The impacts of environmental changes on peatland microbial community structure and function

by

Michelle M. Dart

A thesis submitted in partial fulfillment
of the requirements for the degree of
Masters of Science (MSc) in Biology

The Faculty of Graduate Studies
Laurentian University
Sudbury, Ontario, Canada

THESIS DEFENCE COMMITTEE/COMITÉ DE SOUTENANCE DE THÈSE
Laurentian Université/Université Laurentienne
Faculty of Graduate Studies/Faculté des études supérieures

Title of Thesis Titre de la thèse	The impacts of environmental changes on peatland microbial community structure and function
Name of Candidate Nom du candidat	Dart, Michelle
Degree Diplôme	Master of Science
Department/Program Département/Programme	Biology
Date of Defence Date de la soutenance	August 1, 2019

APPROVED/APPROUVÉ

Thesis Examiners/Examinateurs de thèse:

Dr. Nathan Basiliko
(Supervisor/Directeur de thèse)

Dr. Nadia Mykytczuk
(Committee member/Membre du comité)

Dr. Suzanne Brauer
(Committee member/Membre du comité)

Dr. Steven Short
(External Examiner/Examinateur externe)

Approved for the Faculty of Graduate Studies
Approuvé pour la Faculté des études supérieures
Dr. David Lesbarrères
Monsieur David Lesbarrères
Dean, Faculty of Graduate Studies
Doyen, Faculté des études supérieures

ACCESSIBILITY CLAUSE AND PERMISSION TO USE

Abstract:

Northern peatlands store approximately 30% of the world's soil carbon, and are also responsible for contemporary fluxes of the greenhouse gases carbon dioxide (CO_2) and methane (CH_4), making them important players in the global carbon (C) cycle. These greenhouse gas emissions are mediated by peat-dwelling microbes; however, the environmental factors governing the structures and functions of peat microbial communities are still poorly understood. In order to better understand these dynamics, I examined the effects of two forms of environmental change on peatland microbial communities. Firstly, to gain fundamental knowledge of the drivers of microbial community shift due to natural peatland succession, I examined the effects of long-term peat transplantation from a rich fen to a late-successional poor fen. This allowed me to evaluate the relative effects of solid phase chemistry and substrate (largely determined by the parent material/vegetation) versus aqueous chemistry (influenced more by groundwater or precipitation sources), on peat microbial communities. My results suggest that solid phase chemistry, particularly total nitrogen (TN) and C:N, may be important in determining the makeup of peatland bacterial communities. Secondly, I examined the effects of soil warming simulating projected climate change in a poor and an intermediate fen on peat microbial respiration and CH_4 production as the preliminary stage of a multi-year, large-scale field experiment. Soil warming did not lead to any effects on CO_2 production or CH_4 flux during peat incubation.

Keywords:

Peat, methane, carbon, bacteria, fungi, peatland succession, microbiology, chemistry, climate change

Acknowledgements:

I would like to acknowledge my supervisor, Dr. Nathan Basiliko, for his support and guidance throughout my project. Special thanks go to James Seward for his assistance in fieldwork, data processing and maintaining my sanity. I would also like to thank Dr. Jim McLaughlin and others at the Ontario Ministry of Natural Resources and Forestry for their help with fieldwork and peat chemistry analysis, Drs. Brian Branfireun and Zoë Lindo for their leadership on the BRACE project, Leland Johnston for field support, and Emily Smenderovac for her preternatural coding knowledge.

Table of Contents

List of Tables

List of Figures

General Introduction:

Peat consists of incompletely decomposed organic matter, and forms when decomposition processes proceed more slowly than primary production (Vitt, 2006). In Canada, peatlands are defined as areas of the landscape containing a depth of peat of at least 40 cm (NWWG, 1997). However, in other countries, this minimum depth is reduced to 30 cm (Rydin and Jeglum, 2006). The slow rate of decomposition processes in peatlands results from a combination of cool, anoxic conditions, low nutrient availability, and relatively low microbial number or biomass within peat, as well as the typically high refractory content of plants native to peatland ecosystems (Moore and Basiliko, 2006). Northern peatlands are those that developed following the recession of the ice sheets at the end of the last glaciation (Harden *et al*., 1992). These northern peatlands represent only a small portion of the Earth's surface (approximately 3%) (Rydin and Jeglum, 2006). However, they contain approximately 30% of the Earth's soil carbon, equal to between 210 and 450 Gt, making them important players in the global carbon cycle (Gorham, 1991; Turunen *et al.*, 2002). Globally, Canada comes second only to Russia in containing the largest area of northern peatland, with Ontario containing the largest area of peatland within Canada (Gorham, 1991; McLaughlin, 2004). Globally, northern peatlands sequester CO_2 in the form of partially decomposed plant matter at rates between 8 and 40 g CO_2-C m^{-2} $year^{-1}$, while in North American temperate and boreal peatlands sequestration rates have been shown to be approximately 25 g CO_2-C m^{-2} $year^{-1}$ (Gorham *et al*., 2003; Roulet *et al*., 2007).

In terms of vertical structure, peat consists of two broad zones – a surface layer called the acrotelm, and a deeper layer called the catotelm (Clymo, 1984). The catotelm is a water-saturated, anoxic zone in which anaerobic decomposition takes place, while the acrotelm, in

contrast, can be unsaturated and oxic or saturated and anoxic depending on seasonal fluctuations in water table (Warner, 1996). The acrotelm contains an uppermost productive layer (e.g. with photosynthetic moss tissue), followed by a litter layer and collapse layer as one proceeds deeper into the acrotelm (Warner, 1996). Peatlands also often display a distinct microtopography consisting of alternating raised areas and depressions called hummocks and hollows respectively.

Peatlands can be classified into different types based upon nutrient content and connection to groundwater. On one end of the spectrum are rich fens, which are characterized by high nutrient content and by having hydrologic connections to groundwater (Siegal and Glaser, 1987). These peatlands are said to be minerotrophic. On the other end of the spectrum, one sees poor fens and bogs, which are nutrient-poor with little or no connection to groundwater inputs, getting all or the majority of their hydrologic input from precipitation (Siegal and Glaser, 1987). They also receive most of their nutrients via atmospheric deposition (Glaser *et al*., 1981). Peatlands without hydrologic input from groundwater are termed ombrotrophic (Glaser, 1992). The range of peatland types from rich fens to poor fens and then bogs is also characterized by a shift in pH, with rich fens containing more alkalinity, and ombrotrophic bogs being more acidic, with typical surface water pHs ranging between 3.7 and 4.1 (Glaser, 1992). In terms of plant communities, rich fens tend to be dominated by non-*Sphagnum* bryophytes, sedges and herbs, while poor fens and bogs are dominated by *Sphagnum* moss and ericaceous shrubs (Lai, 2009).

As peatlands age they typically follow a successional pattern from minerotrophic fen to ombrotrophic bog. Apparent exceptions to this pattern have been recorded but are considered rare (Holmquist and MacDonald, 2014). Young peatlands typically initiate as rich fens in low-lying areas of watersheds (Bauer *et al*., 2003). A peat profile aggrades, and low hydraulic conductivity of highly humified organic matter, as well as topographic perching above the local

water table increasingly isolates sites from groundwater that typically contains higher base cations like Ca^{2+} than precipitation. *Sphagnum* mosses eventually become dominant due to their ability to grow in low nutrient conditions, and are responsible for the formation of ombrotrophic bogs at the end of succession, as they further increase porewater acidity by releasing uronic acids, and block the roots of vascular plants from reaching groundwater (Van Breemen, 1995; Pastor *et al*., 2002; Limpens and Berendse, 2003; Dorrepaal *et al*., 2005). *Sphagnum*-based peat is stable and resists decomposition due to its acidifying properties, its resistance to microbial degradation, and its large capacity to hold water and therefore maintain a waterlogged, anoxic environment (Limpens and Berendse, 2003; Dorrepaal *et al*., 2005). Microbial communities shift as peatland succession progresses (Kraigher *et al*., 2006; Jaatinen *et al*., 2007; Lin *et al*., 2012; Seward, 2018), however peat microbial communities are consistently dominated by bacteria, with fungi being much less abundant than is typical in other acidic to circumneutral soil types (Lin *et al*., 2012; Myers *et al*. 2012).

In addition to storing massive amounts of carbon in the form of peat over thousands of years, peatlands also exchange the greenhouse gases CO_2 and CH_4 in contemporary contexts, thus making them complex players in global carbon cycling. In general, peatlands are modest carbon sinks, exhibiting a net ecosystem uptake of 0.1 to 0.5 Pg C yr^{-1} (Roulet *et al*., 2007). This can be compared to a value of roughly 60 Pg C yr^{-1} for the gross terrestrial emission (Schimel, 1995), or between 10 and 50% of the annual estimated net terrestrial C sink. In terms of CH_4 emissions, peatlands are net emitters globally, releasing between 10 and 25 Tg of CH_4 per year (Roulet *et al*., 2007). CH_4 emissions from peatlands are of particular interest, due to CH_4 being an especially potent greenhouse gas; CH_4 traps heat 25 times more effectively over a 100-year time scale than does CO_2 (Lelieveld *et al*., 1998).

Methane emissions from peat result from methanogenesis, a biochemical process performed exclusively by microorganisms from the phylum Euryarchaeota in the domain Archaea, collectively termed methanogens (Woese *et al*., 1990). Seven orders within Euryarchaeota contain exclusively methanogens: Methanobacteriales, Methanocellales, Methanococcales, Methanomassiliicoccales, Methanomicrobiales, Methanopyrales, and Methanosarcinales (Borrel *et al*., 2013). However, it has been suggested via DNA sequencing of the *mcrA* gene, which is a phylogenetic marker used to identify methanogens, that there may be 12 orders within Euryarchaeota containing methanogens (Lever and Teske, 2015). Methanogens are obligate anaerobes that produce CH_4 from acetate, CO_2 and H_2, simple methylated compounds, or alcohols (Garcia *et al.*, 2000). Acetotrophic and hydrogenotrophic methanogens are dominant in peat; the former use acetate as a substrate, producing CO_2 and CH_4 according to the formula:

$$CH_3COOH \rightarrow CH_4 + CO_2$$

while the latter produce CH_4 by using H_2 as an electron donor to reduce CO_2:

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$$

(Lai, 2009). Hydrogenotrophic methanogens typically dominate deeper peat layers, which tend to be more recalcitrant, while acetotrophic methanogens predominate in upper peat layers due to their higher levels of labile organic carbon (Hornibrook *et al*., 1997). Both hydrogenotrophic and acetotrophic methanogens produce CH_4 in an exergonic reaction via the reductive demethylation of methyl-coenzyme M (CH_3-S-CoM) (Ferry, 2010). This reaction is catalyzed by methyl-CoM reductase (Mcr) and relies on electrons donated by coenzyme B (HS-CoB) (Ferry, 2010). Because Mcr mediates all methanogenesis, the *mcrA* gene, which is associated

with the production of this enzyme, is useful as a DNA target sequence for methanogens (Thauer, 1998; Luthon *et al*., 2002).

Methanogens lie at the end point in a sequence of anaerobic decomposition processes that take place in peat. Peat contains complex polymers that cannot be broken down by any one microbial functional group, particularly under anoxic conditions, so peat decomposition occurs via a food web of specialized microbes (Whalen, 2005). The first step in peat decomposition is mediated by hydrolytic microbes which secrete enzymes to break polysaccharides and other polymers into monomeric compounds (Garcia *et al*., 2000). Fermentative bacteria then transform these monomers into organic acids, volatile fatty acids, H_2, CO_2, and alcohols via acidogenesis (Le Mer and Roger, 2001). After fermentation, these products are converted to acetate via acetogenesis. This process can be performed by homoacetogenic bacteria, which degrade sugars and other monomers to acetate, or by syntrophic bacteria, which degrade alcohols and fatty acids to acetate while also producing CO_2 and H_2 (Conrad, 1999). Methanogenesis produces CH_4 from the end products of this sequence of reactions, and this sequence prohibits the accumulation of inhibitory end products during decomposition of organic matter (Garcia *et al*., 2000; Kamal and Varma, 2008).

There are a number of factors that can influence the growth of methanogens. For one, they are poor competitors in the presence of alternative electron acceptors supporting more energetic anaerobic respiration pathways (Achtnich *et al*., 1995). Thus they require a redox potential of less than -300 mV for growth, which indicates that electron acceptors of sulphate, nitrate and ferric iron have been depleted (Kamal and Varma, 2008). In temperate and subarctic peat, pH values between 5.5 and 7.0 have been found to be optimal for methanogenesis (Dunfield *et al*., 1993). Substrate limitation of methanogenesis in peat is also indicated by the

fact that the addition of fermentable substrates such leaf leachate and glucose, or fermentation products such as acetate, H_2, ethanol, formate and methanol, can often result in increased CH_4 production (Amaral and Knowles, 1994; Valentine *et al*., 1994; Bergmann, 1998). Likewise, the addition of the micronutrients Fe, Ni, Co and Na to peat can also increase methanogenesis (Basiliko and Yavitt, 2001).

While methanogens are responsible for CH_4 production in peatlands, peat also harbours other microorganisms which consume CH_4 and thus serve as buffers to lessen the release of CH_4 to the atmosphere. These microorganisms, collectively called methanotrophs, are members of the eubacteria that rely on single-carbon compounds (primarily CH_4 though some can use methanol) that are more reduced than formic acids for the production of energy (Hanson and Hanson, 1996). Methanotrophs sequentially oxidize CH_4 to methanol, formaldehyde, formate, and then CO_2 (Whalen, 2005). Methane monooxygenase (MMO) is the enzyme utilized by all methanotrophs to catalyze methane oxidation (Kamal and Varma, 2008). Methanotrophic activity can be classified into either high affinity oxidation, which takes place when CH_4 concentrations are similar to atmospheric CH_4 concentrations, or low affinity oxidation, which is induced at higher CH_4 concentrations of between 0.07 and 0.65 mg L^{-1} (Bender and Conrad, 1995; Segers, 1998). It is low affinity CH_4 oxidation that primarily occurs in northern peatlands (Bender and Conrad, 1995). The majority of methanotrophic activity generally occurs near the depth of the average water table, where the optimal ratio of CH_4 to oxygen is found (Dedysh, 2002). Methanotrophs also tend to grow at more acidic pHs than methanogens, with Kamal and Varma (2008) finding methanotroph growth to be favoured at pHs of between 4.3 and 5.9.

In addition to aerobic methanotrophy, anaerobic oxidation of methane (AOM) is also responsible for some methane consumption in northern peatlands, as was first observed by

Smemo and Yavitt (2007). However, AOM in northern peatlands remains poorly-understood. The precise methods of carbon assimilation via AOM in peatlands are unknown, and AOM rates do not appear to correlate with rates of CH_4 production (Gupta *et al*., 2013). Fens have been found to have higher rates of AOM than bogs, which suggests that AOM may be limited by electron acceptor availability, however the electron acceptor or acceptors involved are not yet known, with AOM not responding to experimental enrichments of sulphate, nitrate or ferric iron (Gupta *et al*., 2013).

While CH_4 production and consumption are mediated by microorganisms, there are a number of other physical and biological factors that influence the emission of CH_4 from peatlands. The transport of CH_4 from the deeper, anaerobic peat layers where it is produced to the atmosphere can occur through any of three methods: diffusion, ebullition, or via plant aerenchyma. Simple diffusion of CH_4 through waterlogged peat to the atmosphere is the slowest method of CH_4 transport, and provides the greatest opportunity for CH_4 consumption by methanotrophs (Lai, 2009). Ebullition is a faster process, wherein gaseous bubbles of CH_4 form and rise through the peat to the surface, while largely bypassing methanotrophic organisms (Lai, 2009). Higher temperatures can increase ebullition rates by bringing more CH_4 out of solution and into a gaseous state and by increasing possible bubble sizes (Fechner-Levy and Hemond, 1996). Aerenchymal transport is the fastest process by which CH_4 produced in peat can reach the atmosphere, and relies on an anatomical adaptation of some vascular plants, called aerenchymal tissue (Whalen, 2005). Aerenchyma are gas-filled passages within roots and other submerged organs of certain wetland-adapted vascular plants, such as sedges, which allow for gas exchange with the atmosphere and prevent hypoxia in submerged plant tissues (Joabsson *et al*., 1999). CH_4 produced within anoxic peat is able to enter these passages and be rapidly

transported to the atmosphere without interacting with aerobic peat layers where the majority of methanotrophic activity occurs (Whalen, 2005). In peatlands where sedges dominate the plant community, aerenchymal transport may account for up to 90% of CH_4 emissions, and CH_4 emissions from boreal peatlands can be significantly reduced by clipping aerenchymatous sedges (Schimel, 1995; Waddington *et al*., 1996).

Water table level is a major influence on peatland CH_4 emissions, with higher water tables being associated with higher emissions, due to the anaerobic CH_4 production zone being larger in proportion to the aerobic zone where most consumption occurs, and closer to the surface (Bubier *et al*., 1993; Pelletier *et al*., 2007). As fens generally have higher average water tables than bogs, this effect contributes to fens typically being greater emitters of CH_4 (Moore *et al*., 1990). Further, since peatland microtopographic hollows have a shallower water table than hummocks, hollows emit significantly more CH_4 than do hummocks (Clymo and Pearce, 1995; Waddington and Roulet, 1996). However, the magnifying effect of high water table on CH_4 emission often disappears in flooded peatlands, where an oxygenated water column above the peat surface enhances CH_4 oxidation (Bubier, 1995).

Objectives:

The microbial communities within peatlands are subjected to constant environmental change, such as changes in water table, temperature, pH, nutrient availability and vegetation, and they are likely to be subjected to more extreme environmental changes as climate change progresses. However, the effects of environmental change on peat microbial communities are, at present, poorly understood. Research into CH_4 emission from northern peatlands is of global significance in this era of anthropogenic climate warming, especially given the sheer amount of

carbon currently stored within these wetlands. Since peatland CH_4 emission is directly mediated by archaeal methanogenesis and bacterial methanotrophy, it is essential to study the microbial communities of northern peatlands in order to understand their potential impact on the global climate. Further, while it is known that microbial communities differ between different peatland successional stages (e.g. between rich fens and bogs), the drivers of these community shifts are poorly understood. Therefore, the objectives of my thesis were to assess the impact of two forms of environmental change on northern peatland microbial communities. In Chapter 1, I examine the long-term effects of peat core transplantation from a rich fen to a poor fen (mimicking the natural transition from rich fen to poor fen), in an effort to determine the relative effects of substrate and solid phase chemistry (determined in large part by the parent material/vegetation) versus aqueous chemistry, on peat microbial communities. In Chapter 2, I examine the effects of soil warming on peat microbial respiration and CH_4 production via a large-endeavor field experiment, as the preliminary stage to a long-term, large-scale field experiment to assess the future impact of climate warming on northern peatlands.

Chapter 1: The fate of eutrophic rich fen peat in a later-successional poor fen environment

1.1 Introduction:

Peatlands generally begin as rich fens in the low-lying areas of watersheds, and then to transition first into poor fens and then into bogs as peat accumulates and the peatland is colonized by *Sphagnum* mosses, with these factors serving to gradually cut growing plants off from groundwater (Bauer *et al*., 2003; Limpens and Berendse, 2003; Dorrepaal *et al*., 2005). As peatlands transition from rich fens to poor fens to bogs, they become more acidic and more nutrient-poor (Siegal and Glaser, 1987; Glaser, 1992). New peat that accumulates during peatland succession also differs in its structural and chemical make-up, as different forms of vegetation (i.e. the organic soil parent material) dominate different peatland types. Rich fens are typically dominated by sedges and herbs and non-Sphagnum bryophytes, while poor fens and bogs are dominated by *Sphagnum* mosses and ericaceous shrubs (Lai, 2009). Thus, in a peatland that has transitioned from a rich fen to a poor fen or bog, different peat layers will be formed from different vegetation types, with deeper peat layers being formed from sedges and other rich fen vegetation, while newer, shallower layers will be formed mainly from *Sphagnum*. This allows for differences in physical structure and solid phase chemistry between peat layers.

Microbial communities are known to differ between rich fens and poor fens or bogs (Kraigher *et al*., 2006; Jaatinen *et al*., 2007; Lin *et al*., 2012; Seward, 2018). For example, *Acidobacteria* are more prevalent in bogs and poor fens, while *Bacteroidetes* are more prevalent in intermediate and rich fens (Seward, 2018). A number of studies have found pH to be a major driver in archaeal and bacterial community clustering in peat as well as other soil types, indicating that pH likely plays a major role in community differences between peatland types

(Metthies *et al*., 1997; Fierer and Jackson, 2006; Rousk *et al*., 2010; Seward, 2018). However, the direct effects of physical substrate (i.e. peat structure and stable solid phase chemistry) on peatland microbial communities are poorly understood. In November 2011, Preston (2013) conducted an experiment in which peat cores were reciprocally transplanted between a rich and a poor fen, both located near White River, Ontario, within 2.75 km of each other. In June 2012 and October 2012, subsets of these cores were extracted along with replanted peat cores and previously undisturbed peat from both peatlands. Subsequent tests on these cores revealed that by October 2012, pH in the transplanted cores had changed to become similar to the surrounding peat. Additionally, no differences between any treatments were observed in terms of coarse-scale microbial community composition determined via terminal restriction fragment length polymorphism analysis (T-RFLP) using archaeal, bacterial and fungal gene targets. However, microbial community composition and basal respiration rates were also found to be similar between the rich and poor fens at the time, so while this study could have done much to elucidate the relative influences of peat substrate and aqueous chemistry on peat microbial community composition, it is difficult to draw solid conclusions from these findings.

Two possible explanations for Preston's lack of significant findings could be explored. Firstly, the technique used in his study to analyze microbial community composition, T-RFLP, was likely too crude to provide adequate resolution in differentiating different microbial communities. More advanced fingerprinting techniques such as Illumina MiSeq marker gene amplicon sequencing might have revealed community differences not visible using T-RFLP. Secondly, it is possible that the approximately 11 month duration of Preston's transplantation study was too short to produce significant differences in microbial community structure. Fortunately, Preston planted more transplant/replant peat core sets in November 2011 than he

recovered by the end of his study in October 2012. This allowed me to recover Preston's remaining core sets from the poor fen site (the rich fen being inaccessible due to flooding from a downstream beaver dam) in June 2017, approximately five years and seven months after the cores were planted. I analyzed these cores for microbial community structure using the Illumina Miseq platform, after a much longer transplantation duration than Preston's original study. I also performed aqueous and solid state chemical analyses, as well as *in vitro* measurements of $CO_2$and CH_4 production and consumption under both oxic and anoxic conditions, in an effort to discern the relative effects of physical peat substrate versus aqueous chemistry in determining microbial community structure.

1.2 Methods:

1.2.1 Experimental site and sampling:

The experimental site, (48°35'N, 85°33'W), is a poor fen located near White River, Ontario. The site is dominated by *Sphagnum* mosses, ericaceous shrubs such as leatherleaf (*Chamaedaphne calyculata*), tamarack (*Larix laricina*) and black spruce (*Picea mariana*), and has a hummock-hollow microtopography. In November 2011, Michael Preston transplanted peat cores from a nearby rich fen into this poor fen as part of his PhD thesis work (Preston, 2013). This rich fen, located at (48°33'N, 85°35'W), 2.75 km away from the poor fen, was dominated by sedges such as *Eriophorum vaginatum* and *Carex* sp., and had a flat topography. Preston took 30cm long, 2.5cm diameter cores from the rich fen, and placed them in PVC tubes, each with 12 1cm holes drilled into them to permit exchange of water and dissolved substances (Preston, 2013). These were then placed into 500 μm pore size mesh bags and transplanted at various locations into the poor fen, and vegetation placed over top of them (Preston, 2013). Preston also

performed the treatment on cores taken from the poor fen, which were then replanted alongside the transplanted cores as controls (2013).

Following Preston's thesis work, 5 transplant-replant pairs of cores remained at the poor fen site, and these became the subject of my thesis work. These remaining transplanted and replanted cores were located and extracted by hand on June 6th and 7th, 2017. All cores were intact with mesh and PVC casing uncompromised. 30 cm control peat cores were also taken from near each of the transplant/replant core locations (within approximately 2 feet) using a box corer or knife. All cores were placed in plastic resealable bags and stored in a cooler for transplant to the field base, where they were stored at 4ºC. Cores were stored at -20ºC upon arrival at Laurentian University. Transplant and replant cores were removed from their mesh and PVC tubing, and all cores were homogenized by hand prior to analysis. A small number of woody branches and roots were contained in the cores but these were removed during homogenization.

1.2.2 Aerobic and anaerobic incubations:

Methane consumption was measured via aerobic incubation of peat samples. Approximately 2g of peat sample (wet weight) was placed in a 130 mL mason jar, and then each jar lid was firmly closed so as to be airtight. Each mason jar had had a rubber septum inserted into the lid to facilitate injection and extraction of gases using a needle and syringe. At time 0 of the incubation, pure CH_4 was injected into each jar to bring the concentration of CH_4 inside the jar to 50ppm. CH_4 and CO_2 within each jar were measured using an SRI 8610C gas chromatograph at time 0, and also at 4 hours and 24 hours into the incubation. After gas was extracted from each jar for measurement, an equal volume of room air was injected into the jar to

maintain a constant gas pressure within the jar. Jars were kept at room temperature and away from light between measurements.

Methane production was measured via anaerobic incubation. 130 mL mason jars with rubber septa were also used for these incubations. Approximately 2 g of peat sample (wet weight) were placed in each jar and the jar lids tightly closed. All CH_4 and CO_2 was then evacuated from the closed jars via repeated cycles of gas extraction using a vacuum pump and subsequent backfilling with pure N_2. At the end of this cycling (time 0), the atmosphere inside the jar contained only N_2. Gas pressure inside the jar was equalized to approximately that of the atmospheric pressure inside the laboratory. CH_4 and CO_2 measurements were made via SRI 8610C gas chromatograph on days 4, 9, 17, 26 and 57 of the incubation. After gas was extracted via needle and syringe for each measurement, an equal volume of N_2 was injected into each jar to maintain constant gas pressure. Jars were kept at room temperature and away from light between measurements.

Results from the aerobic incubation were expressed as weight of gas consumed/produced per hour per gram dry weight peat. Anaerobic incubation results meanwhile were expressed as net weight of gas produced per gram dry weight peat. Due to data skewness, all incubation data was base-log transformed prior to running one-way ANOVAs. The post hoc test utilized was Tukey's HSD at a significance threshold of $p = 0.05$.

1.2.3 Peat chemistry:

8:1 water:peat extracts were made from each sample. Peat and water were shaken with an oscillating shaker for 1 hour at 200 rpm, and then filtered through 0.5µm disc filters to create water extracts. pH of these water extracts was measured using an Accumet AB150 pH/mV

meter (Fisher Scientific). Water extracts were then sent to the Ontario Ministry of Natural Resources and Forestry laboratory in Sault Ste. Marie, Ontario for analysis of water soluble total C and total N using a Lachat TOC/TN IL550 analyzer (Hach Company, Loveland Colorado, USA), and Ca^{2+}, K^{+}, Mg^{2+}, Na^{+}, HCO_3^{-}, Cl^{-}, NO_3^{-}, and SO_4^{2-} using an ICS 2000 ion chromatograph (Dionex Corp., Sunnyvale, CA), after Myers *et al*. (2012).

A portion of each remaining peat sample was also dried at 60ºC for 24 hours using a Fisher Scientific Isotemp Oven. The resulting dried sample was then ground using a Wiley mill. The dried, milled sample was also sent to the OMNRF laboratory for analysis of solid phase total carbon and total nitrogen using an Elementar Vario Max C and N analyzer (Elementar Analysensysteme, Hanau, Germany), total sulphur using an Eltra Helios C and S analyzer (Eltra GmbH, Neuss, Germany), and Ca, K, Mg and P using a Varian Liberty Series II inductively coupled plasma spectrometer (ICP, Agilent Technologies, Santa Clara, USA) following extraction with 0.1 mL 15.5% SeO_2 in 5 mL concentrated H_2SO_4 digests. All solid phase chemistry analysis was also after Myers *et al*. (2012).

One-way ANOVAs were performed on the resulting chemical data, along with peat chemical data from the rich and poor fens collected by Myers et al. (2012) in July 2008, which had been processed using the same methods and in the same analytical laboratory as in this study. All chemical data (not including those from Myers *et al.*) were base-log transformed prior to running ANOVAs due to data skewness. The post hoc test utilized was Tukey's HSD, at a significance threshold of $p = 0.05$.

1.2.4 DNA extraction:

DNA was extracted from all cores using the PowerSoil DNA Isolation Kit (Qiagen). Extracted DNA was suspended in the supplied elution buffer (10 mMTris), and stored at -20ºC for further use.

1.2.5 Miseq Illumina sequencing and data analysis:

Microbial sequence reads were generated on the MiSeq Illumina Next-Generation sequencing platform (Quail *et al*., 2012). For bacteria communities, the 16S V4 region (515FB (5'-GTGYCAGCMGCCGCGGTAA-3')/806RB (5'-GGACTACNVGGGTWTCTAAT-3')) was targeted, while the internal transcribed spacer (ITS1) (ITS1F (5'-ACCTGCGGARGGATCA-3')/ITS1R (5'-GAGATCCRTTGYTRAAAGTT-3')) region was used for fungal community analysis. Raw sequences data were obtained from Metagenom Bio and was first quality filtered using BBMap package (Bushnell, 2016). Forward and reserve reads were aligned and processed with the DADA2 package (Callahan *et al*., 2016) to produce exact amplicon sequence variants (ASVs). For taxonomic assignment, the Greengenes 2013 database (DeSantis *et al*., 2006) was used for identifying bacterial assemblages, while UNITE (Abarenkov *et al*., 2010) dataset was applied for fungal identification. Phylum-level relative abundance data were base-log transformed prior to running one-way ANOVAs due to data skewness. The post hoc test utilized was Tukey's HSD, at a significance threshold of $p = 0.05$. Phylum richness, evenness and Shannon diversity were calculated in Excel. One-way ANOVAs were run on phylum richness, evenness and Shannon diversity, using Tukey's HSD post hoc test at a significance threshold of $p = 0.05$. Phylum evenness and Shannon diversity were base-log transformed prior to ANOVA due to data skewness.

The R (R Core Team, 2013) based package, phyloseq (McMurdie and Holmes, 2013), was applied to visualize Bray-Curtis beta diversity through non-metric multidimensional scaling

(NMDS) ordination. Chemical gradients, based on collected chemical data which displayed statistically significant differences between treatment conditions, were applied to the NMDS ordination in an attempt to identify the primary drivers of microbial community structure using phyloseq libraries (McMurdie and Holmes, 2013). Ellipses were generated using the R based CRAN package, ellipse (Murdoch and Chow, 2018) and were calculated by means of standard error. For the permutational multivariate analysis of variance using distance matrices (PERMANOVA), the vegan package, Adonis (Oksanen *et al*., 2018) was utilized. This statistical analysis uses a permutation test with pseudo-*F* ratios to partition distance matrices among sources of variation.

1.2.6 Precipitation data:

Monthly and daily precipitation data were obtained from the Government of Canada's National Climate Archives (climate.weather.gc.ca). Precipitation data was from the nearest meteorological stations to the poor fen study site, in Pukaskwa National Park, approximately 62 km from the study site. Complete precipitation data was not available from any one station in Pukaskwa National Park, so two complementary stations were used, located at 48°36'28.000", 86°17'14.000" W (Climate ID 6046768; World Meteorological Organization (WMO) ID 71750), and 48°35'18.000" N, 86°17'41.000" W (Climate ID 6046767; WMO ID 71750). Daily precipitation data from the 30 days prior to the sample dates of June 5th and 6th, 2017 were obtained to look for short-term precipitation inputs that could temporarily raise the water table at the poor fen study site and affect the site's chemistry and microbiology. Monthly precipitation data from 2008-2017 was analyzed in Microsoft Excel to compare 2017's monthly precipitation to the decade mean (note however that complete winter precipitation data was not available from

years prior to 2012, so precipitation means from November to April did not include data from the entire decade). Annual precipitation from 2012-2018 was plotted in Excel and a linear regression was calculated in order to observe any trends in annual precipitation that could affect the site over the long term. Annual precipitation data were not normally distributed and thus were base-log transformed prior to this analysis.

1.3 Results:

1.3.1 Aerobic and anaerobic incubations:

Aerobic incubation did not reveal any significant effect of transplantation or replantation on net CH_4 consumption [$F(2, 12) = 2.40$, $p = 0.133$]. Net aerobic CO_2 production was significantly higher in the control condition than in either the transplant or replant conditions [$F(2, 12) = 17.35$, $p = 2.88 \times 10^{-4}$], indicating effects produced by the experimental conditions, however no significant difference in net CO_2 production was found between the transplanted and replanted cores.

Likewise, no significant effects were found on net CH_4 production during anaerobic incubation [$F(2, 12) = 0.90$, $p = 0.431$]. However, anaerobic net CO_2 production was significantly lower in the transplant condition than either the replant or control conditions [$F(2, 12) = 9.82$, $p = 0.003$], indicating lower overall metabolic activity in the transplanted cores. No significant difference in net CO_2 production was found between the replanted and control cores. Aerobic and anaerobic incubation results are summarized in Table 1.1.

1.3.2 Peat chemistry:

Analysis of peat water extracts revealed no significant differences in soluble total N [$F(4, 17) = 90.27$, $p = 3.23 \times 10^{-11}$], Ca^{2+} [$F(4, 20) = 8.71$, $p = 3.07 \times 10^{-4}$], Mg^{2+} [$F(4, 19) = 13.11$, $p =$

$2.73x10^{-5}$], K^+ [$F(4, 20) = 22.03$, $p = 4.28x10^{-7}$], Na^+ [$F(4, 20) = 9.24$, $p = 2.13x10^{-4}$], HCO_3^- [$F(2, 12) = 0.36$, $p = 0.706$], SO_4^{2-} [$F(4, 20) = 2.45$, $p = 0.080$], NO_3^- [$F(4, 17) = 2.98$, $p = 0.049$], or Cl^- [$F(4, 20) = 9.56$, $p = 1.73x10^{-4}$] content between the transplant, replant or control conditions. Total soluble C was significantly lower in the transplant core samples than in the control [$F(2, 9) = 4.70$, $p = 0.040$], however no significant differences in soluble C:N ratios were found among the different transplant treatment conditions [$F(2, 9) = 0.46$, $p = 0.644$]. No significant differences were found in peat slurry pH between the treatment conditions [$F(4, 20) = 50.86$, $p = 3.33x10^{-10}$]. These soluble chemistry data, along with soluble chemistry data collected from the rich and poor fen by Myers *et al.* (2012), are summarized in Table 1.2 and Figure 1.1.

In contrast, solid phase peat analysis revealed several significant results between the three treatment conditions. Total N was significantly higher in the transplanted cores than in the replanted or control conditions [$F(4, 20) = 20.24$, $p = 8.38x10^{-7}$], while total C was significantly lower [$F(4, 20) = 111.38$, $p = 2.27x10^{-13}$], resulting in C:N ratios of the transplanted cores also being significantly lower than in the replanted or control cores [$F(4, 20) = 32.50$, $p = 1.72x10^{-8}$]. P [$F(4, 20) = 19.62$, $p = 1.07x10^{-6}$] and total S [$F(4, 20) = 34.22$, $p = 1.10x10^{-8}$] were significantly higher in the transplanted cores than in both the replanted or control cores. K was significantly higher in the control cores than in the transplanted or replanted cores [$F(4, 20) = 14.60$, $p = 9.84x10^{-6}$]. Mg was significantly lower in the transplanted cores than in the control cores only [$F(4, 20) = 5.30$, $p = 0.004$]. No significant differences in solid phase Ca content were found between the transplant, replant or control conditions [$F(4, 20) = 16.70$, $p = 3.67x10^{-6}$]. Solid phase peat chemistry data for the transplant, replant and control conditions of this study

are summarized in Table 1.2 and Figure 1.2, along with solid phase chemistry data collected from the poor and rich fens by Myers *et al.* (2012).

1.3.3 16S Illumina sequencing:

Illumina read numbers are summarized in Table 1.3. 16S Illumina sequencing results (Table 1.4; Figures 1.3 and 1.4) showed that phylum-level bacterial/archaeal communities were largely dominated by Proteobacteria across all treatments, with mean fractional abundances of 0.475 in transplant, 0.417 in replant, and 0.709 in control. The second-highest fractional abundances were held by Bacteroidetes in the transplant and control treatments, with mean fractional abundances of 0.309 and 0.143 respectively, and by Acidobacteria in the replant condition, with a mean fractional abundance of 0.149. The Candidate Phyla Radiation (CPR) held third place in highest fractional abundance across all treatments, with mean fractional abundances of 0.083 in transplant, 0.132 in replant, and 0.079 in control. PERMANOVA results indicated that the strongest differences in bacterial/archaeal community composition were between the transplant and replant treatments [$R^2 = 0.36$, $p = 0.03$], and differences were significant, however, interestingly experimental treatments were not significantly different from the in-situ cores (control vs. transplant [$R^2 = 0.23$, $p = 0.112$], and control vs. replant [$R^2 = 0.21$, $p = 0.076$]). One-way ANOVAs on phylum-level fractional abundance data yielded statistically significant results only within the phyla Chloroflexi [$F(2, 8) = 185.76$, $p = 1.97 \times 10^{-7}$] and Verrucomicrobia [$F(2, 8) = 5.71$, $p = 0.029$]. Tukey HSD post hoc testing revealed that, at a 0.05 significance threshold, Chloroflexi was less abundant in the transplant treatment than in either the replant or control treatments, and that Verrucomicrobia was more abundant in the transplant treatment than in either the replant or control. However, Chloroflexi and Verrucomicrobia each

only made up a small portion of the bacterial/archaeal community. The mean fractional abundance of Chloroflexi in each treatment was 0 in transplant, 0.021 in replant and 0.009 in control, while the mean fractional abundance of Verrucomicrobia in each treatment was 0.022 in transplant, and 0 in both replant and control.

One-way ANOVAs revealed no significant differences between treatments in phylum richness [$F(2,8) = 1.33$, $p = 0.318$], evenness [$F(2, 8) = 0.86$, $p = 0.460$], or Shannon diversity [$F(2, 8) = 1.24$, $p = 0.339$] (Table 1.5). However, beta-diversity of all bacterial and archaeal taxonomic classification levels, evaluated via NMDS Bray-Curtis dissimilarity ordination (Figure 1.5), showed tight separate clustering of the transplanted and control samples along the NMDS1 axis, with the replanted samples instead showing a loose distribution, indicating wider variability in the replanted bacterial/archaeal community. NMDS chemistry gradient plots (Figures 1.6A-H) indicated that the chemical analytes with the strongest relationships to 16S community structure were solid phase TN (Figure 1.6C) and C:N (Figure 1.6D). Weaker associations also appeared to be present between bacterial community structure and both solid phase P (Figure 1.6G) and TS (Figure 1.6E).

1.3.4 ITS1 Illumina sequencing:

Illumina read numbers are summarized in Table 1.3. PERMANOVA results indicated a significant difference in phylum-level fungal community composition between the control and replant conditions [$R^2 = 0.24$, $p = 0.041$], but not between the transplant and control [$R^2 = 0.18$, $p = 0.315$], or the transplant and replant conditions [$R^2 = 0.14$, $p = 0.575$]. Illumina sequencing targeting ITS1 showed the phylum-level fungal communities (Table 1.6; Figures 1.7 and 1.8) among the three treatments to be dominated by Basidiomycota, Ascomycota and

Mortierellomycota in differing proportions, though one-way ANOVAs of the fractional abundances of these phyla yielded no statistically significant differences between treatments. The only phylum which displayed any significant differences in fractional abundance between treatments according to one-way ANOVA was Mucoromycota [$F(2, 8) = 6.94$, $p = 0.018$]. Tukey HSD post hoc testing showed Mucoromycota to have a lower fractional abundance under the transplant treatment condition than under either the replant or control treatment condition at a 0.05 significance threshold. Mean fractional abundance of Mucoromycota was 3.01E-04 in transplant, 0.036 in replant, and 0.003 in control.

One-way ANOVA yielded a significant difference in phylum richness between treatments [$F(2, 8) = 5.60$, $p = 0.030$], with a Tukey HSD post hoc test showing a lower phylum richness in the transplant condition (mean = 3.33) than the replant condition (mean = 4.5) at a 0.05 significance threshold. There were no statistically significant differences between treatments in evenness [$F(2, 8) = 0.41$, $p = 0.677$] or Shannon diversity [$F(2, 8) = 1.08$, $p = 0.384$]. Fungal phylum richness, evenness and Shannon diversity are summarized in Table 1.7. NMDS Bray-Curtis dissimilarity ordination (Figure 1.9), visualizing beta-diversity of all fungal taxonomic classification levels, showed clustering together of the transplant and replant treatment samples along the x-axis, while control samples did not display distinct clustering. NMDS chemistry gradient plots (Figures 1.10A-H) failed to illustrate any clear relationship between peat chemistry and ITS1 community structure.

1.3.5 Precipitation data:

Pukaskwa National Park's monthly precipitation data from 2008-2017 showed June to have the highest mean precipitation, with June 2017 being wetter than average but still within

one standard deviation of the mean (Table 1.8; Figure 1.11). However, the bulk of this precipitation came after the sampling dates of June 5th and 6th, and no large precipitation events that would have significantly raised the study site's water table were observed in the 30 days prior to sampling (Table 1.9). Linear regression of annual precipitation data from 2012-2018 showed evidence for a gradual reduction in precipitation over this time period (Log_{10}(Precipitation (mm)) = -0.017 x Year + 2.962; R^2 = 0.546) (Table 1.10; Figure 1.12).

1.4 Discussion:

While Preston (2013) collected only a limited suite of soluble chemistry data and no solid phase chemistry data from his transplantation study sites, Myers *et al.* (2012) sampled and completed a more comprehensive overview of the chemistry of the same poor and rich fen in late July 2008 very near to the current experimental sites. Chemical data from my study were generated using the same protocols as in Myers *et al.*'s study in the same analytical facility at the Ontario Forest Research Institute, allowing the two data sets to be meaningfully compared (Table 1.2; Figures 1.1 and 1.2). Comparison of these data sets indicates that significant changes have occurred in both the soluble and solid phase chemistry of the poor fen since July 2008. The pH of the poor fen as measured in the present study was unusually high compared to values obtained by Myers *et al*., and pH values exceeded what would normally be expected in poor fens in general. The solid phase Ca content of the poor fen peat had increased since sampling by Myers *et al.* (2012), which may account for this higher pH (Table 1.2; Figure 1.2). It was observed that the water table at the poor fen was high during sampling, and it is possible that this may have also contributed to a temporary rise in pH. The other soluble chemistry of the poor fen may have also been affected by this water table flux, however the soluble chemistry between the three

treatments was consistent across all analytes, with the exception of soluble C, suggesting that coring did not greatly impede the exchange of water or soluble substances between the cored peat and the surrounding substrate. Precipitation data from Pukaskwa National Park did not indicate a large influx of precipitation within the 30 days before sampling took place (Table 1.8), so the cause of the high water table is unknown, but it is possible it was due to obstruction of water flow downstream of the poor fen, as happened in the flooded rich fen site. These data give only a rough estimate of precipitation inputs however, given that the distance between the Pukaskwa meteorological stations and the study site is roughly 62 km, and that there are four dates with missing precipitation data in this time span. The negative trend observed in annual precipitation (Table 1.10; Figure 1.12) would suggest that mean water table at the site should actually be decreasing over time based on reduced precipitation inputs, assuming no physical changes to the watershed that could raise the site's water table.

Data obtained by Myers *et al*. suggest that the solid phase chemistry of the transplanted rich fen peat has remained largely stable despite exposure to poor fen aqueous conditions, with the exceptions of total C, K and Mg. In contrast, the solid phase chemistry of both the replanted and control poor fen peat was significantly different from when it was measured by Myers *et al*. in 2008, with only total C, Mg and P remaining the same. Unusually, solid phase total nitrogen has increased substantially in this poor fen since sampling by Myers *et al*., and solid phase C:N has decreased. As peatlands generally follow a pattern of succession from rich fen to poor fen and then bog, with nitrogen accumulation decreasing and C:N ratio increasing as the process progresses, the apparent reversal of this pattern in this poor fen is unusual and may warrant further investigation.

Of the chemical factors analyzed in this study, solid phase TN (Figure 1.6C) and C:N (Figure 1.6D) appeared to have the clearest correlations to bacterial community structure. Nitrogen content is known to influence bacterial community structure across a wide range of soil types (Ramirez *et al*., 2010; Ramirez *et al*., 2012; Hester *et al*., 2018). Acidobacteria reliably show greater abundance in low-nitrogen conditions (Ramirez *et al*., 2010; Ramirez *et al*., 2012; Hester *et al*., 2018), while Bacteroidetes have been shown to increase in abundance in response to increased nitrogen inputs in a wetland soil (Hester *et al*., 2018). There is conflicting evidence for a consistent response of Verrucomicrobia to increased nitrogen input, as this phylum reliably decreased in abundance across a range of soil types in a study by Ramirez *et al*. (2012), but Hester *et al*. (2018) found the inverse to be true. The results of the current study are supportive of the idea that solid phase nitrogen content is an important factor in peat bacterial community composition. Weaker associations also appeared to be present between bacterial community structure and solid phase TS (Figure 1.6E) and P (Figure 1.6G). Phosphorus has also been found to be a driver in peatland microbial community structure (Teurlincx *et al*., 2018), however the effects of phosphorus on peatland microbial communities and functioning are still under-studied (Lin *et al*., 2014; Veraart *et al*., 2015). Sulphur input in the form of sulphate is known to inhibit peatland methane flux (Gauci and Chapman, 2006). This is thought to be due to the presence of sulphate reducing bacteria in peatlands, which use sulphate as a terminal electron acceptor, and are able to outcompete methanogens for electron donors and anabolic carbon substrates (O'Flaherty *et al.*, 1998; Gauci and Chapman, 2006). No clear trends were seen in terms of a relationship between chemistry and fungal community (Figures 1.10A-H). This may be due to the characteristic low abundance and activities of fungi in peatlands, including the present sites (Lin *et al*., 2012; Myers et al., 2012).

That Proteobacteria proved to be the most dominant phylum detected via 16S sequencing across all treatments (Figure 1.4) was expected, given that this phylum is dominant across all peatlands on average (Seward, 2018). The high abundance of Bacteroidetes in the transplanted cores is of note however, as this phylum, after Proteobacteria, tends to dominate peatlands with higher pH and nutrient content (Seward, 2018). This would seem to indicate, since the transplanted rich fen peat retained a high abundance of Bacteroidetes (Figure 1.4) despite being subjected to poor fen aqueous conditions for nearly six years, that the solid phase chemistry and/or physical structure of the rich fen peat played a significant role in preserving aspects of the rich fen's bacterial community. Given that members of the Bacteroidetes are known to metabolize cellulose in neutral pH, eutrophic environments (Pankratov *et al*., 2006), it seems plausible that at least a portion of the Bacteroidetes community in rich fens survives by decomposing cellulose, making them less dependent on the soluble chemistry of an environment, instead metabolizing a solid phase source of carbon. However, Pankratov *et al*. (2006) observed that Bacteroidetes was not present in *Sphagnum*-dominated peat bogs, and that Actinobacteria appeared instead appeared to be responsible for cellulose degradation. It is important to note that the sequencing results of my transplantation study suffer from high within-treatment variability and small sample size, thus producing no statistically significant results in terms of Bacteroidetes relative abundance between treatments. However, the cause of the decline in Bacteroidetes as one goes from rich fen to bog may require further study, as my results tentatively suggest that Bacteroidetes may not lose dominance due to changes to soluble chemistry or pH. Instead they may be outcompeted by Actinobacteria when the peat substrate becomes dominated by dead *Sphagnum*. The Candidate Phyla Radiation (CPR) was also prevalent across all treatments (Figure 1.4). This superphylum is thought to make up more than 15% of the entire domain

Bacteria (Brown *et al*., 2015). Members of the CPR are likely obligate fermenters, and all are characterized by small genomes, restricted metabolic capacities, and incomplete electron transport chains and citric acid cycles (Rinke *et al*., 2013; Brown *et al*., 2015). CPR bacteria in general are thus theorized to be symbionts, with symbiosis having been directly demonstrated in some species (Brown *et al*., 2015; Delafont *et al*., 2015; Nelson and Stegen, 2015; Yeoh *et al*., 2015; Bedree *et al*., 2018).

An unexpected result of 16S and ITS1 sequencing of these long-term transplant cores was that coring itself appeared to have a confounding effect on the bacterial/archaeal and fungal communities of the poor fen peat, despite there being no statistically significant difference in soluble or solid phase peat chemistry between the cored (replanted) and uncored (control) poor fen peat. The fungal community was significantly different between the control and replanted peat according to PERMANOVA. While PERMANOVA of the 16S sequencing data and ANOVAs of individual 16S phyla revealed no statistically significant difference in bacterial/archaeal community between the control and replanted conditions, Bray-Curtis NMDS ordination of the 16S data (Figure 1.5) revealed radically more disparate clustering of replanted communities along the NMDS1 axis than in the control samples, indicating a potential disruption of the bacterial/archaeal community due to coring. It was observed that the replanted core 16S phylum-level communities appeared to follow two distinct patterns, with two of the four samples (R1 and R3) having a community make-up more dominated by Acidobacteria and Actinobacteria, while the other two (R2 and R3) were more similar to the control condition, with very little Acidobacteria and Actinobacteria present, and a higher incidence of CPR bacteria and Bacteroidetes (Figure 1.3). R2 and R4 cluster close to the control samples in the NMDS ordination, while R1 and R3 cluster together, but apart from the control samples (Figure 1.5).

This would tentatively suggest that coring had a strong effect on the bacterial/archaeal communities of specific replanted cores. However, it is important to note that a larger sample size would be necessary in order to confirm this pattern, and these observations should be viewed as an impetus for further investigation. A change to the microbial community due to coring is also implied by the results of aerobic incubation, which indicated higher CO_2 production in the control condition than in either the cored replanted or transplanted peat (Table 1.1). The reasons for these discrepancies between the cored and uncored poor fen peat are unknown, but several possible explanations are put forth here. Firstly, it is possible that the PVC pipe and encasing mesh of the core may have provided an additional substrate for microbial growth or decreased the rate of water flow within the cored peat such that microbes were able to anchor themselves more effectively within the peat substrate. Alternatively, decreased water flow within the cored peat could have reduced flushing of dead cells from the core resulting in a higher concentration of reads of non-living cells. Depending upon environmental conditions, DNA from dead cells can persist in the environment for significant periods of time (Nielsen *et al*., 2007), and DNA from dead bacterial cells has been observed to do so in soil for 70 days (Selenska and Klingmuller, 1991). Differences in apparent microbial community composition between the control and replanted peat could potentially be due to sequencing of trapped DNA from dead microbial cells, with the implication that the microbial community changed significantly prior to sampling. It is also possible that the suite of chemical analytes in this study was not comprehensive enough to capture all factors relevant to microbial community composition, and differences in one or more unmeasured chemical factors between the replanted and control samples are responsible for altering their respective microbial communities. For example, methanogens in peatlands are limited by trace metals and Na (Basiliko and Yavitt, 2001), which

were not analyzed in this study. Another possibility, potentially corroborated by my aerobic CO_2 production results, is that the exterior casing of the replanted and transplanted cores did not allow for adequate exchange of dissolved gases between the core and the surrounding peat, resulting in O_2 depletion within the cored peat. This could explain the lower aerobic metabolic capacity of the cored peat, which indicates a smaller or less active aerobic microbial community. While bacteria are dominant over fungi in peatland microbial communities and are responsible for more CO_2 production (Winsborough and Basiliko, 2010; Myers *et al*., 2012), fungi as a group are more reliant on aerobic respiration, as evidenced by their tendency to decline in prevalence with peat depth (Sjögersten *et al*., 2016). The change and restriction of the fungal community in the replanted peat compared to the control (Figure 1.9) may reflect a dying-off of those fungi which are not facultative anaerobes; however, this cannot be confirmed without measurements of fungal biomass.

1.5 Conclusion:

While a small sample size of transplanted/replanted cores and issues with microbial genetic sequencing quality in these peat transplantation experiments create limitations to reaching definitive conclusions, the results of these experiments highlight several avenues for further investigation, and provide evidence that should help to inform the methodology of future long-term reciprocal transplantation studies. The increase in solid phase TN content and decrease in C:N in the studied poor fen over a period of years, in opposition to the usual pattern of peatland succession, warrants further study in order to ascertain the causes of this change. The results of these experiments suggest that solid phase chemistry, especially TN and C:N, may be significant in determining the makeup of the peat bacterial community. My results also

tentatively suggest that pH is not the major driver of prevalence of Bacteroidetes in peat, with solid phase chemistry and/or physical substrate potentially taking this role, and further study may be warranted to assess this possibility. Finally, the methodology of future reciprocal peat transplantation experiments should take into account the confounding effect on the microbial community produced by Preston's coring methods (2013). Further study is needed in order to determine the best practices for long-term reciprocal peat transplantation experiments.

Tables:

Table 1.1: Results of aerobic incubation for CH_4 consumption and CO_2 production, and anaerobic incubation for CH_4 and CO_2 production. Values are the mean of each treatment, with standard deviation in brackets; n = 5.

Treatment	Aerobic CH_4 Consumption (µg/g dry peat/h)	Aerobic CO_2 Production (mg/g dry peat/h)	Anaerobic Net CH_4 Production to Day 17 (µg/g dry peat)	Anaerobic Net CO_2 Production to Day 17 (mg/g dry peat)
Transplant	0.10 (0.03)	0.04 ($6x10^{-3}$)	1.56 (1.61)	5.12 (0.76)
Replant	0.04 (0.03)	0.06 (0.01)	2.32 (3.34)	9.60 (3.24)
Control	0.08 (0.08)	0.15 (0.08)	13.97 (26.96)	10.36 (2.53)

Table 1.2: Soluble and solid phase chemistry data from transplanted, replanted and control peat, as well as chemistry data collected from the poor and rich fens by Myers *et al.* (2012) in July 2008. Values are treatment means, with standard deviation in brackets. For transplant, replant and control treatments, n = 5 except where marked by $^{\#}$, in which case n = 4. For Myers *et al*.'s data, n = 6.

	Treatment			From Myers *et al.* 2012	
	Transplant	Replant	Control	Poor Fen	Rich Fen
Soluble					
pH	5.91 (0.38)^{d}	5.93 (0.52)^{d}	5.97 (0.09)^{d}	4.08 (0.23)^{abce}	6.26 (0.08)^{d}
TC (mg/g)	0.58 (0.11)$^{\# c}$	0.87 (0.31)$^{\#}$	1.12 (0.23)$^{\# a}$	nd	nd
TN (mg/g)	0.03 (0.02)$^{\# de}$	0.04 (0.01)$^{\# de}$	0.05 (0.01)$^{\# de}$	0.51 (0.08)^{abce}	0.63 (0.16)^{abcd}
C:N	19.85 (5.90)$^{\#}$	22.61 (1.73)$^{\#}$	22.62 (6.55)$^{\#}$	nd	nd
Ca^{2+} (mg/g)	0.05 (0.04)^{e}	0.06 (0.04)^{e}	0.06 (0.04)^{e}	0.03 ($4x10^{-3}$)^{e}	0.24 (0.17)^{abcd}
K^{+} (µg/g)	4.59 (1.56)^{de}	7.71 (4.89)^{de}	16.02 (17.47)^{de}	49.61 (14.82)^{abce}	187.51 (203.00)^{abcd}
Mg^{2+} (mg/g)	4.79 (2.71)^{e}	6.51 (2.98)$^{\# e}$	6.28 ($4x10^{-3}$)^{e}	4.85 (1.01)^{e}	38.45 (29.90)^{abcd}
Na^{+} (mg/g)	0.26 (0.09)	0.37 (0.18)^{de}	0.45 (0.21)^{de}	0.13 (0.04)^{bc}	0.15 (0.06)^{bc}
HCO_3^{-} (mg/g)	0.29 (0.11)	0.31 (0.24)	0.37 (0.20)	nd	nd
Cl^{-} (mg/g)	0.09 (0.02)^{d}	0.20 (0.13)^{de}	0.22 (0.10)^{de}	0.04 (0.03)^{abc}	0.04 (0.02)^{bc}
NO_3^{-} (µg/g)	10.75 (10.06)$^{\#}$	18.86 (13.41)$^{\# d}$	13.58 (15.31)$^{\#}$	2.04 (0.60)^{b}	7.57 (5.57)
SO_4^{2-} (mg/g)	0.35 (0.29)	0.33 (0.24)	0.40 (0.25)	0.13 (0.04)	0.44 (0.39)
Solid Phase					
TC (%)	34.09 (0.05)^{bcde}	48.06 (0.63)^{ae}	49.07 (0.48)^{ae}	49.48 (0.54)^{a}	36.73 (1.62)^{abcd}
TN (%)	2.49 (0.09)^{bcd}	1.43 (0.62)^{ade}	1.44 (0.53)^{ade}	0.84 (0.11)^{abce}	2.40 (0.15)^{bcd}
C:N	16.46 (1.04)^{bcd}	37.41 (11.10)^{ade}	36.82 (9.43)^{ade}	59.37 (6.79)^{abce}	15.33 (1.34)^{bcd}
TS (%)	0.60 (0.05)^{bcd}	0.36 (0.20)^{ade}	0.33 (0.09)^{ade}	0.09 (0.01)^{abce}	0.60 (0.06)^{bcd}
Ca (mg/g)	6.82 (2.07)^{d}	6.84 (2.73)^{d}	6.72 (2.82)^{d}	2.28 (0.56)^{abce}	10.36 (1.51)^{d}
K (mg/g)	0.31 (0.06)^{cde}	0.36 (0.13)^{cd}	0.67 (0.32)^{ab}	0.97 (0.09)^{ab}	0.58 (0.10)^{a}

Mg (mg/g)	0.54 (0.19)[ce]	0.78 (0.16)	0.84 (0.18)[a]	0.60 (0.07)	0.86 (0.11)[a]
P (mg/g)	0.85 (0.18)[bcd]	0.49 (0.10)[ae]	0.53 (0.05)[ae]	0.43 (0.03)[ae]	0.85 (0.14)[bcd]

Significant differences between treatments are indicated using superscripted letters:

[a] Significant difference from Transplant
[b] Significant difference from Replant
[c] Significant difference from Control
[d] Significant difference from Myers Poor Fen
[e] Significant difference from Myers Rich Fen

Table 1.3: 16S and ITS1 read numbers for all samples processed by Metagenom Bio. Samples names with T are transplanted, R are replanted, and C are control. Sample names with the same number indicate that the cores were taken from the same location within the poor fen.

	16S		**ITS1**	
Sample	**Reads In**	**Reads Out**	**Reads In**	**Reads Out**
T1	16046	14773	18454	17001
T2	48605	46124	7447	6442
T3	11601	9925	10922	9950
R1	4591	3658	16124	15504
R2	18761	17612	7997	7502
R3	5343	4158	15533	14914
R4	21698	20663	11661	9356
C1	14129	13384	19635	18725
C2	25891	24844	11303	10583
C3	36652	34944	18306	17501
C4	31235	29497	32803	30797

Note: Due to laboratory error at Metagenom Bio, Illumina reads were only obtained from 4 control, 4 replant, and 3 transplant samples, despite 5 replicates of each being sent in for sequencing, and read numbers for some samples were relatively low.

Table 1.4: 16S bacterial/archaeal phylum fractional abundance across all treatments. Values are treatment means, with standard deviation in brackets.

	Treatment		
Phylum	**Transplant (n = 3)**	**Replant (n = 4)**	**Control (n = 4)**
Acidobacteria	$2x10^{-4}$ ($3x10^{-4}$)	0.15 (0.17)	$2x10^{-3}$ ($3x10^{-3}$)
Actinobacteria	0 (0)	0.07 (0.09)	0 (0)
Bacteroidetes	0.31 (0.25)	0.11 (0.12)	0.14 (0.04)
Chloroflexi	0 (0)	0.02 ($8x10^{-3}$)	$9x10^{-3}$ ($8x10^{-3}$)
Cyanobacteria	$3x10^{-4}$ ($3x10^{-4}$)	$5x10^{-3}$ ($9x10^{-3}$)	$6x10^{-3}$ (0.01)
Fibrobacteres	0 (0)	0.01 (0.03)	0.02 (0.02)
Fusobacteria	0.03 (0.5)	$1x10^{-4}$ ($2x10^{-4}$)	$2x10^{-3}$ ($2x10^{-3}$)
Nanoarchaeaota	0.05 (0.4)	0.07 (0.11)	0.02 (0.02)
Nitrospirae	0 (0.0)	$5x10^{-3}$ ($9x10^{-3}$)	0 (0)
Candidate Phyla Radiation	0.08 (0.14)	0.13 (0.16)	0.08 (0.02)
Proteobacteria	0.47 (0.45)	0.42 (0.12)	0.71 (0.02)
Spirochaetes	0.03 (0.06)	0.01 (0.02)	0.01 ($7x10^{-3}$)
Verrucomicrobia	0.02 (0.04)	0 (0)	0 (0)

Table 1.5: Richness, evenness and Shannon diversity of 16S bacterial/archaeal phyla across all treatments. Values are treatment means, with standard deviation in brackets.

Treatment	**N**	**Richness**	**Evenness**	**Shannon Diversity**
Transplant	3	7 (1)	0.50 (0.34)	0.95 (0.62)
Replant	4	8.5 (1.73)	0.64 (0.06)	1.36 (0.18)
Control	4	8.75 (1.5)	0.46 (0.02)	0.98 (0.06)

Table 1.6: ITS1 fungal phylum fractional abundance across all treatments. Values are treatment means, with standard deviation in brackets.

	Treatment		
Phylum	**Transplant (n = 3)**	**Replant (n = 4)**	**Control (n = 4)**
Ascomycota	0.24 (0.22)	0.24 (0.20)	0.40 (0.37)
Basidiomycota	0.66 (0.27)	0.47 (0.20)	0.26 (0.20)
Mortierellomycota	0.10 (0.05)	0.25 (0.20)	0.34 (0.31)
Mucoromycota	$3x10^{-4}$ ($5x10^{-4}$)	0.04 (0.06)	$3x10^{-3}$ ($3x10^{-3}$)
Rozellomycota	0 (0)	$5x10^{-5}$ ($9x10^{-5}$)	0 (0)
Kickxellomycota	0 (0)	$1x10^{-4}$ ($2x10^{-4}$)	0 (0)

Table 1.7: Richness, evenness and Shannon diversity of ITS1 fungal phyla across all treatments. Values are treatment means, with standard deviation in brackets

Treatment	**N**	**Richness**	**Evenness**	**Shannon Diversity**
Transplant	3	3.33 (0.57)	0.62 (0.25)	0.73 (0.24)
Replant	4	4.5 (0.57)	0.66 (0.04)	0.99 (0.12)
Control	4	4 (0)	0.57 (0.22)	0.79 (0.30)

Table 1.8: Mean monthly precipitation totals from Pukaskwa National Park, Ontario, alongside data from 2017. Standard deviation is shown in brackets next to mean values. Data is taken from the years 2008-2017, however winter precipitation measurements were largely unavailable prior to 2012. Missing years of data are listed here for the affected months: February, 2008-2011; March, 2008-2011; April, 2008; November, 2008-2010; December, 2008-2010.

Month	**Mean Precipitation (mm)**	**2017 Precipitation (mm)**
January	52.2 (23.5)	36.7
February	43.1 (18.4)	71.1
March	44.5 (26.6)	23.6
April	52.2 (38.0)	62.9
May	48.7 (27.7)	58.2
June	87.1 (36.0)	118.8
July	63.1 (44.3)	12.8
August	62.5 (36.4)	48.3
September	72.5 (36.4)	140.4
October	68.5 (46.3)	113.0
November	62.5 (24.4)	76.2
December	61.1 (21.7)	65.0

Table 1.9: Daily precipitation totals from Pukaskwa National Park, Ontario, showing the 30 days prior to peat sampling and the sampling dates (June 5th and 6th, 2017). ND indicates no data available for the specified date.

Date	Precipitation (mm)
May 6, 2017	0
May 7, 2017	0
May 8, 2017	0
May 9, 2017	0
May 10, 2017	0
May 11, 2017	4.1
May 12, 2017	0
May 13, 2017	0
May 14, 2017	0
May 15, 2017	0
May 16, 2017	9.3
May 17, 2017	9.2
May 18, 2017	ND
May 19, 2017	0
May 20, 2017	0
May 21, 2017	4.2
May 22, 2017	ND
May 23, 2017	ND
May 24, 2017	0
May 25, 2017	0
May 26, 2017	0
May 27, 2017	3.4
May 28, 2017	2.1
May 29, 2017	ND
May 30, 2017	6.3
May 31, 2017	2.3
June 1, 2017	0
June 2, 2017	0
June 3, 2017	9.0
June 4, 2017	7.1
June 5, 2017 (sample date)	0
June 6, 2017 (sample date)	0

Table 1.10: Total annual precipitation from Pukaskwa National Park, Ontario for the years 2012 to 2018.

Year	Total Precipitation (mm)
2012	861.7
2013	899.5
2014	810.7
2015	773.1
2016	671.1
2017	827.0
2018	670.1

Figures:

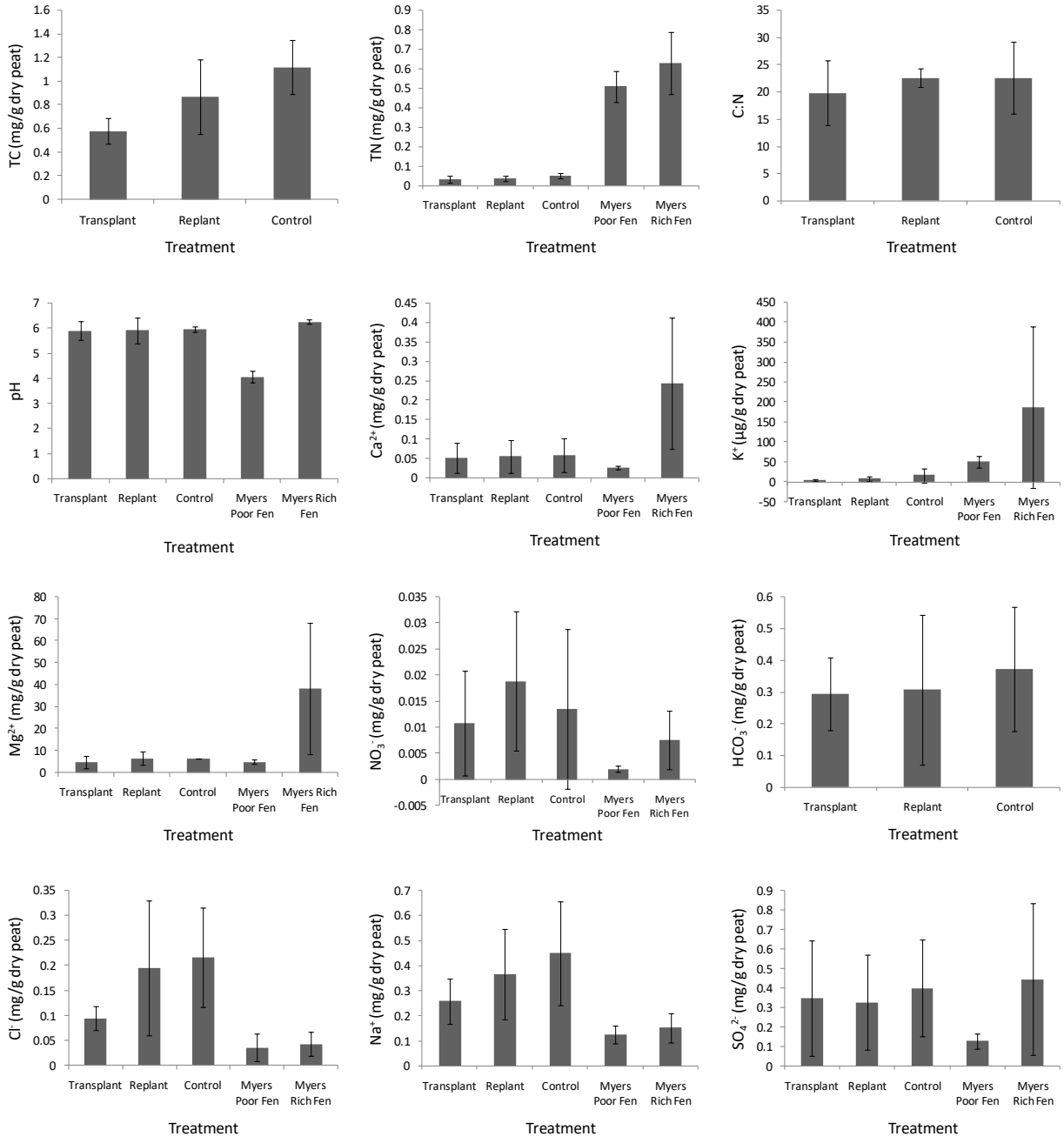

Figure 1.1: Soluble chemistry mean values for transplanted, replanted and control peat. Soluble chemistry data collected by Myers *et al.* (2012) in July 2008 from the poor and rich fens is also shown. Error bars represent standard deviation.

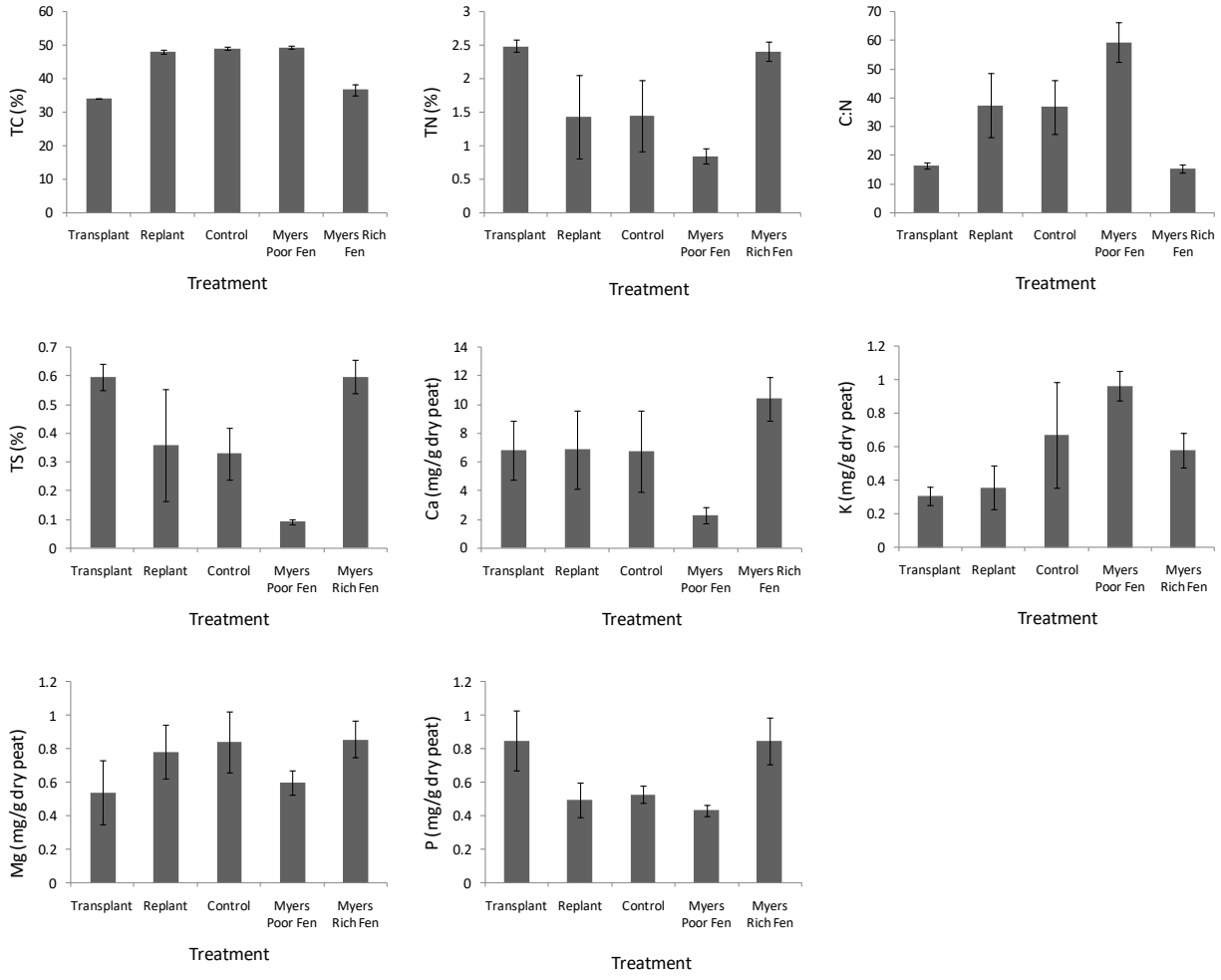

Figure 1.2: Solid phase chemistry mean values for transplanted, replanted and control peat. Soluble chemistry data collected by Myers *et al.* (2012) in July 2008 from the poor and rich fens is also shown. Error bars show standard deviation.

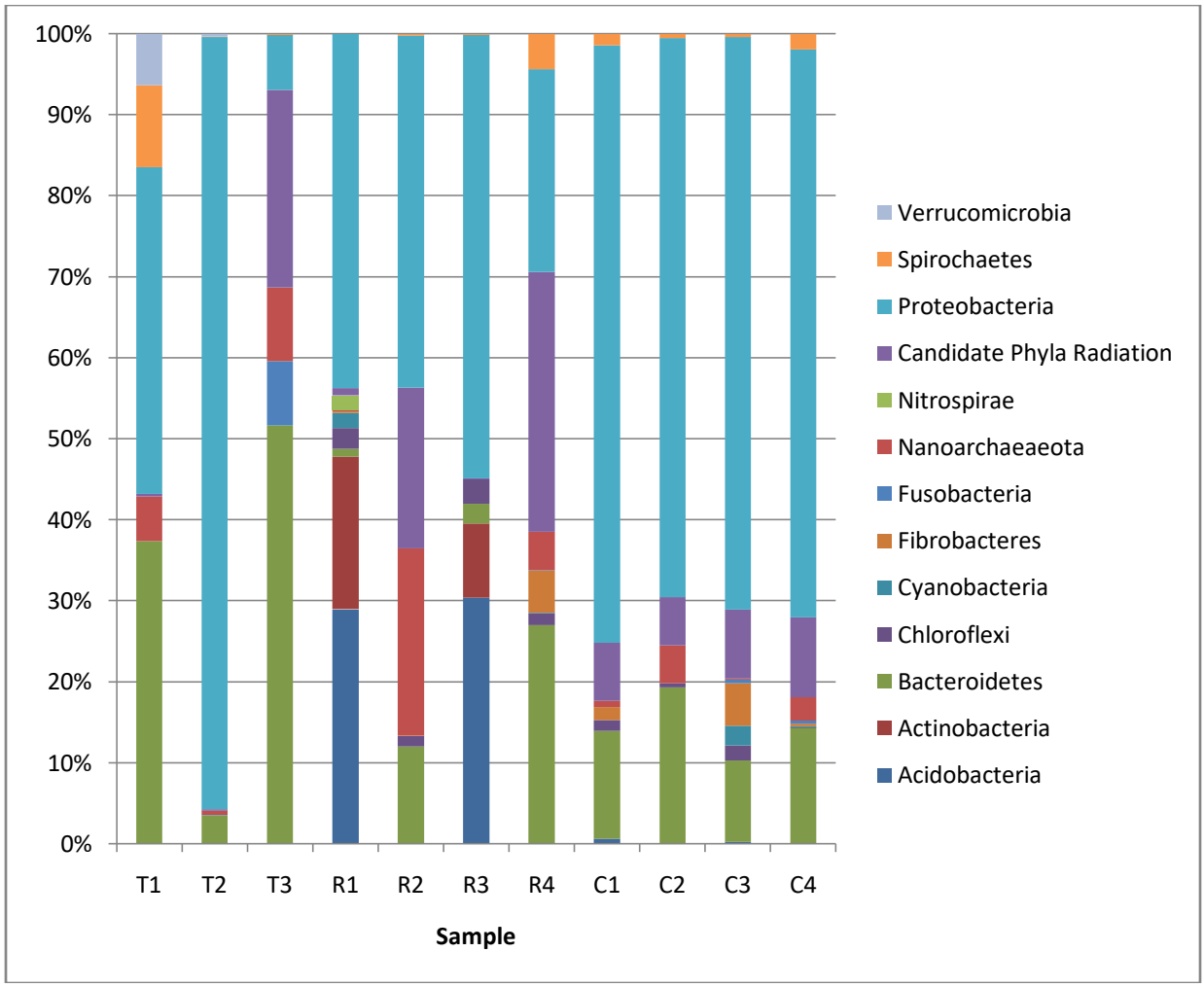

Figure 1.3: Phylum-level 16S bacterial/archaeal community composition across all samples. Samples names with T are transplanted, R are replanted, and C are control. Sample names with the same number indicate that the cores were taken from the same location within the poor fen.

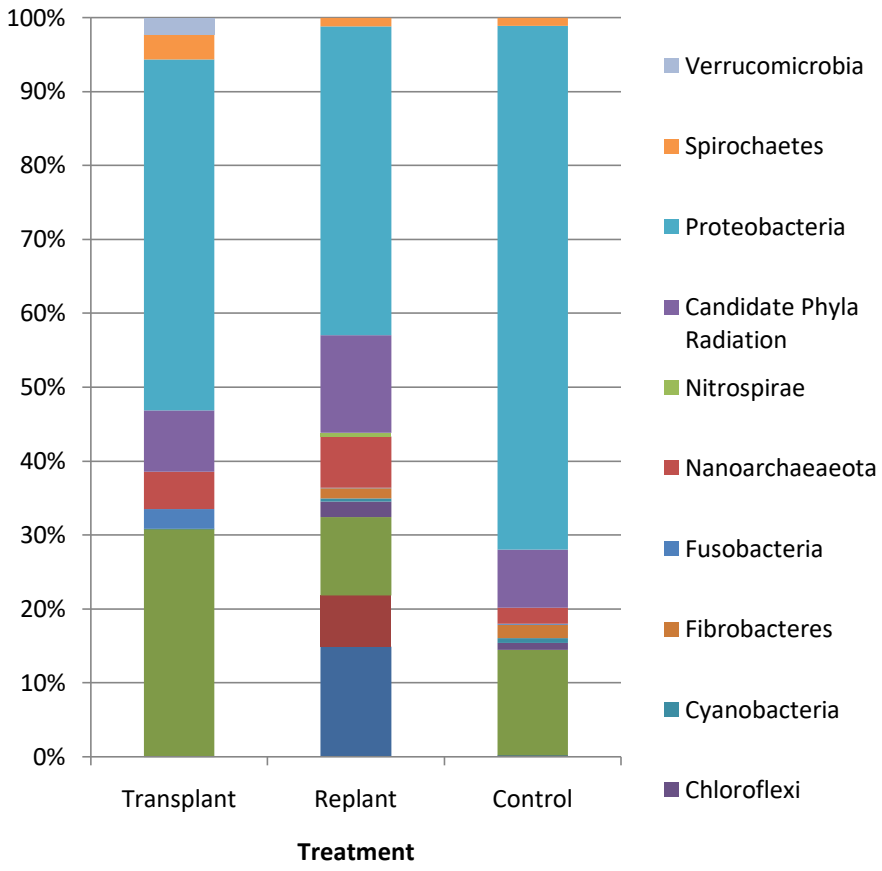

Figure 1.4: Phylum-level 16S bacterial/archaeal community composition. Bars show mean phylum relative abundance for each treatment.

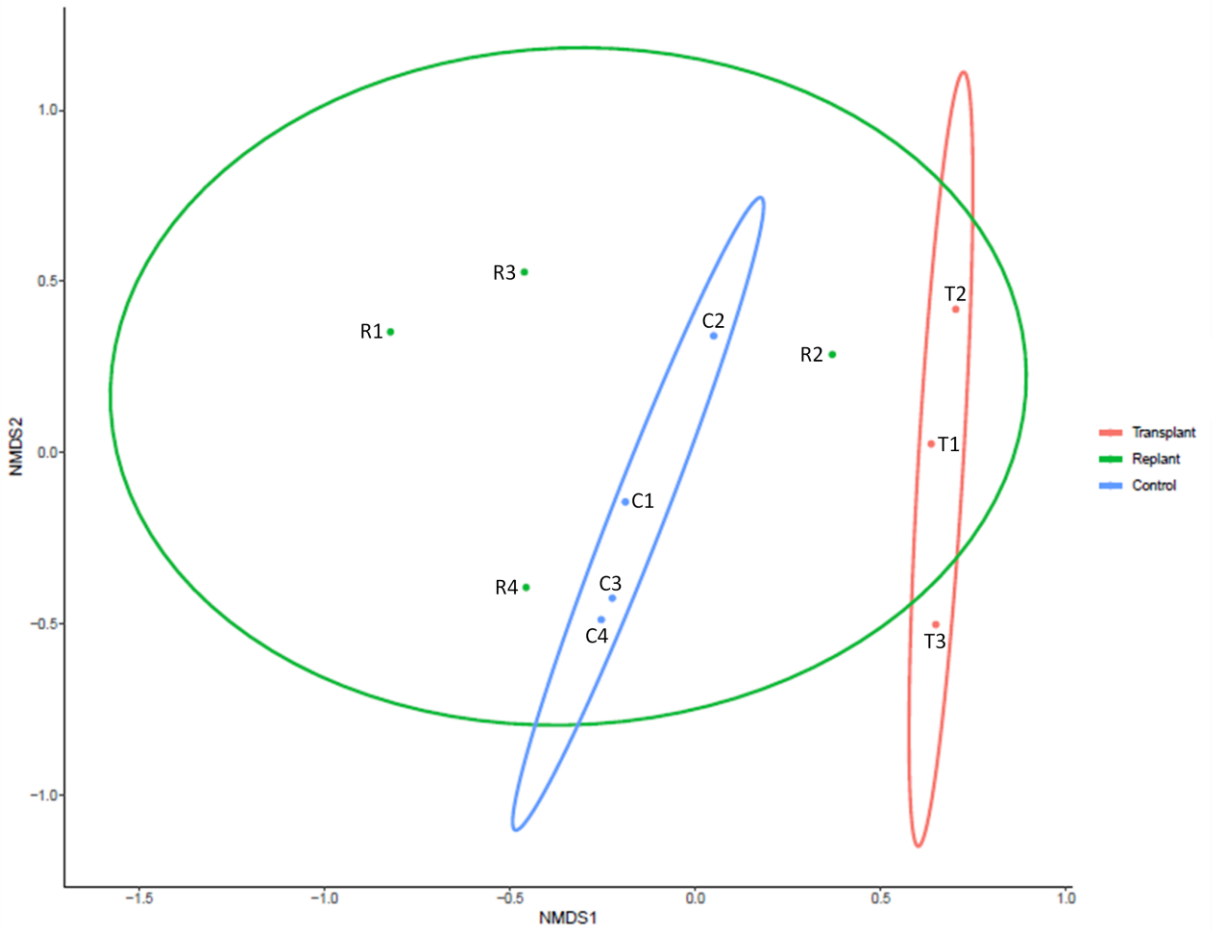

Figure 1.5: NMDS Bray-Curtis dissimilarity ordination of all 16S bacterial/archaeal taxonomic levels. Ellipses represent standard error. Samples names with T are transplanted, R are replanted, and C are control. Sample names with the same number indicate that the cores were taken from the same location within the poor fen.

Figures 1.6A-H: NMDS Bray-Curtis dissimilarity ordinations of all 16S bacterial/archaeal taxonomic levels, colour-coded to show chemical variable measurements for each sample.

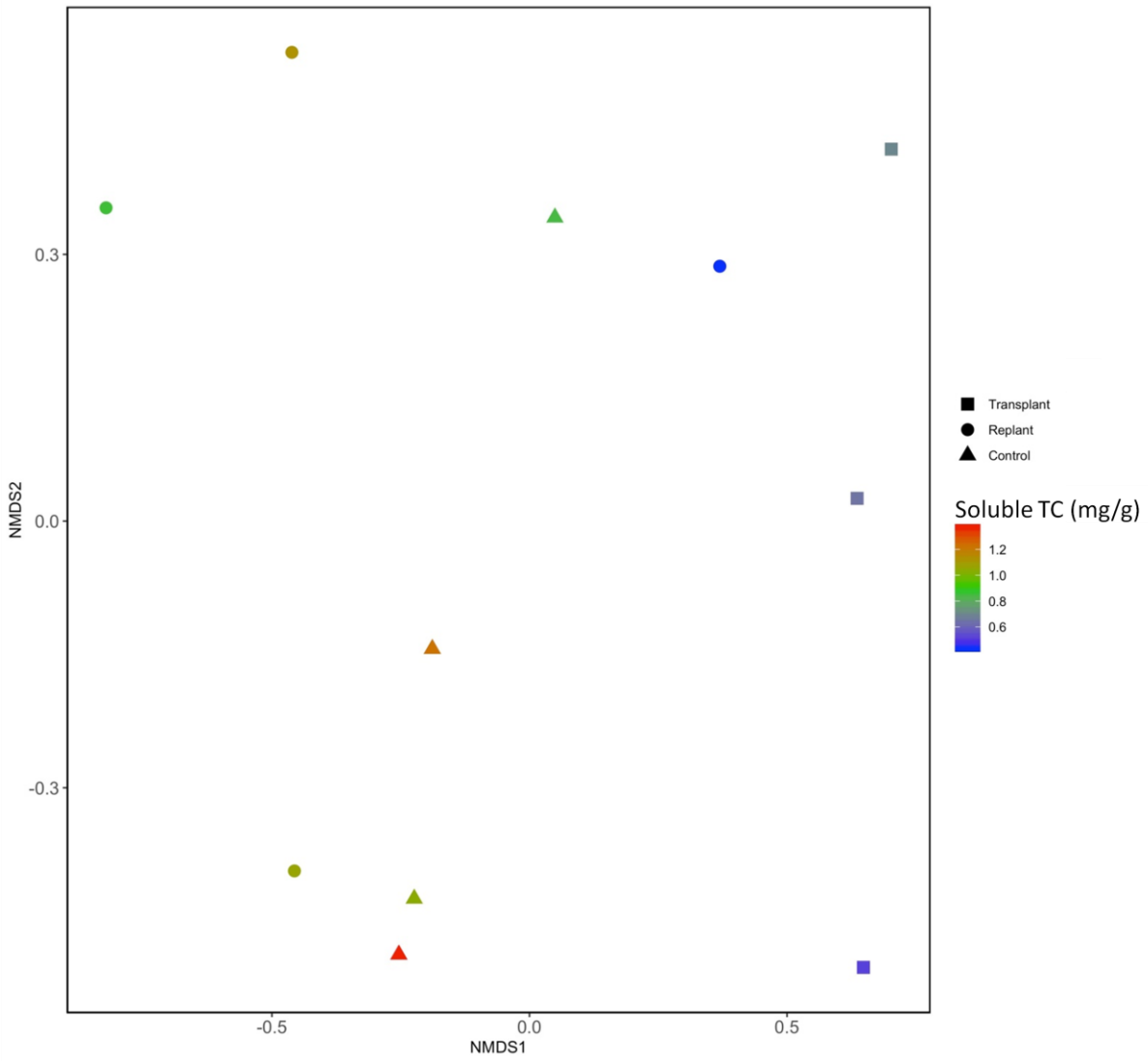

Figure 1.6A: Soluble TC.

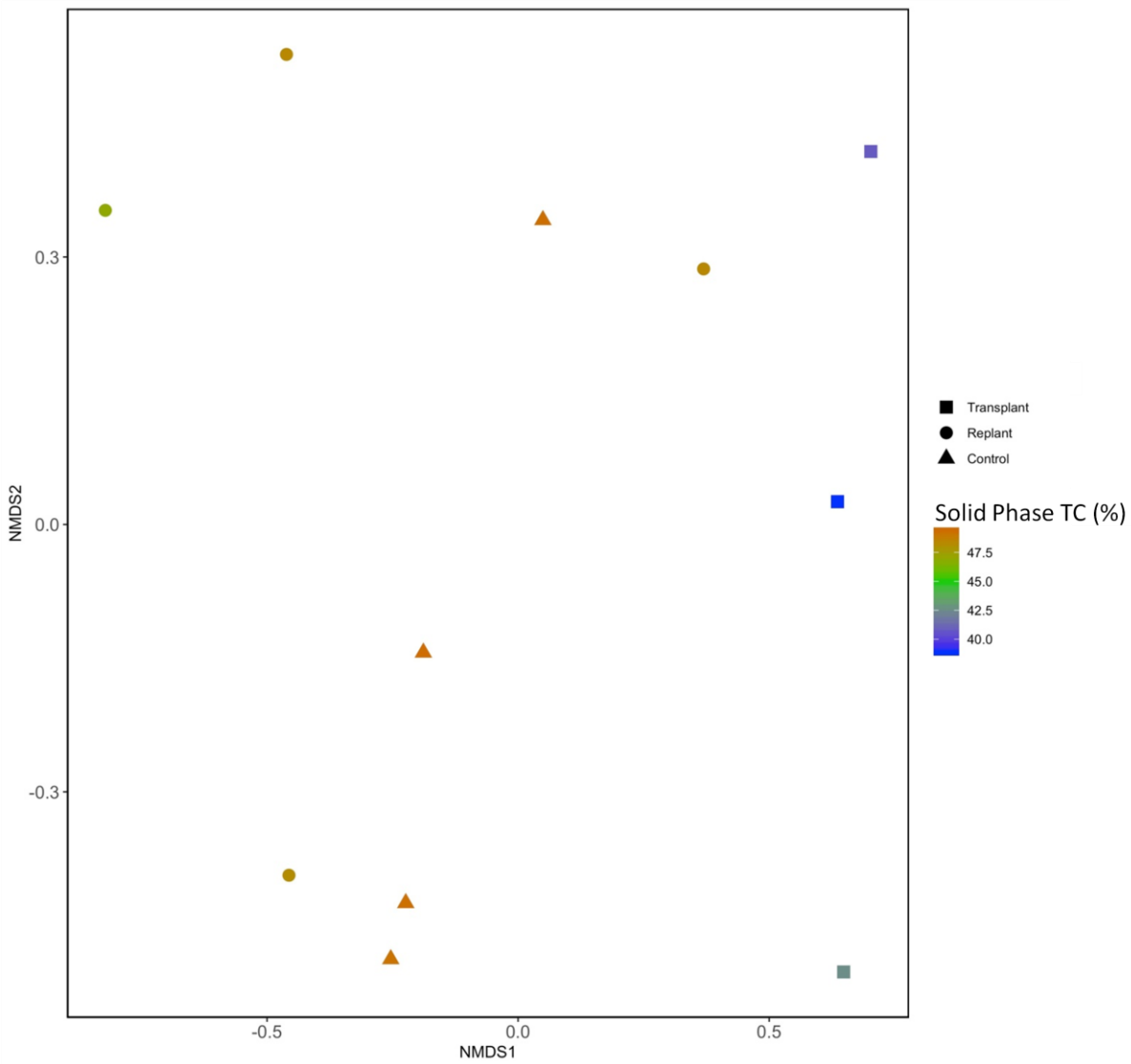

Figure 1.6B: Solid phase TC.

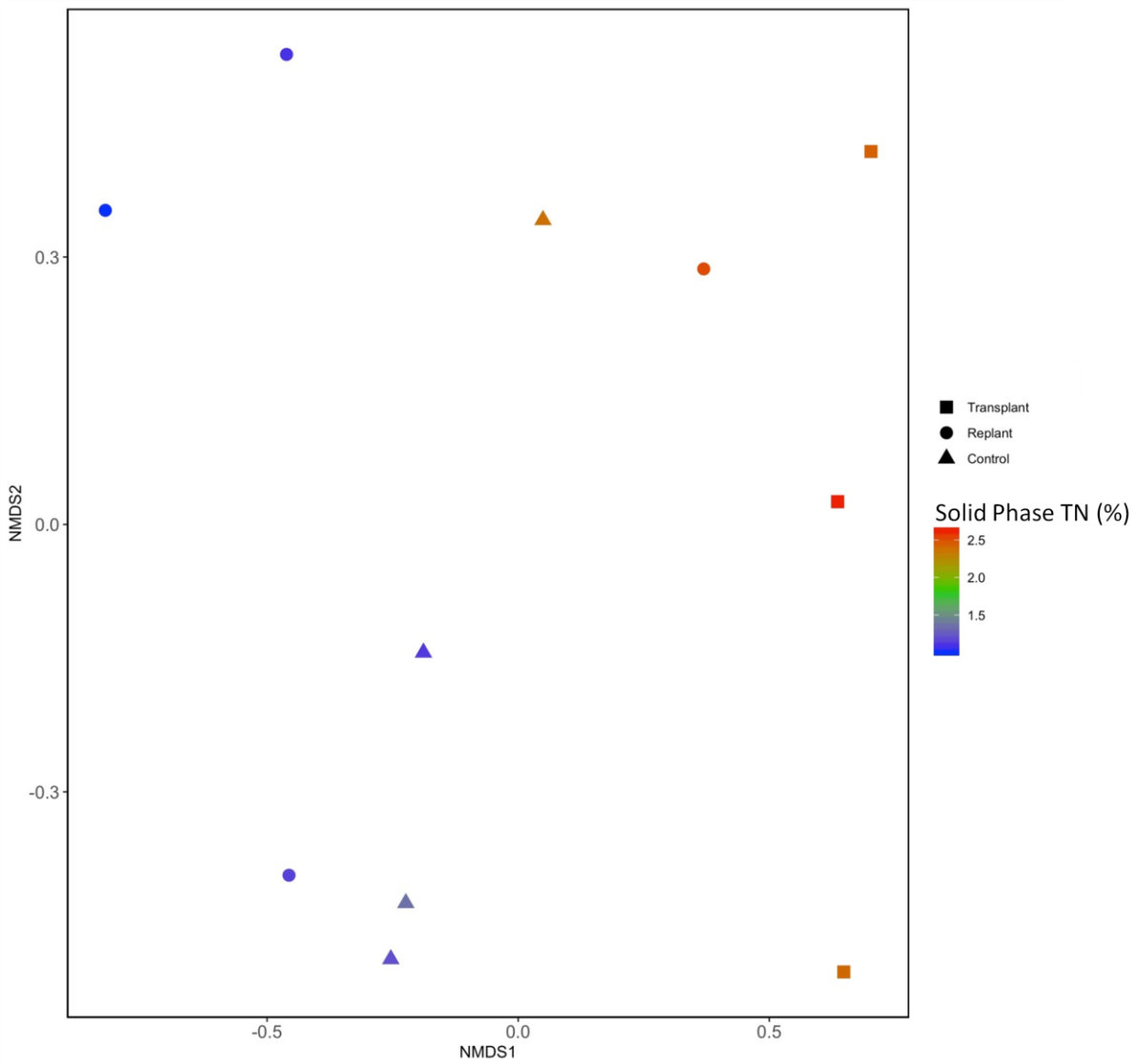

Figure 1.6C: Solid phase TN.

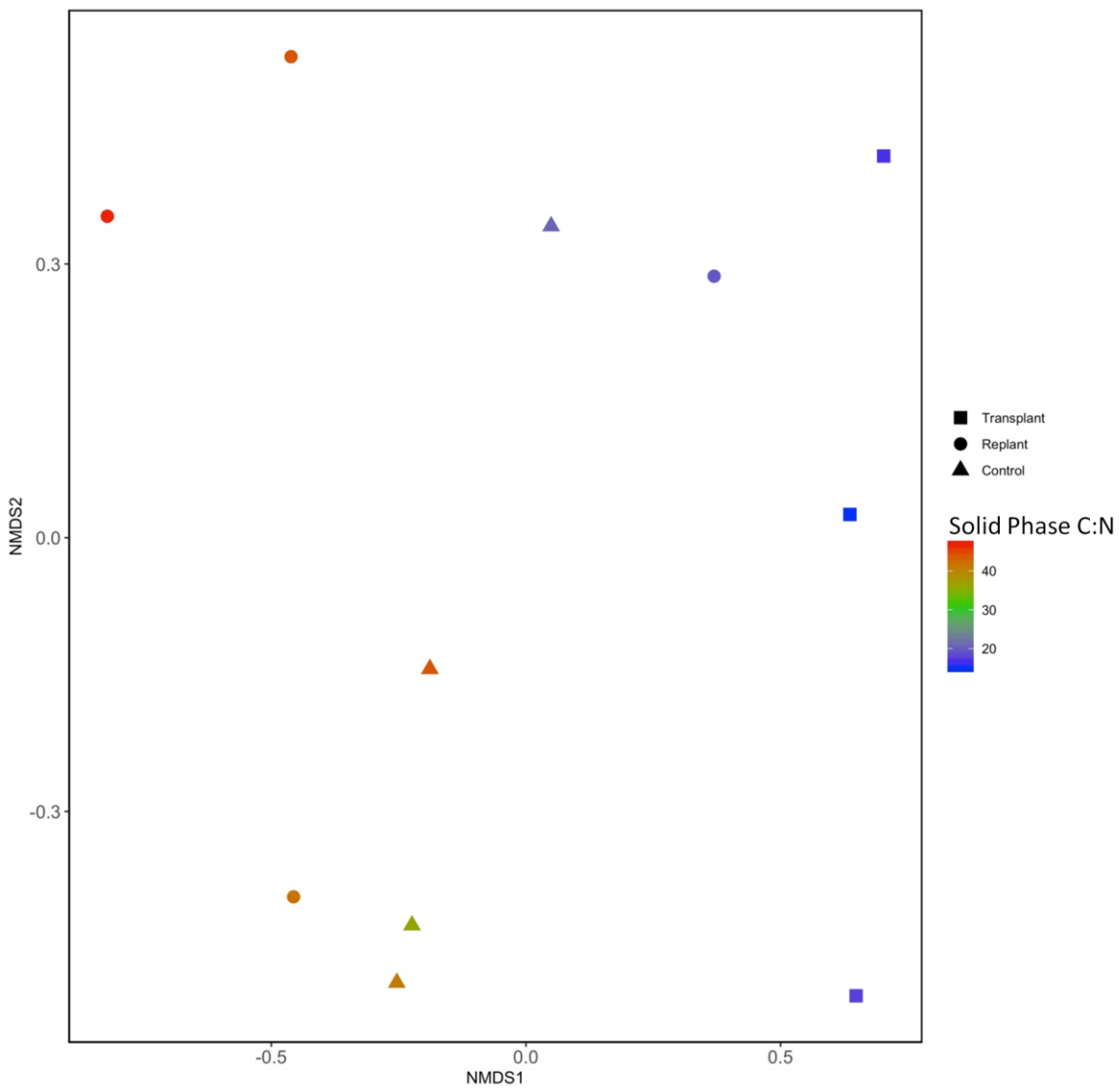

Figure 1.6D: Solid phase C:N.

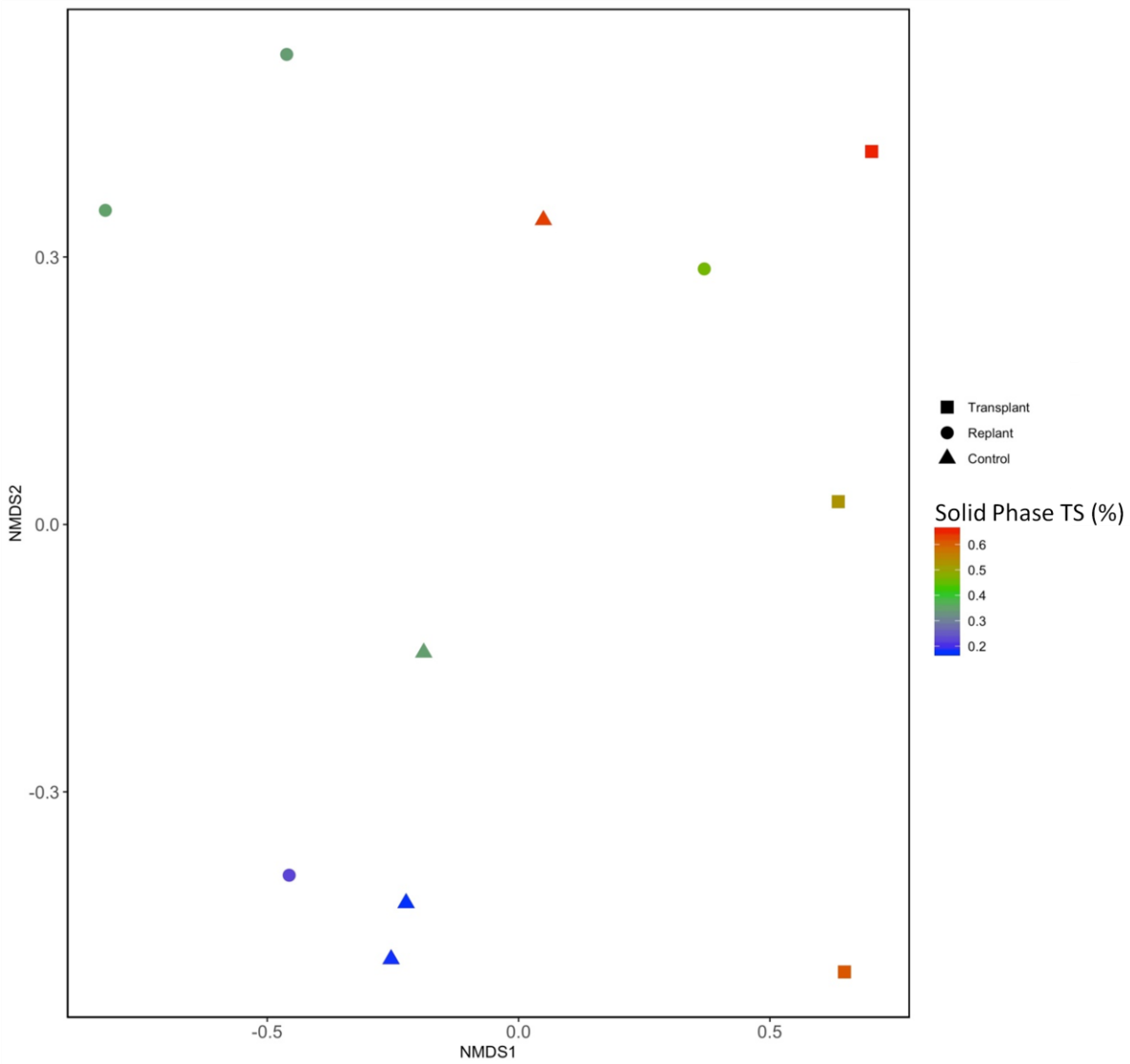

Figure 1.6E: Solid phase TS.

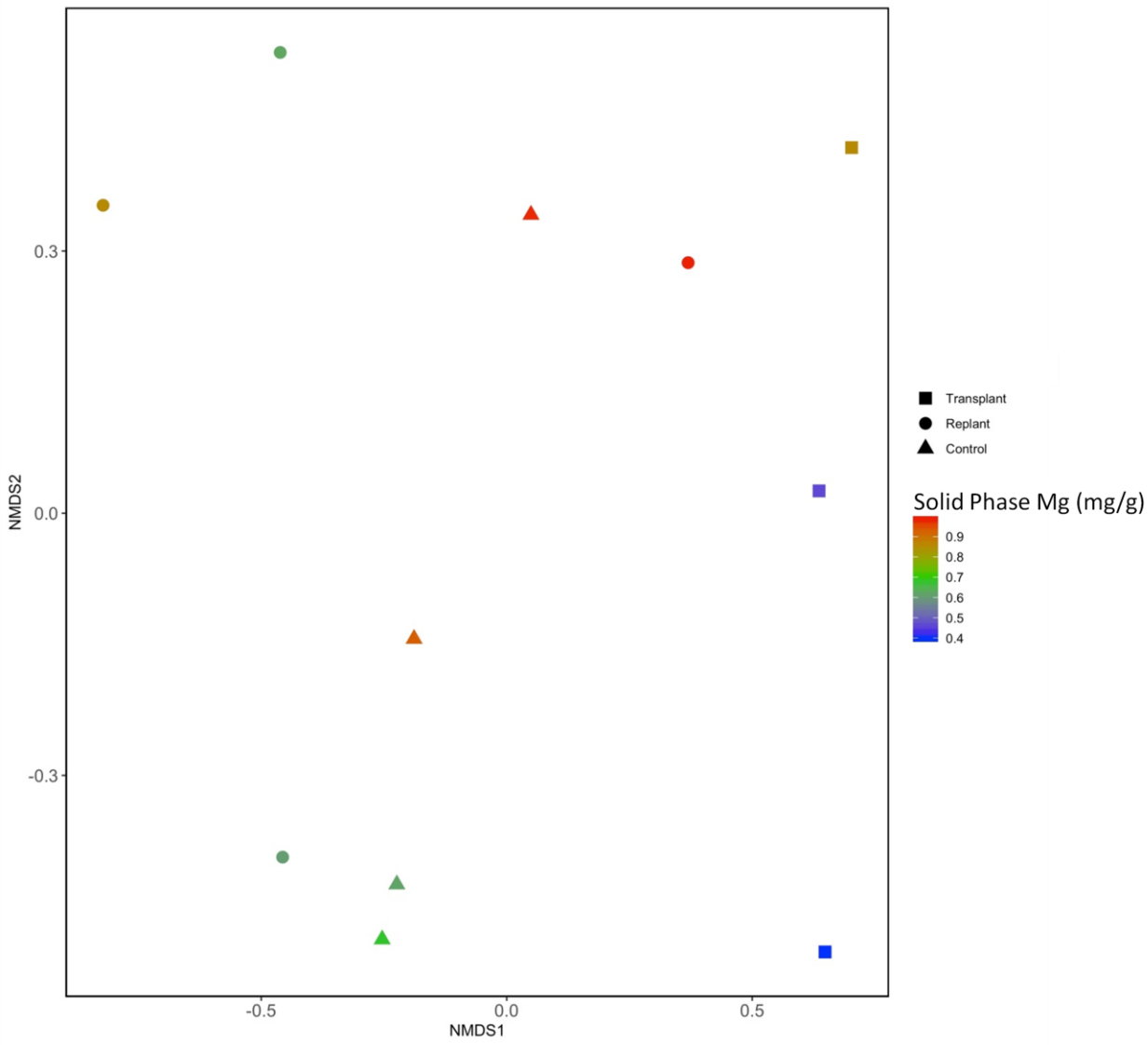

Figure 1.6F: Solid phase Mg.

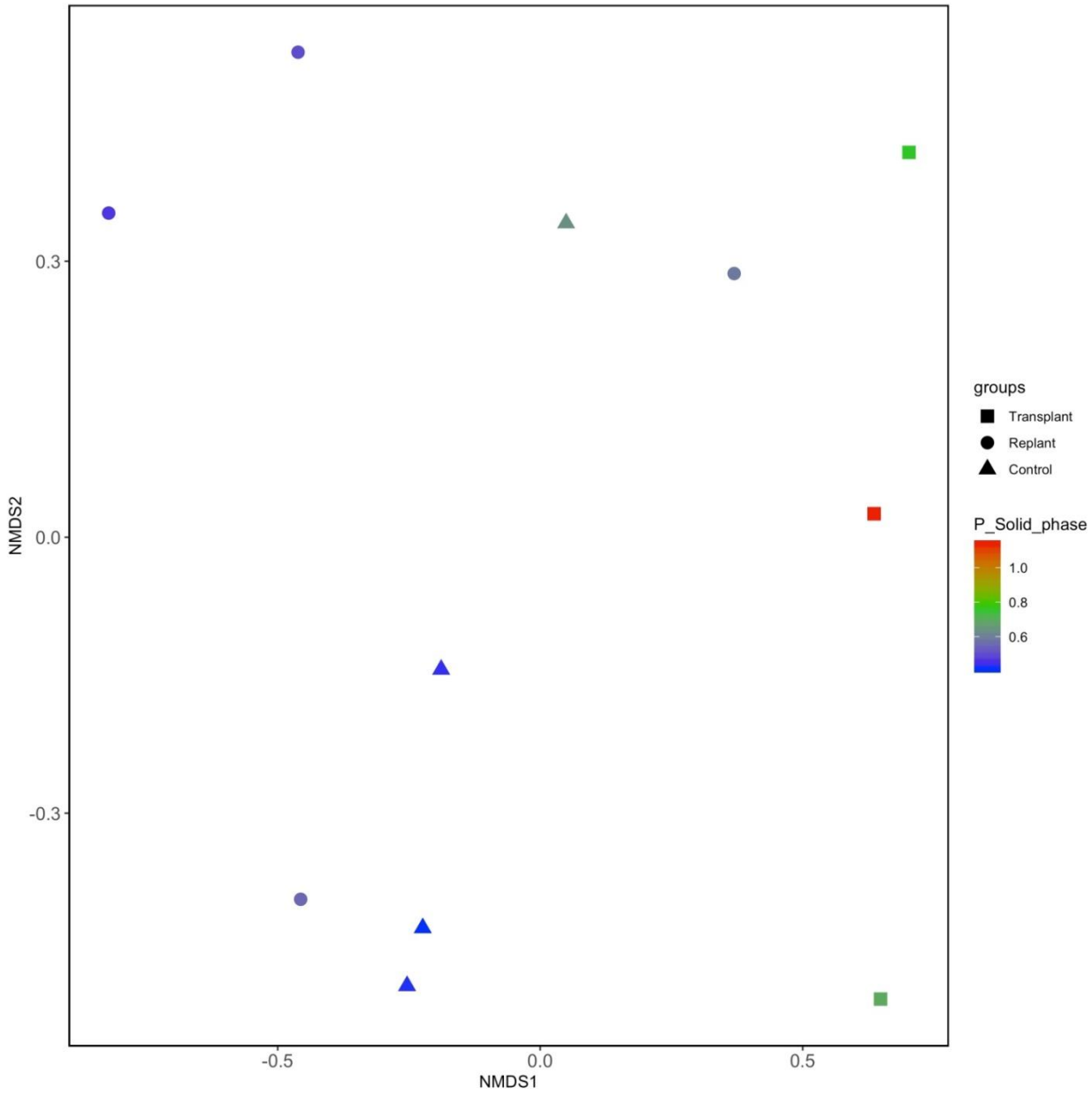

Figure 1.6G: Solid phase P.

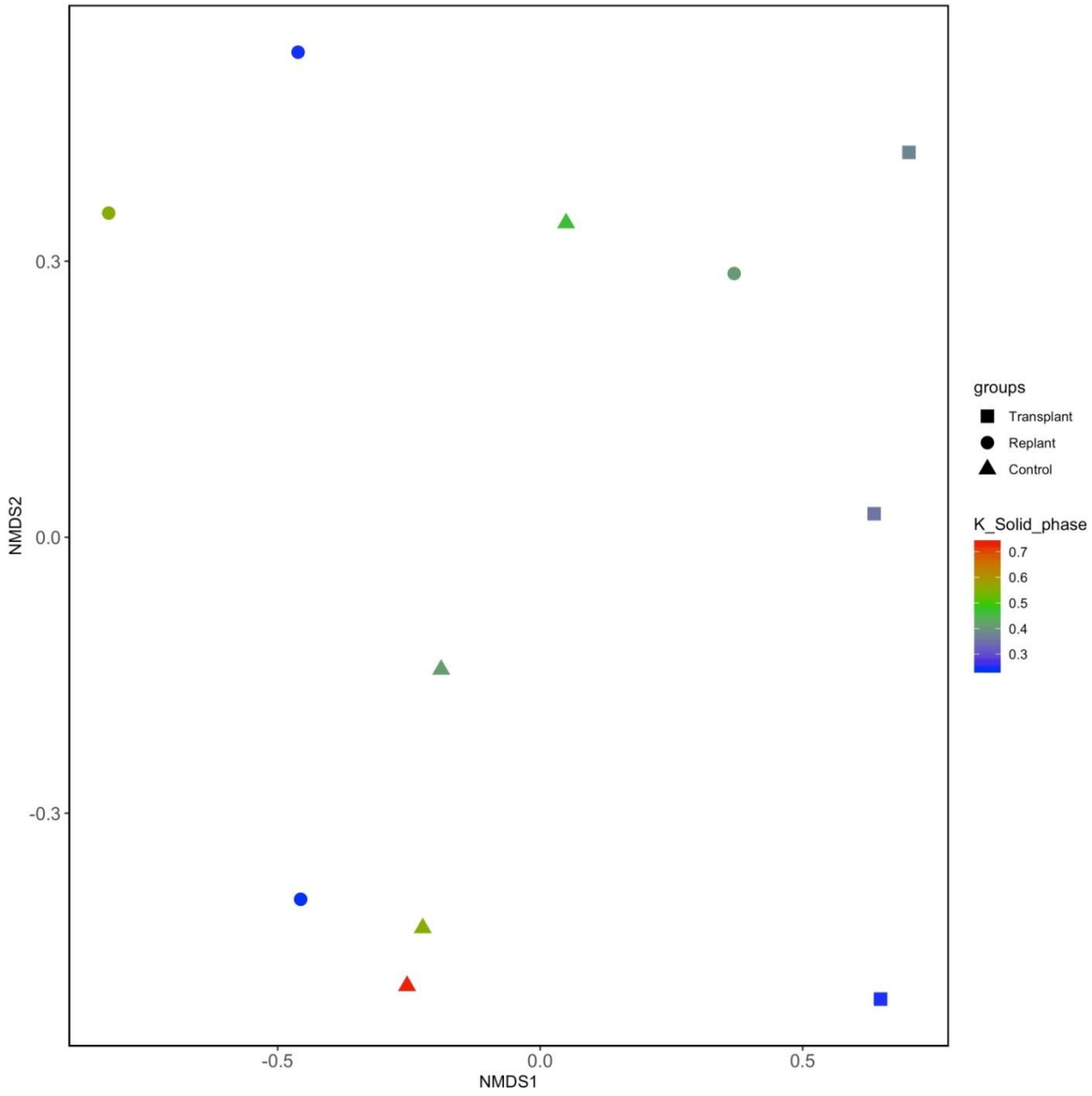

Figure 1.6H: Solid phase K.

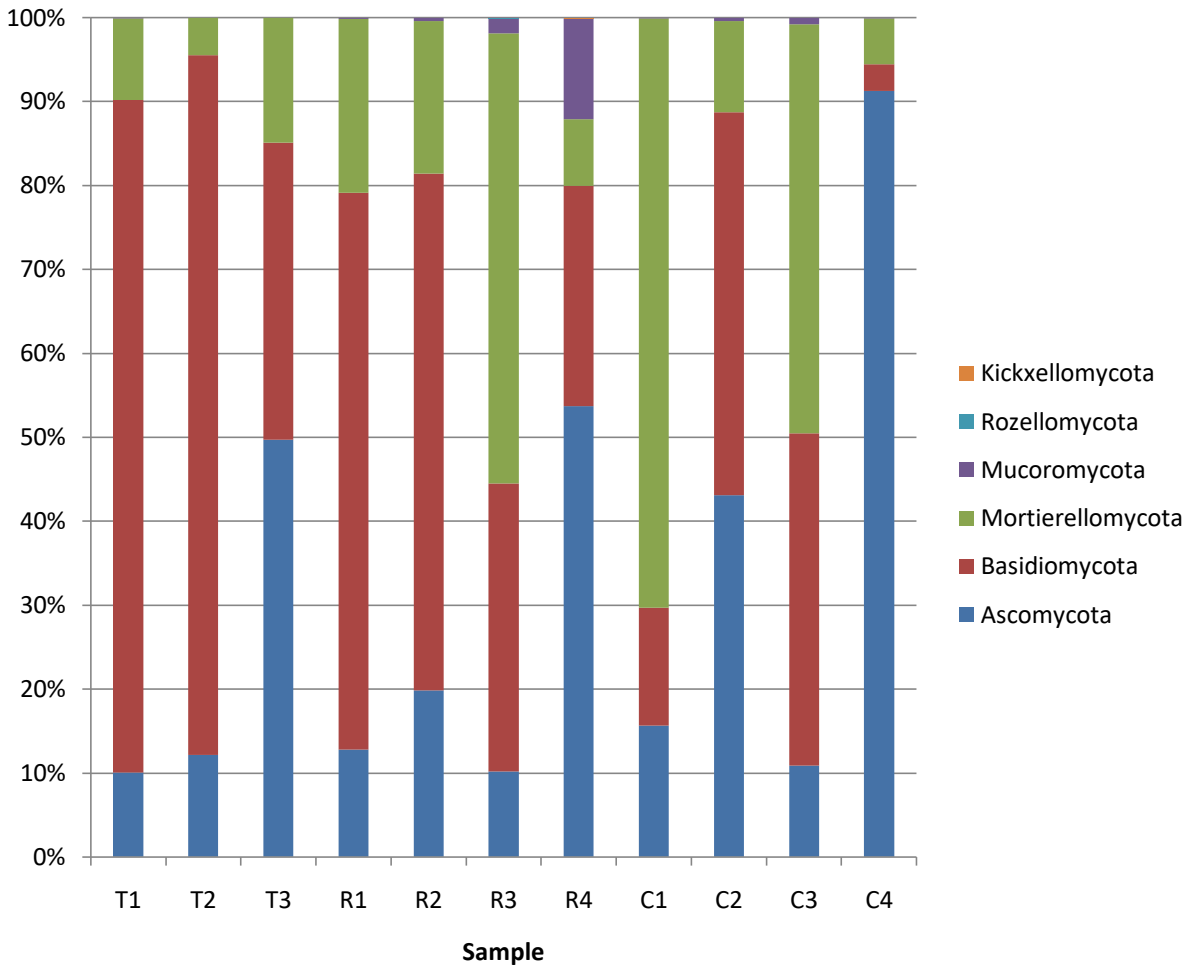

Figure 1.7: Phylum-level ITS1 fungal community composition across all samples. Samples names with T are transplanted, R are replanted, and C are control. Sample names with the same number indicate that the cores were taken from the same location within the poor fen.

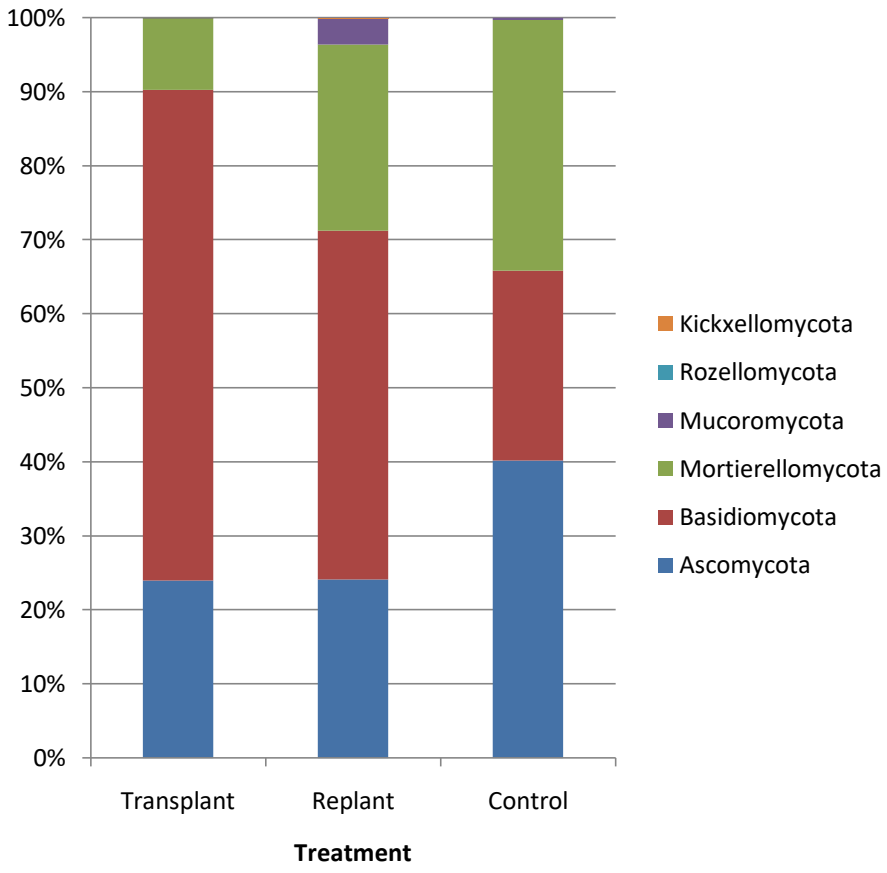

Figure 1.8: Phylum-level ITS1 fungal community composition. Bars show mean phylum relative abundance for each treatment.

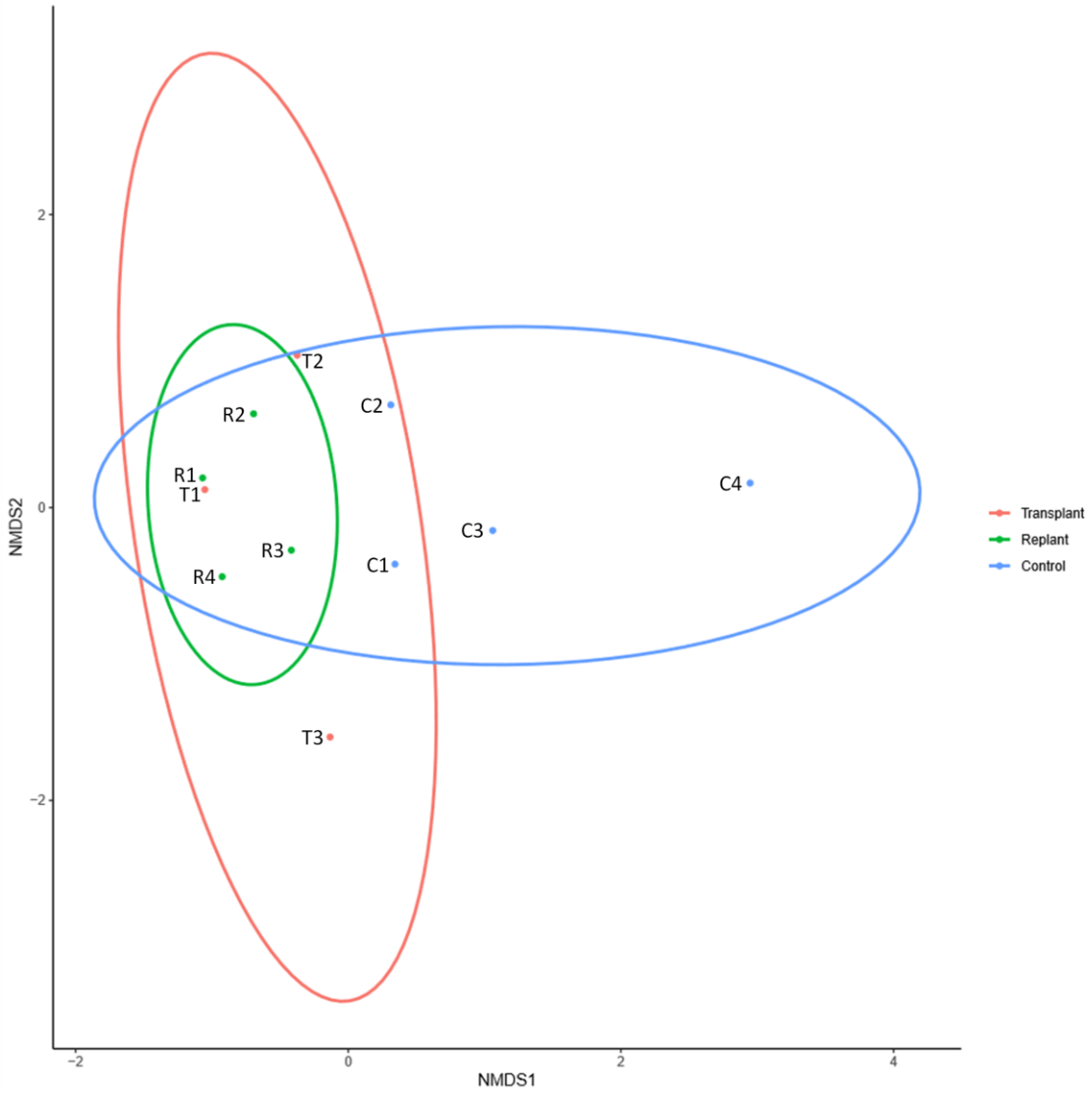

Figure 1.9: NMDS Bray-Curtis dissimilarity ordination of all ITS1 fungal taxonomic levels. Ellipses represent a 95% confidence interval. Samples names with T are transplanted, R are replanted, and C are control. Sample names with the same number indicate that the cores were taken from the same location within the poor fen.

Figures 1.10A-H: NMDS Bray-Curtis dissimilarity ordinations of all ITS1 fungal taxonomic levels, colour-coded to show chemical variable measurements for each sample.

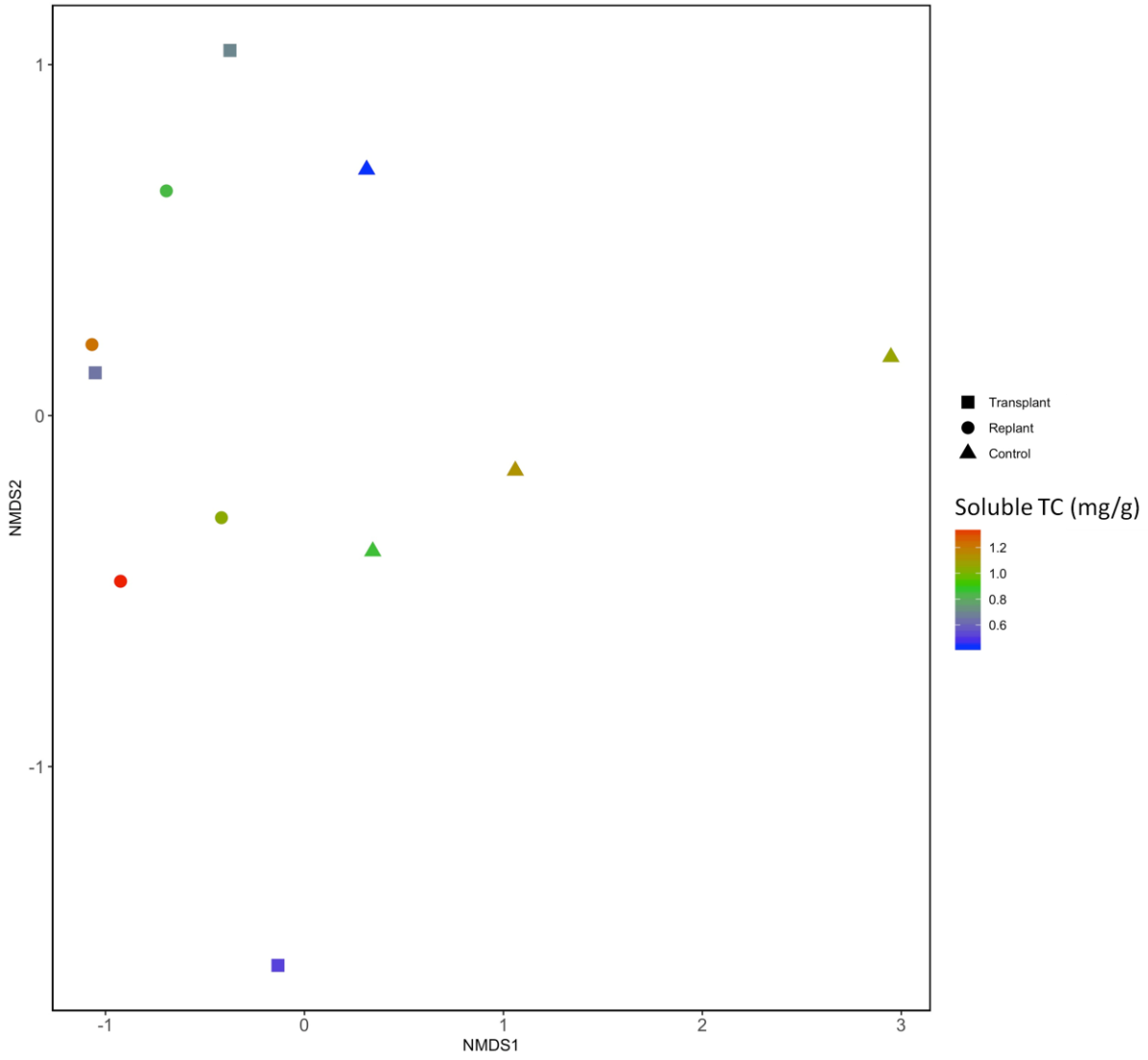

Figure 1.10A: Soluble TC.

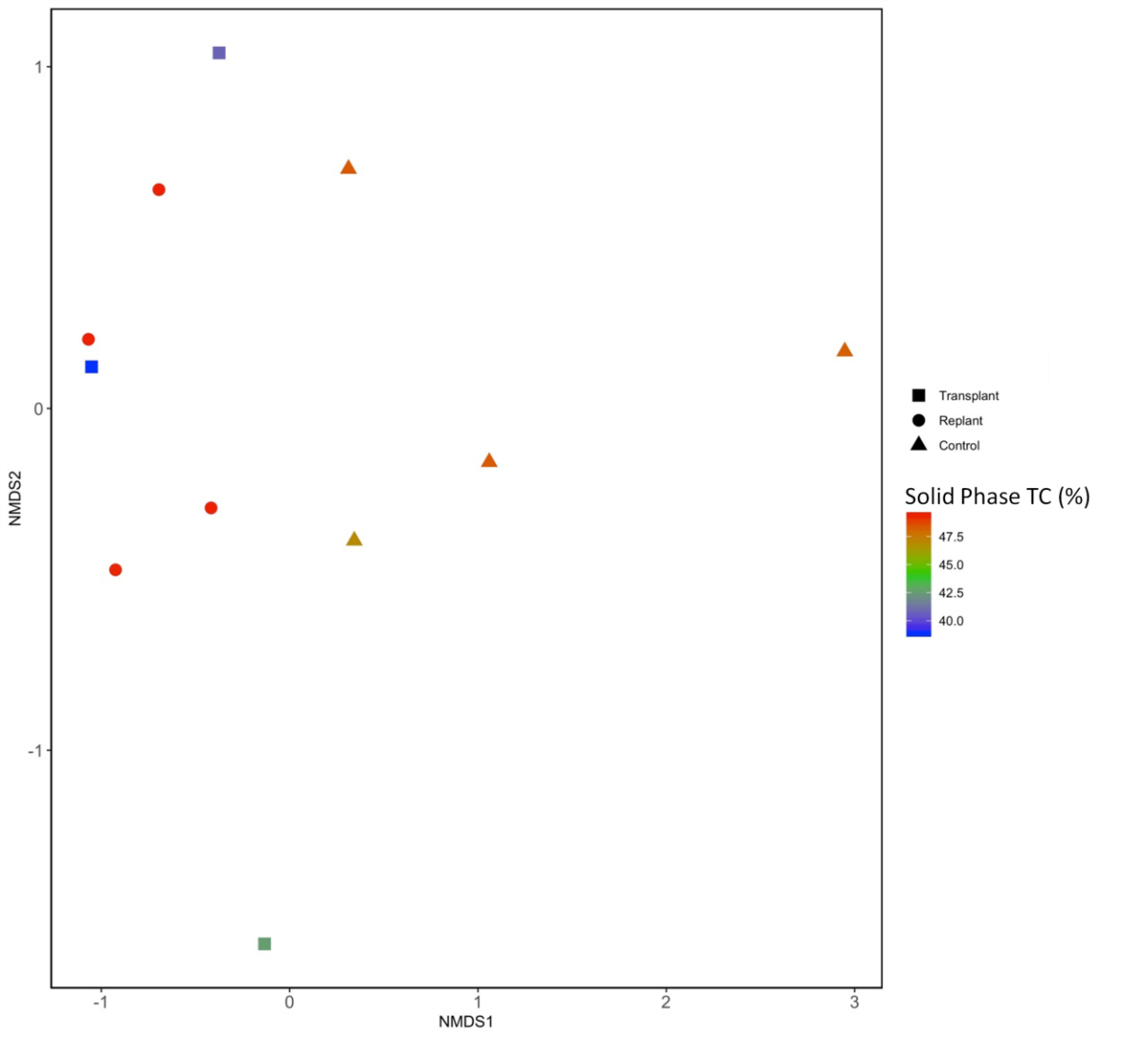

Figure 1.10B: Solid phase TC.

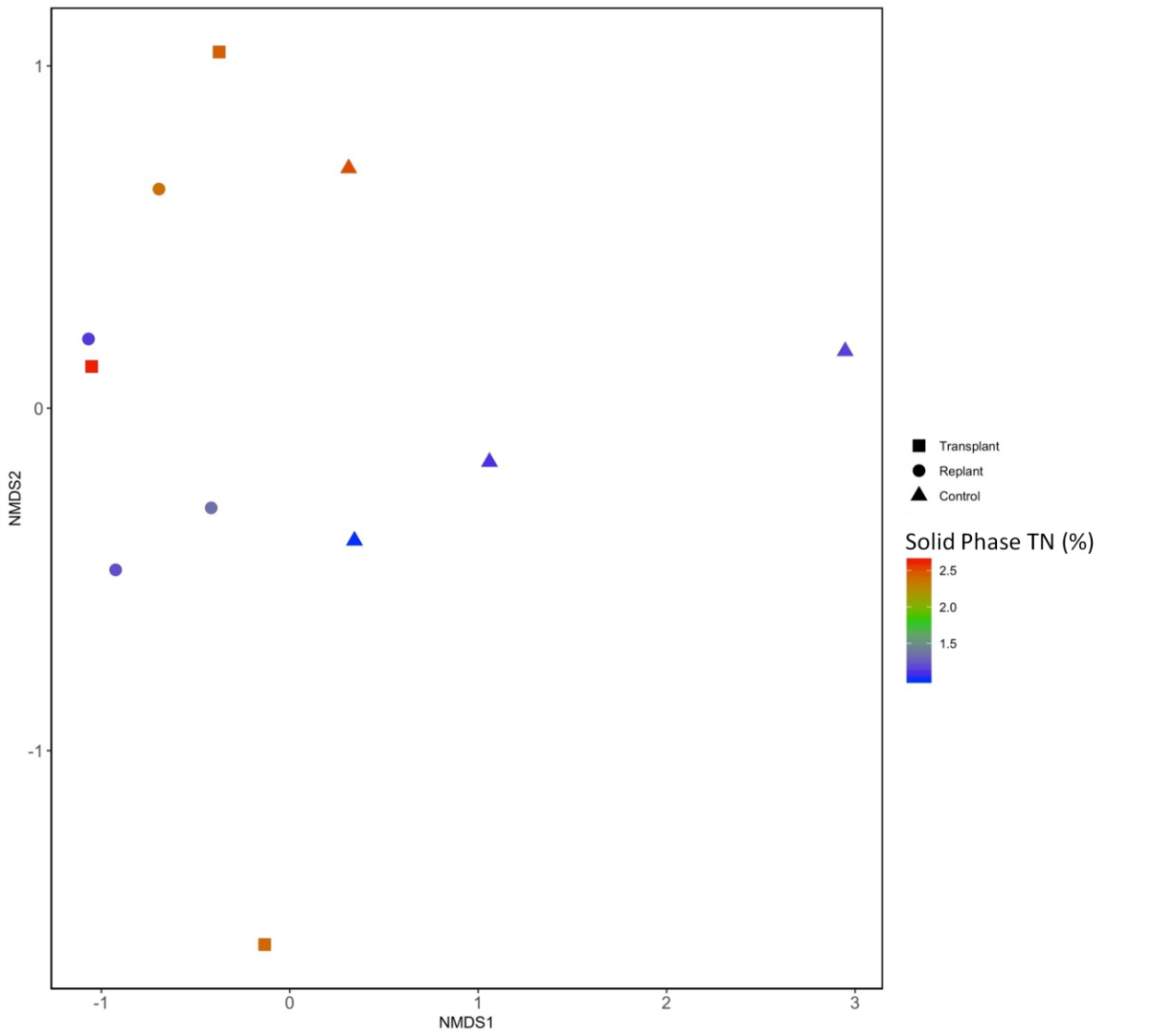

Figure 1.10C: Solid phase TN.

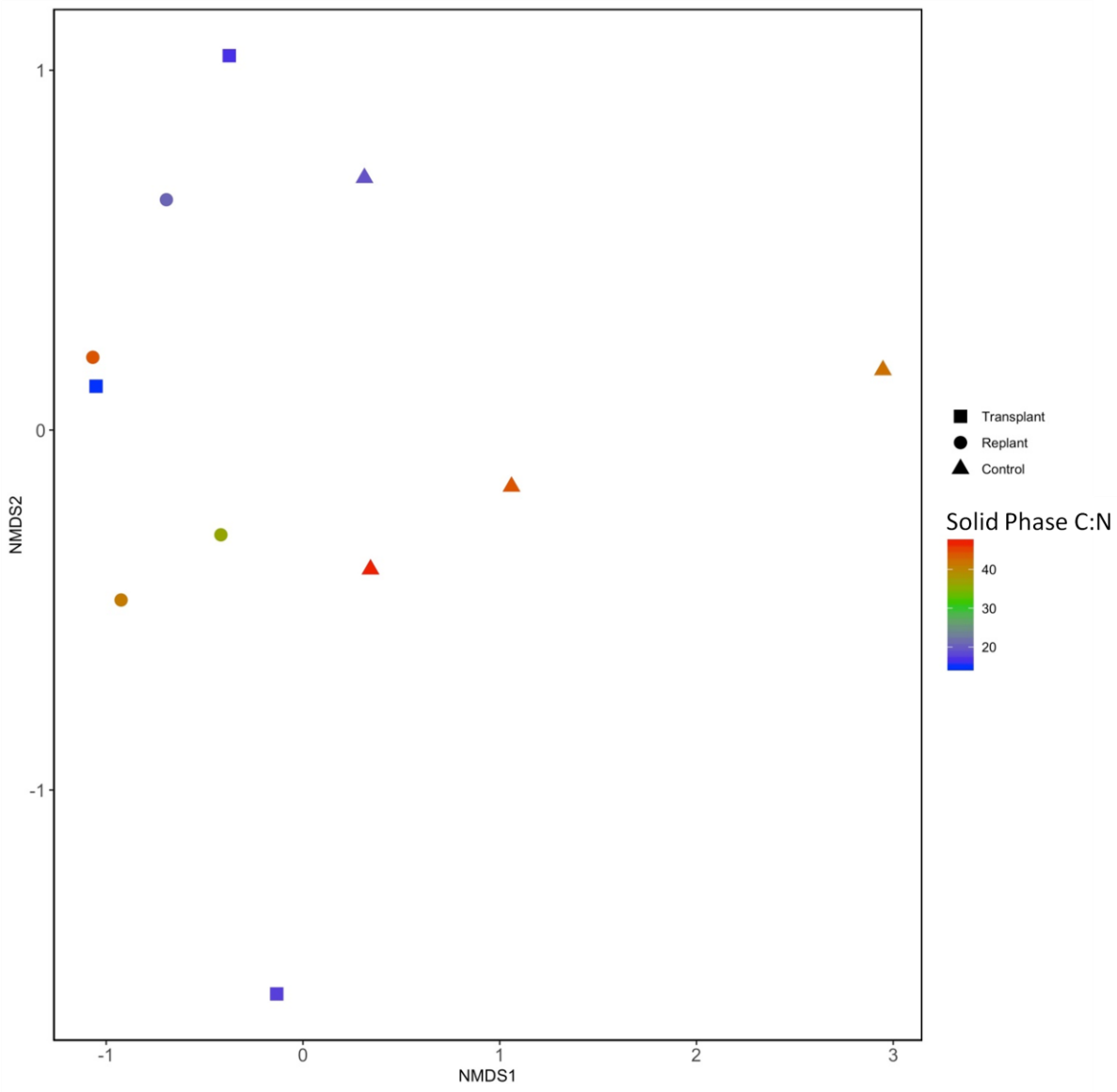

Figure 1.10D: Solid phase C:N.

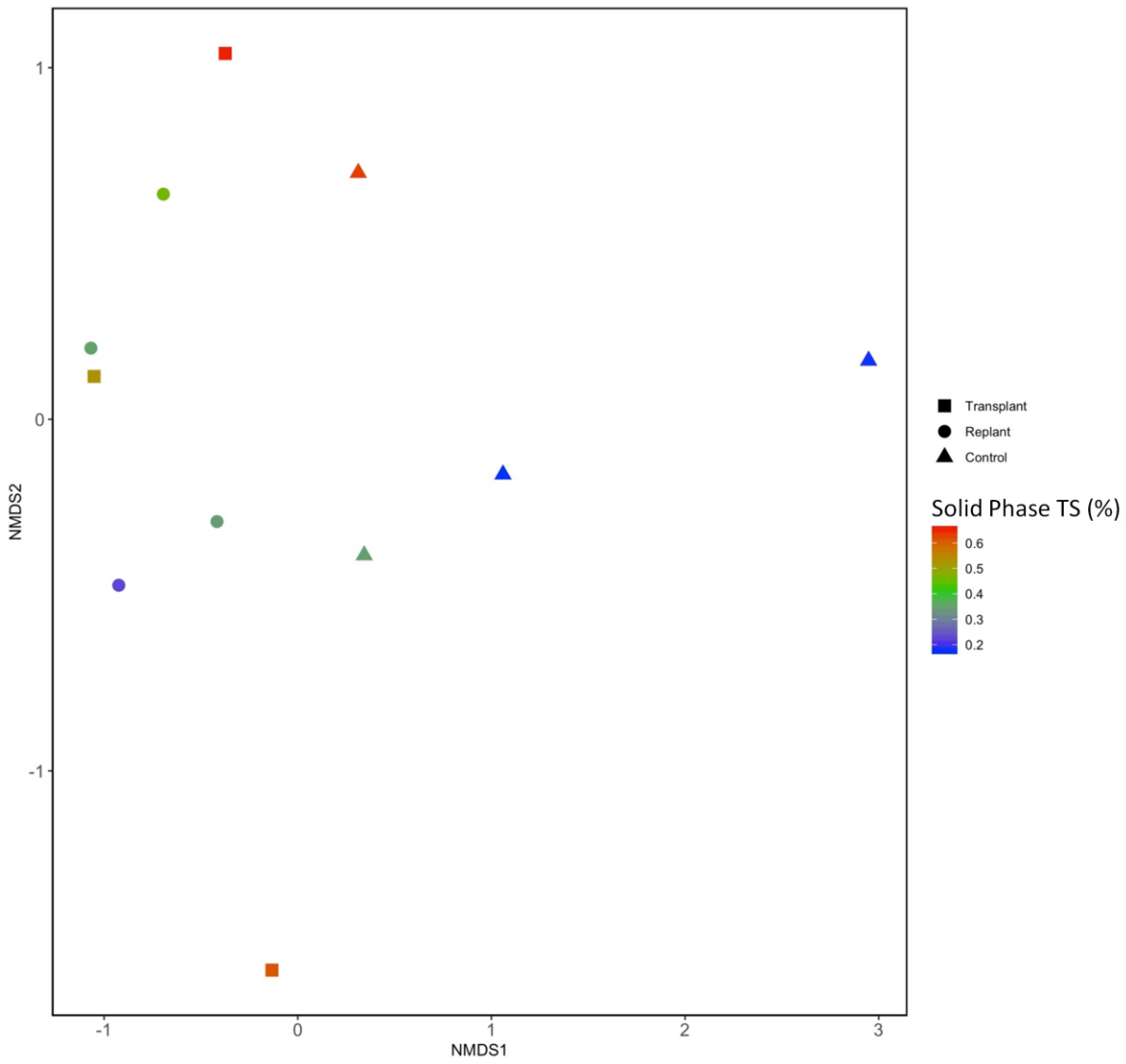

Figure 1.10E: Solid phase TS.

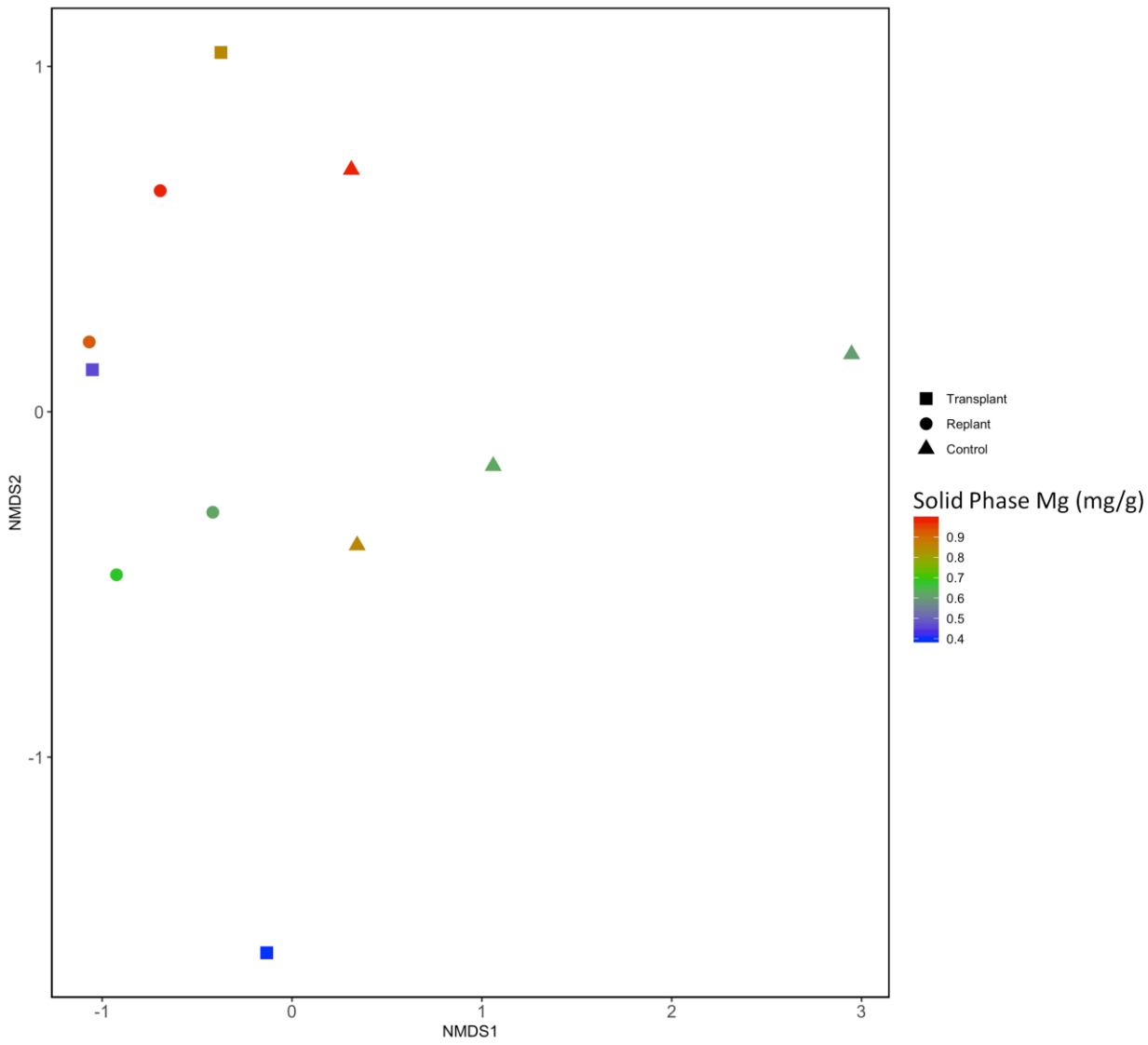

Figure 1.10F: Solid phase Mg.

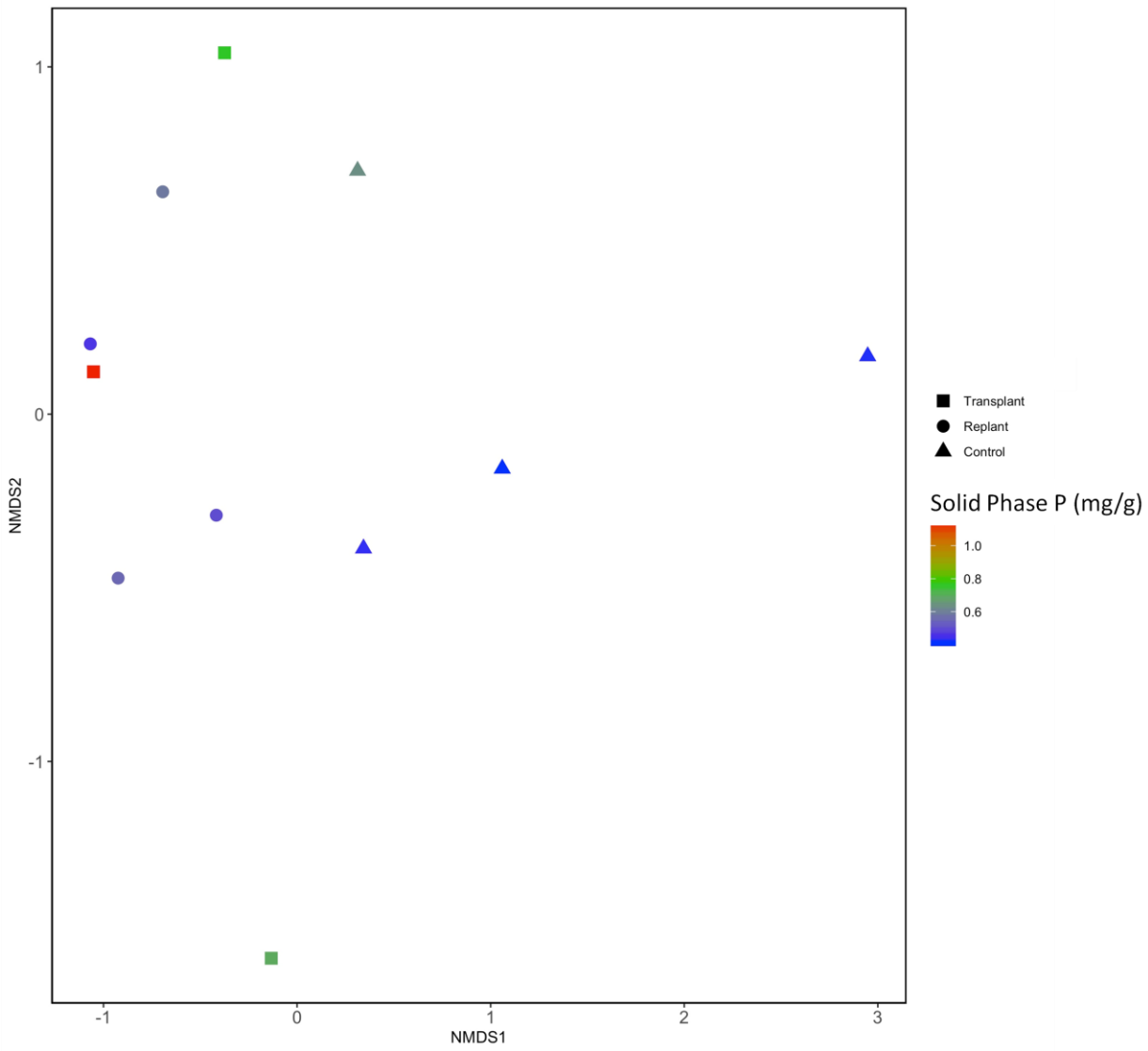

Figure 1.10G: Solid phase P.

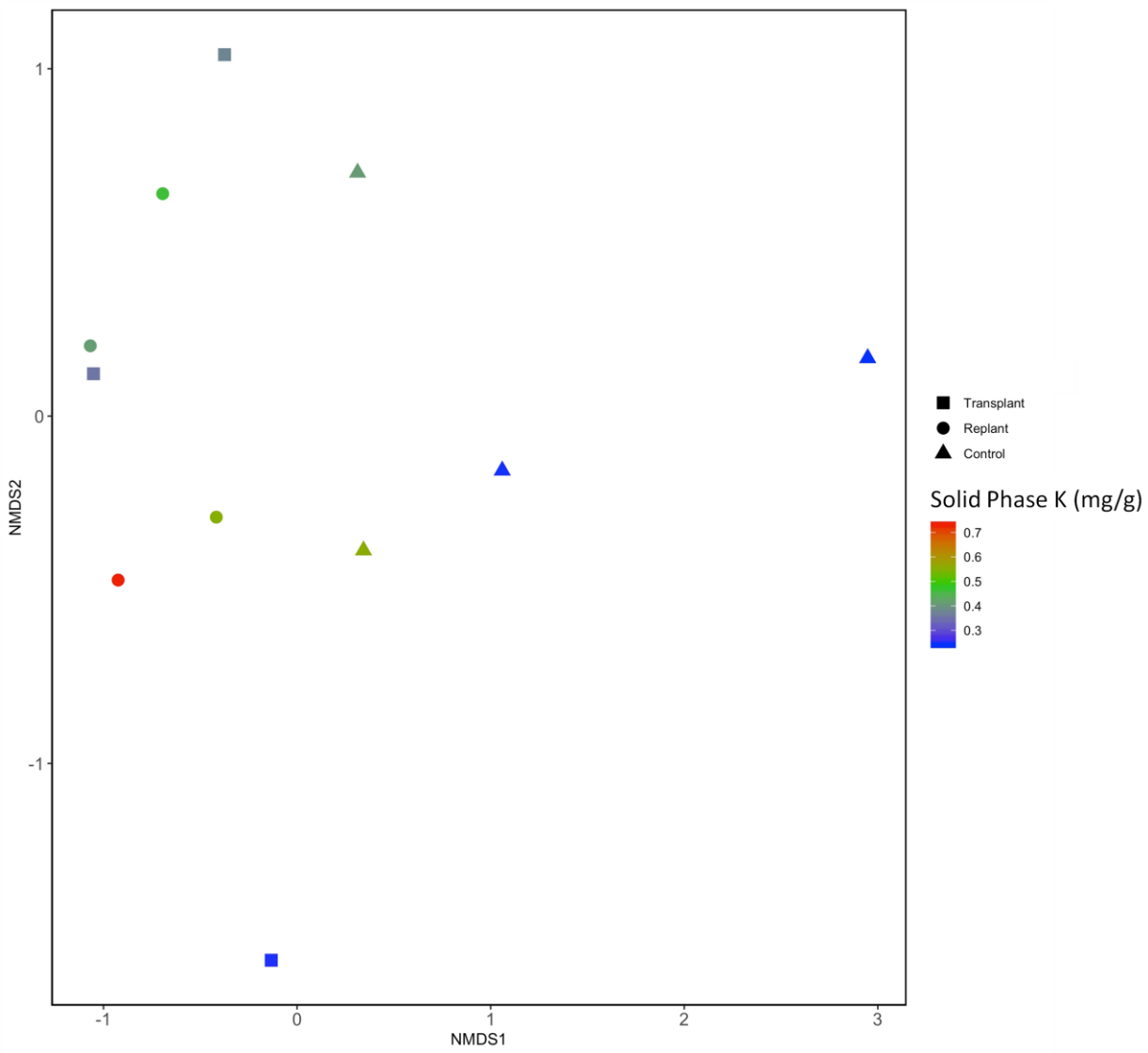

Figure 1.10H: Solid phase K.

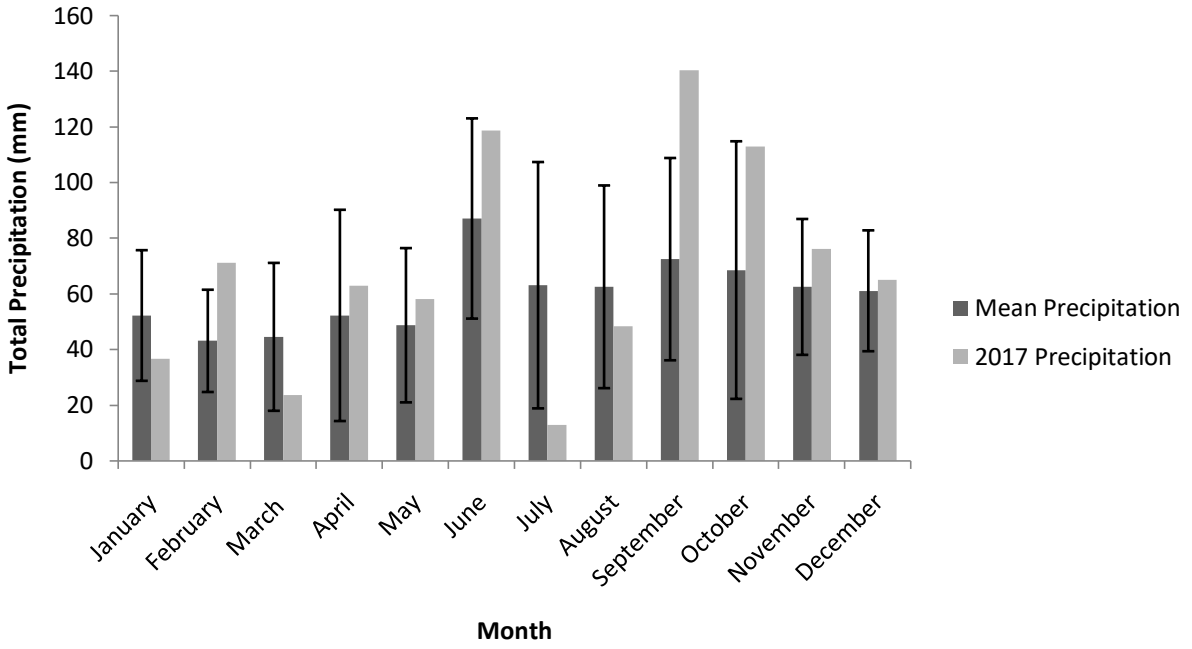

Figure 1.11: Mean monthly precipitation totals from Pukaskwa National Park, Ontario, alongside data from 2017. Error bars indicate standard deviation. Data is taken from the years 2008-2017, however winter precipitation measurements were largely unavailable prior to 2012. Missing years of data are listed here for the affected months: February, 2008-2011; March, 2008-2011; April, 2008; November, 2008-2010; December, 2008-2010.

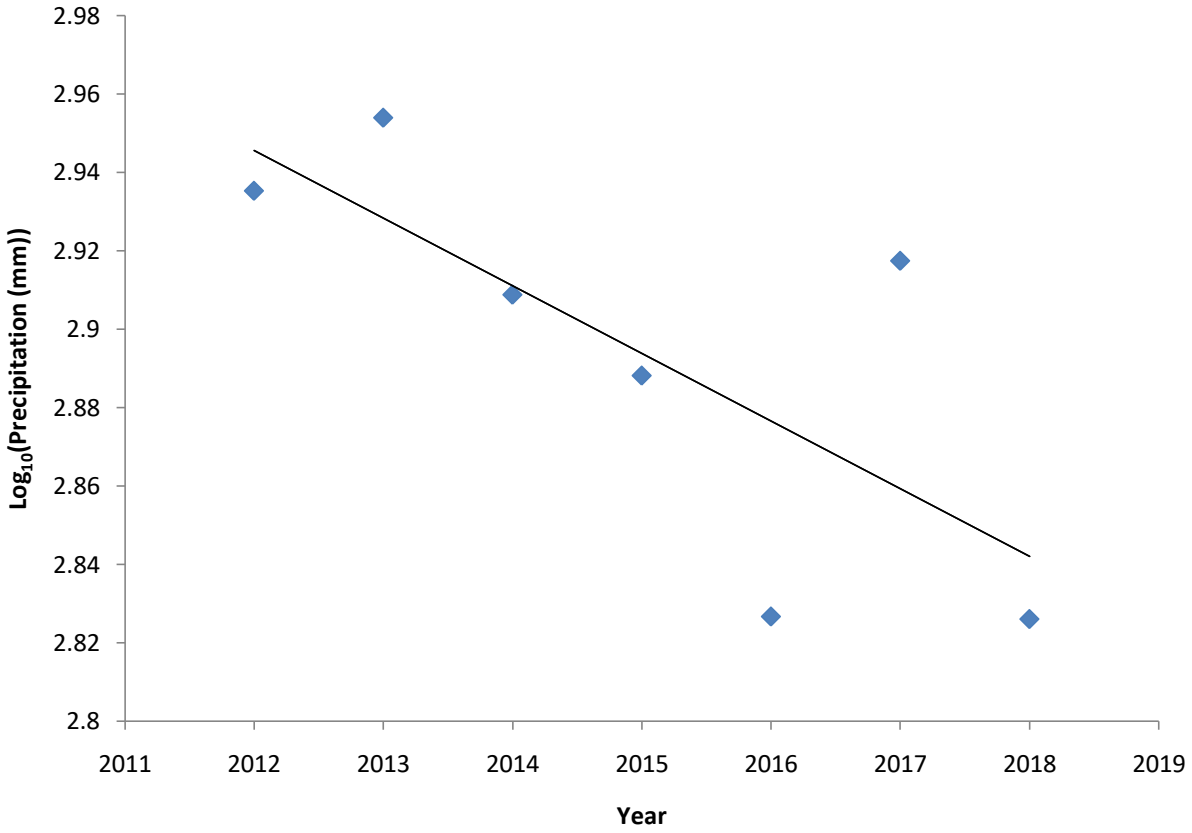

Figure 1.12: Plot of annual precipitation data from Pukaskwa National Park, Ontario from years 2011 to 2018, with line of best fit. Precipitation data has been base 10 log transformed due to non-normal distribution. The formula of the line of best fit is y = -0.017x + 2.962. Linear regression produced an R^2 of 0.546.

Chapter 2: Microbial feedbacks to contemporary climate change in northern peatlands

2.1 Introduction:

Northern peatlands comprise only about 3% of the Earth's surface, yet they contain approximately 30% of the Earth's soil carbon, equal to between 210 and 450 Gt (Gorham, 1991; Turunen *et al*., 2002; Rydin and Jeglum, 2006). However, in addition to being sinks for atmospheric carbon, they also emit methane, a potent greenhouse gas with a global warming potential 23 times higher than CO_2 over a 100 year period (Forster *et al*., 2007). This methane is released due to the activity of archaea called methanogens which thrive in the anoxic conditions found in peat. The dual nature of northern peatlands as both sinks and sources of atmospheric carbon makes them important areas of study when it comes to understanding the global carbon budget and the future course of anthropogenic climate change. According to the Canadian Global Climate Model (CGCM1), average air temperatures over northern Canada, where most Canadian peatlands are located, can be expected to increase by 5-10ºC by 2050 (Tarnocai, 2006). It is expected that this will result in drying of peatlands, as well as increased decomposition rates resulting in higher CO_2 emissions (Moore and Dalva, 1993; Waltzin *et al*., 2000).

Further, Dieleman *et al*. (2015) found that increased temperature and atmospheric CO_2 concentration consistent with projected future conditions can cause shifts in poor fen vegetation within a single growing season, presumably due to increased nutrient mineralization at higher temperatures. In their greenhouse experiments, at a threshold of 4 ºC above ambient conditions, graminoid plant species such as *Carex* sedges increased in abundance, and at a threshold of 8 ºC over ambient, *Sphagnum* began to decline. Introduction of additional atmospheric CO_2 along

with increased temperature amplified these effects, with graminoid species abundance increasing to 15 times that of control. This shift to a vascular plant-dominated community would be expected to entail eventual higher rates of decomposition and greenhouse gas emission, due to the higher nutritional content and decomposability of peat derived from vascular plants (Dieleman *et al*., 2015). An increase in graminoid species abundance would also be expected to increase methane transportation rates, as the aerenchyma of these plants would allow methane to be transported directly from anoxic peat layers to the atmosphere.

Building on these results, an experiment (referred to as Biological Response and Adaptation to Climate and Environment, or BRACE) was designed to test these findings in the field, on a larger scale. The experimental sites, in a poor fen and an intermediate fen located near White River, Ontario, were each equipped with transparent polyethylene chambers designed to increase ground temperature and atmospheric CO_2 within each chamber in a factorial design. However, technical issues regarding the CO_2 fertilization and heating systems meant that only the heating system was operational by the time I needed to take samples, and that only for 4 months prior to my sampling. This shorter timeframe would capture any direct physiological effects on microbial communities, but based on Dieleman *et al*.'s (2015) mesocosm study would not capture indirect changes due to plant community shifts.

2.2 Methods:

2.2.1 Experimental sites and set-up:

Samples were taken from two experimental sites near White River, Ontario, the first in a *Sphagnum*-dominated poor fen located at (48°35'N, 85°33'W) and the second in a nearby sedge-dominated intermediate fen. The experimental set-up at each site consisted of 16 clear

polycarbonate open top chambers (see Hanson *et al*., 2011) arranged around a central boardwalk and solar power supply. Each chamber was 1 m high by 1 m diameter, and was fitted onto a rigid collar that was set 50 cm into the underlying peat, in order to contain the experimental treatments. A circumferential ground heating system was integrated into each chamber, as well as an atmospheric CO_2 injection system, although the CO_2 injection systems were not active prior to sampling.

Treatment was randomly assigned such that the ground heating systems were activated in early June within half of the chambers at each experimental site. The ground heating systems were configured to heat the peat within the chamber to 6.75ºC above ambient temperature, which corresponds to a state shift in vegetation type, as found by Dieleman *et al*. (2015), and to an intermediate temperature treatment in the USDOE SPRUCE experiments located in Minnesota, USA, allowing the results of the present study to be compared to those of the SPRUCE experiments.

2.2.2 Sampling:

Sampling took place on October 1st and 2nd, 2017. Samples were collected from all 32 chambers across both experimental sites, as well as from 4 control locations at each site, which were located outside the experimental chambers, in order to assess any unintended influence of the chambers themselves on the study results. Two samples were taken from each chamber or control location; one sample was taken from the surface peat, being careful to avoid sampling any living plant matter, while another was taken at 10 cm depth. Within-chamber sampling was performed using a pair of long forceps, and a minimal amount of peat was taken for each sample (about 2 to 5 grams wet weight) to minimize disturbance of the peat, while sampling of outside-

chamber controls was performed using a serrated knife. Forceps and knife were cleaned with 70% ethanol between samples to minimize cross-contamination. Samples were placed in resealable plastic bags and put on ice for transport to Laurentian University. Upon arrival at Laurentian University, samples were frozen and stored at -20ºC.

2.2.3 In vitro *anaerobic CH_4 and CO_2 production:*

Samples were thawed and were then homogenized by hand while removing any coarse woody debris and green plant matter. 2g of each sample was measured out using a Sartorius QUINTIX224-1S weigh scale. The weighed samples were then placed in separate, weighed 130-mL mason jars each with a rubber septum inserted in the lid, and the jar lids were closed firmly. Jars were then evacuated via repeated cycles of gas extraction using a vacuum pump and subsequent backfilling with pure N_2. At the end of this cycling (time 0), the atmosphere inside the jar contained only N_2. Gas pressure inside the jar was equalized to approximately that of the atmospheric pressure inside the laboratory. CH_4 and CO_2 measurements were made via a SRI 8610C gas chromatograph on days 10, 20, 30, 45 and 60 of the incubation. After gas was extracted via needle and syringe for each measurement, an equal volume of N_2 was injected into each jar to maintain constant gas pressure. Jars were kept at room temperature and away from light between measurements.

Moisture content was determined post-incubation by drying the incubated peat in the jars for 24 hours at 60ºC, using a Fisher Scientific Isotemp Oven, then weighing the dried peat and jars using a Sartorius QUINTIX224-1S weigh scale and subtracting the jar weight.

2.2.4 Statistical analysis:

Samples were grouped according to experimental location, sample depth, and treatment. CH_4 and CO_2 production were expressed as weight of gas produced per dry weight gram of peat. The CH_4 and CO_2 production data displayed right-skewed distributions and were therefore base-log transformed prior to running a one-way ANOVA. One-way ANOVA was followed by Tukey's HSD post hoc test, with a significance threshold of $p = 0.05$.

2.3 Results:

Incubation results are summarized in Table 2.1. While anaerobic *in vitro* measurements of CH_4 (Figure 2.1) and CO_2 (Figure 2.2) production were made up to Day 60, it was decided that only results up to Day 30 would be used for statistical analysis, due to apparent net consumption of CH_4 after this sample date in some samples after substrate (i.e. CH_4) concentrations became high (c.f. Gupta *et al*., 2013). Anaerobic incubation of the peat samples yielded few significant differences in CH_4 or CO_2 production between samples, and provided no evidence for any effect caused by heating on CH_4 or CO_2 production. Regarding CH_4 production specifically, the control group for the poor fen (10 cm depth) produced significantly less CH_4 than all other sample groups, using a significance threshold of 0.05. The only other significant differences observed in CH_4 production were that the ambient intermediate fen (surface depth) group produced more CH_4 than the ambient poor fen (surface depth) group ($p = 0.039$), and the ambient poor fen (10 cm depth) group ($p = 0.017$).

Results for CO_2 production yielded no significant differences between any sample groups in the poor fen. In the intermediate fen, only significant differences between the surface and depth groupings were observed, with the ambient intermediate fen (surface depth) group producing significantly more CO_2 than the ambient intermediate fen (10 cm depth) group ($p =$

0.009), the heated intermediate fen (10 cm depth) group ($p = 0.001$), and the control intermediate fen (10 cm depth) group ($p = 0.042$). No significant differences were observed between heated and ambient treatments within either the surface or 10 cm depth samples.

2.4 Discussion:

The early results of this field study showed no evidence for a short-term effect of peat warming on anaerobic CO_2 or CH_4 production. However, this study is obviously limited in that peat warming was only in place for four months prior to sampling, rather than the intended full growing season. It is possible that the warming treatment would need to be in place for a more substantial time period in order to observe changes to peat microbial metabolism or community composition. Since no changes to plant communities were observed in the time period between the chamber heating systems being activated and peat sampling, there would have been little to no change in peat nutritional content or cycling due to changes in vegetation type. As shift in vegetation is predicted to be a major driver in increased greenhouse gas production due to peat warming (Dieleman *et al*., 2015), it makes sense that no changes to anaerobic greenhouse gas production were observed as a result of short-term heating. It may also be assumed that changes to vegetation and peat nutrient content would take longer to take effect than the one growing season predicted by Dieleman *et al.* in their greenhouse study (2015), as the BRACE chambers remain hydrologically connected to the surrounding peat, with potential for resulting buffering effects. However it was found that, in the poor fen, the open-top chambers themselves appeared to produce a positive effect on CH_4 production, as CH_4 production was significantly lower in the control samples. I speculate that it may be due to an unintentional greenhouse effect caused by the transparent open-top chambers. If air circulation is insufficient, heat may become trapped in

the chambers, passively warming the peat. Why this only had an effect in the poor fen is unknown, but it may be due to a higher density of trees in the poor fen, which would obstruct wind and thus reduce air circulation within the chambers. It is also possible that differences in microbial community composition between the poor and intermediate fens could produce differences in CH_4 production in response to this passive heating.

CH_4 production responds to peat warming in predictable patterns, which would likely be followed in the BRACE sites in future growing seasons. In previous open-top chamber studies, peat CH_4 emission has responded differently to warming depending upon hydrological conditions, with CH_4 production increasing with temperature under a high water table, and decreasing with temperature under a low water table (Turetsky *et al*., 2008; Munir and Strack, 2014; Peltoniemi *et al*., 2016; Gill *et al*., 2017). However, other peat or soil warming studies suggest that the effects of warming on CO_2 production and the microbial community can be varied. For instance, a +1.5ºC above ambient temperature increase in two boreal, sedge-dominated fens over three years produced a decrease in microbial biomass, but did not affect basal respiration rate in a study by Peltoniemi *et al.* (2015). Further, peat microbial communities from each fen in their study were found to respond differently to warming, as evaluated through phospholipid fatty acid (PLFA) analysis, even though the two fens were from the same habitat classification. This suggests that the basal respiration rates of individual northern peatlands may be somewhat resilient to change due to climate warming, even with warming-dependent change to the microbial community. However, in bog peat/soil warming experiments, CO_2 production has been observed to increase with temperature, both with passive warming via open-top chamber (Dorrepaal *et al*., 2009) and with active heating of deep peat (Gill *et al*., 2017). This would suggest that the response of basal respiration rate to warming may depend upon peatland

type or other factors. Microbial community composition may not be the primary driver in peat CO_2 production. In grassland soils studied by Alster *et al.* (2016), microbial community type was found to account for only 30% of variation in CO_2 production, and instead temperature and soil type were most important in accounting for variation in the size of the labile C pool that this CO_2 was produced from. This principle is also illustrated by a field experiment by Schindlbacher *et al.* (2011), who found that five years of +4ºC warming above ambient during snow-free seasons produced no changes to microbial community or biomass in a mountain forest soil, yet substantially increased microbial metabolic activity. As the reaction of basal respiration rates to peat or soil warming appears to be variable and not well-understood, continued analysis of BRACE samples in subsequent growing seasons will provide important data in the attempt to predict the basal respiration response of Canadian northern peatlands to climate change.

2.5 Conclusion:

Anaerobic incubation of peat samples from the BRACE experiments failed to show any effects on CH_4 or CO_2 production due to 4 months of peat warming in the field. However, the open-top chambers themselves did appear to increase CH_4 production at the poor fen experimental site. This preliminary data will be incorporated into further analysis of the effects of increased temperature and atmospheric CO_2 on peat microbial community structure and function in subsequent studies of the BRACE experimental setup. Observations of future growing seasons and the incorporation of next-generation sequencing data, while beyond the scope of my study, will be crucial to properly assess the effects of the experimental conditions on the peat microbial community, and help to model the potential effects of climate change on greenhouse gas emission from northern peatlands.

Tables:

Table 2.1: Results of anaerobic peat incubation for net CH_4 and CO_2 production to Day 30. Values are treatment means, with standard deviation in brackets; n = 8 for all treatments except control, for which n = 4.

Treatment	Depth	Anaerobic Net CH_4 Production to Day 30 (µg/g dry peat)	Anaerobic Net CO_2 Production to Day 30 (mg/g dry peat)
Poor Fen			
Heat	Surface	33.30 (67.63)	29.30 (40.96)
	10 cm Depth	6.93 (5.18)	17.73 (7.12)
Ambient	Surface	14.14 (12.92)	18.76 (9.33)
	10 cm Depth	11.17 (12.90)	16.55 (4.90)
Control	Surface	16.81 (17.65)	16.15 (1.83)
	10 cm Depth	0.49 (0.92)	16.23 (6.24)
Intermediate Fen			
Heat	Surface	1226.52 (1021.19)	21.25 (6.31)
	10 cm Depth	340.11 (366.71)	12.84 (6.75)
Ambient	Surface	1002.44 (375.89)	29.21 (4.08)
	10 cm Depth	264.38 (284.15)	10.41 (4.09)
Control	Surface	112.32 (208.09)	25.94 (11.74)
	10 cm Depth	4.48 (3.53)	11.80 (5.58)

Figures:

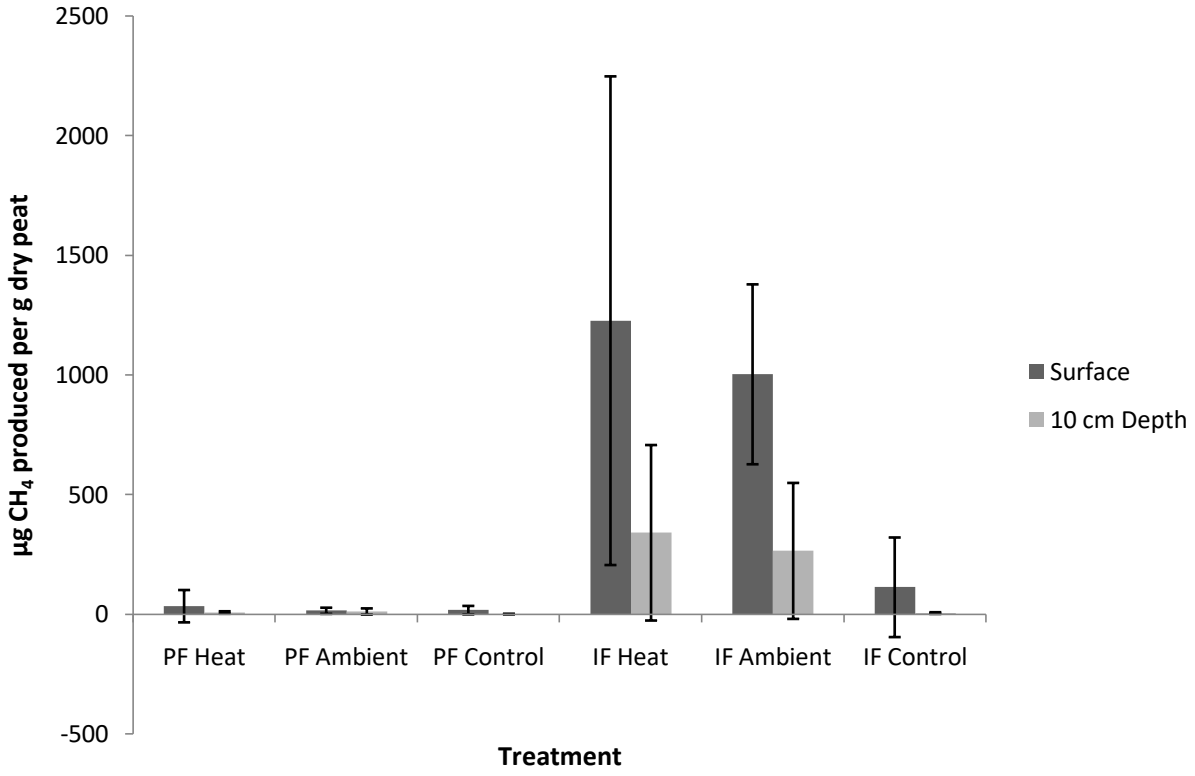

Figure 2.1: Results of anaerobic peat incubation for net CH_4 production up to day 30. Error bars represent standard deviation.

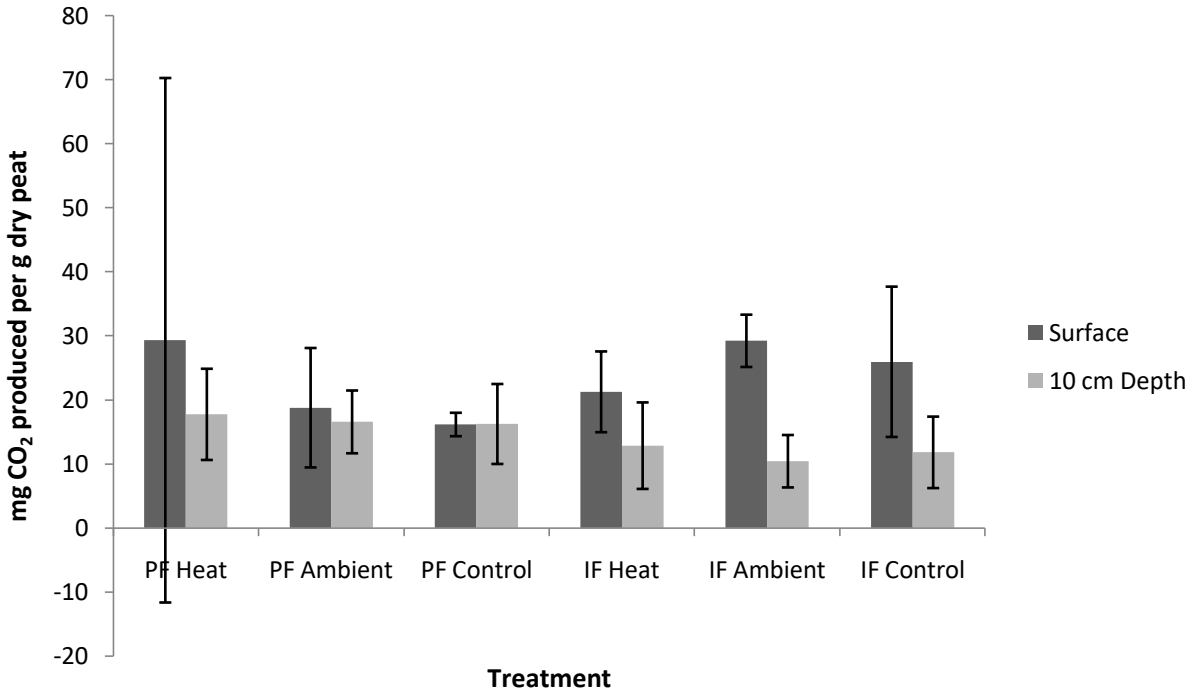

Figure 2.2: Results of anaerobic peat incubation for net CO_2 production up to day 30. Error bars represent standard deviation.

General Conclusion:

Through my thesis work I was able to provide bases for multiple lines of further study in the areas of northern peatland microbiology and chemistry. In my study of rich fen peat transplanted into a poor fen environment, I found that solid phase total nitrogen and C:N ratio correlated with peat bacterial community composition. Additional investigation may reveal the exact relationships between specific bacterial groups and total nitrogen and C:N. Based on my observations of a high abundance of Bacteroidetes remaining in transplanted rich fen peat despite exposure to a poor fen aqueous environment, it may also be worth further study into the drivers of Bacteroidetes abundance in peat. Particularly, the role of peat substrate should be examined to determine whether Bacteroidetes lose prevalence in poor fen and bog environments due to the peat substrate becoming dominated by dead *Sphagnum*. Another area requiring further investigation will be in determining best practices for other long-term peat transplantation experiments, given the confounding effects on peat microbiology produced by the transplantation methods of Preston (2013). Given the limitations of my study due to variability of conditions such as water table, pH and surrounding peat chemistry, in addition to improved transplantation methods, further long-term peat transplantation experiments should attempt to control such variables when possible, or closely monitor them at minimum such that short-term fluxes in these variables can be detected and accounted for. Finally, it was unexpectedly found that the poor fen, which contained both the long-term transplantation experiment and half of the BRACE experimental chambers, had increased in solid phase total nitrogen and pH and decreased in solid phase C:N since sampling by Myers *et al.* (2012), in opposition to usual peatland succession. These changes are consistent with expected results from the BRACE climate change simulation experiments; it appears that this poor fen may be demonstrating the effects of climate change on

its own, and should be subject to further study in this respect. As work on the BRACE experiments continues, the changing environment of the poor fen should be addressed, in addition to the open-top chambers' confounding effect on the poor fen's CH_4 production. In conclusion, my thesis work provides vital information to improve the design and execution of both the ongoing BRACE experiments and any future long-term peat transplantation experiments, and provides new avenues for further research into the interactions between northern peatland microbiology, chemistry and climate,

References:

Abarenkov, K., Henrik Nilsson, R., Larsson, K.H., Alexander, I.J., Eberhardt, U., Erland, S. *et al.* (2010) The UNITE database for molecular identification of fungi--recent updates and future perspectives. *New Phytol.* **186**(2): 281-285.

Achtnich, C., Bak, F. and Conrad, R. (1995) Competition for electron donors among nitrate reducers, ferric iron reducers, sulfate reducers, and methanogens in anoxic paddy soil. *Biol. Fert. Soils* **19**: 65-72.

Alster, C.J., Koyama, A., Johnson, N.G., Wallenstein, M.D., and von Fischer, J.C. (2016) Temperature sensitivity of soil microbial communities: An application of macromolecular rate theory to microbial respiration. *J. Geophys. Res. Biogeosci.* **121**: 1420-1433.

Amaral, J.A. and Knowles, R. (1994) Methane metabolism in a temperate swamp. *Appl. Environ. Microbiol.* **60**(11): 3945-3951.

Basiliko, N. and Yavitt, J.B. (2001) Influence of Ni, Co, Fe, and Na additions on methane production in *Sphagnum*-dominated North American peatlands. *Biogeochemistry* **52**(2): 133-153.

Bauer I.E., Gignac L.D., and Vitt D.H. (2003) Development of a peatland complex in boreal western Canada: Lateral site expansion and local variability in vegetation succession and long-term peat accumulation. *Can. J. Bot.* **81**: 833-847.

Bedree, J.K., Bor, B., Cen, L., Edlund, A., Lux, R., McLean, J.S. *et al.* (2018) Quorum sensing modulates the epibiotic-parasitic relationship between *Actinomyces odontolyticus* and its Saccharibacteria epibiont, a *Nanosynbacter lyticus* strain, TM7x. *Front. Microbiol.* **9**(2049): doi:10.3389/fmicb.2018.02049

Bender, M. and Conrad, R. (1995) Effect of CH_4 concentrations and soil conditions on the induction of CH4 oxidation activity. *Soil Biol. Biochem*. **27**(12): 1517-1527.

Borrel, G., O'Toole, P.W., Harris, H.M.B., Peyret, P., Brugere, J.F. and Gribaldo, S., (2013) Phylogenomic data support a seventh order of methylotrophic methanogens and provide insights into the evolution of methanogenesis. *Genome Biol. Evol.* **5**: 1769-1780.

Bowman, J. (2006) "The methanotrophs—the families Methylococcaceae and Methylocystaceae" *The Prokaryotes, 3rd ed, vol 2.* Dworkin, M., Falkow, S., Rosenberg, E., Schleifer, K.-H. and Stackebrandt, E. (eds.) Springer, New York, NY 266–289.

Brown, C.T., Hug, L.A., Thomas, B.C., Sharon, I., Castelle, C.J., Singh, A. *et al.* (2015) Unusual biology across a group comprising more than 15% of domain Bacteria. *Nature* **523**: 208-211.

Bubier, J. L. (1995) The relationship of vegetation to methane emission and hydrochemical gradients in northern peatlands. *J. Ecol*. **83**(3): 403-420.

Bubier, J. L., Costello, A., Moore, T. R., Roulet, N. T. and Savage, K. (1993) Microtopography and methane flux in boreal peatlands, northern Ontario, Canada. *Can. J. Bot*. **71**: 1056-1063.

Bushnell, B. (2016) BBMap short read aligner.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J. and Holmes, S.P. (2016) DADA2: High-resolution sample inference from Illumina amplicon data. *Nat. Methods* **13**(7): 581-583.

Clymo, R.S. (1984) The limits to peat bog growth. *Philos. Trans. R. Soc. Lond. B*. **303**: 605–654.

Clymo, R. S. and Pearce, D. M. E. (1995) Methane and carbon dioxide production in, transport through, and efflux from a peatland. *Phil. Trans. R. Soc. Lond. A*. **350**(1696): 249–259.

Conrad, R. (1999) Contribution of hydrogen to methane production and control of hydrogen concentrations in methanogenic soils and sediments. *FEMS Microbiol. Ecol*. **28**(3): 193-202.

Dedysh, S.N. (2002) Methanotrophic bacteria of acidic *Sphagnum* peat bogs. *Microbiology* **71**(6): 638-650.

Delafont, V., Samba-Louaka, A., Bouchon, D., Moulin, L. and Héchard, Y. (2015) Shedding light on microbial dark matter: a TM6 bacterium as natural endosymbiont of a free-living amoeba. *Environ. Microbiol. Rep.* **7**(6): 970-978.

DeSantis, T.Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E.L., Keller, K. *et al.* (2006) Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Appl. Environ. Microbiol.* **72(**7): 5069-5072.

Dieleman, C.M., Branfireun, B.A., McLaughlin, J.W. and Lindo, Z. (2015) Climate change drives a shift in peatland ecosystem plant community: Implications for ecosystem function and stability. *Glob. Change Biol.* **21**: 388-395.

Dorrepaal, E., Cornelissen, J.H.C., Aerts, R., Wallén, B. and Logtestijn, R.S.P. Van. (2005) Are growth forms consistent predictors of leaf litter quality and decomposability across peatlands along a latitudinal gradient? *J. Ecol.* **93**:817-828.

Dorrepaal, E., Toet, S., van Logtestijn, R.S.P, Swart, E., van de Weg, M.J., Callaghan, T.V. *et al.* (2009) Carbon respiration from subsurface peat accelerated by climate warming in the subarctic. *Nature* **460**: 616-620.

Dunfield, P., Knowles, R., Dumont, R. and Moore, T. R. (1993) Methane production and consumption in temperate and subarctic peat soils: Response to temperature and pH. *Soil Biol. Biochem*. **25**(3): 321-326.

Dunfield, P.F., Yuryev, A., Senin, P., Smirnova, A.V., Stott, M.B., Hou, S. *et al*. (2007) Methane oxidation by an extremely acidophilic bacterium of the phylum Verrucomicrobia. *Nature* **450**: 879–882.

Fechner-Levy, E. J. and Hemond, H. F. (1996) Trapped methane volume and potential effects on methane ebullition in a northern peatland. *Limnol. Oceanogr*. **41**(7): 1375-1383.

Ferry, J.G. (2010) How to make a living by exhaling methane. *Annu. Rev. Microbiol.* **64**: 453-473.

Fierer, N. and Jackson, R.B. (2006) The diversity and biogeography of soil bacterial communities. *Proc. Natl. Acad. Sci. U. S. A.* **103**:626-631.

Forster, P., Ramaswamy, V., Artaxo, P., Berntsen, T., Betts, R., Fahey, D.W. *et al.* (2007) "Changes in atmospheric constituents and in radiative forcing." in *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change*. Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K.B., Tignor, M. and Miller, H.L. (eds.) Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Garcia, J.L., Patel, B.K.C. and Ollivier, B. (2000) Taxonomic, phylogenetic, and ecological diversity of methanogenic *Archaea*. *Anaerobe*. **6**(4): 205–226.

Gauci, V. and Chapman, S.J. (2006) Simultaneous inhibition of CH_4 efflux and stimulation of sulphate reduction in peat subject to simulated acid rain. *Soil Biol. Biochem.* **38**: 3506-3510.

Gill, A.L., Giasson, M., Rieka, Y. and Finzi, A.C. (2017) Deep peat warming increases surface methane and carbon dioxide emissions in a black spruce-dominated ombrotrophic bog. *Glob. Change Biol.* **23**: 5398-5411.

Glaser, P., Wheeler, G., Gorham, E. and Wright, H. (1981) The patterned mires of the Red Lake peatland, northern Minnesota: Vegetation, water chemistry and landforms. *J. Ecol.* **69**: 575-599.

Glaser, P.H., (1992) Raised bogs in eastern North America – regional controls for species richness and floristic assemblages. *J. Ecol.* **80**: 535-554.

Gorham, E. (1991) Northern peatlands: Role in the global carbon cycle and probable response to climate warming. *Ecol. Appl.* **1**: 182–193.

Gorham E., Janssens J.A. and Glaser P.H. (2003) Rates of peat accumulation during the postglacial period in 32 sites from Alaska to Newfoundland, with special emphasis on northern Minnesota. *Can. J. Bot.* **81**: 429–438

Gupta, V., Smemo, K.A., Yavitt, J.B., Fowle, D., Branfireun, B. and Basiliko, N. (2013) Stable isotopes reveal widespread anaerobic methane oxidation across latitude and peatland type. *Environ. Sci. Technol.* **47**(15): 8273-8279.

Hanson, R. S. and Hanson, T. E. (1996) Methanotrophic bacteria. *Microbiol. Rev*. **60**(2): 439-471.

Hanson, P.J., Childs, K.W., Wullschleger, S.D., Riggs, J.S., Thomas, W.K., Todd, D.E. *et al*. (2011) A method for experimental heating of intact soil profiles for application to climate change experiments. *Glob. Change Biol.* **17**(2): 1083-1096.

Harden, J.W., Sundquist, E.T., Stallard, R.F. and Mark, R.K. (1992) Dynamics of soil carbon during deglaciation of the Laurentide ice sheet. *Science* **258**: 1921–1924.

Hester, E.R., Harpenslager, S.F., van Diggelen, J.M.H., Lamers, L.L., Lüke, C., Lücker, S. *et al.* (2018) Linking nitrogen load to the structure and function of wetland soil and rhizosphere microbial communities. *mSystems* **3**(1): e00214-17

Holmquist, J. R. and MacDonald, G. M. (2014) Peatland succession and long-term apparent carbon accumulation in central and northern Ontario, Canada. *Holocene* **24**(9): 1075-1089.

Hornibrook, E.R.C., Longstaffe, F.J. and Fyfe, W.S. (1997) Spatial distribution of microbial methane production pathways in temperate zone wetland soils: Stable carbon and hydrogen isotope evidence. *Geochim. Cosmochim. Ac.* **61**(4): 745–753.

Jaatinen, K., Fritze, H., Laine, J. and Laiho, R. (2007) Effects of short- and long-term water-level drawdown on the populations and activity of aerobic decomposers in a boreal peatland. *Glob. Change Biol.* **13**: 491-510.

Joabsson, A., Christensen, T. R. and Wallen, B. (1999) Vascular plant controls on methane emissions from northern peatforming wetlands. *Trend. Ecol. Evol*. **14**(10): 385-388.

Kamal, S. and Varma, A. (2008) "Peatland microbiology." in *Microbiology of Extreme Soils. Soil Biology 13.* Dion, P. and Nautiyal, C. S. (eds.) Springer-Verlag, Berlin, Germany, 177–203.

Kraigher, B., Stres, B., Hacin, J., Ausec, L., Mahne, I., Vanelsas, J. *et al.* (2006) Microbial activity and community structure in two drained fen soils in the Ljubljana Marsh. *Soil Biol. Biochem*. **38**: 2762-2771.

Lai, D.Y.F. (2009) Methane dynamics in northern peatlands: A review. *Pedosphere* **19**(4): 409-421, 409-421.

Lelieveld, J., Crutzen, P. J. and Dentener, F. J. (1998) Changing concentration, lifetime and climate forcing of atmospheric methane. *Tellus B*. **50**(2): 128–150.

Le Mer, J. and Roger, P. (2001) Production, oxidation, emission and consumption of methane by soils: A review. *Eur. J. Soil Biol*. **37**(1): 25–50.

Lever, M.A. and Teske, A.P. (2015) Diversity of methane-cycling archaea in hydrothermal sediment investigated by general and group-specific PCR primers. *Appl. Environ. Microbiol.* **81**: 1426–1441.

Limpens, J. and Berendse, F. (2003) How litter quality affects mass loss and N loss from decomposing Sphagnum. *Oikos* **103**:537-547.

Lin, X., Green, S., Tfaily, M.M., Prakash, O., Konstantinidis, K.T., Corbett, J.E., *et al*. (2012) Microbial community structure and activity linked to contrasting biogeochemical gradients in bog and fen environments of the Glacial Lake Agassiz Peatland. *Appl. Environ. Microbiol*. **78**: 7023-7031.

Lin, X., Tfaily, M.M., Green, S.J., Steinweg, J.M., Chanton, P., Imvittaya, A. *et al.* (2014) Microbial metabolic potential for carbon degradation and nutrient (nitrogen and phosphorus) acquisition in an ombrotrophic peatland. *Appl. Environ. Microbiol.* **80**(11): 3531-3540.

Luthon, P.E., Wayne, J.M., Sharp, R.J. and Riley, P.W. (2002) The mcrA gene as an alternative to 16S rRNA in the phylogenetic analysis of methanogen populations in landfill. *Microbiology* **148**(11):3521-3530.

Matthies, C., Erhard, H.P. and Drake, H.L. (1997) Effects of pH on the comparative culturability of fungi and bacteria from acidic and less acidic forest soils. *J. Basic Microbiol*. **37**:335-343.

McLaughlin, J.W. (2004) Carbon assessment in boreal wetlands of Ontario. Ontario Ministry of Natural Resources, Ontario Forest Research Institute, Forest Research Information Paper No 158. Sault Ste Marie, ON, 79.

McMurdie, P.J. and Holmes, S. (2013) phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS ONE* **8**(4):e61217.

Moore, T.R. and Basiliko, N. (2006) "Decomposition in Boreal Peatlands" in *Ecological Studies, Vol. 188. Boreal Peatland Ecosystems*. Wieder, R.K. and Vitt, D.H. (eds.) Springer-Verlag, Berlin, Germany 125-143.

Moore, T.R. and Dalva, M. (1993) The influence of temperature and water table position on carbon dioxide and methane emissions from laboratory columns of peatland soils. *Eur. J. Soil Sci.* **44**:651-664.

Munir, T.M. and Strack, M. (2014) Methane flux influenced by experimental water table drawdown and soil warming in dry boreal continental bog. *Ecosystems* **17**: 1271-1285.

Murdoch, D. and Chow, E.D. (2018) ellipse: Funtions for drawing ellipses and ellipse-like confidence regions. R package version 0.4.1. https://CRAN.R-project.org/package=ellipse

Myers, B., Webster, K.L., McLaughlin, J.W. and Basiliko, N. (2012) Microbial activity across a boreal peatland nutrient gradient: the role of fungi and bacteria. *Wetlands Ecol. Manage.* **20**: 77-88.

Nelson, W.C. and Stegen, J.C. (2015) The reduced genomes of Parcubacteria (OD1) contain signatures of a symbiotic lifestyle. *Front. Microbiol.* **6**(713): doi:10.3389/fmicb.2015.00713

Nielsen, K.M., Johnsen, P.J., Bensasson, D. and Daffonchio, D. (2007) Release and persistence of extracellular DNA in the environment. *Environ. Biosafety Res.* **6**: 37-53.

NWWG (National Wetlands Working Group) (1997) *The Canadian Wetland Classification System*. 2nd ed. Warner, B.G. and Rubec, C.D.A. (eds.), Wetlands Research Center, University of Waterloo, Waterloo, ON., 68.

O'Flaherty, V., Lens, P., Leahy, B. and Colleran, E. (1998) Long-term competition between sulphate-reducing and methane-producing bacteria during full-scale anaerobic treatment of citric acid production wastewater. *Wat. Res.* **32**(3): 815-825.

Oksanen, J., Blanchet, G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D. *et al.* (2018) vegan: Community Ecology Package. R package version 2.5-3. https://CRAN.R-project.org/package=vegan

Pankratov, T., Dedysh, S. and Zavarzin, G. (2006) The leading role of actinobacteria in aerobic cellulose degradation in Sphagnum peat bogs. *Dokl. Biol. Sci.* **410**: 428-430.

Pastor, J., Peckham, B., Bridgham, S., Weltzin, J. and Chen, J. (2002) Plant community dynamics, nutrient cycling, and alternative stable equilibria in peatlands. *Am. Nat.* **160**: 553-568.

Pelletier, L., Moore, T. R., Roulet, N. T., Garneau, M. and Beaulieu-Audy, V. (2007) Methane fluxes from three peatlands in the La Grande Rivière watershed, James Bay lowland, Canada. *J. Geophys. Res*. **112**: G01018, doi:10.1029/2006JG000216.

Peltoniemi, K., Laiho, R., Juottonen, H., Kiikkila, O., Mäkiranta, P., Minkkinen, K., *et al.* (2015) Microbial ecology in a future climate: effects of temperature and moisture on microbial communities of two boreal fens. *FEMS Microbiol. Ecol.* **91**: fiv062, doi: 10.1093/femsec/fiv062

Peltoniemi, K., Laiho, R., Juottonen, H., Bodrossy, L., Kell, D.K., Minkkinen, K. *et al.* (2016) Responses of methanogenic and methanotrophic communities to warming in varying moisture regimes of two boreal fens. *Soil Biol. Biochem.* **97**: 144-156.

Preston, M.D. (2013) Microbial community composition and activities across northern peatlands. Doctor of Philosophy thesis, Department of Geography, University of Toronto, Toronto, ON, Canada

Quail, M.A., Smith, M., Coupland, P., Otto, T.D., Harris, S.R., Connor, T.R. *et al.* (2012) A tale of three next generation sequencing platforms: comparison of Ion Torrent, Pacific Biosciences and Illumina MiSeq sequencers. *BMC Genomics* **13**: 341.

Ramirez, K.S., Lauber, C.L., Knight, R., Bradford, M.A. and Fierer, N. (2010) Consistent effects of nitrogen fertilization on soil bacteria communities in contrasting systems. *Ecology* **91**(12): 3463-3470.

R Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.

Ramirez, K.S., Craine, J.M.and Fierer, N. (2012) Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. *Global Change Biol.* **18**: 1918-1927.

Rinke, C., Schwientek, P., Sczyrba, A., Ivanova, N.N., Anderson, I.J., Cheng, J-F. *et al.* (2013) Insights into the phylogeny and coding potential of microbial dark matter. *Nature* **499**: 431-437.

Roulet, N.T., Lafleur, P.M., Richard, P.J.H., Moore, T.R., Humphreys, E.R., and Bubier, J. (2007) Contemporary carbon balance and late Holocene carbon accumulation in a northern peatland. *Glob. Change Biol.* **13**: 397–411

Rousk, J., Bååth, E., Brookes, P.C., Lauber, C.L., Lozupone, C., Caporaso, J.G., *et al*. (2010) Soil bacterial and fungal communities across a pH gradient in an arable soil. *ISME J.* **4**: 1340.

Rydin, H., and Jeglum, J.K. (2006) *The Biology of Peatlands*. Oxford University Press, Oxford, UK.

Schimel, J.P. (1995) Plant transport and methane production as controls on methane flux from arctic wet meadow tundra. *Biogeochemistry* **28**: 183-200.

Schindlbacher, A., Rodler, A., Kuffner, M., Kitzler, B., Sessitsch, A. and Zechmeister-Boltenstern, S. (2011) Experimental warming effects on the microbial community of a temperate mountain forest soil. *Soil Biol. Biochem.* **43**: 1417-1425.

Segers, R. (1998) Methane production and methane consumption: A review of processes underlying wetland methane fluxes. *Biogeochemistry*. **41**(1): 23-51.

Selenska, S. and Klingmuller, W. (1991) DNA recovery and direct detection of Tn5 sequences from soil. *Lett. Appl. Microbiol*. **13**: 21–24.

Seward, J.D. (2018) Microbial community patterns reveal differences from bogs to intermediate fens in North American peatlands along a latitudinal gradient. Master of Science thesis, Appalachian State University, Boone, NC, USA

Siegel, D.I. and Glaser, P.H. (1987) Groundwater flow in a bog fen complex, Lost River Peatland, northern Minnesota. *J. Ecol*. **75**: 743-754.

Sjögersten, S., Caul, S., Daniell, T.J., Jurd, A.P.S., O'Sullivan, O.S., Stapleton, C.S. *et al.* (2016) Organic matter chemistry controls greenhouse gas emissions from permafrost peatlands. *Soil Biol. Biochem.* **98**: 42-53.

Smemo, K.A. and Yavitt, J.B. (2007) Evidence for anaerobic CH_4 oxidation in freshwater peatlands. *Geomicrobiol. J.* **24**(7-8): 583-597.

Steinberg, L.M. and Regan, J.M. (2008) Phylogenetic comparison of the methanogenic communities from an acidic, oligotrophic fen and an anaerobic digester treating municipal wastewater sludge. *Appl. Environ. Microbiol.* **74**: 6663–6671.

Tarnocai, C. (2006) The effect of climate change on carbon in Canadian peatlands. *Glob. Planet Change* **53**: 222-232.

Teurlincx, S., Heijboer, A., Veraart, A.J., Kowalchuk, G.A. and Declerck, S.A.J. (2018) Local functioning, landscape structuring: Drivers of soil microbial community structure and function in peatlands. *Front. Microbiol.* **9**(2060): doi: 10.3389/fmicb.2018.02060

Thauer, R.K. (1998) Biochemistry of methanogenesis: a tribute to Marjory Stephenson: 1998 Marjory Stephenson Prize Lecture. *Microbiology* **144**: 2377-2406.

Turetsky, M.R., Treat, C.C., Waldrop, M.P., Waddington, J.M., Harden, J.W. and McGuire, A.D. (2008) Short-term response of methane fluxes and methanogen activity to water table and soil warming manipulations in an Alaskan peatland. *J. Geophys. Res.* **113**(G00A10)

Turetsky, M.R., Kotowska, A., Bubier, J., Dise, N.B., Crill, P., Hornibrook, E.R.C, *et al*. (2014) A synthesis of methane emissions from 71 northern, temperate, and subtropical wetlands. *Glob. Change Biol.* **20**: 2183-2197.

Turunen, J., Tomppo, E., Tolonen, K. and Reinikainen, A. (2002) Estimating carbon accumulation rates of undrained mires in Finland—application to boreal and subarctic regions. *Holocene* **12**: 69–80.

Valentine, D.W., Holland, E.A. and Schimel, D.S. (1994) Ecosystem and physiological controls over methane production in northern wetlands. *J. Geophys. Res.* **99**: 1565-1571.

Van Breemen, N. (1995) How *Sphagnum* bogs down other plants. *Trends Ecol. Evol.* **10**: 270-275.

Veraart, A.J., Steenbergh, A.K., Ho, A.H., Kim, S.Y. and Bodelier, P.L.E. (2015) Beyond nitrogen: The importance of phosphorus for CH_4 oxidation in soils and sediments. *Geoderma* **259-260**: 337-346.

Vitt, D.H. (2006) "Functional Characteristics and Indicators of Boreal Peatlands." in *Ecological Studies, Vol. 188. Boreal Peatland Ecosystems*. Wieder, R.K. and Vitt, D.H. (eds.) Springer-Verlag, Berlin, Germany, 9-24.

Waddington, J. M. and Roulet, N. T. (1996) Atmosphere-wetland carbon exchanges: Scale dependency of CO2 and CH4 exchange on the developmental topography of a peatland. *Glob. Biogeochem. Cy.* **10**(2): 233-245.

Waddington, J. M., Roulet, N. T. and Swanson, R. V. (1996) Water table control of CH_4 emission enhancement by vascular plants in boreal peatlands. *J. Geophys. Res*. **101**(D17): 22775–22785.

Warner, B.G. (1996) "Vertical gradients in peatlands." in *Wetlands, Environmental Gradients, Boundaries, and Buffers*. Mulamoottil, G., Warner, B.G. and McBean, E.A. (eds.) Lewis Publishers, Boca Raton, Fla., U.S.A.

Weltzin, J.F., Pastor, J., Harth, C., Bridgham, S.D., Updegraff, K. and Chapin, C.T. (2000) Response of bog and fen plant communities to warming and water-table manipulations. *Ecology* **81**:3464-3478.

Whalen, S.C. (2005) Biogeochemistry of methane exchange between natural wetlands and the atmosphere. *Environ. Eng. Sci*. **22**(1): 73–94.

Winsborough, C.L. and Basiliko, N. (2010) Fungal and bacterial activity in northern peatlands. *Geomicrobiol. J.* **27**:315–320.

Woese, C.R., Kandler, O. and Wheelis, M.L. (1990) Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. *Proc. Natl. Acad. Sci.* **87**: 4576–4579.

Yeoh, Y.K., Sekiguchi, Y., Parks, D.H. and Hugenholtz, P. (2015) Comparative genomics of candidate phylum TM6 suggests that parasitism is widespread and ancestral in this lineage. *Mol. Biol. Evol.* **33**(4): 915-927.