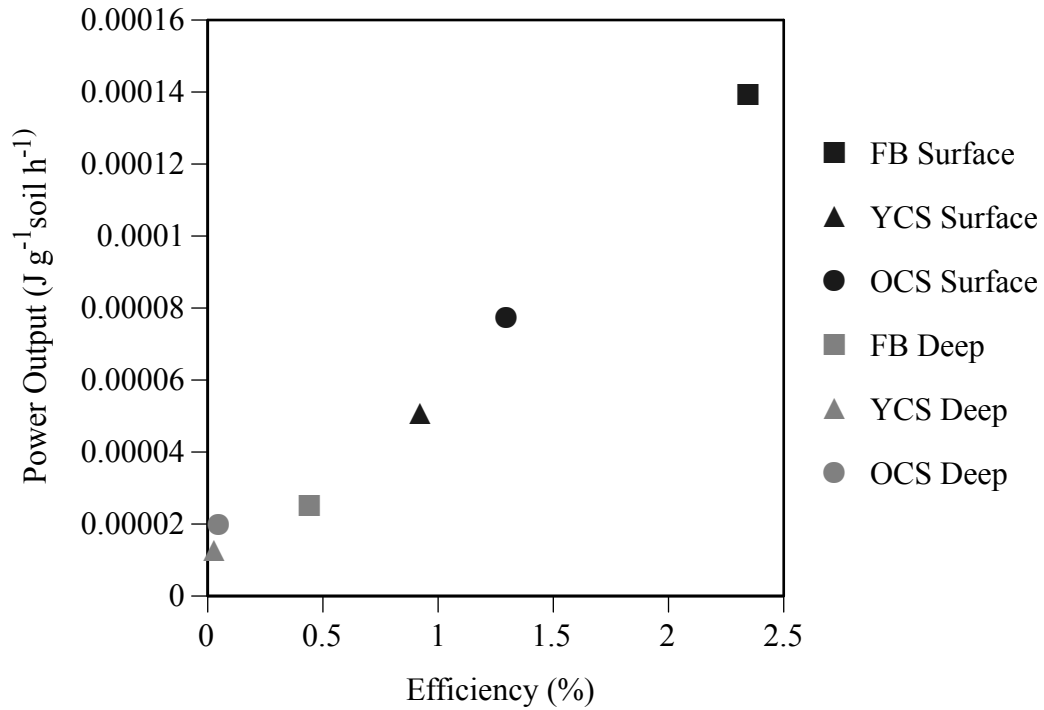


Figure 7. Power output as a function of soil system efficiency. The higher the system efficiency, the higher the power output. There is a clear distinction between the aerobic surface soils (black), and the anaerobic deep sections (gray). Data from surface soil sections incubated anaerobically and deep surface sections incubated aerobically are not shown.

Environmental characteristics of each site

Site	Ice Depth (cm)	SE	Water Table Depth (cm)	SE	Eh (mv)	SE	Temperature (°C)	SE	pH	SE
FB	27	±2.42	N/A	N/A	312	±112	8.02	±1.21	4.81	N/A
YCS	N/A	N/A	5.8	±0.8	-153	±57	12.58	±1.38	5.16	±0.24
OCS	N/A	N/A	8	±0.71	-4	±44	10.2	±0.50	5.17	±0.33

Table 1. Forested bog (FB) was the only site with ice present at the time of sampling. Following permafrost degradation and subsequent wetland formation, there were changes associated with depth to water table and redox potential (eh). Values reported are means ± SE. YCS: young collapse scar, OCS: old collapse scar.

CHAPTER 4

SOIL MICROBIAL COMMUNITIES AND GREENHOUSE GAS EMISSIONS IN BOREAL WETLANDS OF DIFFERENT AGES

Abstract

Northern ecosystems, particularly peatlands, play a disproportionately large role in the global carbon cycle. Over thousands of years, northern peatlands have sequestered carbon (C), as ecosystem production is greater than ecosystem respiration and disturbance losses of soil C. However, these ecosystems are undergoing extensive and rapid transformations as a result of climate change; their role as C sinks may be reversing as stored C is increasingly being lost via accelerating greenhouse gas emissions. In this study, I used laboratory soil incubations to investigate the production of CH₄, CO₂, and N₂O, because of their global warming potentials, along a chronosequence of wetlands that formed as a result of permafrost thaw. Because soil microbes catalyze metabolic reactions that produce greenhouse gases, I quantified the abundance of a gene that produces an enzyme involved with methanogenesis to link the microbial community to ecosystem level processes. I used the 16s rRNA gene to quantify total microbial abundance and the *mcrA* gene to quantify the abundance of Archaeal methanogens along the chronosequence of ecosystem development. I found that CO₂, and N₂O production was generally lower in anoxic soils compared to oxic soils— that is, in soils below the water table and CH₄, was generally higher in anoxic soils. Additionally, observed N₂O fluxes were of the same magnitude as CH₄ fluxes suggesting that N₂O fluxes may play a larger role in boreal ecosystems greenhouse gas production than previously thought. Along the gradient of

wetland development, I found that the young collapse scar site (YCS)—formed ~60-100 years following thaw—produced the highest levels of CH₄ when compared with a forested bog site (FB) that was unimpacted by thaw disturbance. At the old collapse scar bog site (OCS), that collapsed and formed ~400 years ago, I observed much lower rates of CH₄ compared to YCS. This suggests that as wetlands form from permafrost degradation, there is a large but transient pulse of CH₄ emissions. Methanogen abundances exhibited a strong positive relationship with CH₄ production rates. This study provides centuries-scale insights into the production of CH₄, CO₂, and N₂O emissions along a gradient of wetlands that developed from degradation of permafrost and suggests that large changes in functional group contributions to the soil microbial community likely happen after a permafrost thaw disturbance.

I. Introduction

High latitude peatlands play a disproportionately large role in the global carbon (C) cycle. Owing to thousands of years of net organic matter accumulation, northern peatlands store ~500 Gt C, roughly equivalent to 20% of the global soil C on earth, but only occupy ~3% of the global land area (Maltby and Immirzi 1993; Yu 2011). Direct and indirect effects of climate change appear to be altering the role that northern ecosystems play in the global C cycle (Belyea and Malmer 2004; Davidson and Janssens 2006; Gorham 1991; McGuire et al. 2009; Schuur et al. 2008). For example, recent studies have found increases in arctic thaw season length (Markus et al. 2009), which has implications for the C cycle as discussed by Chapin et al. (2005), shifts in genes that regulate C metabolism in soil microbial systems following permafrost degradation (Mackelprang et al. 2011), and an increase in permafrost degradation leading to wetland formation in ice rich permafrost (Jorgenson et al. 2001). These large-scale changes in boreal peatland ecosystems may be reducing the role of these ecosystems in the global C cycle as net C sinks (Zhuang et al. 2007).

Despite functioning as net C sinks over the long term, peatlands are also important sources of greenhouse gas emissions, such as CH₄, and to a lesser extent N₂O (Chivers et al. 2009; Euskirchen et al. 2014; Repo et al. 2009; Turetsky et al. 2008; Turetsky, et al. 2002). Because of their role in the global carbon cycle and their impact on the global climate, quantification of these greenhouse gas fluxes is essential. Though CH₄ and N₂O emissions from peatlands are typically lower than CO₂ releases, their global warming potential is 25 times and 298 times greater at the century time scale,

respectively, than CO₂ (Forster et al. 2007). The production of CO₂, CH₄, and N₂O are largely driven by enzymatic reactions catalyzed by microbes. Changes in ecosystem-level characteristics, including ecosystem change induced by permafrost degradation, are often accompanied by changes in microbial community composition and function (Mackelprang et al. 2011; Ohtonen et al. 1999; Panikov 1999; Zogg et al. 1997). These microbial community changes have large potential impacts on ecosystem function. For example, Hawkes et al. (2005) found that plant invasion changed how nitrogen cycled in a California grassland by modifying the component of the soil microbial community responsible for nitrification. The large role that the microbial community plays in ecosystem function—particularly with C and nitrogen (N) metabolism and the production of greenhouse gases—has been extensively documented. However, fewer boreal peatland studies have examined soil microbial community changes and resulting impact on greenhouse gas emissions following a thaw disturbance (Christensen et al. 2004; Hultman et al. 2015; Waldrop et al. 2012). This is the primary goal of this paper.

Climate change has increased the vulnerability of permafrost in high latitude ecosystems to thaw because of warmer soil temperatures (Schuur et al. 2008). Permafrost governs both the structure and function of these C-rich ecosystems (Grosse et al. 2011; Harden et al. 2006; Jorgenson et al. 2010; Yang et al. 2010). For example, the degradation of permafrost and subsequent formation of wetlands in these northern ecosystems has large implications for how both C and N are cycled (Chivers et al. 2009; Jorgenson et al. 2001). In areas of interior Alaska where ice-rich permafrost thaws, vegetation above the permafrost often collapses into relic ice space. This typically results

in wetland formation as the soil collapse means that more of the soil is below the water table. This formation of wetlands is expected to stimulate the production of CH₄, while diminishing CO₂ emissions (Moore and Roulet 1993). The relationship between boreal wetland ecosystems and their emissions of CH₄ is one of positive feedbacks (Figure 1). For example, in general, warmer atmospheric temperatures lead to increased permafrost thaw. As previously mentioned, lowland areas that experience permafrost degradation are favorable areas for wetland formation and the amount of soil exposed to anoxic conditions increases. These wetland soil conditions lead to an increase in the size of the methanogen community, resulting in enhanced emissions of CH₄. Higher CH₄ emissions increase the global concentration of atmospheric greenhouse gases, further warming the atmosphere (Figure 1). In a ecosystem-scale peatland water table manipulation study, Turetsky et al. (2008) found large increases in CH₄ fluxes in experimentally flooded peatlands that were warmed and the lowest CH₄ fluxes in an unwarmed peatland that had its water table experimentally lowered. The position of the water table relative to the soil surface was found to be the strongest predictor of CH₄ emissions. Because water table position governs emissions of CH₄, and because permafrost degradation and subsequent soil collapse increase the relative position of the water table, wetland formation from permafrost degradation should increase emissions of CH₄ from these ecosystems.

Owing to their large role in the global C cycle, many studies have quantified rates of greenhouse gas releases from northern peatlands. Studies have examined greenhouse gas fluxes from peat slurries using laboratory incubations (Moore and Dalva 1997), from peat mesocosms exposed to water table manipulations (Blodau et al. 2004; Blodau and

Moore 2003), after ecosystem-scale water table manipulations (Turetsky et al. 2008), and at the landscape scale using eddy covariance techniques (Euskirchen et al. 2014). In addition to these empirical quantifications of greenhouse gas emissions, Blodau (2002) reviewed the mechanisms of C cycling in peatlands and discussed fluxes of CO₂ and CH₄ and controls on these fluxes.

Despite the breadth of studies that have quantified greenhouse gas emissions from peatlands at many scales, as well as review articles that have examined the factors that control C emissions from these ecosystems, few studies have compared greenhouse gas emissions across a gradient of wetland development following permafrost thaw disturbance. As such, my goal was to quantify greenhouse gas emissions across a chronosequence of wetland development and to relate abundances of functional genes, and thus key microbial communities, in soils with the production of greenhouse gases. I used a space-for-time substitution to assess long-term changes in greenhouse gas fluxes following permafrost degradation and concurrent changes in microbial functional gene abundances, as a surrogate for the abundance of key functional groups in the soil microbial community.

The objectives of this study were: 1) to measure elemental constituents of peat soil as an indicator of substrate lability; 2) to quantify aerobic and anaerobic emissions of CH₄, N₂O, and CO₂ using a laboratory incubation study; 3) to quantify the abundances of 16s and mcrA microbial genes, and; 4) to relate microbial functional gene abundances to ecosystem processes by linking Objectives 2 and 3. I addressed all four objectives at three sites arrayed along a chronosequence gradient of wetland development.

Across the gradient of wetland ecosystem development, I hypothesized that the subsequent inundation that created anoxic conditions would lead to enhanced emissions of CH₄ and N₂O. Because microbial metabolic processes are inextricably linked to these gas fluxes, I expected to see higher abundances of genes responsible for the production of CH₄ (*mcrA*) in soils from the newly inundated site compared to the site with intact permafrost. I also expected to see higher CO₂ fluxes at my undisturbed forested bog site. Finally, I expected to find lower rates of CH₄ production and lower abundances of methanogens at the oldest wetland site compared to the more recently inundated site because the greater distance to the water table in the oldest bog decrease suitable habitat for methanogens and methanogenesis.

II. Methods

Site Description

My study was conducted in the Alaska Peatland Experiment (APEX) sites, situated near the Bonanza Creek Experimental Forest in interior Alaska, roughly 35 km southeast of Fairbanks. I sampled three sites along a post-thaw gradient: a forested bog with intact permafrost (FB), a young collapse scar bog where permafrost degraded and the soil collapsed roughly 60-100 years ago (YCS), and an older collapse scar bog where permafrost degraded and soils collapsed about 400 years ago (Figure 2; OCS). Both YCS and OCS are characterized by a relatively high water table through the season: both have extensive black spruce tree (*Picea mariana*) dieback as well as *Sphagnum* and *Carex* species indicative of wet, waterlogged soils (Camill et al. 2001; Jones et al. 2012). At

each site I measured a suite of environmental variables, including water table depth, pH, redox, and temperature (Table 1).

Soil Sampling

I collected five soil cores each from the FB, YCS, and OCS sites using a sharpened steel core tube (i.d. 5.4 cm). Soil cores were approximately 30 cm in length. The exact core locations were haphazardly chosen, but represented the microtopographical features at each site: Hummocks, hollows, and carpets. All cores were sectioned in the field at the water table depth. The bottom section of soil core that fell below the water table was stored in bog water to maintain anaerobicity. All soil cores were stored in sealed bags on ice and were shipped on ice to Arizona State University for further analysis. Once there, samples were stored in at 4°C until the soil incubation study was conducted. While still in the field, I also collected a subsample of each soil core from the surface and deep sections for molecular analysis; these subsamples were treated the same as the cores from which they were taken.

Soil Incubation Study and Greenhouse Gas Quantification

To measure greenhouse gas emissions across the gradient of wetland ecosystem development, I conducted a short-term aerobic and anaerobic soil incubation study with homogenized peat soil cores. After removing the soil cores from the freezer and allowing a three-day equilibration period, the soils were homogenized by hand using scissors. The three-day equilibration period allowed the soils to reach ambient temperature conditions

(Dilly and Nannipieri 2001). Roughly 5g of homogenized soil was placed into each 120 mL glass Wheaton vial for the incubations. The aerobic soils were incubated in vials that were covered with parafilm to maintain field moisture conditions and to allow diffusion of O₂ across the permeable membrane. Prior to each gas-sampling period, I removed the parafilm from the vials and placed a rubber butyl stopper on top of the vial for 1 h. The head gas in the vials was sampled at 1, 24, 48, 72, and 192 hours using a plastic syringe with a 22-gauge needle. Gas samples were stored in pre-evacuated 20 mL Wheaton vials until analysis. After sampling, I replaced the parafilm on the Wheaton vials. For the anaerobic soil incubations, prior to the beginning of the experiment, I flushed the vials fitted with butyl rubber stoppers and aluminum crimp rings with N₂ gas for 3 minutes. I analyzed the gas samples on a Varian CP-3800 gas chromatograph fitted with an autosampler and a flame ionizing detector column. Standard curves were generated using purchased standard gases. Gas fluxes were calculated as the slope of the gas concentration curve over the 192 h sampling period (per Hall et al. 2008).

Carbon and Nitrogen Content

To quantify total carbon and nitrogen, I oven-dried soil subsamples for 1 week at 65°C, then ground the soils using a Mini-Wiley Mill first (Thomas Scientific, Swedesboro, NJ) and then with a 8000D Dual Mixer/ Mill (SPEX CentiPrep, Metuchen, NJ). This second grinding step maximized the homogeneity of the samples prior to analysis. Carbon and N content were quantified on a Perkin Elmer 2400 CHN Analyzer (Waltham, MA).

DNA Extractions and Quantitative PCR

I extracted DNA from each of the 5 cores and 2 soil sections at each site as duplicates from soil subsamples using a PowerSoil DNA extraction kit (MoBio). I quantified the concentrations of extracted DNA using a QuBit 2.0 Fluorometer (Invitrogen), then diluted the duplicate DNA extraction with the highest concentration of DNA to $\sim 5 \text{ ng uL}^{-1}$ for use in downstream analyses.

I used quantitative PCR (qPCR) to assess the relative and absolute abundances of methane-producing Archaea and the total microbial community size across the chronosequence of wetland development. For Objective 3, I targeted *mcrA*, an Archaeal gene that releases methane while catalyzing the reduction of a methyl group attached to coenzyme-M (Thauer 1998). To account for the potential effects of different sizes of microbial communities along this gradient, I also quantified the absolute and relative abundances of the 16s rRNA gene.

To quantify absolute abundances of 16s rRNA and *mcrA* genes, I developed qPCR standards by PCR-amplifying the 16s rRNA gene and *mcrA* gene using primers specific to those genes. I amplified the 16s rRNA gene from bacteria and Archaea using the following universal primers: 5' GTGCCAGCMGCCGCGGTA 3' (515f; Reysenbach et al. 1992) for the forward primer and 5' CCCCGYCAATTCMTTTRAGT 3' (909r; Colquhoun et al. 1998) for the reverse primer. I used the following thermocycler conditions for 16s rRNA PCR: 98° for 30 seconds, then 25 cycles of 98° C for 10 seconds, 63° C for 15 seconds, and 72° C for 15 seconds, and finished with a final 72° C 2 minute annealing step.

To amplify *mcrA* I used the following primers: 5' GGTGGTGTMGGDTTCACMCARTA 3' (Steinberg and Regan 2008) for the forward primer and 5' CGTTCATBGCGTAGTTVGGRTAGT 3' (Steinberg and Regan 2008) for the reverse primer. I optimized the PCR for *mcrA* with the following conditions: 98° for 30 seconds, then 25 cycles at 98° C for 10 seconds, 58°C for 15 seconds, and 72°C for 15 seconds and I completed the final annealing reaction at 72°C for 2 minutes. The 16s rRNA gene and *mcrA* PCR amplicons were inserted into a pCR4-TOPO vector containing a gene for ampicillin resistance and transformed into competent *E. coli* cells. I plated the *E. coli* cells on LB agar with ampicillin and those that were successfully transformed with the amplicon inserts conferred resistance to ampicillin and grew on the agar plate. I verified that the colonies that grew on the agar plate had the plasmid by performing colony PCR. That is, I used transformed *E. coli* cells that grew on LB ampicillin plates as template DNA for my PCR reactions to verify that the plasmids contained the correct insert. I visualized these PCR products using 1% agarose gel electrophoresis and verified that the gene fragment was the correct size. I inoculated one colony for each standard in liquid LB medium and extracted and purified the plasmid DNA using a plasmid purification kit (MP Biomedicals). I quantified the concentration of the extracted and purified plasmid and linearized each plasmid to create standards using a PstI restriction digest. These linearized plasmids were used directly in qPCR as template DNA. I calculated the number of gene copies and generated a 6-point standard curve by serially diluting the linearized plasmids containing the genes of interest from 46 – 460,000 gene copies, including a no template control.

Using the linearized plasmids containing the genes of interest as standards, I conducted qPCR using a 384 well format ABI7900HT thermocycler and Maxima SYBR Green qPCR Master Mix (Thermo Scientific) in 20 μ L reactions. For *mcrA* qPCR reactions, I used 1 ng of template DNA and 0.3 μ M forward and reverse primers. For 16s rRNA qPCR reactions I used 50 pg of template DNA and 0.3 μ M forward and reverse primers. I used a three-step cycling protocol with the following conditions: Initial denaturation at 95°C for 10 minutes, then 40 cycles of denaturing at 95°C for 15 seconds, annealing at 62°C for the 16s rRNA gene and at 58°C for the *mcrA* gene for 30 seconds, and extension at 72°C for 30 seconds. I also performed a melting-curve analysis to verify that I did not observe any primer dimers. I ran all of my samples and standards in duplicate.

Statistical Analysis

I used R to perform an Analysis of Variance (ANOVA) on C:N ratios across site and soil section. I also performed ANOVA tests on the rates of gas production (CO_2 , CH_4 , N_2O) across site, soil section, and oxygen status of the incubation as well as gene copy numbers. Where I found significant results in the ANOVAs, I conducted multiple post hoc analyses using Tukey's HSD test to identify means that were significantly different. I tested assumptions of equal variance and normality assumptions for the ANOVA models in R by using residuals vs. fitted and normal Q-Q plots, respectively. Data that did not meet the assumptions of the ANOVA were log transformed.

III. Results and Discussion

In the following subsections I discuss: 1) C:N ratio results as a measure of soil organic matter quality across depth and site; 2) CO₂ fluxes from surface and deep peat soil incubations; 3) CH₄ fluxes from surface and deep peat soil incubations; 4) N₂O fluxes from surface and deep peat soil incubations, and; 5) 16s rRNA and mcrA gene abundances across the gradient of wetland development. Finally, I relate the abundance of microbial functional genes to ecosystem processes across the gradient of wetland development.

Soil Organic Matter Quality By Section and Site

Carbon:N ratios are often used as general indicators of soil organic matter and litter quality and thus to predict decomposition rates (Taylor et al. 1989). Microbial activity is a major factor in soil decomposition and, given physiological nutrient limitations, soil organic matter with a low C:N ratio will generally decompose more rapidly than a soil with a high C:N ratio (Hu et al. 2001). This is because N availability often limits the rate of microbial decomposition. In northern bog ecosystems, as N limitation is relaxed, rates of peat decomposition increase (Bragazza et al. 2006). The C:N ratios that have been reported for high latitude bog soils range from 20-100, but more typically range from 30-50 (Aitkenhead and McDowell 2000; Hodgkins et al. 2014; Lin et al. 2012). The peat soil C:N ratios were between 20-35—well within these previously reported values. I found that peat C:N ratios varied by depth in the soil profile (Figure 3; 1st panel), with lower ratios in deep peat compared to surface soil peat; I found

no differences in C:N ratios among the sites (Figure 3; 2nd panel). Additionally, peat C:N ratios did not vary significantly by depth within a site except at FB, where the surface peat had a significantly higher C:N ratio (Figure 3; 3rd panel). This lower C:N ratio at depth may be attributed to the lower position of the water table at the FB site. This may be attributed to higher loading of organic matter from plant matter at the soil surface at FB or carbon being lost to the atmosphere from the deep peat soil (Kuhry and Vitt 1996; Kuhry and Vitt 1992; Malmer and Holm 1984). These results suggested that either the higher water table at YCS and OCS compared to FB inhibited rates of decomposition and carbon mineralized to the atmosphere thus decreasing C:N ratio or surface soil at each site experienced organic matter loading.

CO₂ Fluxes

In general, CO₂ production did not significantly vary across the sites. However, I found significant differences in CO₂ production by peat depth and oxygen status of the soil incubation experiment (Figure 4). The mean aerobic CO₂ production across the surface soils from the three sites was 16.63 mg g⁻¹ soil d⁻¹ and mean aerobic CO₂ production the deep soil sections was 7.44 mg g⁻¹ soil d⁻¹. CO₂ production was significantly lower in bottom soil sections ($p < 0.001$) and in anaerobic incubations ($p < 0.001$). The rate of anaerobic CO₂ production was two orders of magnitude lower than in the aerobic incubations. In their study that quantified the effects of permafrost thaw on carbon emissions that included thermokarst wetlands and bogs underlain by permafrost, Wickland et al. (2006) also found that *in situ* soil respiration rates did not differ among

ecosystem type. Deep anaerobic sections of peat soil cores showed significantly lower CO₂ production regardless of whether the soil was incubated with or without oxygen.

Soils were collected at two depths: above and below the water table and incubated under aerobic conditions and anaerobic conditions. The mean CO₂ production rate across all depths and sites was also two orders of magnitude higher in the aerobic incubations relative to anaerobic conditions (12.03 mg CO₂ gdw⁻¹ d⁻¹ and 0.1449 mg CO₂ gdw⁻¹ d⁻¹, respectively). Similar results have been observed in other peat soil incubation studies. Moore and Dalva (1997) found that anaerobic CO₂ production rates were less than two orders of magnitude lower aerobic CO₂ production rates, though they observed lower rates of aerobic CO₂ production and higher rates of anaerobic CO₂ production during their study than I did. Moore and Dalva (1997) found that aerobic CO₂ production was related to the origin of the plant matter as well as location relative to the water table. For example, they found the highest CO₂ production rates from soils that were located at the water table. They found intermediate CO₂ production rates from soils from above the water table and the lowest CO₂ production rates from soils below. In regards to the origin of the plant matter, Moore and Dalva (1997) found highest rates of aerobic CO₂ production using herbaceous peat followed by bryophyte and ligneous peat. In their anaerobic incubations they found similar rates of CO₂ production from peat of herbaceous and bryophyte origin and lower CO₂ production from ligneous peat. As observed by Moore and Dalva (1997), I found higher rates of anaerobic CO₂ production from peat soil of bryophyte origin (YCS and OCS) compared to more the more ligneous peat at the black spruce tree dominated FB site.

Generally, I found lower CO₂ production rates from deep soil sections (i.e. those below the water table) compared to CO₂ production rates from surface soils; however, across the gradient of wetland development this pattern was not consistent. In my anaerobic soil incubations I observed higher CO₂ production rates from surface soil despite it being collected from above the water table, though this pattern was not significant at the FB or YCS site. Similarly, with the exception of the FB site, I observed similar rates of CO₂ production from deep and surface sections during aerobic soil incubations.

Across the gradient of wetland ecosystem development, I found a suggestion of lower rates of CO₂ production from YCS compared to OCS and FB, although these differences were not significant. During permafrost thaw and subsequent wetland formation, newly formed anaerobic zones depress CO₂ production rates while enhancing CH₄ emissions (Blodau et al. 2004; Turetsky et al. 2008). Conversely, it has been documented that lowering the water table of a peatland enhances CO₂ emissions (Moore and Knowles 1989). In a large-scale peatland water table manipulation study Kane et al. (2013) measured higher CO₂:CH₄ ratios from a drained peatland compared to a control peatland. This phenomenon was driven by higher CO₂ emissions from the oxic soils, and deeper oxic soils in the drained system.

In addition to the long-term effects of permafrost thaw on greenhouse gas production, boreal peatlands are also subject to short-term seasonal changes in water table position that influence microbial activity as well as greenhouse gas fluxes (Blodau et al. 2004; Christian and Moore 2003). For example, Bubier et al. (1998) found the

highest CO₂ emissions from northern peatlands as the water table dropped with increasing summer evapotranspiration. In their study, the position of the water table was a strong predictor of the rate of CO₂ emissions. I sampled early in the growing season, when the position of the water table was relatively close to the soil surface and I maintained water table field conditions during my anaerobic incubations. Notably, the water table was much deeper at the FB site compared to YCS and OCS. My observation of higher anaerobic CO₂ fluxes from YCS and OCS surface soils compared to FB suggested that the microbial community at these wetland sites was better acclimated to a higher water table and to anaerobic conditions. Boreal peatlands are dynamic ecosystems that experience short-term and long-term environmental variation that influences microbial activity and thus greenhouse gas fluxes.

CH₄ Fluxes

Although CH₄ production is typically associated with anaerobic conditions, I observed CH₄ fluxes in both aerobic and anaerobic incubations (Figure 5). My incubation studies used soils collected and incubated under water table field conditions; given the water-saturated state of these soils it is highly likely that they contained micro-anaerobic zones even in my aerobic incubations, and this is thus a plausible explanation for the CH₄ production I saw in the aerobic incubations. In the anaerobic soil incubations I found significantly higher rates of CH₄ production at YCS relative to OCS and FB (mean CH₄ flux rates, regardless of soil section, were 0.134, 33.7, and 2.53 μg CH₄ g⁻¹ d⁻¹ at FB, YCS, and OCS, respectively). These production rates were similar compared to other

peat soil incubation experiments. For example, in their peat soil laboratory incubation study Moore and Dalva (1997) reported a range of 0.1-100 $\mu\text{g CH}_4 \text{ g}^{-1} \text{ d}^{-1}$, with the median rate of CH_4 production to be around 10 $\mu\text{g CH}_4 \text{ g}^{-1} \text{ d}^{-1}$. Kettunen et al. (1999) measured anaerobic CH_4 production from 0.1-2.4 $\mu\text{g CH}_4 \text{ g}^{-1} \text{ h}^{-1}$.

Across sites, rates of CH_4 production were significantly higher at YCS compared to OCS and FB ($p < 0.001$). Additionally, CH_4 production rates in anaerobic incubations were significantly higher from the surface soils of YCS compared to the deep soils ($p < 0.01$). In addition to anaerobic conditions being an important control on the production of CH_4 , organic substrate supply plays an important role on CH_4 production. (Crill et al. 1988; Lai 2009; Segers 1998). Updegraff et al. (1995) examined environmental and substrate controls on the production of CH_4 and found that rates of methanogenesis were positively related to substrate lability. I found higher rates of methanogenesis in soils from my YCS site from less decomposed substrate. That is, the rate of methane production was higher in soil receiving the highest rates of fresh organic matter. Along the wetland age gradient, I found that rates of methane production were very low at the undisturbed site (FB), and were highest at the newest wetland site (YCS).

These results suggest that as wetlands continue to develop from permafrost thaw in northern latitudes, increases in water table position and shifts in vegetation type from forests, with more recalcitrant organic inputs, to wetlands, with more labile inputs of soil organic matter will favor conditions that lead to further methane emissions for [at least] the first several decades after ecosystem state change. In addition to these long-term changes in water table position that affect CH_4 emissions from boreal bogs, seasonal

changes in the water table also affect their CH₄ production. As such, CH₄ fluxes are subject to the same seasonality in water table location as CO₂ emissions. Roulet et al. (1993) found that seasonal decreases in water table position negatively impacted CH₄ emissions by increasing the size of the oxidation zone in the peat soils, but Kettunen et al. (1999) demonstrated that methanogens and CH₄ emissions were resilient to seasonal shifts in water table position. This suggests that CH₄ emissions may be sensitive to both long-term ecosystem development and short-term seasonal water table fluctuations.

N₂O Fluxes

N₂O is produced both from the incomplete microbial conversion of NO₃⁻ to N₂ in the anaerobic process of denitrification and from NH₄⁺ to NO₃⁻ in the aerobic process of nitrification (Firestone and Davidson 1989). Rates of denitrification in boreal bogs are generally regarded as low; ombrotrophic bogs rely solely on atmospheric inputs of nitrogen, thus NO₃⁻ concentrations and denitrification rates tend to be low (Martin and Holding 1978; Urban and Eisenreich 1988; Wieder and Vitt 2006). Rates of nitrification are also generally low in boreal bogs; low pH, high water tables and anaerobic conditions, and cool temperatures limit nitrification rates (Wieder and Vitt 2006). However, several studies have demonstrated the importance of nitrification as a source of NO₃⁻ to boreal wetlands (Wrage et al. 2001; Wray and Bayley 2007).

In my incubations, I found that anaerobic production rates of N₂O were two orders of magnitude lower than aerobic N₂O production rates, and N₂O fluxes were significantly higher in surface soil sections ($p < 0.05$) and in aerobic soil incubations ($p <$

0.001). This suggested that N₂O production resulted from incomplete nitrification and not denitrification, although it is possible for conditions for denitrification and nitrification to exist at the same time (Repo et al. 2009). I found significantly higher ($p < 0.05$) rates of N₂O production at the YCS site compared to the FB and OCS sites from my aerobic incubations of surface soils (Figure 6). Additionally, observed N₂O fluxes were of the same magnitude as CH₄ fluxes (compare Figures 5 and 6) suggesting that N₂O fluxes may play a larger role in boreal ecosystems greenhouse gas production than previously thought. Elberling et al. (2010) thawed, drained, and rewet wetland permafrost soils using meltwater and found large increases in N₂O production. This demonstrated that boreal wetland soils have the potential to produce large amounts of N₂O following permafrost thaw. Their chemical analyses also showed NH₄⁺ concentrations that were 60 times greater than those in the active layer in permafrost soils (Elberling et al. 2010). My study focused on a centuries-scale time frame of permafrost thaw and subsequent wetland development, so it is unlikely that the YCS and OCS soils had such high concentrations of NH₄⁺. The N₂O flux results add to the small, but growing body of literature suggesting that N₂O production by boreal wetlands may be more important than previously thought.

16s rRNA and mcrA gene abundances

Quantitative PCR of the 16s rRNA gene and other functional genes is often used to assess the overall abundance of the bacterial and Archaeal communities as well as the abundance of functional genes of interest (Fierer et al. 2005; Henry et al. 2006; Leininger et al. 2006). I found that the size of the microbial community did not vary across site or

soil section (Figure 7). In other words, the size of the microbial community as determined by the number of copies of the 16s rRNA gene ng^{-1} of soil DNA was comparable across sites and soil sections (mean numbers of 16s rRNA gene copies were $\sim 730,000 \text{ ng}^{-1}$ of soil DNA in surface sections and $\sim 675,000$ gene copies ng^{-1} of soil DNA in deep sections, for an overall mean of $\sim 705,000$ copies ng^{-1} of soil). For the most part, the number of copies of the 16s rRNA gene was statistically similar per gram of soil (Figure 7). However, the surface soil at the FB site did have significantly lower copies of 16s rRNA gene per gram of soil relative to the surface soil at the YCS site (Figure 7). On a per gram dry weight basis, my abundances of bacterial and Archaeal gene copies were comparable to other studies that used qPCR to quantify abundance of microbial communities in bogs (Kim et al. 2008; Waldrop et al. 2012).

Waldrop et al. (2012) found that both bacterial and Archaeal abundances varied along a gradient of soil moisture, vegetation type, and differing permafrost regimes. Generally, they found higher abundances of bacteria and Archaea per gram of soil at wetter sites (fens) versus sites underlain by permafrost sites (black spruce forest). My soil samples were collected within the same field site as Waldrop et al. (2012). They attributed higher bacterial abundances to higher soil pH and soil N whereas higher Archaea abundances were positively related to soil moisture. My study did not differentiate between Archaeal and bacterial abundances; however, the higher number of microbial 16s rRNA gene copies that I found at the YCS site across my chronosequence may be associated with higher number of Archaea (Figure 7). My data is consistent with the soil moisture pattern that Waldrop et al. (2012) found.

In addition to quantifying total bacterial and Archaeal abundances, I measured methanogen abundance by targeting the *mcrA* gene. Across my gradient of wetland development, *mcrA* gene abundance was significantly higher at the YCS site (Figure 8). Within a few decades of permafrost degradation and subsequent inundation, the abundance of methanogens significantly increased relative to the site with intact permafrost (FB) and the older wetland site (OCS). The abundance of methanogens at this site was an order of magnitude larger than that reported by Kim et al. (2008) in bog, fen, and riparian ecosystems and several orders of magnitude higher than Steinberg and Regan (2008) found in acidic peat ecosystems. Waldrop et al. (2012) found that the number of methanogens increased as soil moisture increased. That is, following permafrost thaw the entire ecosystem atop permafrost collapsed into relic ice space, causing a relative increase in the water table height. The increased water table height led to an increase in soil moisture and anaerobic conditions that are required for methanogens and methanogenesis.

I assessed the approximate extent of methanogens relative to whole soil microbial community. To normalize for the different sizes of microbial communities across the gradient of wetland development, I quantified the percentage of *mcrA* gene abundance relative to the total number of bacterial and Archaeal gene abundances (Figure 9). At the FB site that was underlain by intact permafrost I found that *mcrA* gene abundance accounted for only ~ 0.035% of the total gene abundance compared to ~ 2.8% at the YCS site and 0.35% at the OCS site, and this difference was significant (Figure 9). Furthermore, at the YCS site where I found the highest percentage of methanogens

relative to the rest of the microbial community, the number of methanogens was positively related to the rate of methane production (Figure 10). Similar positive relationships between Archaeal methanogen abundances and the production of methane have been observed in several other studies (Turetsky et al. 2008; Waldrop et al. 2012). Across the gradient of wetland ecosystem development from permafrost degradation, these results suggest that following permafrost thaw there is a several decade-long pulse of CH₄ production. After more time, though, and the bogs slowly infill and become less wet, both abundances of methane-producing Archaea and rates of methane production decrease.

Synthesis and Conclusions

In this study I used a space-for-time substitution approach to investigate how soil processes and characteristics vary along a gradient of wetland ecosystem development. I quantified soil organic matter C:N ratios, greenhouse gas fluxes (CO₂, CH₄, and N₂O), and bacterial, Archaeal, and methanogen abundances in the soil microbial communities. Following the degradation of permafrost and subsequent wetland formation, rates of CO₂ production did not decrease while rates of CH₄ production increased in the first few decades after ecosystem state change. At the young wetland site I also found higher abundances of methanogens and these abundances were positively related to CH₄ flux. These results validate the positive feedback of warming temperatures and permafrost thaw on wetland formation and greenhouse gas production that I conceptualized at the beginning of my study (and of this paper). As these bogs age, fill in, and become less

wet, methanogen abundances and CH₄ fluxes decrease. I also observed higher rates of aerobic N₂O production across each site—on the same order of magnitude as CH₄ production rates in some cases—compared to anaerobic N₂O production, suggesting that incomplete nitrification may be substantially contributing to the production of greenhouse gases in boreal bog wetlands. Understanding dynamic ecosystem responses to permafrost thaw and wetland development, including critical positive feedback loops, may better inform how to mitigate or adapt to the impacts of climate change on boreal ecosystems. Additionally, studies such as this that focus on process-driven understanding of ecosystem function will better inform models of, and policy responses to, climate change effects on high latitude ecosystems.

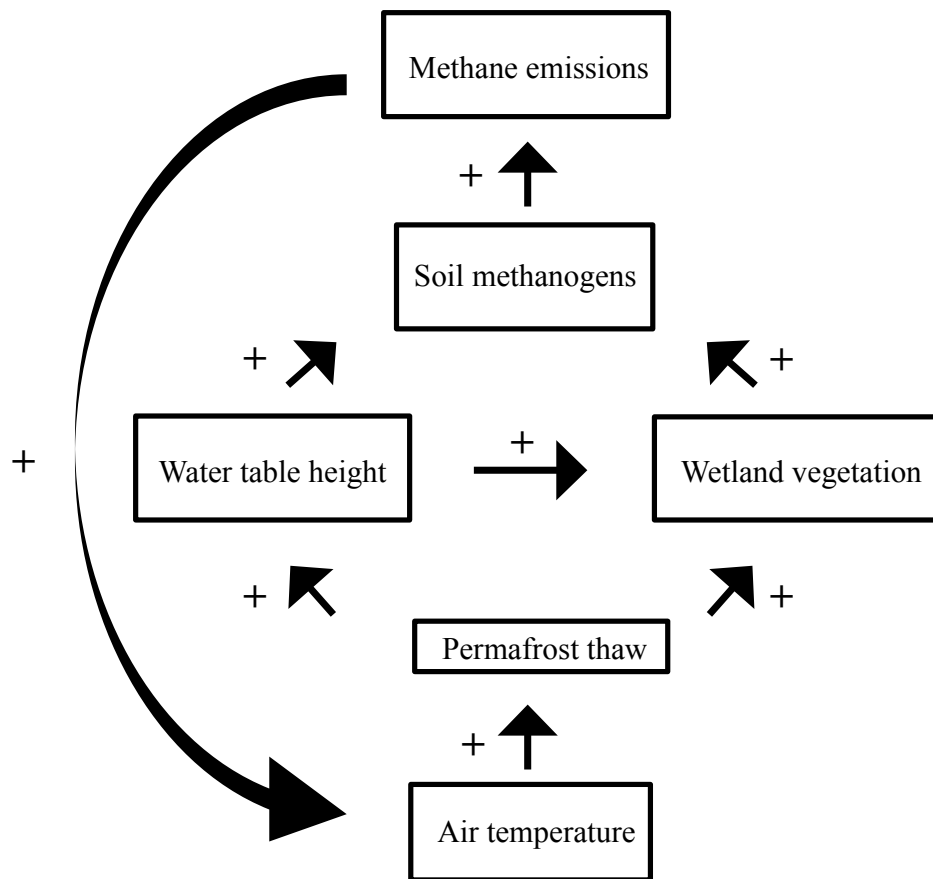


Figure 1. Simplified positive feedback loop of warmer air temperatures on the methane emissions from wetlands forming from permafrost thaw.

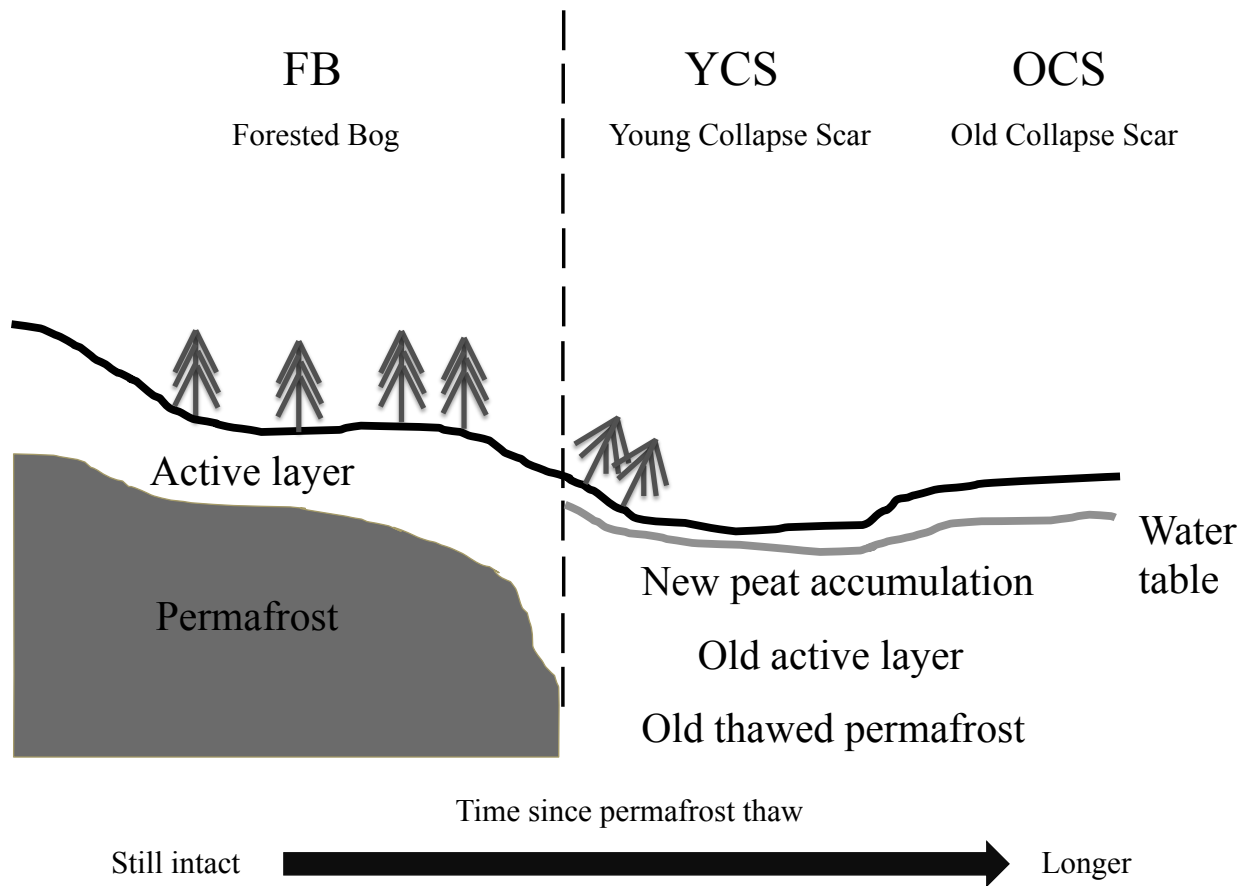


Figure 2. Cartoon schematic of study sites. FB is underlain by intact permafrost, YCS bog was most immediately formed following permafrost thaw and had the highest water table, and OCS formed after undergoing infilling and peat accumulation. Dotted line depicts permafrost thaw disturbance.

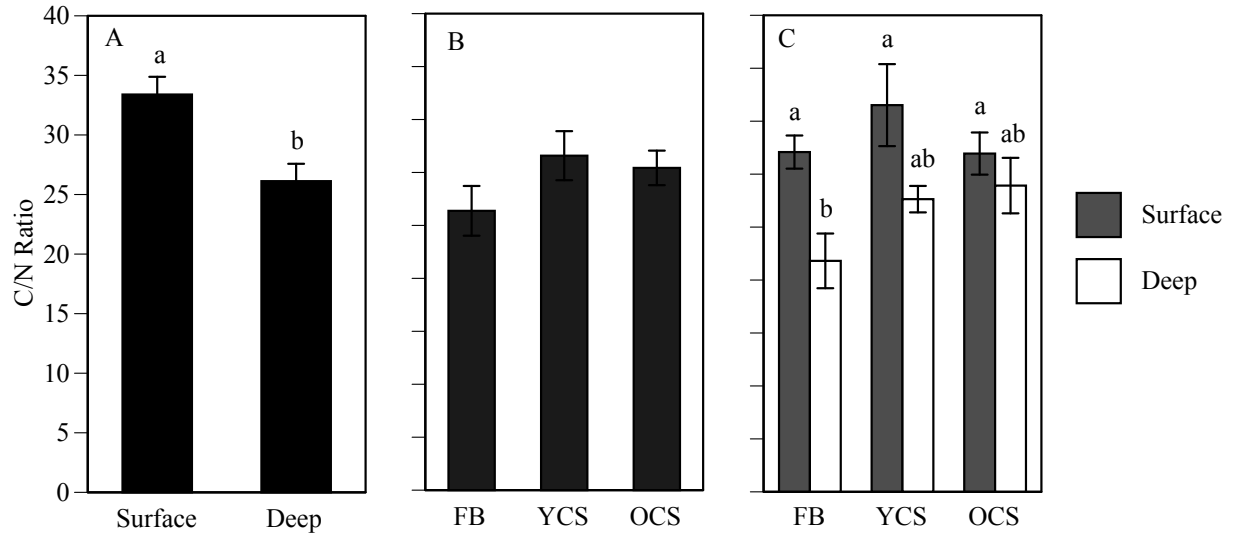


Figure 3. C:N ratio of peat soil from field sites. A) C:N ratio of soil above the water table (Surface), and below the water table (Deep); B) C:N ratio of total vertical soil profile by site; C) C:N ratio of each soil section of each site. Error bars are \pm SEM and different letters within the same figure panel above error bars depict statistically significant differences ($p < 0.05$).

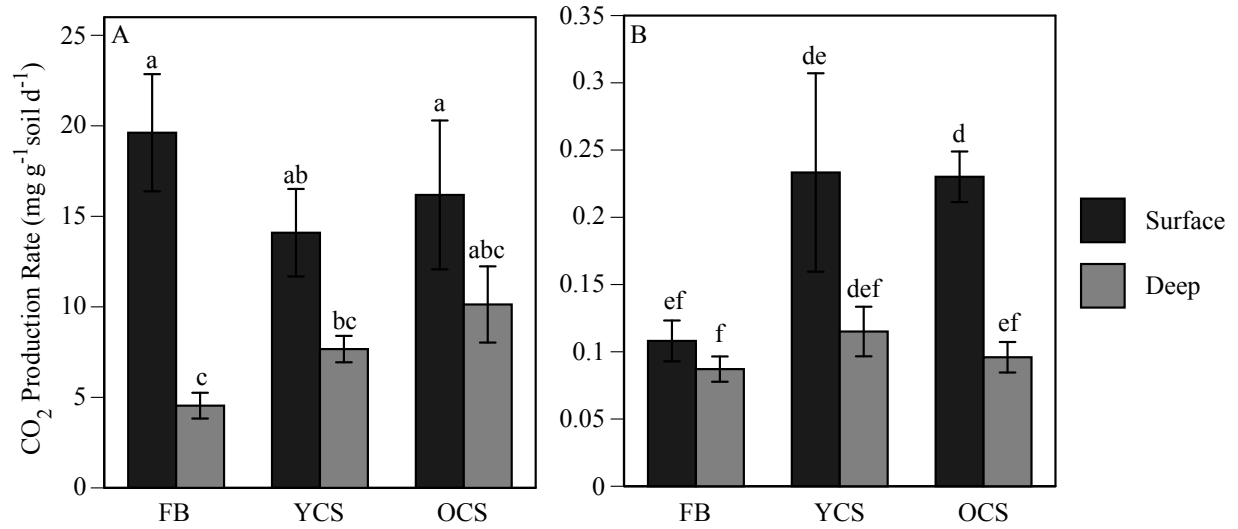


Figure 4. CO₂ production rate during aerobic laboratory soil incubation experiment across site and peat soil section (A), and anaerobic incubation (B). Error bars are ± SEM and different letters across both figure panels above error bars depict statistically significant differences ($p < 0.05$).

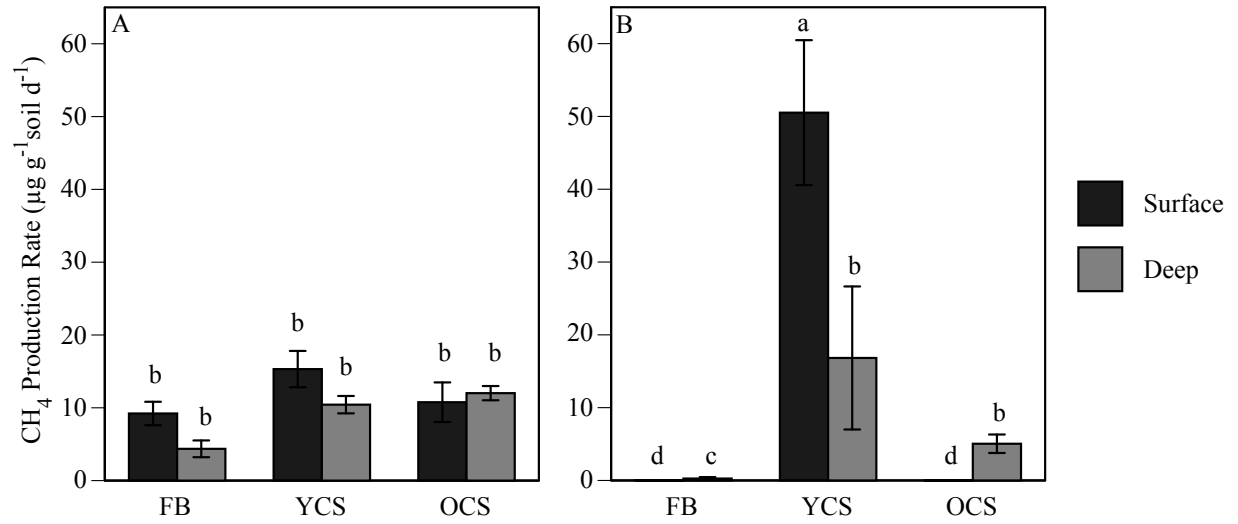


Figure 5. CH₄ production rate during aerobic laboratory soil incubation experiment across site and peat soil section (A), and anaerobic incubation (B). Error bars are ± SEM. FB surface anaerobic CH₄ production rate: 0.004 ± 0.004 , FB deep anaerobic CH₄ production rate: 0.264 ± 0.199 , and OCS surface anaerobic CH₄ production rate: 0.016 ± 0.012 .

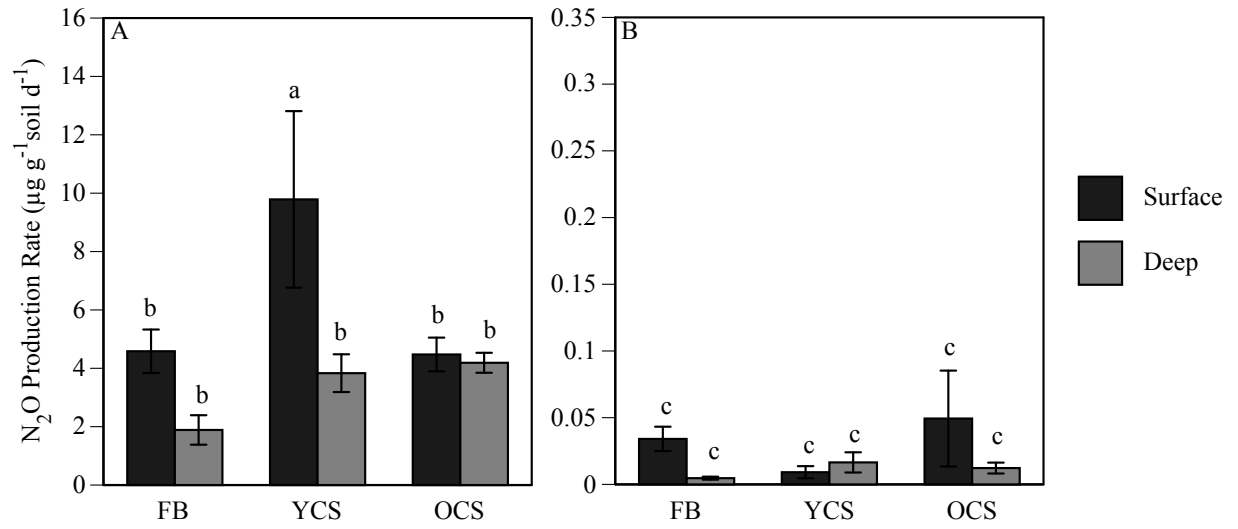


Figure 6. N₂O production rate during aerobic laboratory soil incubation experiment across site and peat soil section (A), and anaerobic incubation (B).

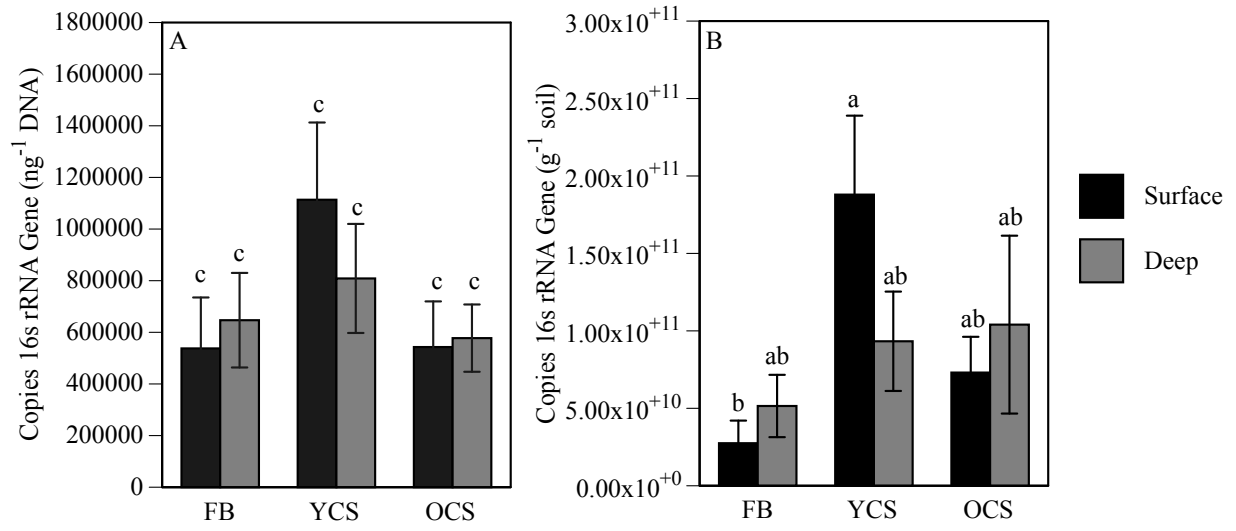


Figure 7. Copies of 16s rRNA gene ng⁻¹ DNA across site and section (A), and copies of 16s rRNA gene g⁻¹ soil across site and section (B).

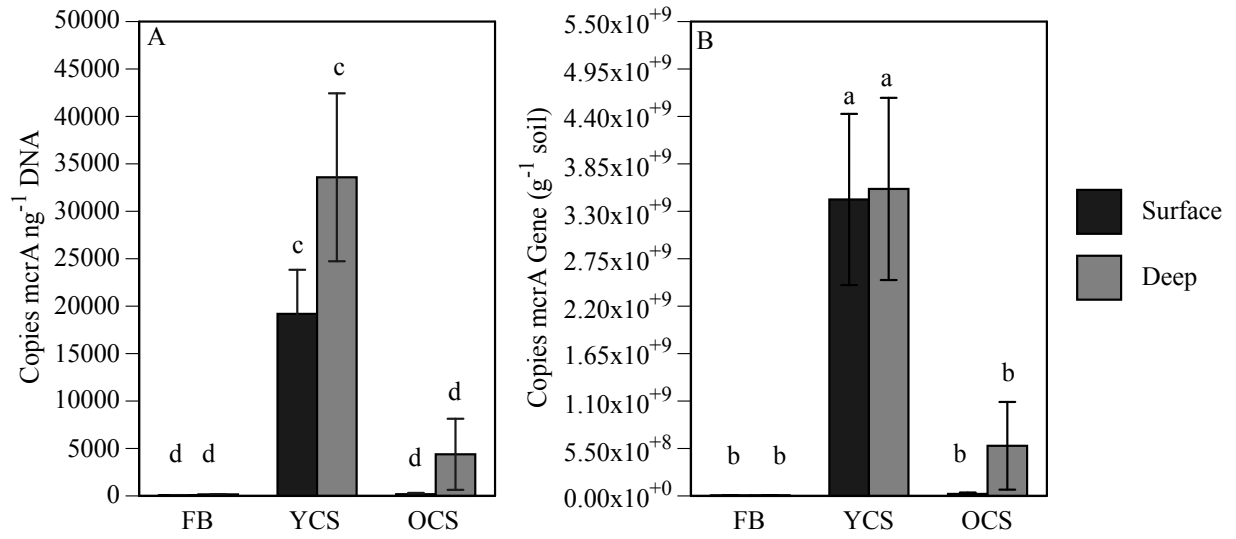


Figure 8. Copies of *mcrA* gene ng⁻¹ DNA across site and section (A), and copies of *mcrA* gene g⁻¹ soil across site and section (B).

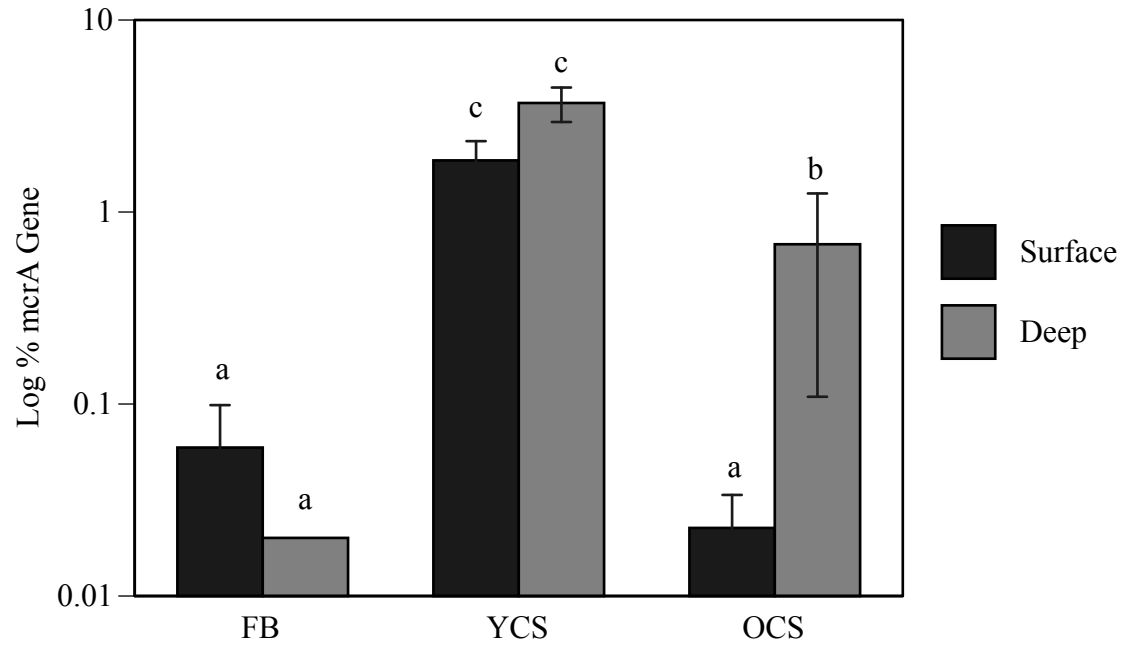


Figure 9. Log plot of mcrA gene as a percentage of overall microbial genes when compared to the 16s rRNA gene across site and depth in peat soil.

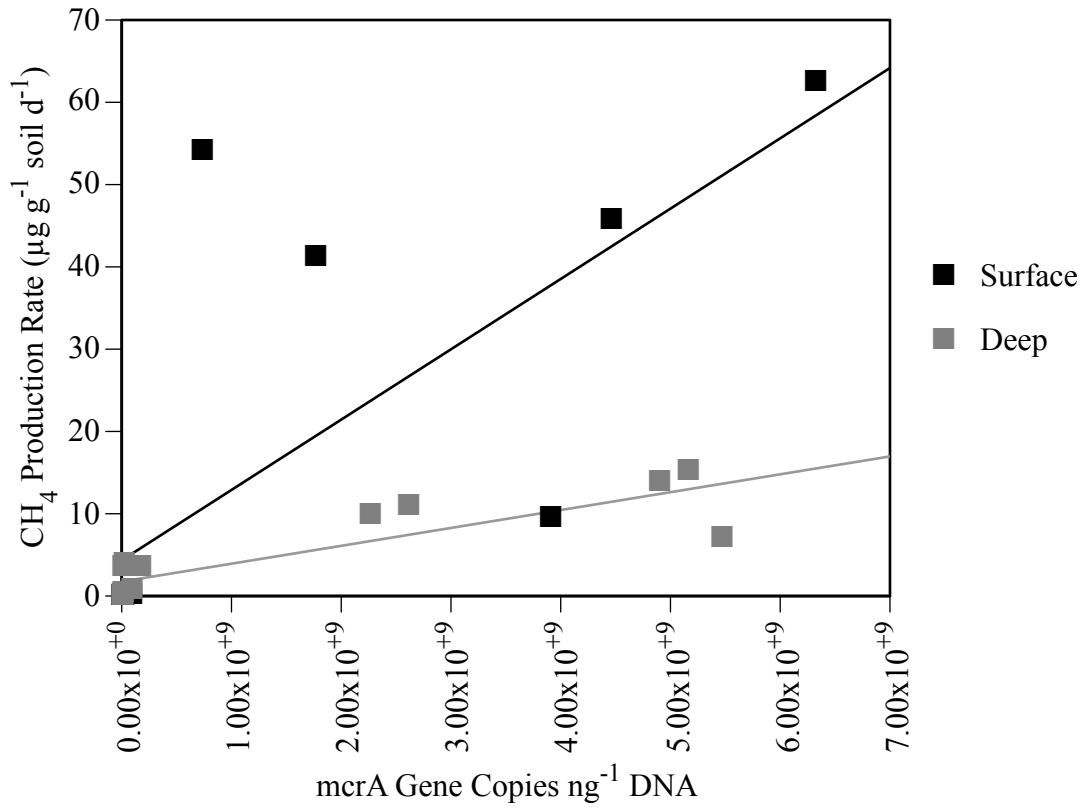


Figure 10. Linear regression of CH₄ production rate as a function of mcrA gene copies ng⁻¹ DNA by depth of peat soil. Surface peat $R^2 = 0.58$, $p < 0.001$, deep peat $R^2 = 0.73$, $p < 0.001$.

Site	Ice Depth (cm)	SE	Water Table Depth (cm)	SE	Eh (mv)	SE	Temperature (°C)	SE	pH	SE
FB	27	±2.42	N/A	N/A	312	±112	8.02	±1.21	4.81	N/A
YCS	N/A	N/A	5.8	±0.8	-153	±57	12.58	±1.38	5.16	±0.24
OCS	N/A	N/A	8	±0.71	-4	±44	10.2	±0.50	5.17	±0.33

Table 1. Environmental characteristics of each site. Forested bog (FB) was the only site with ice present at the time of sampling. Following permafrost degradation and subsequent wetland formation, there were changes associated with depth to water table and redox potential (eh). Values reported are means ± SE. YCS: young collapse scar, OCS: old collapse scar.

CHAPTER 5

A DNA SEQUENCED-BASED INVESTIGATION OF WETLAND SOIL MICROBIAL COMMUNITY RESPONSE TO WETLAND ECOSYSTEM DEVELOPMENT IN A BOREAL ECOSYSTEM

Abstract

Microbial communities play an integral role in biogeochemical cycles and knowledge about their composition helps provide a mechanistic, process-based understanding of ecosystem-level phenomena. Thus, investigating microbial community composition may shed light on ecosystem processes such as carbon and nitrogen cycling. Advances in next-generation sequencing technologies have led to an increasing amount of studies investigating microbial community structure, but many focus on community-scale patterns without regard for broader implications. Boreal wetlands play a disproportionately large role in the global carbon cycle and are often nitrogen limited. I used next generation sequencing approaches to investigate changes in ecosystem relevant microbial communities during ecosystem development in northern boreal wetlands. Extensive permafrost degradation in northern ecosystems is leading to pronounced changes in ecosystem structure and function, including wetland formation. In this study, I used a gradient of wetland development following permafrost degradation to characterize changes in the soil microbial communities that mediate carbon and nitrogen cycling: a forested bog underlain by intact permafrost (FB), a young wetland formed following permafrost thaw ~ 60-100 years ago (YCS), and an old wetland formed in the same

manner ~400 years ago (OCS). I used reference 16s rRNA databases and several diversity indices to assess taxonomic differences in these communities along the space-for-time continuum, to assess relationships between soil microbial community composition and various environmental variables. Along the gradient of ecosystem development I found shifts in the microbial carbon degraders as well as nitrogen fixers. More specifically, I found similar abundances of soil organic carbon degraders and N fixers in an undisturbed forested bog underlain by permafrost (FB) compared to the oldest wetland site that formed from permafrost degradation (OCS). The intermediate young wetland formed most immediately after permafrost thaw (YCS) had strikingly different abundances of several soil organic carbon degraders and N fixers. Analyses of beta diversity trees and alpha diversity plots highlight similar differences in soil microbial communities between FB and YCS and YCS and OCS. That is, I found similar levels of alpha diversity at FB and OCS compared to YCS. Interestingly, alpha diversity was highest at YCS. Alpha diversity was highly correlated with pH, and to a lesser extent redox potential. FB and OCS sites represented relatively mature ecosystem states for these ecosystem types and these results suggest that ecosystem structure is dependent more on ecosystem maturity than ecosystem type. Soil microbial communities play an important role in ecosystem development through influencing ecosystem processes such as carbon and nitrogen cycling.

I. Introduction

Climate change is modifying ecosystem structure and function worldwide (Chapin et al. 2011; Doney et al. 2012; Walther et al. 2002). Many studies that assess the impacts of climate change have focused on ecosystem-level processes (e.g. Davidson and Janssens, 2006; Dixon et al. 1994; Melillo et al. 1993). For example, Cramer et al. (2001) examined ecosystem response to elevated CO₂ levels through simulation modeling of net primary production and net ecosystem production. These studies have undoubtedly advanced our understanding of the impacts of climate change on the planet, and research on the specific processes and mechanisms behind responses to climate change will only enhance this knowledge.

One way to approach a more mechanistic approach to investigating climate change response is by focusing on the soil microbial community. Soil microbial communities play an integral role in the global carbon cycle and the metabolism of other nutrients, such as nitrogen and sulfur (Falkowski et al. 2008). For example, the production of methane (CH₄), a potent greenhouse gas, is mediated by a cohesive group of microbes within the domain *Archaea* (Liu and Whitman 2008). Similarly, nitrous oxide (N₂O), another strong greenhouse gas, is an intermediate byproduct of microbial metabolism across multiple microbial groups including by denitrifying bacteria or nitrifying bacteria or *Archaea*. A better understanding of the microbial-scale conditions and processes that produce these greenhouse gases will better inform models that operate at the ecosystem scale. Despite the large role that soil microbial communities play in global nutrient cycles, less is known about microbial responses to climate change

(Bardgett et al. 2008; Melillo 2002). This is of particular concern because of the potential positive feedbacks that may enhance further climate warming (CHAPTER 4). In this chapter, I investigated changes in soil microbial C and N and diversity during wetland ecosystem development in boreal ecosystems.

Besides more studies focusing on exploring the poorly understood responses of soil microbial communities to climate change, advances in molecular biology technologies have made sequencing microbial soil community endeavors a cost-effective approach to investigating changes in microbial communities for a host of reasons. Researchers can investigate and analyze metrics of soil microbial communities independently of culturing microorganisms, circumventing the expense and difficulty of culturing soil microbes (Ekblom and Galindo 2011; Shokralla et al. 2012). Advances in quantitative tools developed specifically for analyzing soil microbial communities have streamlined workflow for analyzing millions of DNA sequences (Caporaso et al. 2010). For example, using a next generation sequencing approach Mackelprang et al. (2011) showed a rapid change in several microbial genes involved with ecosystem processes, such as, carbon and nitrogen cycling following permafrost thaw. Mackelprang et al. (2011) highlighted shifts in chitin and cellulose processing microbial genes as well as nitrogen fixation, denitrification, and ammonification genes. Advances in next generation sequencing technologies have enhanced researchers abilities to link microbial scale mechanisms and processes with ecosystem scale processes. In this study I used these techniques to investigate soil organic carbon degrading and nitrogen-fixing microbe communities across a chronosequence of wetland ecosystem development.

This study focused on the soil microbial community in ecosystems of high significance in the global carbon cycle: developing Alaskan boreal wetlands. Owing to thousands of years of carbon accumulation and slow rates of decomposition, boreal peatlands have been historical sinks for atmospheric carbon. Direct and indirect effects of climate change are causing extensive degradation of permafrost in interior Alaska (Jorgenson et al. 2001). Because ice occupies more volume than water, when ice rich permafrost thaws, soil collapses into relic ice space leading to ecosystem state changes. These conditions lead to inundation in low-lying areas and subsequently wetland formation. Given the anoxic and waterlogged conditions of these ecosystems, boreal wetlands are a significant source of atmospheric CH₄ and CO₂ and to a lesser extent N₂O (Moore and Roulet 1993; Roulet et al. 1992; Turetsky et al. 2002). Despite peatland's long-term carbon storing past, some models predict that Alaska will be a net source of greenhouse gases by the end of the current century (Zhuang et al. 2007).

In this study I used next generation sequencing technologies to investigate soil microbial community response to indirect and direct effects of climate change. I asked: how does the soil microbial community change as a result of permafrost thaw and subsequent wetland formation? Although more is known about microbial response to climate change than a few years ago, microbial responses to climate change have proven to be unpredictable (Graham et al. 2012). This is one of the first studies using a next generation sequencing approach to quantify changes in the soil microbial community along a century-scale gradient of wetland development to link mechanistic approaches in microbial ecology with ecosystem-scale processes. The objectives of this study were: 1)

To investigate how soil microbial community structure responded to permafrost thaw and wetland development along a space-for-time chronosequence of sites; 2) To indirectly assess changes in microbial functional groups, and; 3) To quantify soil microbial community diversity to assess how microbial community complexity changed during wetland ecosystem development following permafrost degradation. Because wetland formation following permafrost thaw leads to ecosystem state change, with the state change largely due to increased soil waterlogging and vegetation change a key metric of ecosystem state change, I expected to see shifts in microbial community abundance that reflected changes in the water table height and vegetation community. In other words, I expected to see the abundance of different bacterial and Archaeal phyla be tightly coupled to ecosystem type.

II. Methods

Site Description

I located my study sites along a gradient of wetland ecosystem development in peatlands 35 km southeast of Fairbanks, AK. My sites were in the Tanana River floodplain and contained a large number of wetlands that have developed in the last decades to centuries after permafrost degradation. The space-for-time substitution allowed me to test microbial soil system responses to ecosystem disturbance and state change (Figure 1). I selected three different peatlands along this gradient: 1) a forested bog with intact permafrost (FB); 2) a Sphagnum bog that formed following permafrost

degradation within the last 100 years (YCS); 3) and a Sphagnum bog that formed following permafrost degradation between 200-400 years ago (OCS).

Soil Sampling and handling

I sampled soils at each of the sites along the chronosequence gradient by collecting five cores using a sharpened steel tube (i.d. 5.4 cm). I extruded the extracted soil cores by carefully pushing the core out of the steel tube. The extracted soil cores were ~ 30 cm length and I subsectioned each core in the field at the position of the water table. Soil samples that were collected above the water table were stored in plastic bags and kept in a cooler on ice for transport to the lab. The samples that were collected below the water table were stored in plastic bags with bog water, to keep them anaerobic and waterlogged; they were also stored in a cooler on ice. In the lab, soil cores were stored at -20°C freezer until they were shipped on dry ice to Arizona State University in Tempe, AZ. Redox potential, pH, and microtopographical origin of peat soil were also collected during soil sampling.

DNA Extractions

Soil cores were thawed from -20°C followed by DNA extraction by duplicate from each of the 5 cores and 2 soil sections at each site using a PowerSoil DNA extraction kit (MoBio). I quantified the concentrations of extracted DNA using a QuBit 2.0 Fluorometer (Invitrogen), then diluted the duplicate DNA extraction with the highest concentration of DNA to ~5 ng uL⁻¹ for use in downstream analyses. All DNA

extractions were completed in the Wetland Ecosystem Ecology Laboratory at Arizona State University in Tempe, Arizona.

Targeted Gene and PCR Amplifications

I used the 16s rRNA gene and 16s rRNA reference databases (Greengenes) with OTU information to investigate microbial responses and changes in OTUs to permafrost thaw and subsequent wetland formation across a chronosequence of wetland development.

To investigate the bacterial and archaeal communities, I used PCR and Illumina DNA sequencing technology. Polymerase chain reactions (PCR) were carried out using the 515F (5'-GTGYCAGCMGCCGCGGTA; Baker et al. 2003) and 909R (5'-CCCGYCAATTCMTTTRAGT; Wang and Qian 2009; Tamaki et al. 2011) primers that cover the V4 and V5 regions of the 16S rRNA gene of *Bacteria* and *Archaea*; unique tags of variable length 6-10 base pairs were attached to the 5' ends of the primers in order to differentiate between samples and any changes to melting temperature due to tags presence was assayed and adjusted in the PCR reaction. Each sample was amplified in triplicate (and in a few cases quadruplicate) technical replicates, and replicates pooled in order to prevent sequence abundance biases resulting from PCR. All amplified PCR products were verified by fragment size using 1% agarose gel electrophoresis.

PCR Amplicon Normalization and Illumina Amplicon Sequencing and Analysis

To reduce the variation across the multiple PCR amplified samples I used a SequelPrep Normalization Plate Kit (Invitrogen) and followed the manufacturer's protocol before pooling all libraries for single run in one lane of Illumina MiSeq sequencing. Libraries were sequenced using a 300 base pair paired-end read MiSeq v2 chemistry on an Illumina MiSeq 2000 instrument. Sequence data were downloaded from Illumina BaseSpace and the raw forward and reverse sequence reads were merged using PEAR (Paired End reAd, mergeR). The output from PEAR was a single merged fastq file that was directly used with Quantitative Insights Into Microbial Ecology (QIIME; Caporaso et al. 2010). I performed a number of data processing steps using the default QIIME workflow, including: assigning multiplexed reads to soil samples based on their unique barcode sequence in my mapping file, determining operational taxonomic units based on 97% sequence similarity, and assigning taxonomy based on reference databases. In addition to using the QIIME workflow for 16s rRNA analysis, I also used phyloseq, an R package to perform further data analyses, data visualization, and presentation (McMurdie and Holmes 2013).

Calculating Alpha and Beta Diversity

I computed metrics of both alpha and beta diversity to assess shifts in the soil microbial community at various stages of ecosystem development. I used diversity metrics to evaluate similarity and differences among the wetland development gradient. Beta diversity measured dissimilarity among sites (FB, YCS, OCS) whereas alpha

diversity compared OTU level diversity within a site. With this approach I was able to assess how similar or dissimilar the sites along the chronosequence were (beta diversity) and how the diversity within sites compared (alpha diversity). Using the QIIME workflow, I assessed phylogenetic beta diversity by using UniFrac distances, a commonly used metric that measures the phylogenetic distance between taxa from a phylogenetic tree (Lozupone and Knight 2005). I used FigTree to visualize the data from distance matrices using a phylogenetic tree software program (<http://tree.bio.ed.ac.uk/software/figtree/>).

III. Results and Discussion

In the following section I present and discuss the results for: 1) Archaeal and bacterial community structure across the gradient of wetland development and soil depth, 2) microbial community composition of those bacterial phyla involved soil organic C degradation and N cycling, 3) diversity metrics used to measure microbial community complexity (number and abundance) as well as the relationships of these indices to several abiotic and biotic factors, including pH and redox potential.

Microbial Community Structure Across Wetland Gradient and Soil Depth

For a coarse assessment of the dominant soil microbes across the chronosequence of wetland development and soil profile, I plotted the 11 most abundant phyla (Figure 2). The 11 most abundant phyla accounted for ~90% of the observed microbial community

at the FB site, ~75% of the total observed microbial community at the YCS site, and ~85% of the total observed microbial community at the OCS site (Figure 2). In general the surface soil microbial community (Figure 2) resembled the deep soil microbial community at the phylum level. The remaining fraction was either unclassified—~9% at FB, ~14% at YCS, and ~9% at OCS—or a low frequency occurring phyla. The most common bacterial phyla were the Proteobacteria, Actinobacteria, and Acidobacteria; these three phyla accounted for 65% of the total community at both soil depths at FB, 70% at YCS, and 65% at OCS (Figure 2). Within the Proteobacteria, Alphaproteobacteria followed by Gammaproteobacteria and Deltaproteobacteria were the most common bacterial classes accounting for ~16%, 5%, and 4% of the total bacterial sequences, respectively. Less common, but still relatively abundant phyla include: Bacteroidetes, Plantomycetes, Chloroflexi, and Cyanobacteria. Additionally, the prominent microbial phyla that I observed in this study has been observed in other studies of boreal peatland microbial communities (Dedysh et al. 2006; Serkebaeva et al. 2013; Tveit et al. 2013). Tveit et al. (2013) observed that Proteobacteria and Actinobacteria accounted for ~55% of the microbial phyla in their assessment of northern peatlands bacterial phyla. Within the Proteobacteria, however, Deltaproteobacteria accounted for ~20% of the bacterial community and were more abundant than Alphaproteobacteria and Betaproteobacteria. A recent 16s rRNA next-generation sequencing study in boreal peatlands showed high numbers of Acidobacteria, Proteobacteria, Actinobacteria, Plantomycetes, and Verrucomicrobia (Serkebaeva et al. 2013). Several other studies including a clone library sequencing study from mineral Northern Norway permafrost soils and pyrosequencing of

the 16s rRNA gene from various boreal and arctic locations show a relatively high abundance of Actinobacteria, Proteobacteria, and Bacteroidetes bacterial phyla (Chu et al. 2010; Hansen et al. 2007).

In addition to quantifying the relative proportion of different phyla within the bacterial microbial community, I also quantified a relatively small fraction of Archaeal phyla. For example, I observed Euryarchaeota, an Archaeal phylum containing methanogens, in all of the peat soils. I also observed a lower proportion of other lesser-studied Archaeal phyla—the Crenarchaeota and Parvarchaeota—in my Alaskan peatlands. Despite being a large source of atmospheric CH₄, low abundances of methanogenic Archaea in boreal wetlands have been previously observed (Tveit et al. 2013; Waldrop et al. 2010). The relative low abundance of methanogens found in this study highlight the importance of a low frequency microbial phylum in the global carbon cycle.

Along the gradient of wetland development following permafrost thaw, I observed a marked change in relative proportion of several bacterial phyla. For example, I observed an increase in Bacteroidetes at YCS relative to FB, a decrease in Actinobacteria, and an increase in Chloroflexi. Several previous studies have documented higher abundances of Actinobacteria in permafrost soils compared to active layer soils (Wagner et al. 2009; Wilhelm et al. 2011; Yergeau et al. 2010). In addition to finding a higher abundance of Chloroflexi in active layer soils especially at depth, I observed higher proportions of Chloroflexi in the peatland soils compared to other 16s rRNA gene sequencing studies. For example, while I found the proportion of Chloroflexi to be in the

top 11 most abundant phyla in my northern peatlands, Serkebaeva et al. (2013) considered Chloroflexi to be a rare phylum in their study of northern peatland rare OTUs. Additionally, the microbial community proportion of Chloroflexi at YCS is considerably higher than has been previously documented in other soil and clone sequencing studies, though Chloroflexi have been previously observed in active layer soils in another peatland soil microbial community study (Dedysh et al. 2006; Mackelprang et al. 2011; Serkebaeva et al. 2013).

Microbial Community and Carbon Cycling

Soil Organic Carbon Degrading Microbial Community

Because 16s rRNA gene databases are taxonomically robust and contain sequence information from studies not only on soil metagenomes but also on bacterial isolates and clones, many bacterial groups have been described in terms of their ecosystem function. That is, certain bacterial and Archaeal groups are known to have potential ecosystem roles within the broader soil microbial community. For example, many studies describe the bacterial groups of Bacteroidetes, Actinobacteria, Verrucomicrobia, Alphaproteobacteria, and Planctomycetes in terms of their role in the carbon cycle and their ability to degrade polysaccharides, including cellulose and hemicellulose (Kotiaho et al. 2013; Pankratov et al. 2006;. Pankratov et al. 2011). While recent studies have shown a wide range of bacterial groups capable of degrading soil organic carbon, another recent study found that the majority of bacterial genes encoding soil organic carbon

degradation enzymes are found in Bacteroidetes, Actinobacteria, and Verrucomicrobia (Tveit et al. 2013).

Across the gradient of wetland development, my sequencing data revealed a high proportion of these soil organic carbon degraders; Bacteroidetes, Actinobacteria, and Verrucomicrobia were present across all sites and soil depths and represented ~20% of the overall proportion of the microbial community (Figure 3). Bacteroidetes and Actinobacteria were a larger proportion of the SOC degraders compared to Verrucomicrobia and Verrucomicrobia represented ~2% of the overall microbial community. These results generally agree with a previously published study on microbial communities and SOC degradation in peatlands; however, I observed a smaller proportion of Verrucomicrobia that was five times smaller relative to the whole microbial community (Tveit et al. 2013).

Interestingly, the total proportion of SOC degraders across the gradient of wetland development and soil depths was not significantly different with the exception of the FB deep soil section. However, the relative proportion of each different SOC-degrading phylum shifted across the gradient of wetland development. For example, at the FB site, there were higher proportions of Actinobacteria compared to YCS and OCS. Additionally, Bacteroidetes were in a higher proportion at the YCS site compared to the other sites along the wetland development gradient, and at OCS there was a higher proportion of Verrucomicrobia. In the deep soil section at the FB site, the higher proportion of Actinobacteria—often associated with permafrost soil—slightly elevated the total SOC degrading population at the FB deep soil site. Following fungi,

Actinobacteria are one of the main decomposers in peatlands (Peltoniemi et al. 2012; Thormann 2006).

The degradation of permafrost and subsequent wetland formation from soil surface collapse in boreal peatlands changes plant community composition (Camill et al. 2001; Jorgenson et al. 2001). For example, along the gradient of wetland development the black spruce and woody boreal shrub dominated FB site had remarkably different vegetation types compared to the sphagnum dominated YCS site and the sphagnum and boreal shrub dominated OCS site. Given these large plant community shifts along the gradient of wetland development it is not surprising that there were shifts in the SOC-degrading bacterial community. Previous peatland succession studies have shown shifts in the SOC-degrading microbial community (Artz et al. 2007; Merilä et al. 2006; Putkinen et al. 2014). For example, a recent study showed that during the succession of a minerotrophic fen to an ombrotrophic peat bog in Finland, the methanotroph community was different depending on the sphagnum moss species that were present in each ecosystem (Putkinen et al. 2014). In a peatland recovering from extensive peat harvesting, Artz et al. (2007) found shifts in the fungal carbon-degrading community along the gradient of succession. These results suggest that following permafrost thaw and subsequent wetland formation changes in ecosystem and vegetation type alter the SOC degrading bacterial community.

Anaerobic Respiration and Fermentative Microbial Community

Given the complex relationship between microbial community composition and

anaerobic respiration, it is difficult to attribute particular taxa to a certain metabolism. However, several recent gene sequencing studies have attributed the majority of anaerobic respiration in peatlands to microbes in the Proteobacteria, Actinobacteria, and to a lesser extent Acidobacteria phyla (Lin et al. 2014; Lipson et al. 2013; Tveit et al. 2013). While there are metagenomic studies that suggest a relatively high abundance of denitrification genes in peatlands (Mackelprang et al. 2011), other studies have found low abundances of denitrification gene transcripts, suggesting that there are relatively small pools of mRNA to produce enzymes for denitrification (Tveit et al. 2013).

In their study of organic carbon transformations in boreal peatlands, Tveit et al. (2013) attributed the majority of fermentation and fermentative genes—a gene encoding hydrogenase gene (*hydA*) involved with H₂ producing fermentations and a formyltetrahydrofolate synthetase gene (*fhs*) involved with encoding a key enzyme in homoacetogenesis—to the Firmicutes and Actinobacteria bacterial phyla. I found low abundances of the Firmicutes, but Actinobacteria was one of the most abundant phyla, comprised of ~10% of the total microbial population. By creating H₂ and acetate from fermentative pathways and CO₂ from SOC decomposition, northern peatland microbial communities provide the raw substrates needed for methanogenesis and subsequent aerobic and anaerobic methane oxidation from the methanotrophs.

Methanogen and Methanotroph Community

Methanogens play a large role in anaerobic C cycling in boreal wetlands and peatlands (Keller and Bridgham 2007). To assess differences of methanogens along the gradient of ecosystem development I plotted several phyla associated with

methanogenesis (Figure 4). I observed the presence of three different orders of methanogens from the Euryarchaeota phylum: the Methanobacteriales, the Methanomicrobiales, and the Methanosarcinales (Figure 4). Methanobacteriales were the most abundant of the three orders, followed by Methanosarcinales, and finally Methanomicrobiales. Methanobacteriales and Methanomicrobiales produce CH₄ from CO₂ and H₂, whereas Methanosarcinales are more metabolically diverse and can produce CH₄ acetoclastically, hydrogenotrophically, or from methylated compounds. Thus, the composition of different Euryarchaeota orders can reveal important environmental constraints of the ecosystems. The abundance of methanogens varied by site along the gradient of wetland development (Figure 4). Methanogens were almost entirely absent at the FB site and the most abundant at the newly formed wetland site, YCS. I observed an intermediate abundance of methanogens at the older bog site, OCS, but not above the water table. Additionally, all three orders of methanogens that were detected were found at YCS, whereas only two orders of methanogens were found at OCS. The metabolically versatile Methanosarcinales were only found at YCS, suggesting that YCS contained a diverse pool of substrates for methanogens and methanogenesis. Additionally, methanogen abundances were higher in deep peat below the water table, a finding that agrees with several studies (Kotsyurbenko et al. 2004; Tveit et al. 2013; Tveit et al. 2014). Additionally, the relative abundance of methanogens correlates with CH₄ production along the same gradient of wetland development (CHAPTER 4), possibly because of higher concentrations of methane precursors from fermentation products and a lack of oxygen at depth (Beer and Blodau, 2007; Tveit et al. 2013).

In addition to identifying a diverse methanogen peat soil microbial community, I also observed differences in the methanotroph microbial community. Methanotrophs can oxidize methane to produce energy and obtain carbon. Methane can be consumed to obtain carbon and energy by two different types of methanotrophic bacteria that are categorized based on the used pathway of methane oxidation. Type I and type x methanotrophs convert methane to formaldehyde using the ribulose monophosphate pathway, whereas type II methanotrophs use the serine pathway to oxidize methane to formaldehyde (Hanson and Hanson 1996). Formaldehyde is converted into several intermediates until the carbon molecule is bioavailable. In addition to their ability to oxidize methane, type x and type II methanotrophs have the ability to fix nitrogen. The ability to fix nitrogen in the type II methanotrophs supports and drives carbon and nitrogen accumulation in peatlands (Vile et al. 2014). In this study, I found relatively high abundances of Methylocystaceae and lower abundances of Methylococcaceae; both families of methanotrophs belong to the Proteobacteria phylum (Figure 5). Generally, across the gradient of wetland development I found similar abundances of these type II methanotrophs at each site. Deep soil sections at FB and YCS had lower abundances of Methylocystaceae, but moderately higher abundances in the deep soil section of OCS. Methylococcaceae abundances were much lower than Methylocystaceae across the gradient, but the abundances of Methylococcaceae were slightly higher at YCS and OCS compared to FB. There are few studies in boreal peatlands that discuss the abundance of Methylocystaceae and Methylococcaceae, however, in a metagenomic sequencing study on aerobic and anaerobic laboratory incubations on freshwater sediment, Beck et al.

(2013) found that the unamended methanotroph community was evenly split between Methylococcaceae and Methylocystaceae. When incubated aerobically, the ratio of Methylococcaceae to Methylocystaceae increased. Conversely, when incubated anaerobically, Beck et al. (2013) found a higher proportion of Methylocystaceae to Methylococcaceae. This suggests that the majority of my peatland soils along the gradient of wetland development contained anaerobic—or at least microanaerobic zones that favor higher abundances of Methylocystaceae.

Peatland sphagnum-associated methanotrophs undergo changes in activity and community structure during ecosystem succession. However, sphagnum type does not appear to impact methanotroph community composition (Putkinen et al. 2014). Along my gradient of wetland development in peatland ecosystems I did not find any family-level shifts in methanotroph community composition, although I did observe changes in taxonomically unassigned methanotroph genera within the Methylocystaceae bacterial order. Across the chronosequence of wetland development I found highest abundances of Methanogenic microbes at the ecosystem with the highest water table. The vast majority of methanotrophic microbial communities in this study were type II methanotrophs, suggesting that N limitation played a large role in shaping methanotrophic communities.

Microbial Community Composition and Nitrogen Cycling

In ombrotrophic bogs such as those I sampled in this study, N inputs are driven largely by atmospheric inputs and total N concentrations are typically low and often limiting (Bubier et al. 2007). Bacterial N fixed from the atmosphere has a positive effect

on sphagnum growth rates (Berg et al. 2013). Thus, soil microbes play an important role in N cycling and N inputs through N fixation, and also through plant-microbe interaction, especially in aerobic surface peat (Chapman and Hemond, 1982; Waughman and Bellamy, 1980). I quantified the abundance of different taxonomical levels in the microbial N-fixing community using the same comparative approach that was used with the soil microbial SOC-degrading phyla (see above). For example, based on gene sequencing and analysis, the N-fixing bacterial community has been attributed to the following phyla: Actinobacteria, Chlorobi, Chloroflexi, Cyanobacteria, Firmicutes and Proteobacteria (Dos Santos et al. 2012; Hartmann and Barnum, 2010). Additionally, in peatlands, N-fixers associated with peat sphagnum belong mainly to the Alphaproteobacteria class within the Proteobacteria phylum (Bragina et al. 2013). Although recent studies have highlighted the potential importance of methanotrophic-induced N fixation, I excluded considering methanotrophs as N-fixers for the purposes of this study as methanotrophs were a small fraction of the community relative to other N-fixers (Larmola et al. 2014).

To investigate the broadest scope of bacterial N-fixation in my study system, I focused my investigation on the five most abundant taxa associated with N fixation. When I summed the abundance of all the bacterial phyla associated with N fixation, I found similar abundances of the total N fixers across the chronosequence of wetland development (Figure 6A). Additionally, the summed abundance of N-fixing phyla did not differ by soil depth. However, when I separated the N-fixing phyla, I found significant differences across the gradient of wetland development (Figure 6 B-F). Actinobacteria,

Rhizobiales, Cyanobacteria, and Bacteroidetes all vary by site along the chronosequence of wetland development. That is, FB and OCS generally have the highest proportion of Cyanobacteria, and Rhizobiales, whereas YCS has the highest proportion of Bacteroidetes. Actinobacteria are in highest proportion in FB. These results suggest that N fixation is an important source of nitrogen for each of the sites along the gradient of wetland development. The composition of the bacterial community of N-fixers shifts over the course of wetland development, though the total proportion of N-fixers is constant at each site.

Since N fixation is an aerobic process, I expected to see lower abundances of bacterial phyla known to fix N in soils below the water table. I did observe lower abundances of the Proteobacteria phylum, as well as a lower abundance of Rhizobiales, an order within the Proteobacteria phylum and Alphaproteobacteria class (Figure 6). The abundance of the Alphaproteobacteria Sphagnum-associated N-fixers decreased at depth and accounted for over 19% of the total microbial community abundance in surface peat across the gradient of wetland development and only 13% of the total microbial community abundance in deep peat sections (data not shown).

Additionally, I observed small decreases in the N-fixing bacterial community that form close symbiotic relationships with plant roots. The decrease in Rhizobiales at depth coincided with a decrease in plant roots detected in my soil cores. A previous study showed lower rates of N fixation at depth (Kravchenko, 1996), and another study of ombrotrophic peats bogs in Patagonia showed low rates of N-fixation deep in the peat profile though only in micro-oxic conditions of the rhizosphere (Knorr et al. 2014). My

results suggest that N-fixing bacteria are most abundant in aerobic peat soils above the water table.

Microbial Community Composition and Ecosystem Development

Despite shifts in key carbon and nitrogen-processing bacteria and Archaea phyla from FB to YCS, the bacterial and Archaeal microbial community at the intact permafrost site, FB, largely resembled the older bog site, OCS (Figures 2, 3, and 6). Additionally, although there were phyla-specific shifts in SOC-degrading bacteria during bog development following permafrost degradation, the summed total abundance of bacterial phyla associated with SOC degradation did not change. While I observed large shifts across the gradient in bacterial phyla associated with SOC degradation and N fixation, specifically at YCS compared to the other sites; I did not observe large shifts between FB and OCS with the exception of higher abundances of the permafrost associated Actinobacteria. These results suggest that soil microbial community structure is fundamentally a product of ecosystem maturity, not ecosystem type (Odum 1969). Along the chronosequence of wetland development the bog underlain by permafrost (FB) was a mature black spruce forested bog ecosystem. Following the degradation of the permafrost this forest bog underwent an ecosystem state change to a bog wetland, and the older collapse scar bog ecosystem represented another mature ecosystem, particularly relative to the younger collapse scar wetland.

Alpha and beta diversity across chronosequence

My beta diversity analysis reinforced how different YCS was from FB and OCS regarding the C and N cycling microbial community that was discussed above. That is, FB and OCS were more similar to one another than FB to YCS or YCS to OCS (Figure 7). Thus, along the chronosequence of ecosystem development the two sites representing relatively mature ecosystems, FB and OCS, were more phylogenetically similar to one another compared to the transitional intermediate young collapse scar (YCS). My beta diversity analyses also revealed that surface peat sections tended to be more similar to one another compared to subsurface peat sections, a common observation in other peatland microbial diversity studies (Morales et al. 2006).

I used Faith's phylogenetic diversity—a measure of alpha diversity computed as the sum of branch lengths of a phylogenetic tree—for the measure of alpha diversity. Across the chronosequence of wetland development, I found significant differences in alpha diversity across sites (Figure 8). FB and OCS both had significantly lower alpha diversity compared to YCS, but were not significantly different from one another. Considering diversity has long thought to increase with ecosystem maturity (Margalef 1975; Odum 1969), it was a surprising finding that both ecosystems representing relatively mature ecosystems for their ecosystem type (forested bog underlain by intact permafrost and an old collapse scar bog) had lower measures of diversity, possibly suggesting that soil microbial diversity is not equivalent to whole ecosystem species diversity. Because Faith's phylogenetic diversity metric is calculated based on branch lengths of a phylogenetic tree, these results suggest that the intermediate site and recently

formed wetland, YCS, has more distantly related OTUs compared to FB and OCS. Diversity also was higher in deep peat soil though these results were not significant (Figure 9), a finding that contradicts several recent boreal peatland microbial sequencing studies (Frank-Fahle et al. 2014; Serkebaeva et al. 2013). However, microbial diversity is strongly positively correlated with pH (Allison and Treseder, 2008; Fierer and Jackson, 2006) and I observed small increases in pH at increasing peat depths, potentially leading to higher levels of microbial diversity deeper in the soil profile.

Microbial Diversity and Environmental Constraints

Microbial diversity has been shown to be highly positively correlated to ecosystem pH. Fierer and Jackson (2006) used T-RFLP to quantify soil microbial diversity across a half-dozen biomes across the Americas. They found that microbial diversity could be fit with a quadratic regression; diversity was highest at a pH of roughly 7, and decreased as pH became more acidic or alkaline. The pH at the intact-permafrost forested bog varied from less than 4.0 to 5.5, while pH at the two Sphagnum bog sites was less variable (Figure 10). The young wetland was the most acidic site and OCS was the most alkaline. Across the ~ 4-5.5 pH range I observed, I found a linear, increasing relationship between microbial diversity and pH (Figure 10). These findings have been demonstrated by numerous studies across ecosystem types ranging from biomes across the Americas, in arable lands, and hay fields in the UK (Lauber et al. 2009; Rousk et al. 2010; Zhalnina et al. 2014).

I also found considerable variation in redox potential along the chronosequence of wetland development (Figure 11). Given that the position of the water table relative to the peat soil surface was different at each site along the gradient this result is not surprising. Generally, OCS was the most reducing site, YCS had intermediate redox potentials, and FB was the least reducing site. I expected to see the lowest redox potentials (i.e. most reducing environments) at YCS, followed by OCS, and FB based on the position of the water table relative to the soil surface. For example, the FB site was underlain by an intact permafrost plateau and had the lowest water table position relative to the soil surface, and thus the redox potential was the greatest. YCS and OCS both had higher water tables relative to the soil surface and lower redox potentials, indicative of a reducing environment. Because the water table was higher at YCS I expected to see the lowest redox potentials at YCS, a result that I did not observe. Much like the results with pH, YCS and OCS had much less variable redox potentials compared to FB.

Redox potential explained less of the variation in diversity than did pH, although the relationship was still significant ($R^2 = 0.151$; Figure 11). I found higher microbial diversity in more reduced environments compared to those environments with higher redox potential values. Though there are few studies that investigate the role of redox potential on microbial community structure and diversity, Pett-Ridge and Firestone (2005) found that when dominant bacterial OTUs were incubated anaerobically for extended periods of time, microbial diversity decreased relative to field conditions as well as soils that were incubated with fluctuating redox potentials, a finding that differs with my results. In a study investigating microbial diversity at various depths in a

California lake, Humayoun et al. (2003) found that microbial communities were more diverse at depth in more reduced environments: a finding that Humayoun et al. (2003) attributed to a greater potential for niche differentiation in the reduced environments. pH is widely documented as the strongest predictor of microbial community structure on small and large scales across ecosystem types.

Conclusion

Along the chronosequence of wetland ecosystem development I found: 1) shifts in the general microbial community by quantifying changes in dominant boreal peatland bacterial and Archaeal taxonomy using rRNA reference databases; 2) shifts in specific, individual microbial group abundances associated with soil organic C degradation and N cycling, but not on the total summed abundance of soil organic carbon degraders or N cyclers; and 3) differences in microbial diversity within and among the wetland age gradient; and 4) pH is positively related to microbial diversity and negatively related to redox potential. The microbial community at the relatively mature forested bog underlain by intact permafrost more closely resembled the microbial community at the older and relatively mature collapse scar wetland compared to the younger collapse scar wetland. Given that the YCS wetland formed more immediately following the degradation of permafrost at FB than the OCS wetland and I would expect to find that ecosystems that sequentially follow in development are more similar than those more distantly, these results are surprising. These results suggest that ecosystem maturity—whether it is a

mature forested bog underlain by permafrost or a relatively mature collapse scar wetland—is a more important driver for ecosystem structure than ecosystem type.

Along the gradient of ecosystem development I found shifts in the soil microbial organic carbon degraders as well as nitrogen fixers. More specifically, along my chronosequence of wetland development I found similar abundances of soil organic carbon degraders and N fixers in a forest bog prior to permafrost degradation (FB) compared with my oldest collapse scar bog site (OCS). The intermediate young collapse scar bog (YCS) had strikingly different abundances of several soil organic carbon degraders and N fixers. Analyses of beta diversity trees and alpha diversity plots highlight similar differences in soil microbial communities between FB and YCS and YCS and OCS. That is, I found similar levels of alpha diversity at FB and OCS compared to YCS. Interestingly, alpha diversity was highest in the intermediate young collapse scar bog (YCS). Alpha diversity was highly correlated with pH, and to a lesser extent redox potential. FB and OCS sites represented relatively mature ecosystem states for these ecosystem types and these results suggest that ecosystem structure is dependent more on ecosystem maturity than ecosystem type.

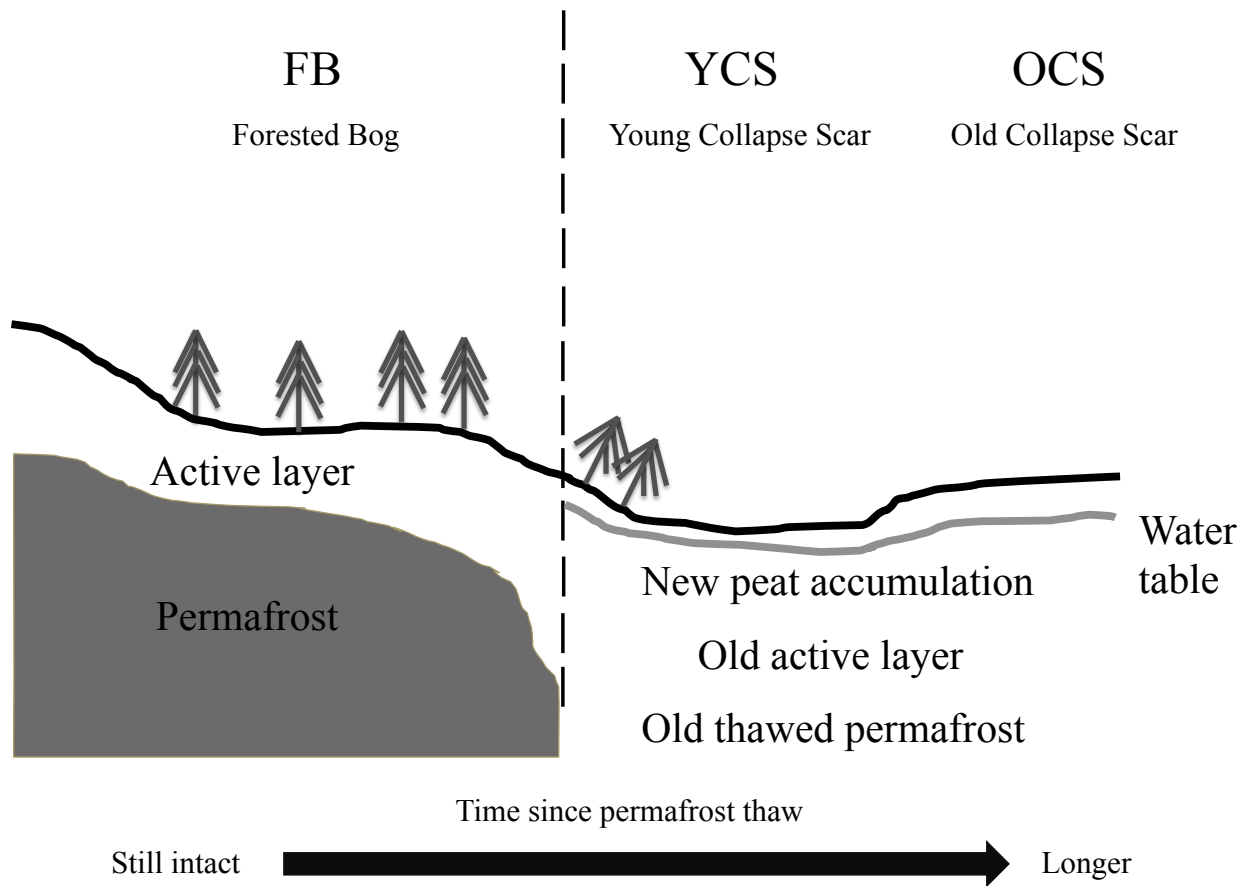


Figure 1. Cartoon schematic of study sites. FB is underlain by intact permafrost, YCS bog was most immediately formed following permafrost thaw and had the highest water table, and OCS formed after undergoing infilling and peat accumulation. Dotted line depicts permafrost thaw disturbance.

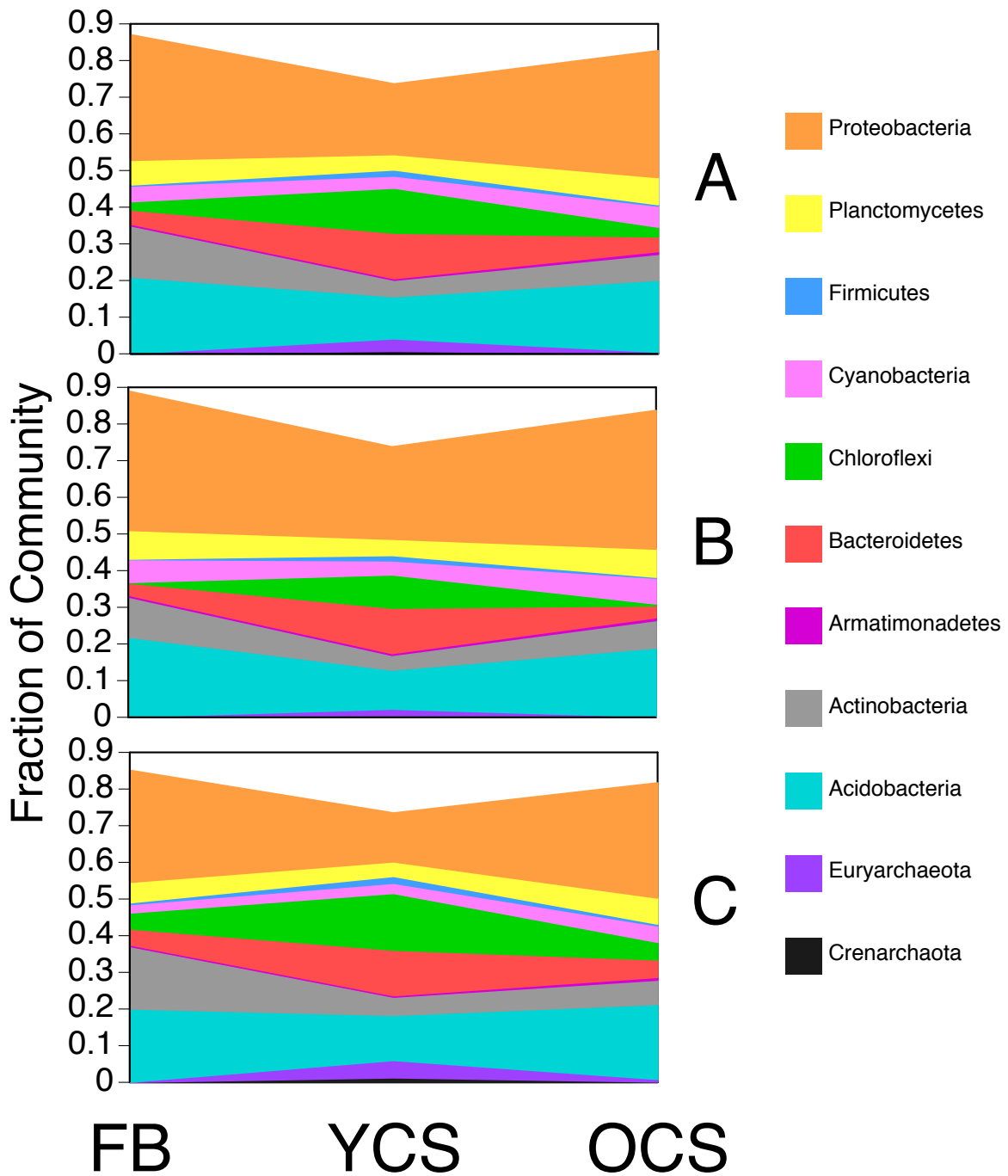


Figure 2. Phyla level microbial community structure at each site along the gradient of wetland ecosystem development on the x-axis. Legend colors are listed in order that correspond to figure.

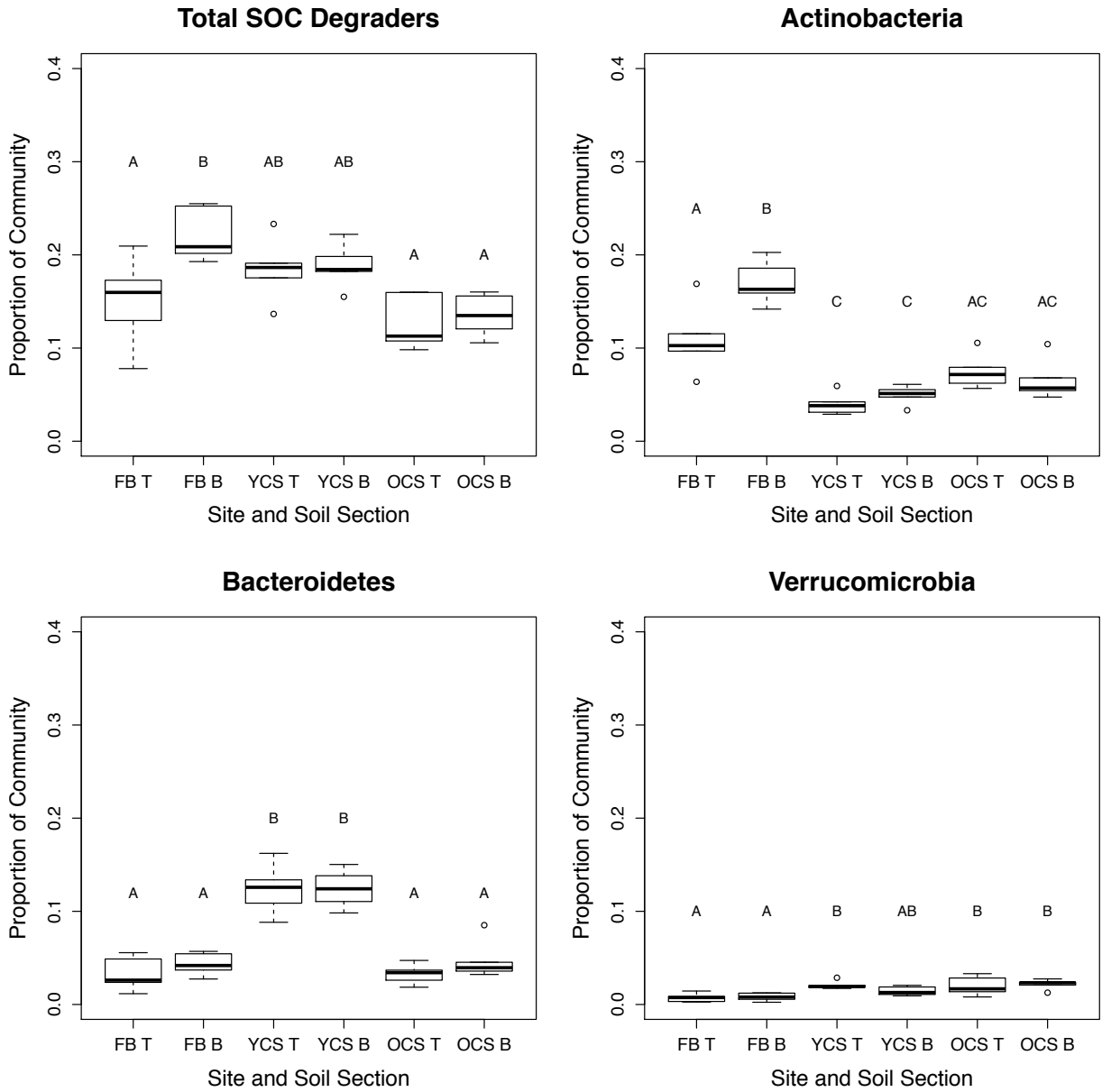


Figure 3. Soil organic carbon degraders across site and peat soil section. Top left panel represents the total summed proportion of the soil organic carbon degraders; top right panel is the proportion of Actinobacteria across site and soil depth; bottom left is the proportion of Bacteroidetes; and bottom right is the proportion of Verrucomicrobia.

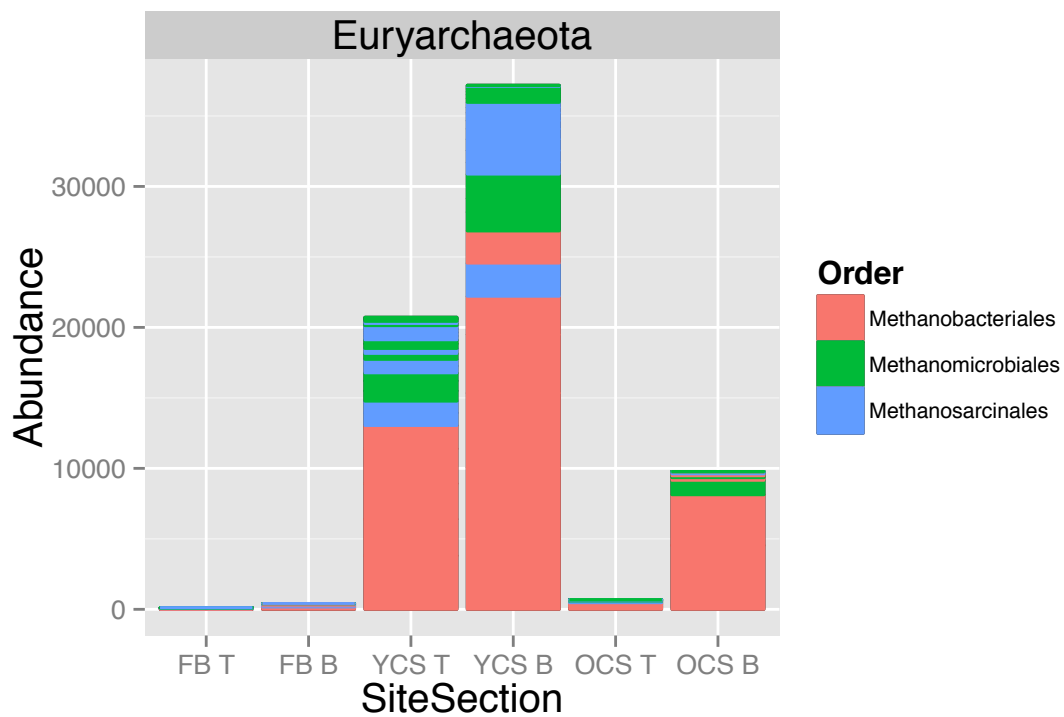


Figure 4. Abundance of the Archaeal methanogens in the Euryarchaeota phylum across site and soil depth. Colors correspond to different orders within the Euryarchaeota phylum.

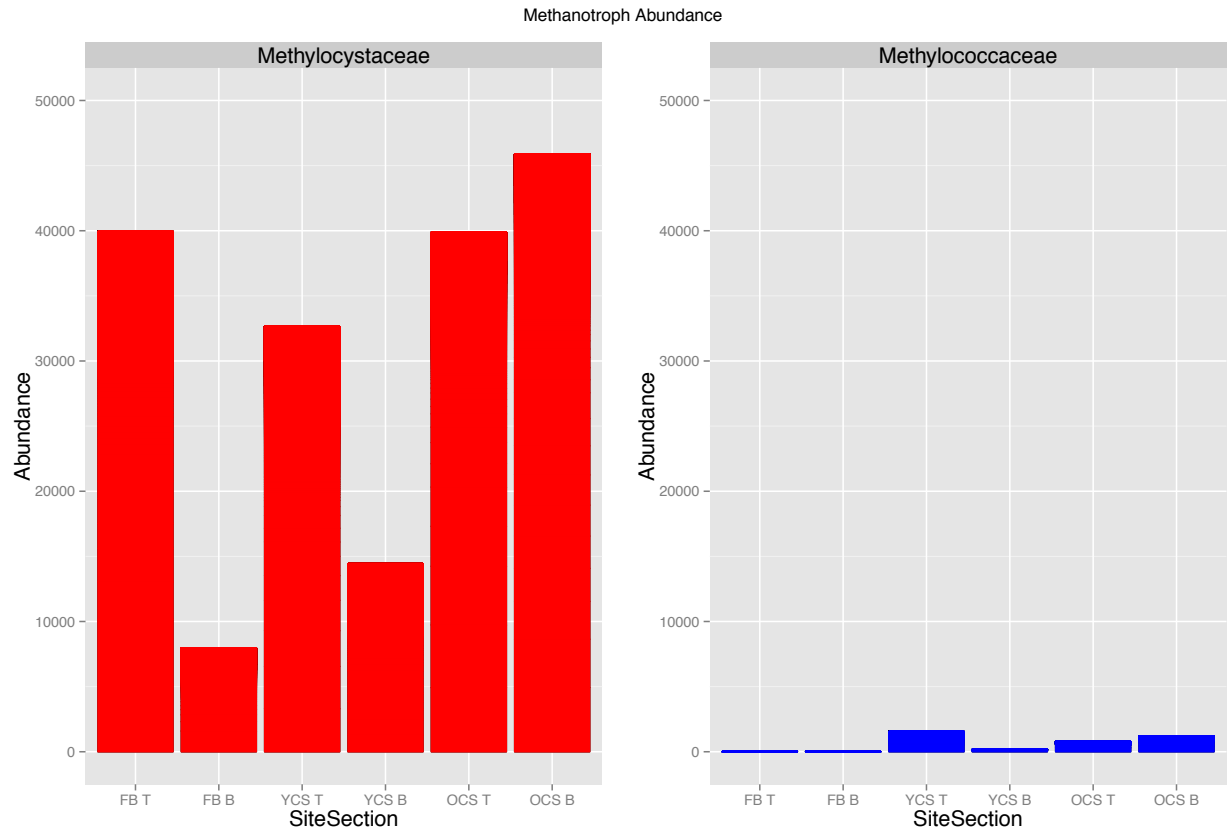


Figure 5. Methanotroph abundance across site and soil depth. Type II methanotrophs (Methylocystaceae) in the left panel and Type I methanotrophs in the right panel (Methylococcaceae). Type I and Type II methanotrophs obtain energy and carbon from methane, however, Type I methanotrophs use the Ribulose Monophosphate Cycle to incorporate formaldehyde, whereas Type II methanotrophs use formaldehyde through the serine pathway.

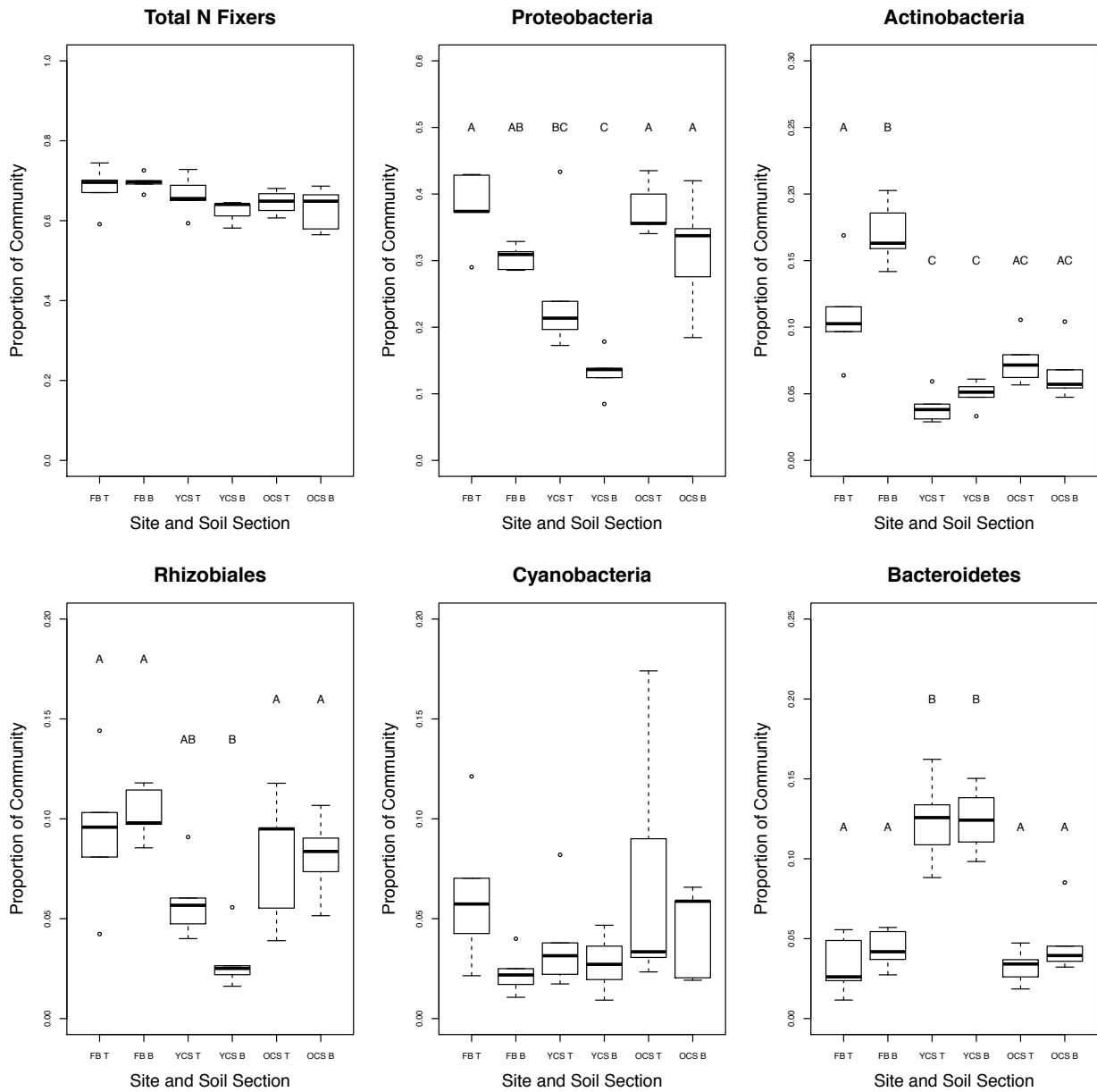


Figure 6. Bacterial abundances of microbes affiliated with N fixation. Top left panel represents the total summed proportion total N fixers; top center panel is the proportion of Proteobacteria across site and soil depth; top right is the proportion of Actinobacteria; bottom left is the proportion of Rhizobiales, bottom center panel is the proportion of Cyanobacteria; and bottom right is the proportion of Bacteroidetes.

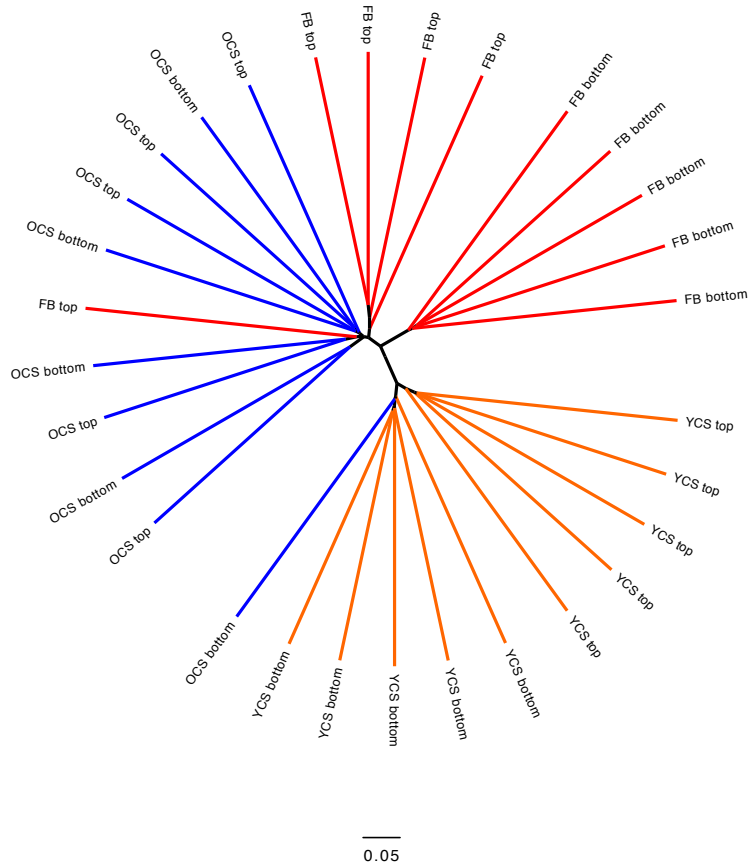


Figure 7. Beta diversity across site and soil depth. FB is represented by red branches, YCS by orange branches, and OCS by blue branches. Length of bar in bottom center of the figure represents 0.05 nucleotide substitutions per site.

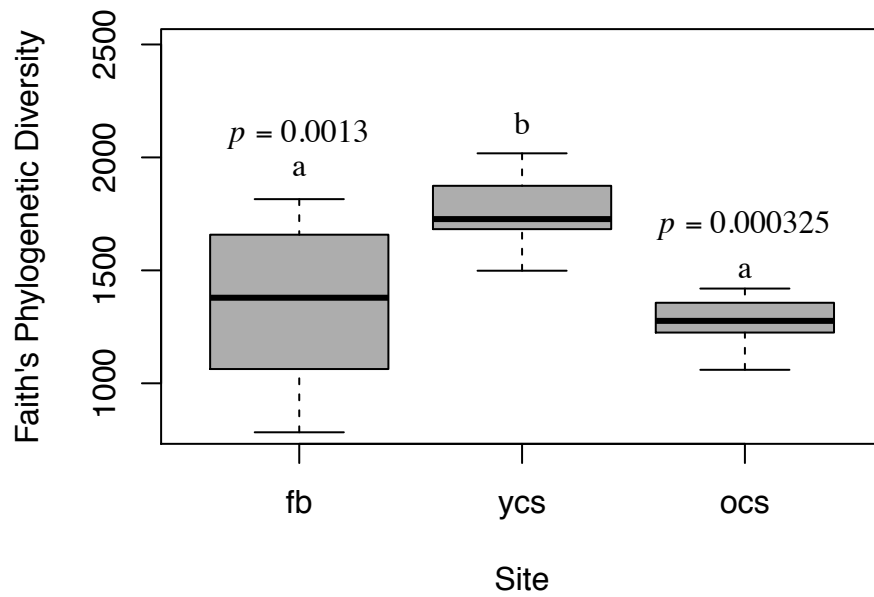


Figure 8. Alpha diversity across site as measured with Faith's Phylogenetic diversity. Letters above whiskers on box plots represent statistically different relationships. P value above whiskers designates the significance of those different relationships.

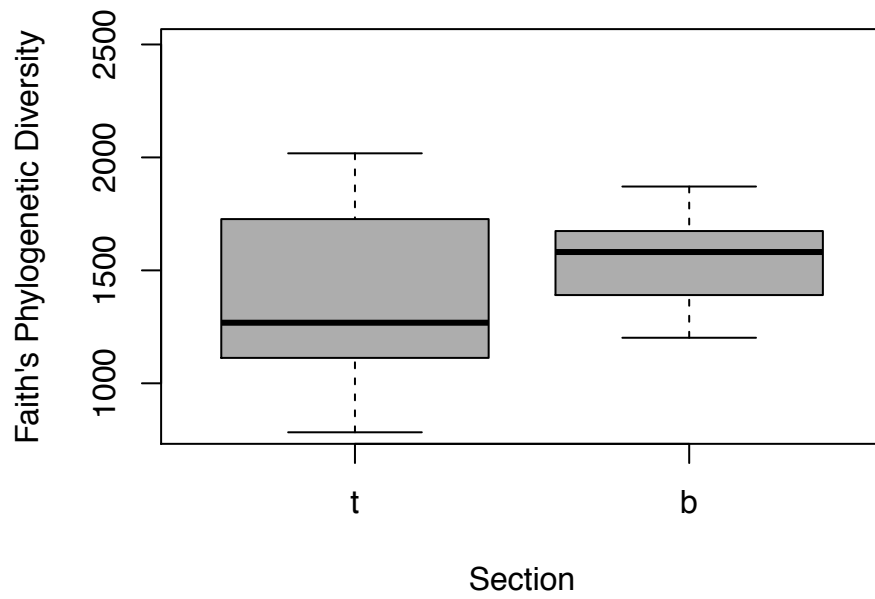


Figure 9. Alpha diversity across soil depth as measured with Faith's Phylogenetic diversity. The alpha diversity at the surface and subsurface sections was not statistically different.

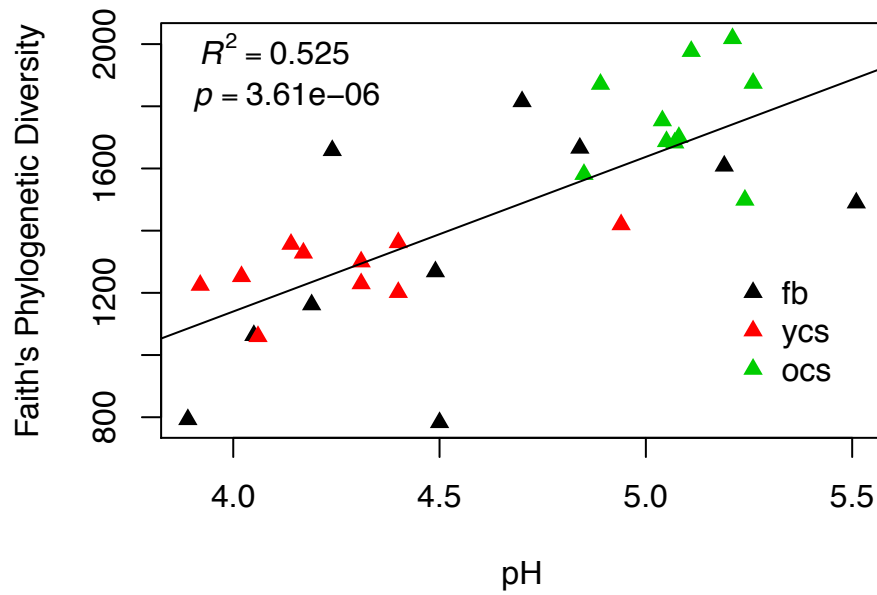


Figure 10. Alpha diversity across soil pH as measured with Faith's Phylogenetic diversity. Linear regression explained 52% of the variation of the data points and was significant ($P < 0.0001$). Sites were color-coded: FB was black, YCS was red, and OCS was green.

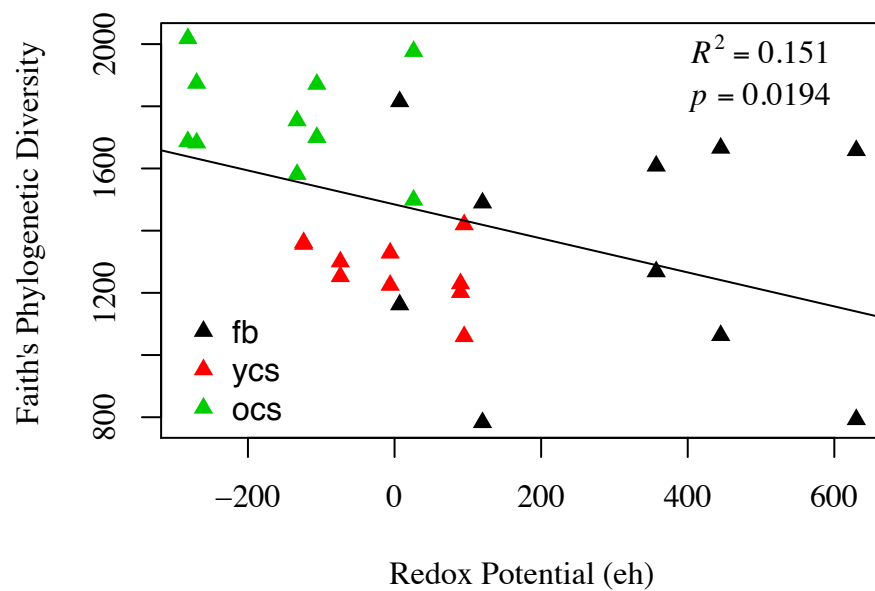


Figure 11. Alpha diversity across soil redox potential as measured with Faith's Phylogenetic diversity. Linear regression explained 15% of the variation of the data points and was significant ($P = 0.0194$). Sites were color-coded: FB was black, YCS was red, and OCS was green.

Illumina adapted primer sequences for the 16s rRNA gene.

Gene	Direction	Primer Sequence (5' – 3')
16s rRNA	F	ATCACGGTGYCAGCMGCCGCGGTA
16s rRNA	F	CGATGTGTGYCAGCMGCCGCGGTA
16s rRNA	F	AGTCAAGGTGYCAGCMGCCGCGGTA
16s rRNA	F	AGTTCCAGTGYCAGCMGCCGCGGTA
16s rRNA	F	GTTTCGTTCGTGYCAGCMGCCGCGGTA
16s rRNA	F	CGTACGATGTGYCAGCMGCCGCGGTA
16s rRNA	F	CATGGCATAAGTGYCAGCMGCCGCGGTA
16s rRNA	R	TCGAAGTGTGCCCCGYCAATTCMTTTRAGT
16s rRNA	R	CATTTTCCTGCCCCGYCAATTCMTTTRAGT
16s rRNA	R	ATGAGCAGCCCCGYCAATTCMTTTRAGT
16s rRNA	R	CAACTAGGCCCCGYCAATTCMTTTRAGT
16s rRNA	R	ATGTCAGCCCCGYCAATTCMTTTRAGT

Table 1. To multiplex all samples in a single lane on a Miseq 2000 run, I used 7 forward (F) and 5 reverse (R) primers for each gene. In the primer sequences the following letters represent a mix of the following nucleotides: Y = C, T, M = A, T, R = A, G, D = A, G, T, K = G, T, V = A, C, G, B = C, G, T, S = C, G.

CHAPTER 6

CONCLUSION

I. Introduction

Long before the coining of the term “Ecosystem” by Arthur Tansley in 1935, studies in ecosystem ecology have sought to explore the interactions among living and non-living ecosystem components. Ecosystem ecology has a rich history of seminal observational and empirical studies that provide an understanding of ecosystem structure and function. For example, early work by Clements (1916) and Gleason (1926) explored changes in ecosystem structure during succession. Though they proposed radically different ideas for how ecosystems organize, these early studies influenced a generation of ecologists that explored mechanisms for how ecosystems develop. After work on the concept of the ecological niche by Elton (1927), Lindeman (1942) highlighted the importance of the flow of energy and trophic dynamics for structuring lacustrine ecosystems that influenced ecosystem ecology.

These early observational and empirical studies have been complimented with theoretical work by Odum and Pinkerton (1955) and Odum (1969) that sought to provide an underlying theory of ecosystem development. Despite their early efforts, this underlying theory remains elusive—to a large extent from the lack of empirical work testing these theoretical constructs. My dissertation work sought to empirically test the Maximum Power Principle (MPP), a principle developed by Odum and Pinkerton (1955) that argues that systems develop to maximize power production or the flow of energy through a system. This dissertation addresses how the MPP relates to wetland ecosystem

development and examines changes in ecosystem structure and function during ecosystem development using the following guiding objectives: 1) To examine the historical origins of the MPP and relation to other prominent goal-oriented ecological principles; 2) To empirically test the MPP using adenosine triphosphate (ATP) and peat soil incubations; 3) To quantify the dynamics of greenhouse gas emissions across a chronosequence of wetland development, and because of their role with the production of greenhouse gases; 4) To investigate shifts in the microbial community during wetland ecosystem development.

II. Summary of Each Chapter

In CHAPTER 2 I explored the historical origins of the MPP and traced it back to The Second Law of Thermodynamics. The explicit consideration of energy flows in ecosystem ecology made The Laws of Thermodynamics—specifically The Second Law of Thermodynamics, a construct from physics—an appropriate place to start. I showed that the Maximum Entropy Production Principle (MEPP), a goal-oriented principle that has experienced renewed interest from disciplines ranging from physics, atmospheric sciences, and ecosystem ecology, and the MPP are more similar than dissimilar and maximization of power in ecosystems occurs with maximum entropy production. I found that these principles have great potential to explain how systems develop, organize, and function, but there are no widely agreed upon theoretical derivations for the MEPP and MPP, hindering their broader use in ecological research. There is scant work empirically testing these principles, which also hinders their broader use.

In CHAPTER 3 I used adenosine triphosphate (ATP) as a proxy for power in high-latitude wetland soil systems to test the MPP along a gradient of wetlands that have developed following permafrost degradation: a forested bog with permafrost (FB) and a young bog and older bog that have formed since their permafrost thawed (young collapse scar, or YCS and old collapse scar, or OCS, respectively). The results of this chapter suggested that system power decreased temporarily in the ~60-100 year old collapse scar bog (YCS) and that the system reorganized to rates of power production that approached pre-disturbance levels in the ~400 year old collapse scar bog (OCS), but in a markedly different state. This approach allowed me to characterize the outcome of permafrost thaw disturbance on subsequent changes in boreal wetland soil structure and function using an explicitly thermodynamic construct using the MPP.

In CHAPTER 4 I examined the temporal dynamics of greenhouse gas emissions across the same chronosequence gradient of ecosystem development using soil incubation experiments in the laboratory. I found that CH₄, CO₂, and N₂O production were generally lower in anoxic soils— that is, in soils below the water table. Along the gradient of wetland development, I found that the young collapse scar site (YCS)—formed ~60-100 years ago and most recently after permafrost thaw—produced the highest levels of CH₄ when compared with a forested bog site (FB) that was unimpacted by thaw disturbance. At the old collapse scar bog site (OCS), that collapsed and formed ~400 years ago, I observed much lower rates of CH₄ compared to YCS and FB. This suggested that as wetlands form from permafrost degradation, there is a large but transient pulse of CH₄ emissions. I also found that methanogen abundances were significantly higher at YCS

compared to the other sites and exhibited a strong positive relationship with CH₄ production rates.

In CHAPTER 5 I used the same gradient of wetland development following permafrost degradation to characterize changes in microbial communities involved with carbon and nitrogen cycling. I used reference 16s rRNA databases to assess taxonomic changes in the soil microbial community, and several diversity indices as metrics to assess soil microbial community changes in response to ecosystem development. Along the gradient of ecosystem development I found shifts in important ecosystem-level processes driven by the soil microbial community. I found evidence of large shifts along the chronosequence in individual phyla associated with soil organic carbon (SOC) degradation as well as in nitrogen fixers, although overall the SOC degraders and nitrogen fixers remained a constant fraction of the overall community. The FB and OCS sites represented relatively mature ecosystem states for these ecosystem types—forested bog and wetland bog, respectively—and these results suggested that ecosystem structure is dependent more on ecosystem maturity than ecosystem type.

III. Synthesis and Dissertation Contribution

Synthesis

As a whole, the chapters of this dissertation highlight several important changes that take place in soils during ecosystem development: 1) based on this work, ecosystem age was a more important factor for ecosystem structure and function relative to ecosystem type; 2) inundation and wetland formation in northern ecosystems favors

methanogens and methanogenesis; an important consideration given the potential positive feedbacks to further climatic warming, and; 3) studies that use goal-oriented principles to describe ecosystem structure and function have experienced a renaissance in the ecosystem ecology literature.

The importance of ecosystem maturity—not ecosystem type—for ecosystem structure and function was observed in CHAPTER 3 with rates of ATP production: The mature forested bog underlain by permafrost (FB) was more similar to OCS, the old collapse scar bog than to the younger intermediate site: the collapse scar bog (YCS). I also observed this pattern in CHAPTER 4 where FB and OCS were more similar than YCS with respect to greenhouse gas production. Additionally, I observed this pattern with microbial community composition in CHAPTER 5. Although YCS and OCS were of similar ecosystem type, FB and OCS were more mature ecosystems and were often more similar in terms of energy production (ATP production), greenhouse gas production, and microbial community composition.

Dissertation Contribution

By operationalizing a theoretical concept, such as ecosystem power, this dissertation contributes a novel approach and perspective to studying ecosystem development. This dissertation reports several important changes during ecosystem development, such as changes in greenhouse gas emissions, shifts in soil microbial communities, and changes in ecosystem power production. I also contributed to the advancement of theoretical ecology by explicitly documenting the development and

relation of several prominent goal-oriented principles in ecosystem ecology to the Laws of Thermodynamics. Given the profound importance of energy flows and materials cycling, explicitly grounding principles in ecosystem ecology to foundational physical laws represents an important advancement and strong link between power, physics, and ecology.

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