Rebuilding the boreal: analyzing the replicability of the bacterial community structure and soil functioning of forest floor mineral mix with peat subsoil admixtures

By

William A. Kirby

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Department of Biological Sciences

University of Alberta

Abstract:

Alberta law requires reclamation of lands disturbed by surface mining operations, such as those occurring at the Athabasca Oil Sands Region, however, reclamation rates lag far behind continued disturbance rates. Due to cost, mining companies must make use of the materials on site, of which the majority is mineral subsoil and peat. The far less abundant forest floor material is the most suitable base for a reclamation substrate, however its quantity is limited. The bacterial community of the soil is inherently linked to soil functioning, and can potentially be used as a proxy to investigate the likelihood of success of a reclamation regime. To investigate if peat and subsoil could be mixed to produce a FFM-like bacterial community, we supplemented FFM, as well as peat and subsoil mixed at different ratios, with/without biochar and/or a trembling aspen seedling and incubated for 12 weeks in a greenhouse. We measured bulk soil respiration throughout the incubation period, and performed end-point high throughput sequencing of the 16s rRNA gene and bacterial community analysis. The overall diversity of the peat containing admixtures was indistinguishable from that of FFM, while that of subsoil was lower than all other admixtures. However, this was not reflected in the soil respiration, as the respiration rate was indistinguishable for all peat containing admixtures, which was lower than for the FFM and higher than for the pure subsoil. The trends seen in the soil respiration rate correlated to the community composition, where there were three distinct groups; peat containing admixtures, FFM, and subsoil. Biochar and tree additions had minimal effects on any of the admixtures. These results show that peat cannot be used if the goal is to approach a FFM-like community and that subsoil can be used to "dilute" the peat microbial community without an effect on its composition.

Preface

Acknowledgments

First and foremost I would like to thank Dr. Brian Lanoil for enthusiastically accepting me into his lab, despite my mediocre academic experience. His patience was almost continually tested over the course of this thesis study, and without his efforts to help me in the ways I needed help, the last 28 months would have seen me grow much less. I would also like to thank my colleagues, Sina Kazemi, Ann Hammad, and Ido Hatam for their guidance in the first half of this study, and Patrick Neuberger and Alireza Saidi-Mehrabad for their assistance and friendship in the second half.

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Table of Contents

List of Tables

List of Figures

Chapter 1: Literature Review

1.1. Introduction

The nation which destroys its soil destroys itself

- Franklin D Roosevelt

Never before in the history of the Earth has a species been capable of dramatically altering the environment in such a short time. While much of the focus of human induced changes to the Earth is rightly so on the climate, human activities on the ground have significantly altered many large areas of ecosystems. Nowhere is this more evident than at surface mining sites, such as the ongoing mining operations at the Northern Alberta Athabasca Oil Sands Region (Government of Alberta, 2014). While we have learned how to move massive amounts of Earth to get at a target resource, so too have we learned much about this Earth that we move. Perhaps not as a direct result of mining operations, but in the last century mankind has learned a great deal of the enormous value of soils, which form the basis of many aspects of society (Blum, 2005). Many of our regular actions have adverse effects on soil systems, which in turn will have adverse effects on society. As Franklin D. Roosevelt recognized, the continuation of our standard of living is dependent on our ability to protect, and in some cases rebuild, the soils of the land.

Soils play an important role in the biogeochemical cycling of all terrestrial ecosystems, and thus they are the base of these ecosystems (Quinton et al. 2010). Soil bacterial communities are responsible for this much of this biogeochemical cycling (Beare et al. 1995). The soil bacteria are sustained through organic materials supplied by plants, such as dead materials and root

exudates (Zak et al. 2003). These organic materials are broken down and transformed into nutrients that are available for plants, fungi, and animals (Zak et al. 2003).

These biochemical transactions are a central component of soil functioning. Soil functioning is a broad term encompassing many chemical and physical services the soil performs; including nutrient cycling, water and air filtration, and contaminant immobilization (Larson and Pierce, 1991; Doran and Parkin, 1994; Harris et al. 1996; Daily 1997). Some definitions of soil functioning include these services, but also account for soil as a habitat and growth platform for plants and animals, as well as the soil's ability to support human habitation (Harris, 1996; Seybold et al. 1997). For the purpose of this thesis, I will be using the term "soil functioning" as interpreted by Andren and Balandreau (1999), who interpret the definition of soil quality by Doran and Parkin (1994) to be a strong definition of soil functioning as well. Doran and Parkin define soil quality as "The ability of a soil to function within land use and ecosystem boundaries to sustain biological productivity, maintain environmental quality, and promote plant, animal, and human health". I have chosen to use this definition as it allows for measurement of actual soil functions (Andren and Balandreau, 1999), and is a widely adopted definition (US Department of Agriculture, 2011). The biogeochemical cycling that the soil bacteria provide is a necessary service to all other soil organisms, and is strongly linked with terrestrial ecosystem health (Chang et al. 1995; Nannipieri et al. 2003). Soil functioning has been shown to be dependent on the biomass, biodiversity, and activity of the microbial community (Insam and Domsh, 1988; Beare et al. 1995; Tilman et al. 1997; Hooper et al. 2005; Xu et al. 2008). Many human activities damage the soil microbial community, which can have significant consequences for the greater ecosystem health. Surface mining activities, such as those in the Alberta oil sands, are a highly destructive disturbance, resulting in the complete removal of all soil and vegetation

above a target resource. Thus, land reclamation, i.e. the rebuilding of a functioning ecosystem at highly disturbed sites is necessary. It is nearly impossible to recreate an already existing ecosystem (such as the boreal forest); thus, the Alberta Environmental Protection and Enhancement Act (the Act) states that "the objective of Land Reclamation is to return the specified site to an equivalent land capability" (Government of Alberta, 2014). Equivalent land capability is not defined in the Act, but has been assumed to involve equivalent ecosystem functioning.

Land reclamation is limited by on site materials, involving the construction of soils from material removed before mining operations. These constructed soils are known as anthrosols, which is defined as any soil that has been significantly modified by human activity (FAO United Nations, 2007), and can range in properties depending on their parent material. The available materials are altered from their original physical, chemical, and (presumably) biological conditions by the removal process (MacKenzie and Quideau, 2012). Currently, efforts to restore disturbed sites are lagging behind the rate of continued disturbance of boreal forest and peatland ecosystems in Northern Alberta (Government of Alberta, 1993; pg 6). In order to develop more efficient and effective reclamation practices, an understanding of the microbiology of mining associated disturbances and current and potential reclamation practices is necessary. This literature review serves as a summary and analysis of the characteristics of the bacterial communities associated with soils, how they are impacted by surface mining disturbances, and how soil bacterial communities can affect reclamation outcomes.

1.1.2. Ecology of the Athabasca Oil Sands Region

The Athabasca Oil Sands Region (AOSR) covers a large area in Northern Alberta, and is located in the heart of the vast boreal forest of Canada. The boreal forest of Northern Alberta can be divided into two broad ecotypes, the upland forests and the low-lying wetlands (Moss, 1955). The uplands are mixed-wood forests and are densely populated with conifers such as white and black spruce, balsam fir, and jack pine, and deciduous such as balsam poplar, paper birch, and trembling aspen (Moss, 1955). Shade tolerant shrubs also inhabit the southern parts of the boreal. Low-lying areas with poor drainage develop as wetlands, and much of these wetlands in Northern Alberta are peatlands (Warner and Asada, 2006). Peatlands consist mostly of peat, which is a mixture of partially decomposed plant matter that has accumulated in a water-logged, anaerobic environment (Warner and Asada, 2006). The accumulation of this partially decomposed plant matter occurs because the anaerobic conditions of the water-saturated peat slow microbial decomposition enough that it is exceeded by the deposition of new dead plant matter (Waddington and Roulet. 2000). Thus, the long-term water saturation of these peatlands is what drives their physicochemical, and thus microbiological, parameters. The slow degradation of plant matter results in peat having different soil chemistry than boreal forest soils. Features of peaty soils include substantially higher total carbon and nitrogen content, as well as much lower pH, and rates of N mineralization and nitrification relative to boreal forest soils (Devito et al. 1999). One of the biggest differences in the bacterial communities of peatlands and forest soils is the diverse methanogenic and methanotrophic communities of peatlands (Dedysh, 2009; Andersen et al. 2013), a feature that implies a broadly different microbial community structure and function in peatlands.

Natural fires regularly burn large areas of boreal forest, which results in the transformation of aboveground vegetation into char, much of which is eventually incorporated into the soil (Knicker, 2007). These regular fires have a temporary effect on several properties of the soil, such as decreasing the depth of the A horizon and changes, altering micromorphology of the soils, and decreasing organic matter content (Phillips et al. 2000). When char is introduced to the soil environment, several physicochemical properties of the soil are affected, sometimes resulting in a soil community shift in response to the disturbance (Preston and Schmidt, 2006). Microbial community recovery time following fire can vary widely, but the burn history of the soil is a key factor as soil bacteria communities appear to be affected by and adapt to the regular influx of new biochar (Mabuhay et al. 2006). For example, Khodadad et al. (2011) found that the microbial community of a soil with a history of annual burns responded differently to the addition of biochar than the community of an unburned soil. When amended with biochar, the community of the unburned soils increased activity significantly more than did the community of the burned soils (Khodadad et al. 2011).

1.2. Soil quality and microbial communities

Soil quality has long been an important idea in agriculture, as higher quality soils tend to be more productive (Doran et al. 1997). Several reports have proposed definitions (Larson and Pierce, 1991; Acton and Gregorich, 1995; Karlan et al. 1997). The United States Department of Agriculture adopted a definition of soil quality derived from that of Doran et al. (1996) which states: "soil quality is the capacity of a specific kind of soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance

water and air quality, and to support human health and habitation" (US Department of Agriculture, 2001; pg 3). This definition is broad and inclusive to the many functions of soil, but Carter et al. (1997) argue to further broaden the definition of soil quality to take into account two different concepts; the inherent quality, which describes the natural composition and activity, as well as the dynamic quality, which is the description of soil qualities which can change and how they change in response to human input. Carter et al. (1997) note that the measurement of soil quality involved placing a value on certain functions and the soil's fitness to perform them. Soil quality has historically been linked with production, but due to the increased focus of environmental preservation and monitoring in recent decades and the continued advancement of understanding of the role soil plays in terrestrial ecosystems, the concept of soil quality has been recognized as a key indicator of overall ecosystem health. Because of this, soil quality has become an important concept in forestry (Council of Europe, 1992; Howard, 1993; Doran and Parkin, 1996; Moffat, 2003). Soil quality is sensitive and is often adversely effected by human activities such as recreational activities (Batey, 2009), agriculture (Grieve, 2001), and mining operations (Insam and Domsh 1988; Schwenke et al. 2000). While it is now recognized that soil quality is a central aspect of any terrestrial ecosystem, exactly how to monitor soil quality remains a challenging task (Nortcliff, 2001). A number of different soil quality indices have been developed and successfully implemented (Arshad and Coen, 1992; Andrews et al. 2002;Qi et al. 2009), but different soil indices appear to be most effective in the soil environment in which it was developed (Nortcliff, 2001; Zvomuya et al. 2008; Askari and Holden, 2014). While a universal method of describing soil quality has yet to be established, discussions of soil quality are generally centered around soil functioning (Nannipieri et al. 1990; Chang et al. 1995;Totsche et al. 2010; Li et al. 2010; Laishram et al. 2012).

Soils harbor the most diverse communities in the world, where one gram of soil can contain tens of thousands of different bacterial species, as well as hundreds of fungal species and dozens of soil invertebrate species (Giller, 1996; Roesch, 2007; Fierer et al. 2007). It is this enormous diversity that is key to the biogeochemical transformations that take place in the soil (Wagg et al. 2014). This diversity exists because of the heterogeneous nature of soils, which leads to a large diversity of substrates and niches. The soil bacterial community has evolved to utilize the many substrates of the soil, and it is this biogeochemical cycling that provides ecosystem services that sustain other soil organisms (Topp, 2003; Dance, 2008; Hayat et al. 2010). As the biogeochemical cycling of the soils is performed by the microbial community, community parameters such as biodiversity, biomass, and activity therefore can often have a large impact on soil function and overall soil quality (Insam and Domsh, 1988; Beare et al. 1995; Tilman et al. 1997; Hooper et al. 2005; Xu et al. 2008). This connection between microbial community parameters and soil functioning; however, is not always the case, as some studies have observed little to no effect of decreasing biodiversity on soil function (Wertz et al. 2006; Wertz et al. 2007). Due to the inherent complexity of the soil ecosystem and the limitations of technologies prior to next generation sequencing (NGS), there has been comparatively little research into developing a microbial community-based index for soil quality. Since the advent of next generation sequencing however, characterizing the microbial community and its effects on the soil has become more in focus.

1.2.1 Microbial community structure and effects on soil functioning

Biodiversity is an important concept in ecology, as the biodiversity of an ecosystem is linked to the overall health of the ecosystem at every trophic level (Catizzone et al. 1998). Biodiversity is a multidimensional concept with many definitions and measures, and its exact relationship with

ecosystem health is complex (Purvis and Hector, 2000). While diversity is sometimes used to describe species richness (i.e. the number of different species in a system), it can also include species evenness (the relative abundance of species in a system), morphological, functional, and phylogenetic diversity. Some definitions are conceived with a global perspective, such as that first proposed at the 1993 Convention of Biological Diversity, which defines biodiversity as "the variability among living organisms from all sources including terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems" (Downes, 1993). This definition is widely accepted as one of the most inclusive and useful, and has been used in policy making (Mac et al. 2012). In terms of simple definitions, however, much of the research into how diversity effects ecosystem functioning has used diversity to describe some mixture of species richness, which is the number of species in a system, and evenness, which describes the equality of representation of a species in the system (Griffiths et al. 2000; Michel and Williams, 2011; Deng, 2012; Baumann et al. 2013). The two most commonly used examples of this are the Simpson and Shannon diversity indexes (Shannon, 1947; Simpson, 1949). The Shannon index measures entropy of a system, and describes the uncertainty of the identity of an unknown individual. The Simpson index reports the probability that two random samples of a population will be of the same species. Regardless of the definition in use, most interpretations of biodiversity attempt to link biodiversity to ecosystem functionality (Purvis and Hector, 2000; Gaston and Spicer, 2004; Kim and Byrne, 2006). Most studies find a positive relationship between biodiversity and ecosystem function. This positive relationship has been ascribed to several factors, including resource partitioning (Loreau and Hector, 2001), the insurance hypothesis (Yachi and Loreau, 1999), and increased likelihood of the presence of keystone species in diverse ecosystems

(Schulze and Mooney, 1994). Resource partitioning results when multiple species compete for the same resource and "partition" it into smaller, more specific niches; thus, resources are utilized more thoroughly and efficiently in systems with higher biodiversity. The insurance hypothesis states that the functional redundancy that results from overlapping potential resource utilization (i.e. overlapping potential niches) in more biodiverse systems creates increased resistance and resilience to disturbance. While the likelihood that a disturbance will lead to loss of species in a system does not change, the chance that some other species that is resistant to the disturbance will be able utilize the same resource in the system is higher in biodiverse systems, resulting in little if any loss in system function following disturbance. High biodiversity also increases the likelihood of keystone species being present in the system. A keystone species is any member of the community that has a significant effect on ecosystem function (Griffiths, 1997). High diversity ecosystems are more likely to harbor multiple keystone species. Microbial ecosystems, and especially soil ecosystems, tend to be amongst the most biodiverse in the world (Roesch, 2007) and the relationship between biodiversity and functioning in microbial ecosystems is not fully understood. Below I describe different aspects of biodiversity and their impacts on ecosystem functioning in microbial ecosystems.

1.2.1.1 Richness

The number of different species in an ecosystem is nearly always one of the primary components of biodiversity (Griffiths et al. 2000; Michel and Williams, 2011; Deng, 2012; Baumann et al. 2013). Measuring species richness of bacteria in soil ecosystems presents unique challenges as compared to measuring the richness of larger organisms: limitations of traditional microbiology technologies resulting in the inaccessibility of most of the community (discussed in more detail

in section 5.3) and the difficulty in defining species with respect to bacteria. The species idea is one of the most debated in biology (Wilkens, 2009). The species concept for plants and animals has historically relied on morphological and phylogenetic analyses, which are difficult to implement on prokaryotes. Since molecular techniques have been developed to allow the sequencing of whole genomes, new definitions of species have been proposed; however, these definitions remain controversial (Gevers et al. 2005; Chan et al. 2012; Caro-Quintero et al. 2012). One of the currently most widely used definitions equates bacterial species as 'ecotypes', which are populations residing within the same ecological niche (Cohan, 2001). Analyzing ecotypes is often difficult, as measurement of specific niches and determining the identity of the resident population is complex and often impossible, so operational taxonomic units (OTU) are often substituted for species in studies of microbial ecosystems. OTUs are defined by an operational parameter, such as 16s rRNA or ITS gene similarity at some arbitrarily determined cut-off or functional parameter (Blaxter et al. 2005). These functional parameters do not necessarily correlate with biological or ecological species concepts, but they are easily measureable and relate to the overall diversity within an ecosystem. Because these cut-offs are arbitrarily determined, they can be used to examine diversity at a variety of similarity levels; thus, they describe taxon richness (which must be defined by the researcher) rather than species richness. Commonly used cut-off levels for 16S rRNA gene similarity is 97%-99% similarity (sometimes referred to as "species-level"), 93%-95% similarity (sometimes referred to as "genus level"), or ~85% similarity (sometimes referred to as "phylum level"). These groupings are only loosely related to the taxonomic groupings that they are correlated with and are based entirely upon DNA sequence and thus sequences assigned to these groups can only be considered as a

candidate grouping. Thus, different levels of taxon richness are related to, but distinct from, species richness.

One of the primary mechanisms by which taxon richness is related to ecosystem functioning is through resource partitioning, or niche differentiation (Tilman et al. 1997; Loreau and Hector, 2001). While most research into niche differentiation has been conducted with macro-organisms, this principle holds true in microbial systems as well (Bell et al. 2005; Langenheder et al. 2010). In their study investigating soil bacterial richness and ecosystem functioning by directly manipulating richness, Bell et al. (2005) found that increasing richness resulted in an increase of ecosystem functioning. As taxon richness increased, however, the effect each OTU addition has on functioning decreased (Bell et al. 2005). This is likely due to saturation of the niches in the experimental environment, known as the complimentary effect (Naeem et al. 1999). Different bacteria are capable of utilizing different resources, and different species contribute more to ecosystem functioning. As ecosystem functioning is dependent on the rate of chemical turnover, ecosystem functioning increases as more substrates are utilized. Through higher richness, the likelihood that a keystone species is present will also increase. Langenheder et al. (2010) investigated the effect of increasing complexity of substrates and species richness had on functioning, finding that increasing substrate complexity as well as species richness independently increased ecosystem functioning. However, no environment can be infinitely complex, so there is a limit to how many substrate niches are available for utilization. The incremental increase of system functioning towards an asymptote is not unexpected in high diversity systems such as soil. In an environment with low species richness, there is a higher likelihood that a newly introduced species would be able to utilize a substrate that was not in use before, which would increase ecosystem functioning significantly. On the other hand, in an

environment with high species richness, most niches would likely already be filled; therefore, it would be unlikely that a newly introduced species would be capable of utilizing an unused niche, meaning the new species would have to compete for resources. This results in systems that can observe a reduction in diversity, but not a subsequent reduction in functioning. For example, Griffiths et al. (2000) found that soil systems with differential diversities prepared from the same parent community through serial dilutions, did not exhibit different functioning levels. Similarly, in an experiment that decreased species richness through removal of less abundant taxa, Wertz et al. (2006) found that removing even a very large portion of the soil community, up to 99.99% of taxa, resulted in no change in soil functioning, showing that only a very small percentage of the functional diversity of the soil community investigated is necessary to maintain functioning. This point of niche saturation differs depending on the complexity of the soil. Ecosystem complexity refers to the total number of niches available in an ecosystem (Cadenasse et al. 2006), and soil complexity is a major determining factor in functional diversity (Yin et al. 2000; Gomez et al. 2006). Even as the species richness approaches niche saturation, a continued increase in richness does not decrease stability (Bell et al. 2005). In fact, a high species richness gives a system greater stability through functional redundancy.

When an ecosystem undergoes some disturbance significant enough to alter the abundance or ability to function of an organism that contributes to overall ecosystem functioning, if the species richness of this ecosystem is high, there is a higher likelihood that some species that is less affected by the disturbance will be capable of performing the same function, and the ecosystem will recover (Naeem and Li, 1997; Yachi and Loreau, 1999). Functional redundancy occurs in an ecosystem when two or more species are capable of performing the same function (Wohl et al. 2004). Wertz et al. (2006) demonstrated high functional redundancy within the ammonia

oxidizer, denitrifier, and heterotroph functional groups. This finding indicates a large number of overlapping potential niches. The potential niche is the combined total of the niches which a particular species is capable of occupying in addition to the role the species plays with relation to others, and is bound by the species' tolerance of environmental factors (Kylafis and Loreau, 2008). However, different species utilize resources at different rates under slightly different conditions, or can exhibit other competitive adaptations in an environment in order to secure more of a resource where the potential niches of different species may overlap. This competition shrinks the potential niche of a species into a realized niche (Kylafis and Loreau, 2008). The realized niche is the niche where a given species has a positive growth rate given environmental and biological constraints (Pearman et al. 2008). Environmental factors, such as pH optimal or nutrient utilization efficiency, can also shrink the potential niche into a realized niche (Kylafis and Loreau, 2008). This resource partitioning leads to functional redundancy within a system. If a species utilizing a resource in its realized niche is removed from a system, higher diversity increases the likelihood that other species with overlapping potential niches will be present, and can expand their realized niche to utilize that resource in place of the lost species, thereby mitigating decreased ecosystem functioning. The soil microbial community is intimately linked to the soil physicochemical parameters (Baath, 1996; Bramley et al. 1989; Fierer and Jackson, 2006; Pietri and Brooks, 2008; Lauberet al. 2009; Rousk et al. 2010) and because the functioning of a soil is dependent on the microbial community, even a subtle disturbance of the soil has potential to negatively impact soil health. However, a higher diversity results in an increase in both resistance and resilience to disturbances in an ecosystem.

1.2.1.2 Evenness

Another dimension of biodiversity is species evenness. Species evenness, which is the measure of how equally represented each member of a community is, plays an important role in ecosystem functioning and stability by increasing the representation of each species' functional traits (Wittebolle et al. 2009; Lemieux and Cusson, 2014; Wang et al. 2015; Daly et al. 2015). Wittebolle et al. (2009) showed that not only does ecosystem functioning increase with species evenness, but also ecosystem functioning is less likely to be negatively impacted by a selective environmental disturbance. In a selective disturbance, some species are affected, while others are not. The magnitude of this effect on ecosystem functioning depends on which species were affected. Some species contribute more to ecosystem function than others, and if a population with a large impact on functioning dominates a highly uneven ecosystem, then any disturbance that affects this population results in a significant decrease in ecosystem functioning. As shown by Wittebolle et al. (2009), if the system has higher taxon evenness, the effect of the selective disturbance is decrease because the system is not as likely to be dominated by a small number of high impact species. Similarly, species evenness also stabilizes the ecosystem. In a study conducted by Daly et al. (2015), competitive systems with higher initial species evenness maintained stable coexistence of more taxa (i.e. maintained higher taxon richness) for a longer period of time than did systems with a lower species evenness. The effects of species evenness of an ecosystem can also interact with the effects of species richness. Wang et al. (2015), investigated the affect of evenness on the effect of richness on light resource use and found that species richness has a positive relationship with light resource use, but only in systems that had a high initial evenness.

1.2.1.3 Community composition

Just as different microbial species perform can different ecosystem services, so too can more than one microbial species perform the same function (Wohl et al. 2009). However, the identity of the taxon utilizing a resource matters, as different taxa that perform the same function do so with greater or lesser efficiency (Waldrop and Firestone, 2006b; You et al. 2014). Community composition is strongly associated with specific functions, but that the tremendous abundance and diversity of bacteria may obscure the sometimes subtle link between composition and function when looking at broad parameters. For instance, Fierer et al. (2012) found a community shift across a nitrogen (N) gradient that was more pronounced in soils amended with fertilizer and that the soils amended with more fertilizer had a different catabolic profile than those with amended with intermediate or low levels. Cavigelli and Robertson (2000) investigated how the different denitrifier communities of a successional soil and an agriculture soil influenced denitrification. They found that the differences in the community composition of the soils accounted for the differences in denitrification rates. Eilers et al. (2010) found changes in the relative abundances of certain taxa in response to the addition of specific C compounds. Similarly, Waldrop and Firestone (2006a) found a decrease in soil respiration with a transplant disturbance associated community shift. On the other hand, some studies have found a disconnect between broad soil functioning and community composition. For example, Sattin et al. (2009) found no difference in the community composition of differently aged glacial foreland soils, but saw a significant difference in N mineralization. In the species evenness study performed by Wittebolle et al. (2009), the identity of the dominant species had an effect on functioning; however, these differences were minor compared to the effect of species evenness.

Soils are the most diverse ecosystem in the world and changes to soil biodiversity may have substantial impacts on the soil functioning. How changes in the community parameters effect soil functioning depends on the response of community structure and which members of the community are affected. It is impossible to predict *a priori* which species are keystone species, or how sensitive a keystone species may be to a disturbance. The loss of biodiversity in an ecosystem has negative effects on ecosystem functioning, and large disturbances are more likely to affect the biodiversity of a community more dramatically. This significant loss in biodiversity may include the loss of one or more keystone or otherwise abundant or impactful species, thus further negatively effecting ecosystem functioning. A more diverse community would be better able to recover from a disturbance, because other species might not be effected that can functionally replace a lost keystone species. Because of these reasons, based on ecological theory (functional redundancy, the insurance hypothesis, niche differentiation), the diversity of the system is the most influential factor on the soil functioning. Because soils are extremely diverse, community composition does play a role in soil functioning; however, this role appears to be relatively minor compared to the richness and evenness of the system.

1.2.2 Environmental influences on soil microbial community composition

While the soil microbial community largely determines soil health, the microbial community diversity, composition, and activity is in turn determined by the soil physicochemical parameters, collectively termed edaphic parameters (Fierer and Jackson, 2006). Edaphic parameters are in soil parameters such as pH, soil structure, and nutrient availability, as opposed to climate-driven

variables such as sunlight or annual mean temperature or rainfall, or biological variables such as plant coverage, composition, or productivity. These effects can manifest as a positive or negative influence on soil functioning. Furthermore, microbial activity can also affect soil physicochemical parameters; thus, microorganisms can act as "ecosystem engineers", i.e. manipulating their own environments, and "niche constructionists", i.e. the iterative process of ecosystem engineering and evolutionary adaptation to the modified environment (Odling-Smee et al. 2013). Alternatively, these environmental impacts can degrade the soil conditions for these microbes, i.e. niche degradation. For example, nitrification produces protons as a by-product of ammonia oxidation, which leads to the conversion of the nitrification substrate ammonia to the non-substrate ammonium (Nichol, et al. (2008)). This process can lead to niche partitioning between ammonia oxidizing bacteria and ammonia oxidizing archaea (Zhang et al. (2012). Thus, reciprocal interactions between microorganisms and soil edaphic parameters are a primary mechanism of control on soil function.

1.2.2.1 pH

pH is one of the most significant soil physicochemical parameters regulating soil function because it has both direct and indirect influence on most other soil properties. Soil pH is largely determined by the fine soil particles and their exchangeable ions, such as Al and Na, which decrease and increase pH respectively (Nyle and Ray, 1999). pH not only directly influences the soil community, particularly plants, fungi, and microbes, but also the nutrient availability including key nutrients such as N, S, P, and K (Nyle and Ray, 1999). Microbial community composition is significantly affected by soil pH: even a small change can alter several

community parameters (Baath, 1996; Bramley et al. 1989; Fierer and Jackson, 2006; Pietri and Brooks a, 2008; Lauber et al. 2009; Rousk et al. 2010). Rousk et al. (2010) investigated differences between microbial communities along a pH gradient, and found that bacterial biomass and diversity increased with pH. Bacterial communities shift with pH both at a local scale (Rousk et al. 2010) and a transcontinental scale across different soil types (Lauber et al. 2009). This effect of pH on members of the soil community has consequences on key soil chemical cycles (Kemmitt et al. 2006). One such cycle is the N cycle. The effect pH has on the N cycle in soils is complex, but due to the importance of N cycling to agricultural industry, it is well studied. The N cycle, which is the responsible for the majority of bioavailable N in soils, is a performed by a community of bacteria in a step-by-step process. This community is strongly influenced by soil pH, and thus the concentrations of N-cycle intermediates and products (Simmons et al. 1996). For example, denitrification is performed by a diverse set of taxa (Linn and Doran, 1984), and as such the relationship between pH and denitrification is complex. A change in soil pH can hinder denitrification for a short period (Baggs et al. 2010), but due to the diversity of denitrifiers, in a soil with high richness of denitrifiers were one subset of denitrifiers activity may be hindered at a certain pH, another subset may undergo increased activity, thereby stabilizing denitrification and adjusting the optimum denitrification pH (Simek et al. 2002). While Simek et al. (2002) found that denitrifier communities quickly shifted in response to a change in soil pH and adjusted denitrification rates; they also found a dramatic shift in denitrification products, where N_2O emissions were heavily reduced in neutral to alkaline soils. Similar to denitrification, increasing pH generally increases rates of nitrification as well (Stevens et al. 1998; Islam et al. 2006; Pietri and Brooks, 2008). In a long-term study of acid pasture soil the nitrification optimum pH matched the indigenous soil pH (Islam et al. 2006).

The effects of pH on bacterial communities are broad and complex, as pH influences many other soil physicochemical parameters. Because of the large influence of pH on the rest of the soil environment, it appears that the soil communities have evolved a functional resilience to pH changes, in that nutrient cycling optimums are shifted along with the community. In the previously discussed study by Islam et al. (2006), liming the soil shifted the nitrification optimum pH towards the new elevated soil pH; an effect that lasted for several years. Similarly, Simek et al. (2002) found in pH adjustment experiments that the optimum pH for denitrification in different soils was that of the natural soil prior to any adjustment.

1.2.2.2 Soil porosity

Soil porosity is an important factor in multiple soil factors, such as soil moisture, soil density, and pore space oxygen. What determines the porosity of a soil is the particle size and distribution. Soils with higher clay content have a very low porosity, due to the tiny particle size which defines clay. Another determining factor in soil porosity is the organic content. Humic substances, which the carbon products of microbial degradation of complex plant and animal tissues, are argued to be the majority of the organic content of the soils (Nyle and Ray, 1999; Lehmann and Kebler, 2015) and are somewhat 'sticky', forming humic-clay complexes and binding multiple clay particles together, increasing the particle size of the soil (Nyle and Ray, 1999). In general, a larger particle size results in a higher porosity, which increases air and water movement through the soil. Soils that are more porous are better aerated, and are more easily susceptible to dessication.

Droughts are a significant stress on microbial populations that can affect several parameters of the soil microbial community. Both C-cycling and respiration (Wang et al. 2013) and N-cycling (Larsen et al. 2011; Hartmann et al. 2013; Fuchslueger et al. 2014) have been shown to undergo a functional shift in response to decrease in soil moisture. Wang et al. (2013) argue that there are several mechanisms by which desiccation can inhibit soil organic matter (SOM) degradation, namely a slower mobility and rate of substrate uptake. Hartmann et al. (2013) saw a large concentration in extractable NH_4^+ and a very low concentration of NO_3^- in fertilized soils under drought conditions, demonstrating inhibition of nitrifiers. Frank and Goffman (1998) found denitrification enzyme activity decreased with soil moisture content. Denitrification occurs in anoxic regions of the soil. Soils with low water content have greater aeration and thus fewer of these anoxic regions, thereby reducing N cycling.

On the other hand, soils with smaller particle sizes are usually less porous, and water is retained more easily (Nyle and Ray, 1999). More moisture in a soil creates more anoxic regions, which are the primary sites of denitrification, and thus can have an effect on the N cycling community. Yu and Ehrenfeld (2009) investigated the response of N cycling to different soil moisture regimes, and it was found the N cycling of wetland soils was affected by soil moisture, and was most affected if the change in moisture is maintained for a longer period of time. While soil moisture is a significant parameter in N cycling, it has implications on other chemical cycles and community parameters as well. For example, in their study looking at community influencers across soil types along a regional climate gradient, Brockett et al. (2012) found that soil moisture is one of the most influential parameters, having more of an impact than even vegetation type. They also found enzymatic activities differ with soil moisture.

1.2.2.3 Soil organic matter and nutrient availability

Nutrient availability is a key determinant in the quality of soil. While the microbial community is responsible for a majority of soil nutrient cycling, the nutrient content of soils has significant effect on the microbial communities.

Next to pH, SOM is arguably the second most influential soil parameter to the soil community. SOM is the sum total of organic substances in the soil, which amounts to approximately 1395 x 10^{15} g of carbon across all soils on the globe (Post et al. 1982). One of the most important biochemical services soil bacteria perform is the transformation of C and the contribution to total SOM (Singh et al. 2010; Seaker and Sopper, 1988). Much of this carbon may be locked away in the recalcitrant form of humic substances, however, the proportion of SOM that is humic in nature remains in debate (Lehmann and Kleber, 2015). One of the primary sources of SOM is plants, and different species of plants will input different carbon compounds into the soil, and bacteria have been shown to respond differently to different SOM compounds (Hertenberger et al. 2002; Gomez et al. 2006; Eilers et al. 2010 Jiang et al. 2012; Prescott and Grayson, 2013; Aponte et al. 2013; Dimassi et al. 2014). One of the clearest examples of this is within the rhizosphere, where root exudes from plants dominate the SOM pool. El Zahar Haichar et al. (2008) studied how the species of plant impacts the bacterial community, and found that different plant species exudates stimulate different bacterial groups.

Carbon is not the only nutrient important to the health of the soil community. N is also a nutrient of substantial importance in the soil bacterial community. The role of bacteria in supplying plants with bioavailable N through the nitrogen cycle is critical for the plant community, but bacteria, especially those associated with N cycling processes, are also sensitive to

concentrations of N, and communities can differ in structure depending on the C/N ratio (Eiland et al. 2001; Deng et al. 2016). Community shift in response to an influx of soil N can have an impact on carbon cycling as well. Fierer et al. (2012) found that in response to N amendment, the community shifted from a predominately oligotrophic community to a more copiotrophic one. Copiotrophic microbes tend to have a higher activity level than do oligotrophs, but are less able to utilize recalcitrant forms of SOC (Miki et al. 2010).

While a number of studies have found that the bacterial community can be affected by the nutrient availability of the soil, the reverse is also true, in that the soil community is important in determining the nutrient availability. In their investigation into the communities and enzyme activities of mountainside soils, Xu et al. (2015) found that while the microbial communities were strongly correlated with various edaphic parameters, the enzyme activities of these soils were strongly correlated with SOM content and decomposition. Similarly, Baumann et al. (2013) found that soils with a lower biodiversity were less capable of degradation of sugar and lignin decomposition, resulting in a different SOM pool than a more biodiverse system.

Chemical properties, such as nutrient availability and chemistry, of the soils can have an effect on the soil community, and the soil community subsequently has an effect on SOM content (de Vries et al. 2012; Leff et al. 2015; Jeanbille et al. 2016). The literature on nutrient concentrations and soil microbial communities is extensive, as microbial community parameters and nutrient availability to plants are inherently linked (Miransari, 2013). The primary focus of much of the literature is on agricultural soils, which investigate very high concentrations of nutrients and show an exaggerated community response. The more subtle effects of nutrient concentrations on the soil community in natural soils are relatively understudied, as a number of other edaphic

parameters may also effect soil communities more strongly, confounding results. The strongest influencer on soil community with regards to SOM, however, appears to be plant species.

1.2.2.4 Other parameters

Soils are a highly complex, dynamic environment. There are numerous parameters and environmental influences which can affect the microbial community aside from the parameters described above. Some of these parameters are associated with human activities, such as contamination of soils with inorganic contaminants such as metals (Nunes et al. 2016) or with organic contaminants like petroleum products (Sutton et al. 2013). Natural environmental variables can also affect the soil microbial communities, either directly or indirectly through their effects on other variables. Temperature is one such indirect variable, as it can influence a large number of other edaphic parameters (Zhou et al. 2016). Other natural variables are precipitation (Evans and Wallenstein, 2012), herbivore activity (Yang et al. 2013), and salinity (Rath and Rousk, 2015), among others.

1.3: Oil Sands land reclamation

1.3.1: Current reclamation status

Alberta law requires mining companies restore disturbed land to an equivalent capability as the pre-disturbed state, but rate of disruption far exceeds the rate of reclamation (Government of Alberta, 2014). As of 2012, 767km^2 had been disturbed, and this is projected to double in 10-15 years (Government of Alberta, 2014). Of this, 77km^2 is undergoing reclamation, and 1.04 km^2 has been certified reclaimed. Trembling aspen is capable of establishment in all of the soils being

used in oil sands reclamation (Pinno et al. 2012), but tree establishment in the field has been inconsistent (Barbour et al. 2010). The development of a reliable long-term reclamation regime has been the subject of extensive long-term projects, particularly with a focus in landscape and soil reconstruction (Barbour et al. 2010). Soil is a complex environment, and returning the land to an equivalent capability requires the reconstruction of entire landscapes with the broad goal of stimulating the growth of a natural boreal forest. While the large scale focus of much of the oil sands reclamation research is obviously important, so too is the relatively understudied biotic response to soil reconstruction and treatments and subsequent effects on soil functioning. Much of the overburden material available for land reclamation at the Alberta oil sands is the mineral subsoil of the lower horizons (Barbour et al. 2010). Due to the inherent low nutrient content of subsoils, reclamation efforts must use a capping material. This cap is constructed from the topsoil layers. The two primary topsoils available for reclamation are peat from the wet lowlands and forest floor soil (LFH) from boreal forest highlands (MacMillan et al. 2007). These top soils are physicochemically different in nature, and these differences are reflected in their properties as a reclamation substrate (MacKenzie and Quideau, 2012; Beasse et al. 2015). Peat, which is the thick topsoil layer of many lowlands and wetlands, consists primarily of decomposed organic matter. During the process of removal, the peat and LFH top soils will mix with the mineral subsoil. The resulting anthrosols, the peat mineral mixture (PMM) and the forest floor mineral mixture (FFM) are physicochemically distinct from their parent soils (Barbour et al. 2010; MacKenzie et al. 2012; Sorenson et al. 2011). PMM and FFM are currently used as high organic cap materials in oil sands reclamation sites (Barbour et al. 2010). The depth of the cap has been shown to affect the likelihood of reclamation success, where the application of a thinner cap may see the mining company have to return to further treat the site (Barbour et al. 2010); however,

thicker caps require more materials and are more expensive than thinner caps. Research into the performance of reclamation substrates has revealed the FFM consistently performs better than other organic caps with regards to vegetation growth (McMillan et al. 2007; Barbour et al. 2010; MacDonald et al. 2015). These reclaimed soils have different nutrient profiles than the natural neighboring forest floor, but the FFM is often considered the most suitable reclamation substrate due in part to it doubling as a natural seed and propagule bank, allowing for a faster establishment of a richer plant community than PMM (MacKenzie and Naeth, 2009). Furthermore, it is possible that the microbial communities found in the aerobic, upland LFH that is the organic component of FFM are better adapted to establishment in the upland cap soils than are the microbial communities found in anaerobic, lowland peat used for PMM (e.g. McMillan et al. (2006) J. Environ. Qual. 36:1470-1478). Three local tree species are used in reclamation capping study sites in order to promote the growth of a natural boreal forest; Trembling Aspen, Jack Pine, and White Spruce.

1.3.2: Soil function and microbial processes

Due to the physicochemical differences between them, the microbial communities differ between PMM, FFM, and their parent materials (Dimitriu et al. 2010; MacKenzie and Quideau, 2012; Beasse et al. 2015). These differences are marked by differences in soil functioning as well. Beasse et al. (2015) note a significantly higher respiration rate in FFM than in PMM. On the other hand, MacKenzie and Quideau (2012) found PMM has a higher rate of nitrogen mineralization, comparable to agricultural soils, with more plant available nitrogen species. Microbial communities are also significantly affected by reclamation material, as demonstrated

by McMillan et al. (2007), who found that a mixture of PMM and FFM resulted in a higher microbial biomass and activity than PMM alone. However, McMillan et al. (2005) had previously observed that the microbial C and N of PMM and FFM were lower than that of neighboring undisturbed forest soils.

Dimitriu et al. (2010), report the microbial community was more strongly influenced by plant community composition than by age, showing that if plant colonization were to occur, the bacterial communities of PMM and FFM should increase in similarity. Similarly, MacKenzie and Quideau (2010) do observe an age effect in community composition, but it is likely due to changes occurring in the soil from overhead vegetation. Confirming this further, Sorenson et al. (2011) found that once tree establishment has occurred, the microbial community becomes more affected by the plant community than the soil type.

While current research demonstrates a clear link between plant community, soil physiochemistry, and microbial community, an in-depth analysis of bacterial community parameters of oil sands reclamation substrates has not been performed. As previously discussed, the biodiversity of a soil can have a significant impact on soil functioning and overall soil health. A deeper investigation into the bacterial community dynamics of these soils would likely provide insight of value to the optimization of current reclamation programs.

1.3.3: Biochar as a potential soil amendment

Biochar, or charcoal, is a potential amendment for reclamation substrates. The benefits of biochar as a soil amendment appear to be plentiful, and there have been many studies focusing on its potential use in agriculture (Liu et al. 2013; Graber et al. 2010; McHenry, 2011). Biochar

alters the physicochemical properties of the soil, primarily through its extraordinarily high surface area and porosity. The presence of biochar in a soil has been shown to alter soil pH (Ventura et al. 2012; Wang et al. 2013; Jones et al. 2012), increase water holding capacity (Peake et al. 2014; Devereux et al. 2012), and increase nutrient retention (Altland and Locke, 2013; Alling et al. 2014). While some research has found that the addition of biochar can reduce soil functioning (Zimmerman et al. 2011), the general trend seen is that biochar amendment increases soil quality, and subsequently, soil productivity (Jeffery et al. 2011; McHenry, 2011). The biochar-soil interactions have been shown to affect the soil microbial community through an increase in activity, biomass, or a community shift (Steiner et al. 2008; Steinbeiss et al. 2009; Khodadad et al. 2011). Soil quality is intimately linked with the health of the microbial community, so by positively affecting the community, biochar can act to increase soil quality. Because of this, biochar may prove to be a relatively inexpensive aid in land reclamation

While there have been several trials and investigations into the potential uses and benefits of biochar for use in mine reclamation, biochar has yet to be included into large-scale practice. The mechanisms of action leading to these potential benefits remain poorly understood, and a large variability of the effects of biochar amendment exists in the literature. Notably, while there have been some field trials for the effects of biochar on soils and plant productivity for use in agriculture, no large scale studies have been conducted looking into its potential as an aid in surface mine reclamation. Further research into the large-scale effects of biochar for land reclamation purposes could shed light on the variability seen in the biochar related literature, and perhaps accelerate biochar's implementation into common reclamation practice.

1.4. Mining activity disturbances to soil

1.4.1 Soil community degradation at mine sites

Surface mining requires the removal of the overburden, which is all material above the target resource. This consists of not just vegetation and soil layers, but also rock. Alberta law states that lands that have been disturbed through mining operations must be reclaimed to an equivalent land capability (Government of Alberta, 2014). Due to the scale of the surface mining operations, reclamation requires the use of overburden materials to reconstruct the landscape. The majority of the material available for the soil portion of a reclamation plot is mineral subsoil, while the remainder is from the topsoil layers. Topsoil is a fertile, biologically active, complex environment, and it is therefore difficult to reproduce, and thus is of significant value to reclamation efforts. Much of the topsoil is therefore relocated and stored for later application to the disturbed site during reclamation. During this removal, topsoil is inadvertently mixed with some of the mineral subsoil, altering the physicochemical parameters of the mixture such that it is distinct from either of the two parent soils (MacKenzie and Quideau, 2012). Because there is not enough topsoil material for use in the full soil column, it is typically used as an organic rich cap in reclamation regimes (Barbour et al. 2010). This upheaval, relocation, storage, and redeposition is a dramatic disturbance of the soil system, significantly altering the functional community, and may complicate development of active and stable microbial communities during reclamation.

1.4.1.1 Changes in microbial communities and abundance in overburden soils

The process of removal and storage of the overburden mixes different soil layers together, altering the properties of the resulting soil (MacKenzie and Quideau, 2012). The soil microbial communities at disturbed sites typically have impacted microbial parameters, including decreased microbial biomass, activity, and diversity (Insam and Domsh. 1988; Peacock et al. 2001; Barbhuiya et al. 2004; Lewis et al. 2010; Poncelet et al. 2014). This disruption in soil functioning due to the disturbance of the bacterial community may reduce the potential for reclamation success if these materials are used while in their disturbed state. Overburden materials are stored for use in reclamation regimes, sometimes for years or decades. Poncelet et al. (2014) investigated microbial responses within overburden, and found a decrease in microbial biomass and activity, as well as a large community shift. In the overburden soils some groups experienced an increase in relative abundances, but all groups experienced a decrease in abundance. The community shift in overburden soils appears to have a significant effect on the chemical cycling within these soils as well, as overburden soils have been shown to have a lower nutrient content (Insam and Domsh 1988; Schwenke et al. 2000; Bendfeldt et al. 2001; Banning et al. 2008). Banning et al. (2008) related the lower nutrient content and nutrient cycling in recently disturbed soils to several parameters of the soil microbial community. Within the chronosequence studied by Banning et al. (2008), nutrient cycling of reclaimed soils increased progressively with age. However, Poncelet et al. (2014) also found that the microbial biomass, activity, and community composition of an older overburden stock more closely resembled that of undisturbed subsoil, suggesting microbial recovery within the overburden.

1.4.2 Microbial recovery in mining-associated disturbed soils and reclamation soils

The ability of an ecosystem to recover quickly from a disturbance is known as ecosystem resilience. Since soil is among the most biologically diverse systems on Earth (Nyle and Ray, 1999; Michel and Williams. 2011), one can expect a high degree of resilience and a relatively fast recovery in response to disturbances, depending on the type and severity of the disturbance. For instance, freshwater, hard substrate surface bacterial communities have been shown to recover from mechanical disturbance in 3-14 days (Railkin, 1998), whereas soil crust bacterial communities took more than a month to recover to a pre-disturbance community structure from livestock grazing associated disturbances (Concostrina-Zubiriet al. 2014). Mining associated disturbances are likely one of the most devastating to any ecosystem, rivaling that of a glaciation event, and an understanding of the microbial succession processes at reclamation sites is critical to the design of successful reclamation regimes.

1.4.2.1 Microbial biomass, community, and nutrient turnover

While large disturbances have a substantial effect on many parameters of the microbial community, several studies have shown successional processes within these disturbed soils (Insam and Domsh 1988; Ingram et al. 2005; Banning et al. 2011; Poncelet et al. 2014). Banning et al. (2011) saw the nutrient turnover of reclaimed soil across a chronosequence increase with microbial biomass. However, Lewis et al. (2010) saw a different trend. It was found that, of the reclaimed sites studies, the oldest site, reclaimed in 1987, had a significantly lower biodiversity, biomass, and microbial activity than a similar site reclaimed 10 years later (Lewis et al. 2010). A third site, reclaimed in 2007, had a significantly lower biodiversity, biomass, and microbial activity than either of the older reclaimed sites (Lewis et al. 2010). Similarly, Mummey et al.

(2002) found that a site reclaimed 20 years earlier had a 54% lower microbial biomass C than an undisturbed neighboring site. As there are marked differences in the properties of the reclamation substrates, these studies show that microbial recovery is dependent on substrate properties and not just time (MacKenzie and Quideau. 2010). McMillan et al. (2007) found differing N cycling rates in Alberta oil sands boreal forests reclaimed using different mineral substrates. Dimitriu et al. (2010) saw no discernable trend in microbial community succession in aged oil sands reclamation soils, but note that this is likely due to several differing factors of the different reclamation sites; primarily the heterogeneity of reclamation material used, cap depth, and fertilization regime. However, Dimitriu et al. (2010) also note that the study areas were well covered with vegetation, therefore it appears that soil quality has improved enough that plant colonization can occur, showing that ecosystem functioning has returned to the reclaimed sites, despite differences in the microbial community between the reclaimed sites and undisturbed forest soil.

1.5. Current study: bacterial community structure of a peat dilution series amended with a tree and/or biochar

1.5.1 Scientific questions and purpose of study

Alberta law requiring oil sands companies restore disturbed land to an equivalent land capability, but the rate of disturbance far exceeds the rate of reclamation (Government of Alberta, 2014). In order to minimize the need for follow up treatments to reclamation sites, a reliable reclamation program where native plants are quick to establish and soil functioning is maintained at a level comparable to natural soils must be incorporated. Previous research demonstrates that reclamation would be most successful if the FFM substrate were to be used, but the relatively small amount of FFM available prevents its widespread use (Barbour et al. 2010). PMM treatments have had mixed success (Barbour et al. 2010), but PMM is more plentiful due to the abundance of peat in the region. We therefore investigated whether the more abundant resources available, namely peat and subsoil, can be used to approximate FFM as a reclamation substrate.

The primary aim of this study is to investigate the effect of a series of peat:subsoil admixtures on the soil bacterial community, and compare these communities to that of the FFM reclamation substrate. Our goal is to establish a formula for oil sands reclamation using a treatment mixture of on-site materials. If a mixture of peat and subsoil can reproduce the FFM bacterial community, this will provide insight on reclamation regimes that may replicate the functionality of FFM, while using the more abundant materials.

The central hypothesis for this study is that the physicochemical differences in the peat:subsoil admixtures will significantly affect the bacterial community and intermediate ratios of peat:subsoil will shift it to become more similar to FFM. It is further hypothesize that this effect will be enhanced by amendment with biochar and planting of trembling aspen (*Populus tremuloides*) trees.

1.5.2 Experimental design

To test the hypothesis, a greenhouse experiment was set up using materials gathered from the Aurora mine site of the Athabasca oil sands deposit. Nine replicates of each treatment were mixed with peat-derived biochar, while another nine were left without. Within these sets, *Populus tremuloides* saplings were planted in six of the replicates, leaving three without. The samples were incubated for 12 weeks, with respiration measurements taken weekly and soil collection for DNA analysis occurring at the end of the experiment. DNA was extracted directly from the soil. NGS was performed on the 16s rRNA gene using Illumina Miseq. Ecological analysis of the sequences was performed using the Mothur (Schloss et al. 2009) pipeline.

1.5.3 Methodology:

Until very recently, the ecological characteristics of the soil bacterial community have been largely inaccessible due to the inherently challenging nature of molecular research in the soil environment. For much of the 20th century, soil microbiology had to rely on the traditional cultivation of bacteria as an attempt at characterizing some of the community. However, only a very small percentage of bacteria are cultivable, and little information could be gained about the broader community through culturing (Rappé and Giovannoni, 2003). Because of this, DNA extraction from the soil is necessary to access the community. However, the humic substances in soils can be co-extracted with DNA and interfere with downstream applications, and extensive purification was required, increasing the complexity and cost of extractions (Tebbe and Vahjen. 1993). With the development of low cost DNA extraction methods that can reliably remove humic substances and give access to the DNA straight from the soil (Yeates et al. 1998), many of the limitations of isolation and culturing could be bypassed. Despite this, the tremendous

diversity and abundance of soil bacteria proved to be a challenge to its own characterization. Community fingerprinting methods, such as terminal restriction fragment length polymorphism (T-RFLP), automated ribosomal intergenic spacer analysis (ARISA), and denaturing gradient gel electrophoresis (DGGE), are biased in their analysis of communities and anything but the top most abundant species in an environment go undetected (Bent et al. 2007). The first generation sequencing technology, Sanger sequencing, is expensive, slow, and low throughput, making in depth studies into the soil community difficult. Despite this, Sanger sequencing was the dominant sequencing method for decades. As advancements were made in molecular biology, analytical chemistry, and computational power, the need for more efficient sequencing technologies was met with the development of several methods of high throughput genetic sequencing, collectively termed 'Next Generation Sequencing' (NGS) (Metzker. 2010). These new methods allow the production of a volume of data several orders of magnitude larger than Sanger sequencing from a single run, allowing entire genomes of many species to be sequenced simultaneously and relatively inexpensively. The development of NGS and subsequent analysis pipelines has allowed a level of characterization of the soil community that was impossible only ten years prior to this study. The tools are now in place that allow researchers to apply ecological theory to the soil environment and assess community structure and how it impacts the broader ecosystem. Since the development of these technologies, there have been few studies investigating the bacterial communities of oil sands reclamation substrates, and this study is the first to look at the bacterial community structure of anthrosols in comparison to that of FFM. The utilization of the NGS to focus on the community structure and the soil microbial ecology may provide insight on how to solve some of the persistent problems of oil sands reclamation.

1.5.4 Impact of this study and potential benefits to oil sands land reclamation

This study investigates the effects of multiple factors on the bacterial communities of oil sands reclamation substrates. Ecosystem disruption in the oil sands mining regions is happening at a rate far exceeding the rate of restoration. Oil sands companies are legally required to return disturbed land to pre-disturbance condition, and a stronger understanding of the soil functioning and bacterial community of reclamation substrates will aid in the design of reliable reclamation regimes. FFM has been shown to be a very effective reclamation substrate, but it is in limited supply. Peat has been found to be a poor substrate, but its efficacy is improved when mixed with subsoil. If a viable, economical alternative is found that can achieve similar results to FFM, oil sands reclamation could greatly benefit. Biochar has been shown to have positive effects on soil quality, and several studies have looked into its use as a reclamation soil amendment (Chen et al. 2011; Jiang et al. 2013). However, results are highly variable and biochar amendment can potentially have negative effects, inadvertently decreasing the soil quality (Mukherjee, 2014). If this study demonstrates that peat-derived biochar causes a microbial community shift and increases soil quality, it would provide insight on management regimes for how to increase the efficiency of reclamation practices in the Alberta oil sands. The microbial community differences between the peat:subsoil treatment mixes will still provide insight on reclamation soil health and inform future studies into bettering reclamation practices.

Chapter 2: Comparisons of bacterial community structure and soil functioning in peat containing admixtures with pure subsoil and forest floor mineral mix

2.1: Abstract:

Alberta law requires reclamation of lands disturbed by surface mining operations, such as those occurring at the Athabasca Oil Sands Region, however, reclamation rates lag far behind continued disturbance rates. Due to cost, mining companies must make use of the materials on site, of which the majority is mineral subsoil and peat. The far less abundant forest floor material is the most suitable base for a reclamation substrate, however its quantity is limited. The bacterial community of the soil is inherently linked to soil functioning, and can potentially be used as a proxy to investigate the likelihood of success of a reclamation regime. To investigate if peat and subsoil could be mixed to produce a FFM-like bacterial community, we supplemented FFM, as well as peat and subsoil mixed at different ratios, with/without biochar and/or a trembling aspen seedling and incubated for 12 weeks in a greenhouse. We measured bulk soil respiration throughout the incubation period, and performed end-point high throughput sequencing of the 16s rRNA gene and bacterial community analysis. The overall diversity of the peat containing admixtures all indistinguishable from that of FFM, while the overall diversity of subsoil was lower than all other admixtures. However, this was not reflected in the soil respiration, as the respiration rate was indistinguishable for all peat containing admixtures, which was lower than for the FFM and higher than for the pure subsoil. The trends seen in the soil respiration rate correlated to the community composition, where there were three distinct groups; peat containing admixtures, FFM, and subsoil. Biochar and tree additions had minimal effects on

any of the admixtures. These results show that peat cannot be used if the goal is to approach a FFM-like community and that subsoil can be used to "dilute" the peat microbial community without an effect on its composition.

2.2 Introduction:

Surface mining operations, such as those conducted in the Athabasca Oil Sands Region (AOSR) in Northern Alberta, necessitate the removal of all material above a target resource. However, Alberta law requires mining companies reclaim the land and return a disturbed site "to an equivalent land capability" (Government of Alberta, 1993). Rebuilding a landscape is a complex task that has proven to be a challenging, expensive process, and reclamation rates lag far behind disturbance rates (Barbour et al. 2010). An important consideration for mining companies when designing programs to return the land to an equivalent capability is the soil quality of their reclamation substrates. Soil quality, while not yet having a universally accepted definition, is largely related to the general productivity and fertility of the soil (Parr et al. 1992). Ongoing discussions of how to describe soil quality often center around soil biogeochemical cycling, also known as soil functioning (Nannipieri et al. 1990; Chang et al. 1995; Totsche et al. 2010; Li et al. 2010). As the microbial community, in large part, is responsible for soil functioning, microbial community parameters such as biodiversity and activity can have a large impact on overall soil quality (Insam and Domsh, 1988; Beare et al. 1995; Tilman et al. 1997; Hooper et al. 2005; Xu et al. 2008). Soils are the most biologically diverse environment on the planet, containing tens of thousands of species per gram of soil (Nyle and Ray, 1999; Michel and Williams. 2011);

connecting biodiversity and composition to functional properties such as biogeochemical cycling can thus be complex.

The bacterial community is a potential marker for reclamation success because it responds quickly to environmental changes (Ranjard et al. 2006) and is strongly associated with many soil biogeochemical parameters (MacKenzie and Quideau, 2010). A number of microbial analysis parameters could act as a means of assessing the development of a reclaimed site, including microbial biomass (Machulla et al. 2005), biodiversity (Mummey et al. 2002), and activity (Sourkova et al. 2005).

Due to the scale of operations, all reclamation efforts must be performed with on site materials. Thus, the fertile topsoils of the target sites are stored for use as reclamation substrates. The majority (approximately 70%) of the topsoil in the AOSR is in the form of peat from lowlands, while the remainder is the upland boreal forest floor soil. During removal, these topsoils are mixed with lower mineral subsoil layers, creating materials that are physicochemically different from their parent materials (McKenzie, 1979). These two topsoils, when mixed with mineral soils, have been tested as reclamation substrates. While both have proven somewhat effective, the forest floor mineral mix (FFM) has consistently had a higher rate of success, and is considered the most suitable substrate (McMillan et al. 2007; MacKenzie and Naeth, 2009; Barbour et al. 2010). However, because of the scarcity of the upland forest soils, there is not enough FFM to meet reclamation needs for the entire AOSR, therefore, finding an economical alternative can assist in reclamation programs. Mixing the much more abundant peat with mineral subsoil, resulting in peat mineral mix (PMM), has been shown to result in a reclamation suitable substrate (Dimitriu et al. 2010; MacKenzie and Quideau, 2012; Beasse et al. 2015). Furthermore, boreal forest soils naturally contain biochar (charcoal) from fires, which are a

natural feature of boreal ecosystems (Larson, 1997), and the presence of biochar has been found to increase soil quality (Jeffery et al. 2011; McHenry, 2011). Thus, it is also possible that biochar plays a role in soil functioning.

One site where microbial analysis could be used to assist in determining reclamation progress is Syncrude's Aurora North Capping Study (ANCS). The ANCS is a large scale multidisciplinary field investigation into the suitability of a variety of combinations of different subsoil mixtures, capping materials, capping depths, and tree supplementation. The study presented here serves to complement the ANCS, as the microbial community parameters of capping materials FFM and peat mineral mix in use at the Aurora North site are being investigated here. This is the first in depth investigation into the microbial community structure of these reclamation materials Looking at the community structure of these reclamation substrates may provide valuable insight into their effect on soil functioning. The purpose of this study is to investigate whether or not peat and subsoil can be mixed to approach the bacterial community of the FFM. We hypothesized that an intermediate mixture of peat:subsoil, when supplemented with a tree and biochar, would develop a bacterial community most similar to that of FFM.

2.3: Methods:

2.3.1 Experimental Design and Soil Sampling:

Peat (p), mineral subsoils (s), and forest floor mineral mix (FFM) were collected in bulk from the ANCS and supplied by Syncrude. Peat and subsoil were mixed at different ratios using a soil mixer (Table 1). Each admixture was used to fill 10cm x 20cm potting pots to an 8cm depth, and the soils were gently packed down. Treatment pots incubated in a greenhouse for 2 weeks before

10cm *Populus tremuloides* saplings were transferred into individual pots, for those pots receiving trees. For pots receiving biochar, the biochar was produced from peat by gradual carbonization in a muffle furnace in a reduced oxygen environment (tinfoil and sand bed). The peat was charred by fast ramping (30 minutes) to a temperature of 500°C where it was held for one hour. The resulting biochar was of fine structure and had a BET surface area of 23 m^2*g^{-1},the total carbon (C) content was 23.58%, the hydrogen (H) content was 0.78%, the nitrogen (N) content was 0.51%, and oxygen (O) was 5.78%. Each pot received 32.2 g of biochar, equivalent to 10 MT biochar/ha mixed evenly into the treatment soils prior to potting.

The sample positions were randomized and incubated for 12 weeks in a greenhouse kept at approximately 20°C-23°C and were watered daily with approximately 150 ml deionized H_2O. The positions of the samples were randomized weekly.

Table 1: Experimental design and number of replicates for each experimental group. + denotes the presence of a variable, and – denotes the absence. Biochar was added during potting, and *Populous tremuloides* saplings were grown in plugs of potting soil, and were planted 48 hours after the soils were potted and placed in the greenhouse.

Admixture (p = peat, s = subsoil, FFM = forest floor mineral mix)	Biochar +		Biochar -		Total samples incubated
	Tree +	Tree -	Tree +	Tree -	
1p:0s	6	3	6	3	18
8p:1s	6	3	6	3	18
4p:1s	6	3	6	3	18
1p:1s	6	3	6	3	18
1p:4s	6	3	6	3	18
0p:1s	6	3	6	3	18
FFM	6	3	6	3	18

2.3.2 Bulk soil respiration:

Bulk soil CO_2 emissions were measured using a Li-Cor Li8100a automatic multiplex infrared gas analyzer system (Li-Cor, Nebraska, USA). Each of the samples were outfitted with a PVC collar. Caps were fitted with fixtures and tubing to allow connection to the Li8100a. Measurements of the headspace CO_2 concentration were taken weekly for the duration of the experiment as per the manufacturers' recommendations. Atmosphere in the measurement chamber was cleared and equilibrated with ambient atmosphere for 45 seconds before and after measurement. Two observations were taken during the measurement cycle, between which the measurement chamber was cleared and equilibrated with ambient atmosphere. Each observation period was 45 seconds. Observations were averaged. The curve fitting was performed using a first order kinetics equation known to reliably work for decomposition of SOM compounds from natural soil ecosystems (Stanford and Smith, 1972). We integrated the area beneath the curve to calculate the total CO_2 evolution from each sample.

2.3.3 DNA extraction and 16S rRNA gene PCR amplification:

Bulk soil subsamples were collected at the end of the experiment. Soils were cautiously removed by hand from the pots. After removing roots by hand, the remaining soil was roughly homogenized by hand, and 200-300g of bulk soil were collected in a sterile Whirlpak® bag (Fisher) and frozen at -80°C until extraction. DNA extraction was performed in triplicate for each treatment. For treatments where a tree was present, soil from two replicates of the treatments were combined, homogenized by hand, then extracted, resulting in three DNA extractions for each treatment. DNA extraction of 0.5 g of sample per replicate was performed

with FastDNA® SPIN Kit for Soil (MP Biomedicals, Ohio, USA) as instructed by the manufacturer. DNA purity was confirmed and quantified fluorometrically using a Qubit fluorometer (Version 1, ThermoFisher Scientific, Massachusetts, USA). The Genome Quebec Innovation Centre at McGill University performed amplicon library preparation from genomic DNA and sequenced the PCR products using an Illumina Miseq with the v2 reagent kit (Illumina Inc, San Diego, CA). The V3 region of 16s rRNA was amplified with PCR using the barcoded primers 341F and 518R under the following conditions for 30 cycles: 94ºC for 3 minutes, followed by 28 cycles of 94ºC for 30 seconds; 53ºC for 40 seconds and 72ºC for 1 minute with a final elongation step at 72ºC for 5 minutes (Muyzer et al. 1993). The V3 region was used because of the small size of the fragment which enabled sufficient coverage during sequencing, and also because it is among the most widely used regions for bacterial community characterization, thereby allowing comparison of our results to other studies (Wang & Qian 2009). Across a total of 96 samples, a total of 381120 reads were returned. Samples had a range of 67-11980 reads. Samples were subsampled to 2500 random reads to normalize sequencing depth across samples, and samples with less than 2500 were excluded from further analysis. A subsampling depth of 2500 reads was chosen because it gave a high coverage while eliminating only a few samples from analysis.

2.3.4 16S rRNA gene sequence data analysis:

Library assembly, quality control, filtering, and OTU clustering were done with USEARCH (version 7) according to the UPARSE pipeline (Edgar, 2013). Briefly, as suggested by the UPARSE pipeline, sequences with either 2-bp mismatches with the primer, 1-bp mismatch with the barcode, homopolymers longer than 8bp or a maximum expected error probability >.05 were

removed from the analysis. The remaining sequences were then trimmed to 120bp and sequences shorter than 120bp were omitted from further analysis. OTUs were clustered using the UPARSE greedy algorithm using a 97% OTU sequence similarity definition. The UPARSE greedy algorithm removes chimeras, however, the UPARSE pipeline recommends a secondary chimera removal step. Therefore, chimeras were removed using UCHIME with self-reference (Edgar et al. 2013). Global singleton OTUs (OTUs represented by a single sequence in the entire data set) were removed. The bacterial OTUs were classified to phylum, class, family and/or genus level using the Ribosomal Database Project classifier (train set 10) with a 60% confidence threshold (RDP; http://rdp.cme.msu.edu/). The sequences of eukaryotic, mitochondrial, chloroplast, archaeal, or unknown origin were removed from the data set. The assembled dataset was imported into Mothur (version 1.36.1, Schloss et al. 2009) for community structure analysis. We used six measures of alpha diversity as implemented in Mothur (version 1.36.1, Schloss et al. 2009): Shannon's H-index (H; Shannon, 1947) and the Inverse Simpson index (1/D; Simpson, 1949) were used to measure overall biodiversity; Shannon Evenness (SEI) and Simpson Evenness ($E_{1/D}$) were used as measures of community evenness (Smith and Wilson, 1996); taxon richness was estimated using the Chao1 richness estimator (a nonparametric measure of taxon richness) (Chao, 1984) and the number of unique OTUs observed as implemented in Mothur . Differences between alpha diversity measures were tested for significance using a two-tailed Student's T-test in Microsoft Excel. Values with a p-value < .05 were deemed significantly different. Comparisons of the communities between samples were made using the θ_{YC} dissimilarity index and the Jaccard similarity coefficient (Jaccard, 1901; Yue and Clayton, 2005). The θyc dissimilarity index is a measure of how dissimilar two communities are based on presence/absence of community members, as well as the abundances of those members. The

Jaccard similarity coefficient is calculated only with the presence/absence of the community membership. Non-metric multidimensional scaling (NMS) ordinated distances between the treatments in multidimensional space using 250 runs of real data based on the θ_{YC} and Jaccard distance matrixes. Multiresponse permutation procedure (MRPP) tested the significance of the differences between compositions of treatments (McCune et al. 2002). Multiresponse permutation procedure (MRPP) was used to determine significant differences in community composition between the admixtures (McCune et al. 2002). The MRPP delivers three statistical values: the test statistic (T) shows the separation between pre-defined groups, where more negative values indicate stronger separation; the chance-corrected within-group agreement (A), where A=0 when within-group heterogeneity equals that expected by chance, A<1 when within-group heterogeneity is greater than expected by chance, A=1 (max value) when all samples within a group are identical; and finally a probability (p) value given for all comparisons, including multiple comparisons, which indicates the likelihood that the comparison groups are significantly different from each other (McCune et al. 2002). NMS coordinates and MRPP tests were calculated in PC-Ord (Version 6) (McCune and Mefford, 2011). Sigmaplot (Version 13, Systat Software, San Jose CA) was used to construct beta diversity ordinations.

2.4: Results:

Sequence sets were randomly subsampled to 2500 sequences, which gave a high coverage for each sample with an average of 95% (Good's coverage estimate), ranging from 92% to 99.8%. Rarefaction analysis confirmed high coverage (Supplementary Figure 1). Subsampling to 2500 sequences resulted in the omission of a total of 6 samples from further analysis.

2.4.1 Admixtures

We expected an intermediate mixture of peat and subsoil to most resemble FFM. However, the pure peat had similar levels of overall diversity as FFM, mixing any amount of peat with subsoil at any ratio did not alter the biodiversity community significantly (Table 2). The pure subsoil had a significantly lower diversity than the peat containing admixtures or the FFM (Table 2). While the evenness of the pure subsoil community was not significantly different from that of any other admixture, the OTU richness was significantly lower for both the Chao1 richness estimator and the # OTUs observed (Table 2). However, adding peat to the subsoil, even to a final concentration as low as 1 peat:4 subsoil, increased diversity of the mixture to resemble that of pure peat. This is true for all measures of OTU richness and overall diversity used in this study (Table 2). Furthermore, the addition of any amount of peat to the subsoil increased the richness and overall biodiversity such that it was not statistically different from that of FFM. This resulted in two statistical groupings for biodiversity; the peat containing admixtures grouped with the FFM, and the pure subsoil was significantly lower.

We expected that the soil function, as measured by respiration rates, would be similar amongst the peat containing admixtures and between these admixtures and FFM because they had similar levels of richness and overall diversity. There were no significant differences in respiration rates between the admixtures; however, the respiration rate in the admixtures was significantly higher than the pure subsoil and significantly lower than the FFM (Figure 1, T-B-). Thus, overall diversity, taxon richness, or taxon evenness were not strongly correlated with soil functioning in this system.

The significant difference in respiration rates between the FFM and the peat containing admixtures does appear to be correlated with the composition of the communities. We expected that an intermediate mixture of peat and subsoil would result in a community that was more similar to FFM than the more unbalanced mixtures. The community composition of the peat containing admixtures were indistinguishable from each other. However, FFM communities and subsoil communities formed groups that were clearly distinguishable from the peat containing admixtures (Figures 3 and 4). It appears that the peat bacterial community dominates that of the subsoil, as both the membership and composition of the communities of the peat containing admixtures do not differ significantly from each other (Figure 3). Because the communities of all the peat containing admixtures were indistinguishable, no mixture of peat and subsoil resulted in a community more similar to that of the FFM (figure 3).

2.4.2 Biochar additions

The Northern Alberta boreal forest soil is subject to regular influxes of natural char due to fire, which is a natural component of this ecosystem (Larsen, 1997). Biochar has significant impacts on soil microbial community structure and function (Chen et al. 2011; Jiang et al. 2013). Therefore, we expected that the microbial communities of the different topsoil materials (peat, FFM, and subsoil) would respond to biochar additions both in activity and in community structure. Surprisingly, the addition of biochar did not have significant effects on any measure of the biodiversity (Table 2), nor on the community membership or composition (Figure 2) in any of the peat containing admixtures or the FFM. Furthermore, the addition of biochar alone had no significant impact on respiration rates in either FFM or peat-containing admixtures. However, we did observe a synergistic effect of the combination of biochar and a tree in the rate of respiration in both the 8p:1s and 4p:1s admixtures, as well as a positive biochar effect on respiration rate in

the 8p:1s admixture. This biochar effect on respiration was not reflected in the biodiversity, membership, or composition of these admixtures.

While not affecting the overall biodiversity of the pure subsoil, the presence of biochar did significantly shift its community composition (Figure 2). The addition of biochar, which likely contains some portion of labile carbon usable to the community, to the pure subsoil, led to a shift in the community structure to be more similar to the peat containing admixtures. The pure subsoil community was initially distinct from that of the FFM community, but the biochar-induced community shift made the resulting community even less similar (Figure 1). This effect was more pronounced in the community composition than the community membership, possibly indicating a stronger effect on the relative abundance of particular OTUs than on their presence/absence.

2.4.3 Tree addition

Because the plant community has a significant effect on the structure and composition of the rhizosphere community (el Zahar Haichar et al. 2008), we expected the presence of a trembling aspen seedling to have an impact on microbial community activity, biodiversity, membership, and composition in peat containing admixtures, pure subsoil, and FFM. However, the presence of the *P. tremuloides* sapling did not have a significant impact on any measure of biodiversity or community composition of any peat containing admixture or FFM. Furthermore, the biodiversity and community composition of pure subsoil were also not significantly affected in these samples. The presence of a tree substantially increased the variability of the pure subsoil community membership and composition; however, this effect was not strong enough to separate the communities of the pure subsoil with and without a tree into two statistically distinguishable

groups. The presence of a tree did increase the respiration rate of two peat containing admixtures, 8p:1s and 4p:1s, but did not alter the respiration rate in FFM or pure subsoil.

Table 2: Summary table of alpha diversity measures of all treatments. Standard deviation is noted in parentheses. 'p' denotes peat, and 's' denotes subsoil. For treatments, A = admixture; T = tree; and B = biochar. * indicates a significant difference ($p<.05$) between treatments. ND denotes treatments which could not be analysed due to insufficient sequences.

Treatment			Diversity		Evenness		Richness	
A	T	B	(H)	(1/D)	SEI	1/EID	Chao1	#obs
1p:0s	+	-	5.22 (.12)	133.04 (19.05)	.87 (.01)	.27 (.02)	615.34 (41.09)	420.67 (27.81)
1p:0s	-	-	5.30 (.09)	119.31 (11.1)	.87 (.01)	.27 (.02)	611.53 (24.72)	435 (29.22)
1p:0s	+	+	5.08 (.17)	92.00 (16.83)	.85 (.01)	.23 (.02)	621.86 (41)	389.67 (42.32)
1p:0s	-	+	5.19 (.19)	112.32 (36.63)	.87 (.02)	.28 (.07)	583.3 (66.34)	412 (41.24)
8p:1s	+	-	5.32 (.13)	119.98 (31.7)	.88 (.01)	.28 (.06)	603.00 (8.08)	436.5 (19.60)
8p:1s	-	-	5.27	133.17	.88	.34	589.26	401
8p:1s	+	+	5.19 (.017)	113.68 (5.40)	.86 (.001)	.28 (.01)	640.70 (6.96)	400.3 (28.05)
8p:1s	-	+	5.21 (.05)	112.88 (9.71)	.87 (.01)	.29 (.03)	609.95 (58.88)	406.3 (28.05)
4p:1s	+	-	5.14 (.14)	109.06 (14.51)	.86 (.01)	.28 (.01)	635.75 (20.64)	397.67 (26.95)
4p:1s	-	-	5.18 (.13)	108.58 (16.22)	.86 (.02)	.26 (.04)	610.18 (67.29)	405.67 (29.15)
4p:1s	+	+	5.09 (.05)	107.34 (107.34)	.86 (.003)	.29 (.01)	624.82 (25.97)	381.3 (31.26)
4p:1s	-	+	5.18 (.10)	106.34 (18.09)	.86 (.01)	.27 (.04)	614.67 (75.52)	409.67 (56.60)
1p:1s	+	-	5.27 (.11)	117.74 (15.23)	.87 (.01)	.28 (.01)	632.26 (82.64)	424.67 (28.08)
1p:1s	-	-	5.12 (.04)	108.38 (16.64)	.86 (.01)	.29 (03)	619.0 (61.89)	381 (52.67)
1p:1s	+	+	5.29 (.15)	123.89 (6.28)	.88 (.01)	.30 (.02)	559.13 (18.27)	409 (47.44)
1p:1s	-	+	5.28 (.15)	125.41 (29.97)	.89 (.01)	.31 (.05)	556.28 (103.05)	407 (52.67)
1p:4s	+	-	5.38 (.16)	143.85 (22.42)	.89 (.02)	.33 (.03)	612.00 (15.86)	413 (47.44)
1p:4s	-	-	5.22 (.12)	118.17 (22.64)	.88 (.01)	.32 (.02)	598.88 (40.4)	400.67 (45.50)
1p:4s	+	+	5.27 (.14)	126.38 (19.12)	.89 (.01)	.34 (.02)	586.45 (16.93)	373.33 (7.41)
1p:4s	-	+	5.17 (.08)	110.56 (11.09)	.88 (.02)	.31 (.01)	562.68 (42.69)	392.67 (15.92)
0p:1s	+	-	4.72* (.28)	78.43* (15.5)	.90 (.03)	.36 (.02)	202.81* (42.10)	176* (15.56)
0p:1s	-	-	4.67* (.05)	63.61* (20.32)	.89 (.02)	.34 (.02)	197.623* (33.58)	184* (14.97)

0p:1s	+	+	4.96* (.05)	87.55* (5.87)	.90 (.02)	.36 (.03)	279.99* (22.55)	253.3* (34.92
0p:1s	-	+	4.56* (.16)	55.32* (22.28)	.86 (.01)	.26 (.02)	219.00* (12.02)	201* (31.26)
FFM	+	-	5.11 (.13)	108.78 (13.62)	.86 (.01)	.23 (.04)	596.45 (10.07)	395 (9.63)
FFM	-	-	ND	ND	ND	ND	ND	ND
FFM	+	+	5.12 (.08)	97.00 (5.86)	.86 (.01)	.22 (.01)	587.9 (15.25)	392 (16.87)
FFM	-	+	5.18 (.09)	108.79 (11.79)	.87 (.02)	.28 (.02)	552.52 (41.46)	401.33 (7.85)

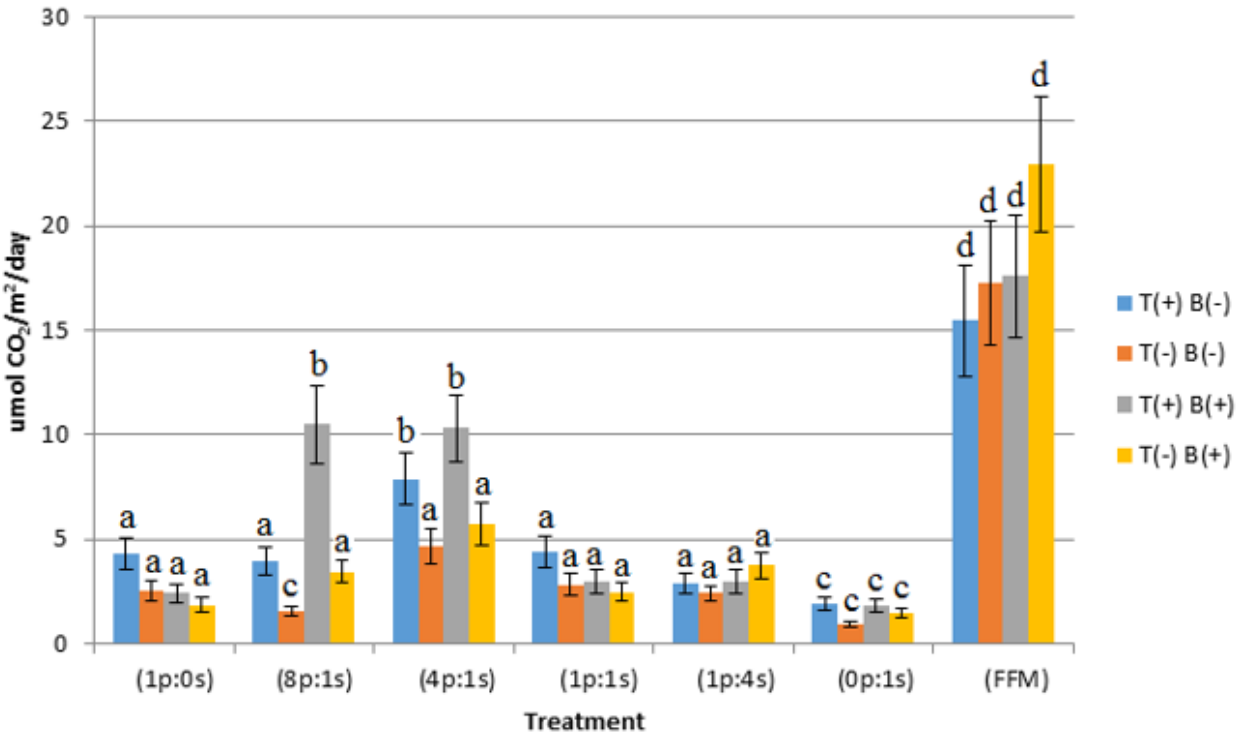

Figure 1: Integrated respiration rate for bulk soil. Data labels indicate statistical groupings. T indicates the presence (+) or absence (-) of a tree and B indicates the presence (+) or absence (-) of biochar.

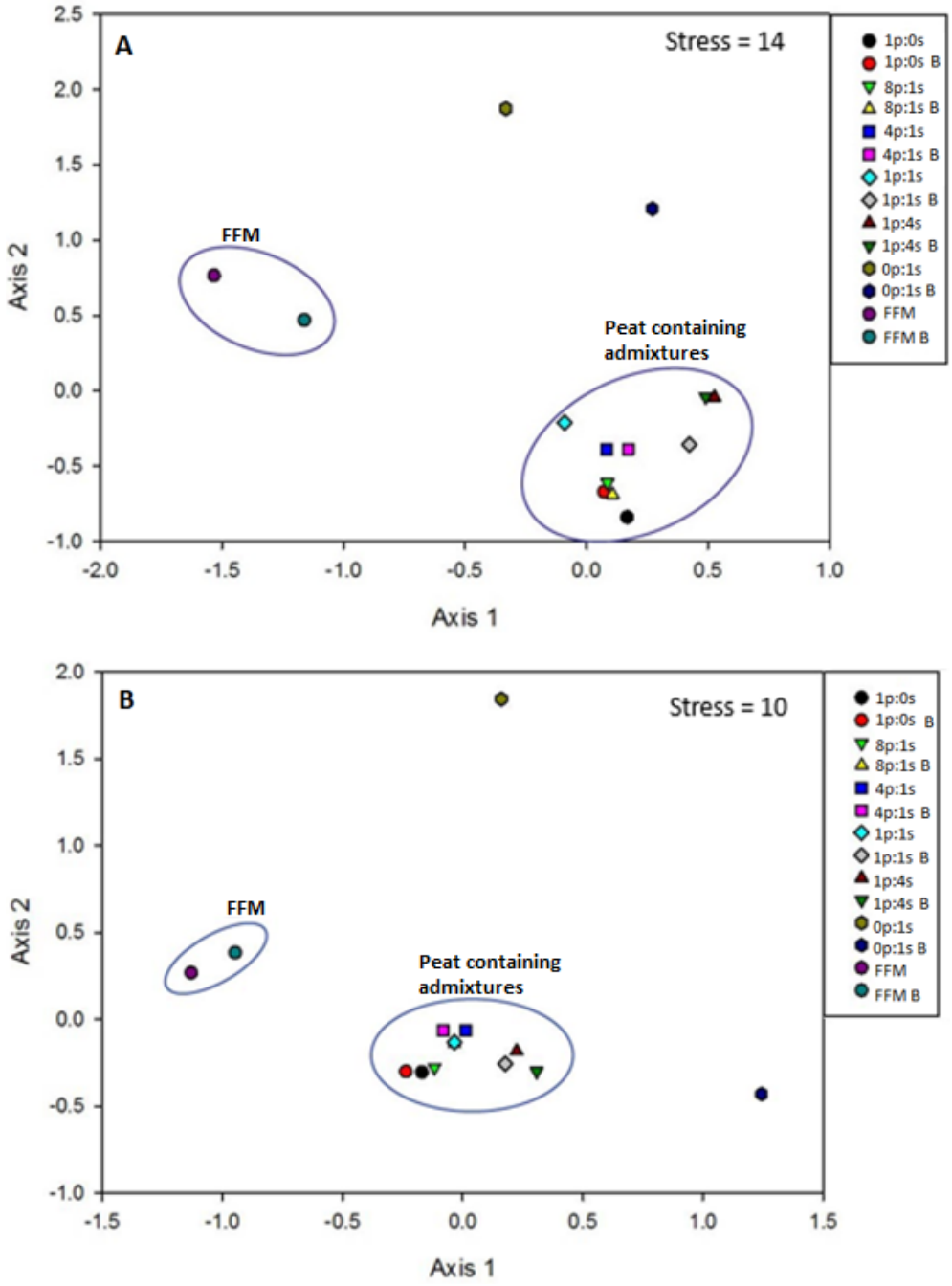

Figure 2: NMDS ordinations of biochar and admixture effects using the A) Jaccard presence/absence dissimilarity index between treatments ("community membership") and B) θ_{YC} abundance based similarity index between treatments ("community composition"). Circles indicate statistically significant groups ($p < 0.05$) based on MRPP. B indicates the presence of biochar.

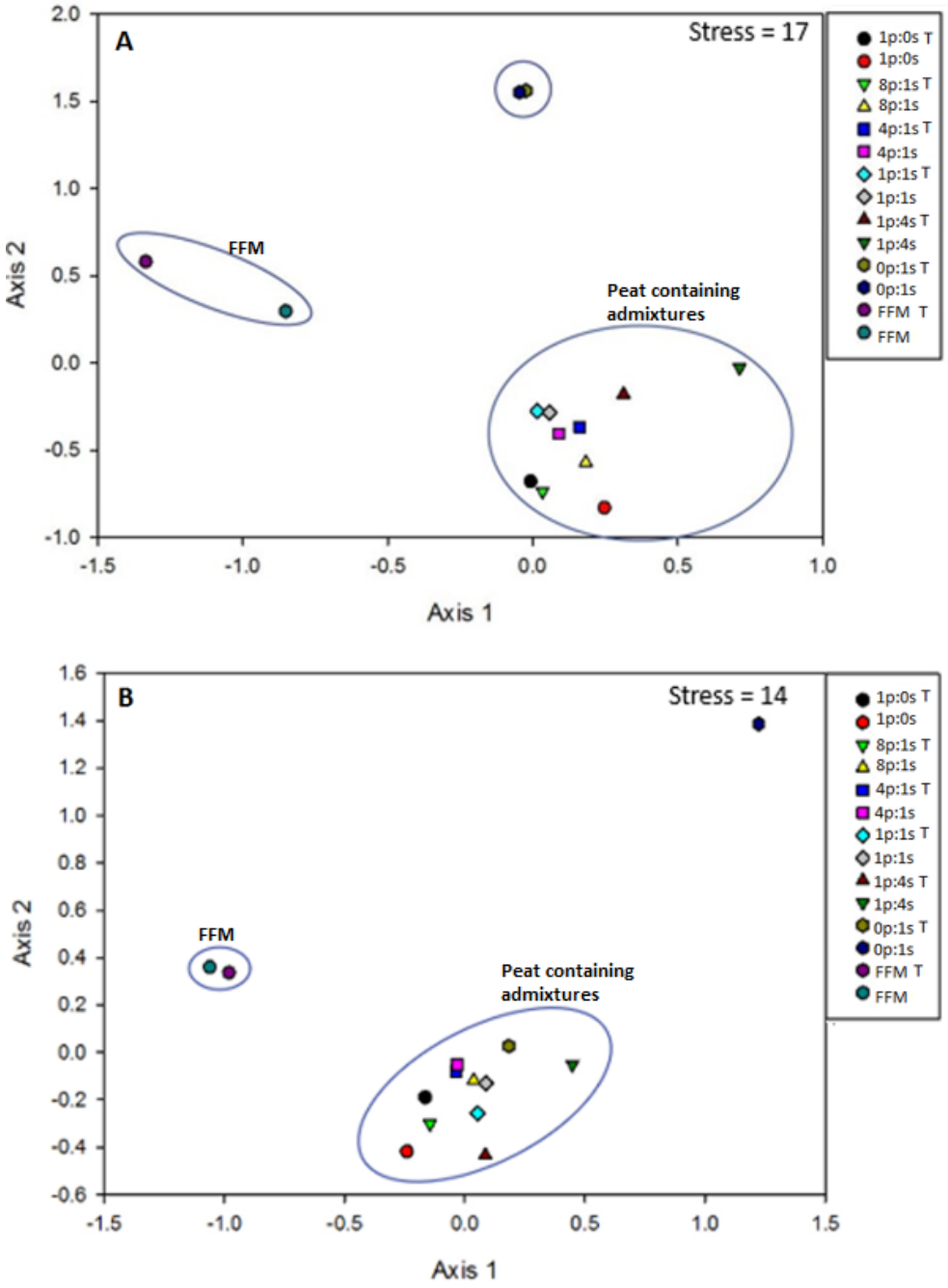

Figure 3: NMDS ordinations of tree and admixture effects using the A) Jaccard presence/absence dissimilarity index between treatments ("community membership") and B) θ_{YC} abundance based similarity index between treatments ("community composition"). Circles indicate statistically significant groups ($p < 0.05$) based on MRPP. T indicates the presence of a single *P. tremuloides* sapling.

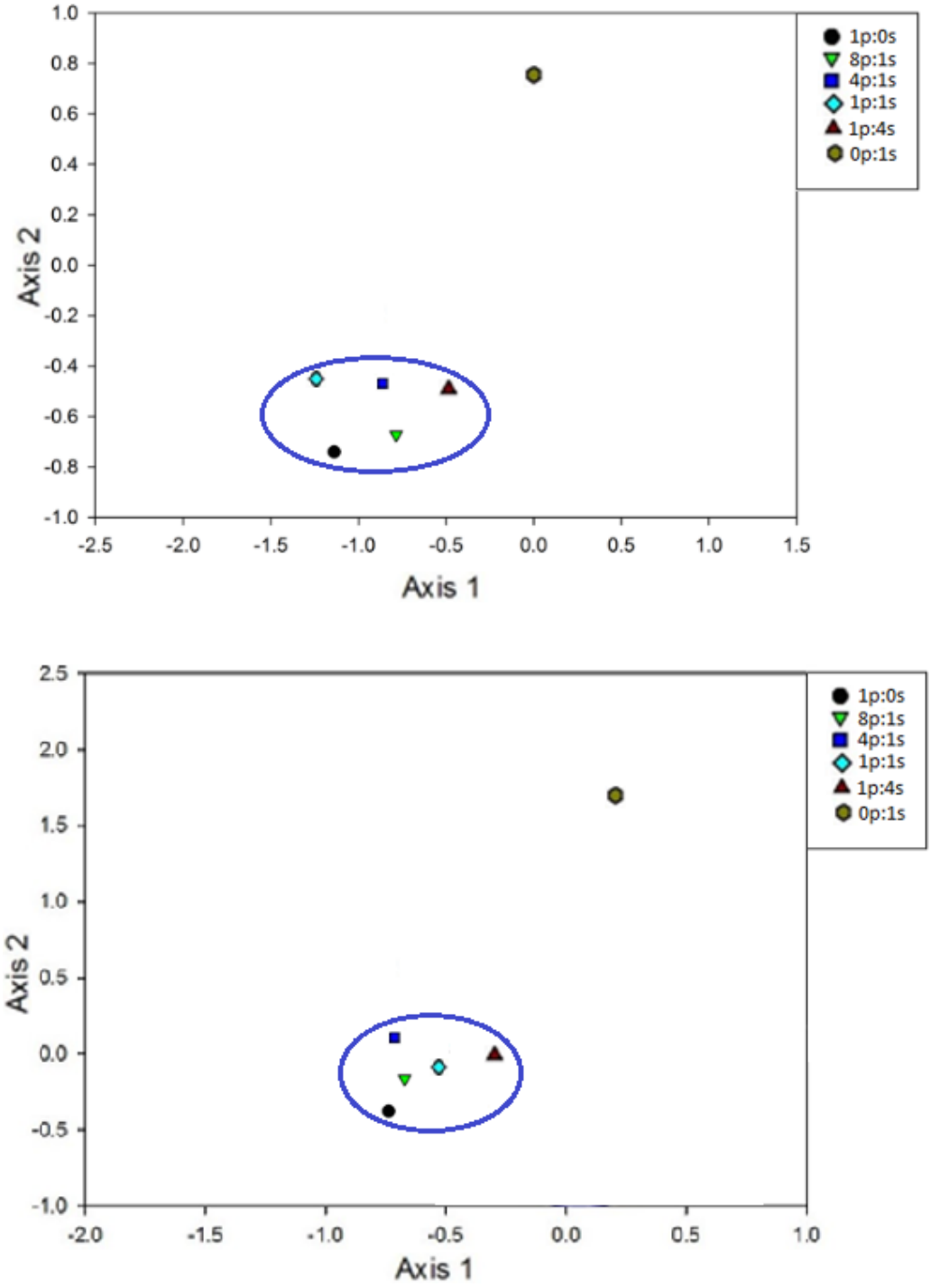

Figure 4: NMDS ordinations of admixture effects in the absence of a tree and biochar using the A) Jaccard presence/absence dissimilarity index between treatments ("community membership") and B) θ_{YC} abundance based similarity index between treatments ("community composition"). Circles indicate statistically significant groups ($p < 0.05$) based on MRPP. P indicates the portion of peat, s indicates the portion of subsoil.

2.5: Discussion:

We found that the diversity measures alone were not enough to predict soil functioning in this experiment. Species richness can positively affect ecosystem functioning primarily through two mechanisms. Firstly, increasing the richness of an environment increases the likelihood that additional nutrient substrates will be utilized, and overall ecosystem functioning will increase (Bell et al. 2005). However, because no environment is infinitely complex, there is a limit on how much ecosystem functioning can be increased by adding community members due to niche saturation (Wohl et al. 2004). Secondly, increased species richness in an ecosystem adds functional redundancy to the system. As the soil community is intimately linked to the soil physicochemical properties (Lauber et al. 2009; Rousk et al. 2010), a disturbance to the soil can have potentially severe effects on the community and thus the soil functioning. The insurance hypothesis states that when an ecosystem undergoes some disturbance significant enough to alter the abundance of an organism that contributes to overall ecosystem functioning, if the species richness of this ecosystem is high, there is a high likelihood that some species that is not affected by the disturbance will be capable of performing the same function (Naeem and Li, 1997; Yachi and Loreau, 1999). Species evenness is the measure of how equally all species are represented in an environment, and has also been shown to have an influence on soil functioning. Ecosystems with a more evenly distributed community have a higher ecosystem functioning than do those with a more uneven distribution (Wittebolle et al. 2009). Furthermore, the ecosystem functioning of a more even ecosystem is less likely to be negatively impacted by a selective environmental disturbance than is a less even ecosystem (Wittebolle et al. 2009). Thus, overall biodiversity (taxon richness and evenness) should, theoretically, have the greatest impact on soil function,

followed by community composition. If this were the case in this system, we would expect that the respiration rate of the peat containing admixtures, which had similar overall diversity, richness, and evenness levels to FFM, would be similar to the respiration rate of the FFM (Table 2). In our system, we observed similar overall biodiversity between FFM and the peat containing admixtures, but the soil respiration rate for peat containing admixtures was significantly lower than for FFM, although significantly higher than for pure subsoil. Thus, in this system, overall biodiversity was not the primary driver of soil functioning.

However, the respiration rates did appear to be correlated to the community membership and composition. Surprisingly, however, there was a significant difference in soil respiration between peat containing admixtures and FFM (Figure 1).

The CO_2 emission rates observed for peat lowlands depend on a number of factors such as temperature (Dioumaeva et al. 2002), water content (Bergman et al. 1999; Yan and Marschner, 2014), and depth and density (Scanlon and Moore, 2000). Peat has a high water holding capacity, and peat degradation is slow under anaerobic conditions (Fisk et al. 2003). The watering scheme used in this experiment kept the samples at a higher than field capacity level, and some anaerobic zones were likely in the admixtures containing peat.

Another explanation for the lower respiration rates in peat containing admixtures is the quality of the organic matter. Peat is known to be recalcitrant with a high humic substance content (Chiou et al. 2000). Comparatively, the SOM in the FFM is much more labile, and the greenhouse environment (warm, wet, aerobic, shallow) would have allowed a high level of functioning in the FFM soils as compared to forest floor soils in nature. The peat containing admixtures and pure subsoil may also have experienced a similar increase to their respiration rates, but due to the low

availability of labile carbon, this was overshadowed by the respiration rate of FFM. The anaerobic conditions coupled with the inherently recalcitrant nature of peat may explain the observed low respiration in peat containing admixtures relative to FFM.

However, the functional difference between FFM and peat containing admixtures was specifically correlated with community composition; the three statistical groups for composition were reflected in part in the respiration rates. Community composition is an influence on ecosystem functioning; however, the impact of the presence/absence of a species on functioning varies within the literature. For example, Cavigelli and Robertson (2001) showed that differences in the community composition of the soils accounted for the differences in denitrification rates in a successional soil and an agriculture soil. Similarly, Eilers et al. (2010) found changes in relative abundances of some taxa associated with supplementation and degradation of carbon substrates. While there are likely other factors playing into the differences in soil function between the peat containing admixtures and the FFM that this experiment did not test for, there appears to be a relationship between the high soil functioning and the community composition of FFM.

The hypothesis of this study, that there would be some mixture of peat and subsoil supplemented with biochar and a tree that would harbour a bacterial community approaching similarity to that of FFM, was refuted.

Although the data from this experiment cannot determine the mechanism of this dominance of peat communities in these admixtures, there are several possible explanations. One possible explanation is that there is a much higher community biomass in the peat than in the subsoil. Peat harbours high bacterial diversity due to the heterogeneous nature of peatlands (Andersen et al.

2013). While we did not measure bacterial abundance or biomass, organic carbon content is a main driver of bacterial biomass in soils, and it is a reasonable assumption that the high carbon peat will have a higher bacterial biomass (Hu et al. 2014). Because of the potentially large difference in biomass, the observed dominance of the peat community over the subsoil community could be due to the peat community simply overwhelming the signal from the subsoil community. Even in the low peat concentration admixture, 1p:4s, the biomass of the peat community may have comparatively high enough to dominate the subsoil community. Alternatively, the source of the carbon is a main driver of the community composition (Hertenberger et al. 2002; Gomez et al. 2006; Eilers et al. 2010 Jiang et al. 2012; Prescott and Grayson, 2013; Aponte et al. 2013). The assemblages of specific organic species present in peat and forest floor soils differ significantly (Qualls and Haines, 1992; Chiou et al. 2000). Since peat is the major source of carbon in the peat containing admixtures, it is possible that the communities of the admixtures converged to a community that is most capable of utilizing peat.

Bulk soil biodiversity, community composition, and activity were not affected by the presence of biochar, a tree, or both together. Two exceptions to this were in the 8p:1s and 4p:1s treatments, where a combination of a tree and biochar as well as a tree alone had a positive effect on respiration in both, and the presence of biochar alone had a positive effect in 8p:1s. However, these two treatments were indistinguishable from the other peat containing admixtures in both diversity and community compositions. These are not the only parameters which can affect soil functioning, but they are the only ones we tested for, so it appears some other parameter is playing a role here in affecting respiration. Typically, C mineralization increases with the presence of a source of organic C. With the addition of either a tree and/or biochar, we expected to see an increase in respiration rate in all admixtures. Because of this, it was surprising to see no

increase in respiration in the carbon poor 0p:1s treatment with either addition. The addition of a plant source of carbon to a nutrient poor soil is usually followed by a large boost in soil respiration (Gartzia-Bengoetxea et al. 2016). However, the greatest increase in respiration with the addition of plants is in the region directly in contact with the root (i.e. the rhizosphere); in this case, the tree root system was fairly limited in extent and the bulk soil communities beyond the rhizosphere were not affected by its presence.

While there was an increase in respiration in the 8p:1s admixture when supplemented with biochar, however this did not correlate to any measurable change in community structure or composition. The response of the microbial community to the presence or addition of biochar varies widely in the literature, ranging from a strong positive effect (increasing diversity, activity, etc.), no effect, or a negative effect (decreasing diversity, activity, etc.) (Borchard et al. 2014). There are a number of different factors that appear to influence biochar effect in the soil: pyrolysis temperature (Mimmo et al. 2014), feedstock source (Purakayastha et al. 2015), and application concentration (Butnan et al. 2015). Some of the primary mechanisms by which biochar alters the soil parameters are correlated to the production temperature, and studies have found that biochar produced under a lower temperature can have a less significant effect on the microbial community than does a biochar produced under high temperatures (Khodadad et al. 2011). On the other hand, biochars produced at a low temperature tend to have a higher labile proportion, resulting in a stronger initial burst of microbial activity. Some studies have found that application rate is also an important consideration to amending a soil with biochar, where too little biochar may not have an effect (Butnan et al. 2015). Regardless of what the reason for a microbial response may be in systems supplemented with biochar, there is currently no way to predict how the microbial community will respond. It is possible that the community did not

respond to the addition of biochar because a low temperature biochar was used, and at a concentration that may not have been sufficient to get a response. A biochar made at a higher temperature and applied at a higher concentration would likely elicit a response from the bacterial community. Alternatively, it is possible that the communities of these admixtures are simply unresponsive to this peat-derived biochar. Further trials would be needed to find what type of biochar would be needed, and how much, in order to elicit a response from the microbial community. Both of these options drive up cost, however, so it does not appear that the use of biochar is an economically feasible aid to land reclamation programs in the AOSR.

The primary findings of this study are subject to some important caveats. First, the study period was 12 weeks in a greenhouse environment. This may not have been a sufficient timeframe to allow the *P. tremuloides* sapling to establish. If more time was allotted to incubation, the root network could have grown more extensively and grown a larger influence on the overall soil community. Whether or not the admixtures had any effect on the rhizosphere community remains unknown.Previous studies have found that successful plant colonization is imperative to the success of a reclamation regime (McMillan et al. 2007; MacKenzie and Naeth, 2009; Barbour et al. 2010; MacDonald et al. 2015). If the rhizosphere community responds positively to these admixtures, plant colonization may be expatiated as an effect. Investigating if the trends observed here hold true for rhizosphere communities will provide further insight for reclamation program design.

In conclusion, our hypothesis was refuted: peat and subsoil admixtures, with or without biochar and/or tree additions, did not approximate the FFM microbial community structure or function, despite having similar overall biodiversity. Therefore the significance difference between the soil functioning of the peat containing admixtures and the FFM could be due to the significant

differences in the community composition, though there are likely other factors that played into this not tested in this experiment. It appears that the FFM community composition is a marker for its suitability as a reclamation substrate; simply replacing the FFM community with another, equally biodiverse community will not suffice. No matter the concentration of the peat, the admixtures containing peat had statistically indistinguishable bacterial communities, indicating that the presence of subsoil had no detectable effect on the peat bacterial community and can be considered as a "blank" that can be used to dilute the peat. This leads to the question of whether or not a similar effect would be seen if FFM were diluted with subsoil, and whether the FFM microbial community could be retained even when heavily diluted. This is an important idea for consideration, because this experiment provides evidence to suggest that the FFM community composition plays a central role in its suitability as a reclamation substrate. FFM is in limited supply for reclamation; however, if the FFM could be diluted without any significant change to its bacterial community, there is potential to create more of it to allow for more extensive use in oil sands reclamation. Future studies, where FFM is diluted with subsoil, will be needed to determine whether this is the case. Because no mixture of peat and subsoil, whether or not they were amended with biochar or a tree, was able to shift the bacterial toward that of FFM, no recommendations on reclamation programs can be suggested as a direct result of this study.

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Chapter 3: Conclusion

Large-scale mining operations in the Athabasca Oil Sands Region (AOSR) in Northern Alberta have severely disturbed more than 800 km^2 of boreal forest. The Government of Alberta requires that disturbed land to be reclaimed to "an equivalent capability" (Government of Alberta, 2014). This requires rebuilding landscapes, from the soil to the topography and vegetation. On-site materials limit reclamation protocols, and rebuilding soils requires the use of the original materials, particularly topsoils, on site. Large-scale reclamation programs are intensive, difficult, and very costly, and will often require follow up work (Barbour, 2010). Because of this, careful attention must be paid to long-term program design in order to minimize downstream work. One ongoing study is Syncrude's Aurora North Capping Study (NorthWind Land Resources Inc, 2013). The Capping Study is a large scale, long-term field study investigating the suitability of a range of reclamation substrates for reclamation at Syncrude's Aurora site (COSIA Land EPA Site Reclamation Report, 2014). The study is looking at a number of soil parameters, including soil water dynamics, hydrocarbon degradation, nutrient retention, mycorrhizal communities, and microbial community parameters. The Capping Study is uses a number of different capping designs and depths, using either peat/mineral mix (PMM) or forest floor mineral mix (FFM) as the capping material.

FFM has been shown to be a more reliable reclamation substrate than PMM (Barbour et al. 2010). However, the large majority of the topsoil material available is peat, and there is not enough FFM available to be used in large-scale reclamation operations. It is therefore important to investigate approaches to replicate FFM reclamation results with other, more abundant materials. An important consideration of building viable reclamation substrates is the soil

quality. Therefore, a reclamation substrate that harbours a bacterial community similar to that of FFM may be a comparable replacement to FFM. Furthermore, microbial community members respond rapidly to changes in their environments; thus, they may serve as indicators of a successful reclamation trajectory well before other ecosystem components (MacKenzie and Quideau. 2010), such as trees or animals. The objective of this experiment was to investigate if some mixture of peat and subsoil, as well as amending the admixture with biochar and/or a *Populous tremuloides* sapling would result in a bacterial community similar to that of FFM. I hypothesized that an intermediate mixture with biochar and a tree would harbour a bacterial community most similar in structure and functioning to that of FFM.

I found that across all measures except evenness, all peat containing admixtures were not significantly different from those of the FFM, all of which were significantly higher than those of the pure subsoil. The literature on community diversity and its effect on soil functioning largely show a positive relationship. Despite what we predicted based on ecological theory, the trends in diversity across the admixtures was not reflected in the soil respiration rate. While FFM and the peat containing admixtures diversity was indistinguishable, respiration was much higher in FFM. Instead, soil respiration correlated with the groupings observed in community composition measures, where the peat containing admixtures were not different from each other, but were different from both pure subsoil and from FFM. This suggests that community composition is a more influential community parameter, or that it is correlated with another parameter that effects soil respiration that was not tested for in this experiment. This demonstrates that an understanding of the relationship between community composition and soil functioning, as well as between diversity and functioning, are critical for a clear understanding of the relationship between community and function. This understanding would inform mining companies which

capping substrates are most suitable for reclamation. The bacterial community of the peat was unaffected by the increasing fractions of subsoil, and the hypothesis was refuted.

Some important conclusions can be drawn from these findings. First, no amount of subsoil added to peat can shift the peat community to become more similar to the FFM community, at least up to a 1:4 peat:subsoil ratio. This demonstrates that the initial material may determine the outcome of the community composition. Because of this, the goal of recreating the FFM bacterial community from peat and subsoil does not appear to be attainable. Secondly, the subsoil appears to act as somewhat of a blank, increasing soil mass while not altering the microbial community composition or bulk activity. While this is true for the peat community, the FFM community has not been tested. What has been accomplished in this study is essentially the creation of a peat dilution series, whereby even a small volume of inoculum is enough to make the community structure, composition, and the soil function, indistinguishable from that of the pure inoculum material, in this case peat. This raises an important question; if subsoil acts as a blank to peat, increasing the volume of this peat mineral admixture without altering any microbial parameters of it, can the same be done with FFM? If so, the dilution of FFM could be a means of creating more of it, allowing a more widespread use in reclamation programs. How the FFM community responds in these treatments is unknown. One of the recommendations to arise from this study is a follow up study investigating the FFM bacterial community response to amendments with the purpose of replicating FFM from an initial inoculum, as opposed to trying to recreate it, as was the case here. This could be done in a similar manner to this experiment, by mixing FFM and subsoil to different ratios. It is also possible that the community may respond positively to a mixture of peat, subsoil, and FFM together.

The finding that the peat community diversity and composition is not altered when mixed with subsoil is surprising, and the data collected here is insufficient to supply an explanation. The processes of collection, storage, experimental set up, and even incubation, are all significant potential disturbances that can affect the bacterial community. It could be that the community data collected may represent only a particularly hardy portion of the entire peat community. Alternatively, a converging community shift is also a possible explanation, where the communities of the admixtures containing peat were initially different, but converged due to incubation conditions within the greenhouse. Furthermore, this experiment had a relatively short incubation time of 12 weeks. It is possible that there was a slow, ongoing community shift occurring in the admixtures, but that there was insufficient time to observe a shift.

These hypotheses could be tested with longer running experiments examining multiple time points. This study did not produce the data required to examine any community dynamics, a problem that can be solved by analyzing the community structure at multiple time points, including one at time point zero; although these samples were collected for this study, they were not analyzed in order to decrease the complexity and cost of the analyses. Future students could capitalize on these preserved samples. Information on the dynamics of the community can offer valuable insight into potential reclamation regimes. This study examined only one time point, at 12 weeks incubation. The possibility of the admixtures undergoing a long-term community shift brings with it the possibility that the soil functioning may also have been undergoing a shift. If multiple time points were analyzed and the experiment were run longer, a shift in community may have occurred and we could expect an increase in functioning, potentially making the peat containing admixtures more similar in community and functioning to FFM. It is impossible to know how long an experiment would need to run in order to observe a community shift in these

admixtures, since it is not known if such a shift was even occurring. However, to keep the admixture communities consistent with field conditions, the duration of such a experiment could be one growing season.

Further follow-up studies could be performed to further investigate if a suitable FFM alternative can be produced using more abundant materials. Dimitriu et al. (2010) found that the microbial community of reclamation soils was strongly influenced by the plant community. In the study described in this thesis, an individual *P. tremuloides* sapling was used, and its presence was found to not have an effect on the bulk soil community. Twelve weeks incubation time did not supply the saplings with enough time to grow a large enough root network to influence most of the soil in the pot, because the size of the rhizosphere extending only a few mm from the root. How the peat content of the soil affects the rhizosphere is unknown; a longer incubation time would have allowed the root system to grow, which might have then influenced the bulk soil community composition. It is possible that, while the bulk soil community was not affected by the peat concentration, the rhizosphere soil was. If the long-term goal of a reclamation program is to grow a self-sustaining ecosystem, then how these reclamation substrates affect the rhizosphere communities is a valuable question to address.

Furthermore, it is likely that if multiple species of plant were used during incubation the bacterial community could have been affected by the presence of the plants, and the plant-effect may shift the FFM and peat dilution series communities towards each other. MacKenzie and Naeth (2009) argue that one of the reasons that FFM is the most effective reclamation substrate is that it naturally contains a large amount of native seeds, allowing plant colonization to occur faster. To test if plant community could aid in shifting the peat community more towards that of FFM, a potential experiment is to plant a mosaic of native plants, including trees that have been used in

the Aurora Capping Study (NorthWind Land Resources Inc., 2013) planted in these peat admixtures. To seed the peat admixtures naturally, a portion of the mixture should be FFM.

However, such an investigation into plant species and peat/FFM/subsoil mixing along a time series is a very complex experiment with many variables. It would be more feasible to split this investigation into multiple trials, however, this would increase the logistical effort and costs. It is then necessary to address the issues that should be of the highest priority. Because of the value gained in potentially replicating FFM through diluting with subsoil, I believe that an experiment investigating this should take precedence. An experimental design much like the one used in this study would suffice, with a few modifications as outlined above, namely a longer experimental duration and analysing multiple time points. Future directions would be discerned based on the outcome of this experiment.

This study demonstrated that the subsoil acted to dilute the peat, not affecting the bacterial community, or the bulk the soil respiration. I also showed that the presence of biochar or a tree did not have an effect on the community structure. While the hypothesis for this study was refuted, the results offer some insight into future directions. FFM has been shown to be a reliable reclamation substrate, but due to its scarcity, it cannot be used wide scale. If peat can be diluted with mineral subsoil with no significant impact on both the soil respiration and the bacterial community, it may be possible to do the same with FFM. This would in essence replicate FFM without altering the bacterial community or activity, and more of it could be used in more reclamation regimes. Similarly, if peat, subsoil, and FFM could be mixed to produce an FFM-like microbial community, that too would allow a greater utilization of FFM.

The objective of Syncrude's Aurora capping study is to determine the most suitable reclamation substrate design, including plant species, capping material, and subsoil mixtures (NorthWind Land Resources Inc., 2013; Barber et al. 2015). The microbial community of the two capping materials in use at the Aurora North site were tested here. This is the first in depth analysis of the microbial community of these reclamation substrates, and provides insight into what future experiments could be designed to further improve the availability and suitability of these reclamation substrates in large-scale land reclamation regimes.

I strongly recommend one or more follow-up studies examining the potential to replicate FFM by admixing it with the peat and/or mineral subsoil in a longer duration, multi time point investigation into the bacterial community's response within the bulk and rhizosphere of these admixtures.

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Supplementary material

Table S1: Classes of OTUs present in each treatment. + denotes the presence of at least one taxa belonging to the class, and – denotes the absence of any taxa belonging to the class. P denotes the peat content, while s denotes subsoil content.

Class	Soil treatment						
	1p:0s	8p:1s	4p:1s	1p:1s	1p:4s	0p:1s	FFM
Gammaproteobacteria	+	+	+	+	+	+	+
Bacilli	+	+	+	+	+	+	+
Betaproteobacteria	+	+	+	+	+	+	+
Proteobacteria_unclassified	+	+	+	+	+	+	+
Actinobacteria	+	+	+	+	+	+	+
Nitrospira	+	+	+	+	+	+	+
Acidobacteria Gp4	+	+	+	+	+	+	+
Bacteroidetes unclassified	+	+	+	+	+	+	+
Verrucomicrobia unclassified	+	+	+	+	+	+	+
Sphingobacteriia	+	+	+	+	+	+	+
Deltaproteobacteria	+	+	+	+	+	+	+
Cytophagia	+	+	+	+	+	+	+
Bacteroidetes incertae sedis	+	+	+	+	+	+	+
Acidobacteria unclassified	+	+	+	+	+	+	+
Actinobacteria unclassified	+	+	+	+	+	+	+
Subdivision3	+	+	+	+	+	+	+
Gemmatimonadetes	+	+	+	+	+	+	+
Flavobacteriia	+	+	+	+	+	+	+
Acidobacteria Gp7	-	+	-	+	+	+	-
Opitutae	+	+	+	+	+	+	+
Negativicutes	+	+	+	+	+	+	+
Planctomycetia	+	+	+	+	+	+	+
Spartobacteria	+	-	+	-	+	+	-
Firmicutes_unclassified	+	+	+	+	+	+	+
Verrucomicrobiae	+	+	+	+	+	+	+
Acidobacteria Gp6	+	+	+	+	+	+	+
Acidobacteria Gp17	+	-	-	-	+	+	-
Spirochaetia	+	+	+	+	+	+	-
Ignavibacteria	+	+	+	+	+	+	-
Latescibacteria_unclassified	-	-	-	-	-	+	-
Acidobacteria Gp2	-	-	-	-	-	-	-
Acidobacteria Gp25	+	-	-	-	-	-	-
Chlamydiia	+	+	+	+	+	+	+

Acidobacteria Gp9	+	-	-	-	-	+	-
Acidobacteria Gp19	-	-	-	-	-	-	-
Deinococci	-	-	-	-	+	-	-
Acidobacteria Gp10	-	-	-	-	-	-	+
Clostridia	+	+	+	+	-	-	+
Elusimicrobia	-	-	-	-	-	-	-
Bacteroidia	-	-	-	-	-	-	-
Acidobacteria Gp15	-	-	-	-	-	-	-
Planctomycetes unclassified	-	-	-	-	-	-	-
Holophagae	-	-	-	-	-	-	-
Alphaproteobacteria	-	-	-	-	-	+	-
Acidobacteria Gp18	-	-	-	-	-	-	-

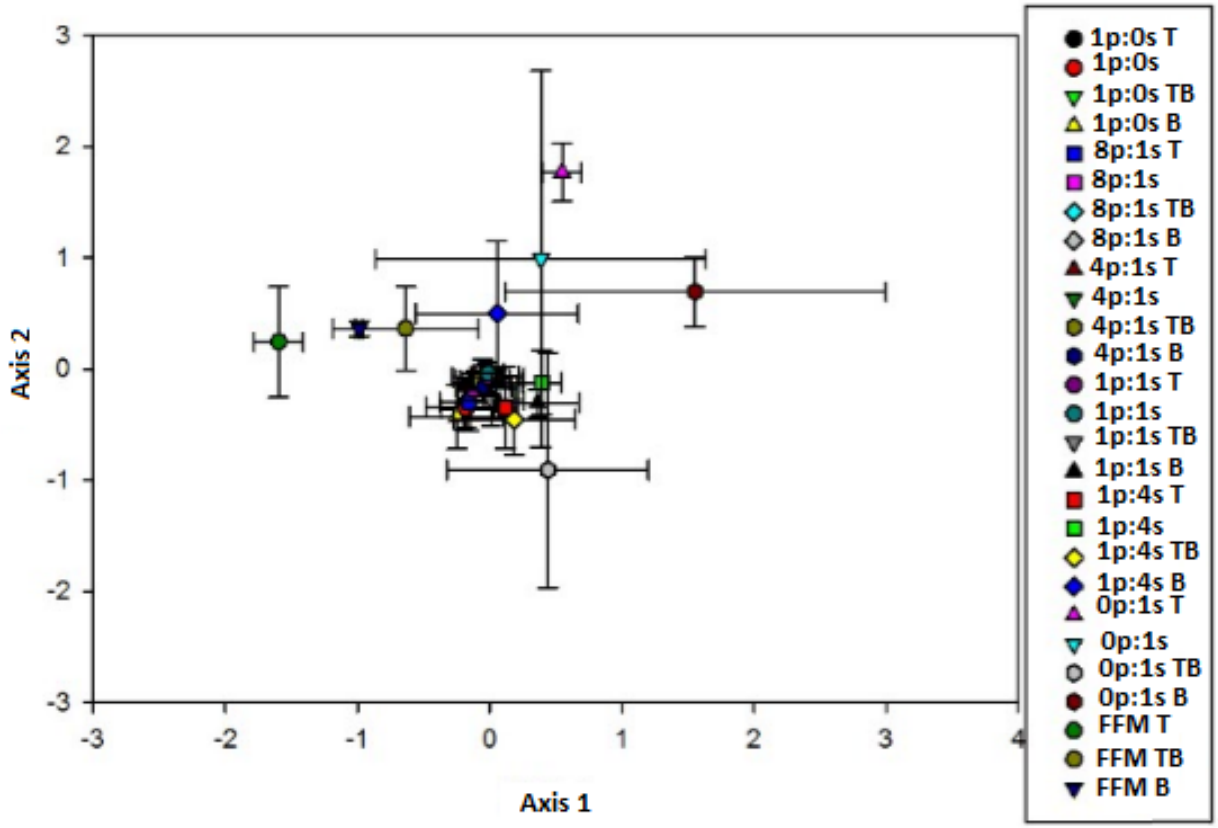

Figure S1: NMS ordination of the θ_{YC} abundance based similarity index between treatments ("community composition"). Error bars denote standard error. P indicates the peat content, while s indicates the subsoil content. T indicates the presence of a single *P. tremuloides* sapling, while B indicates the presence of biochar.

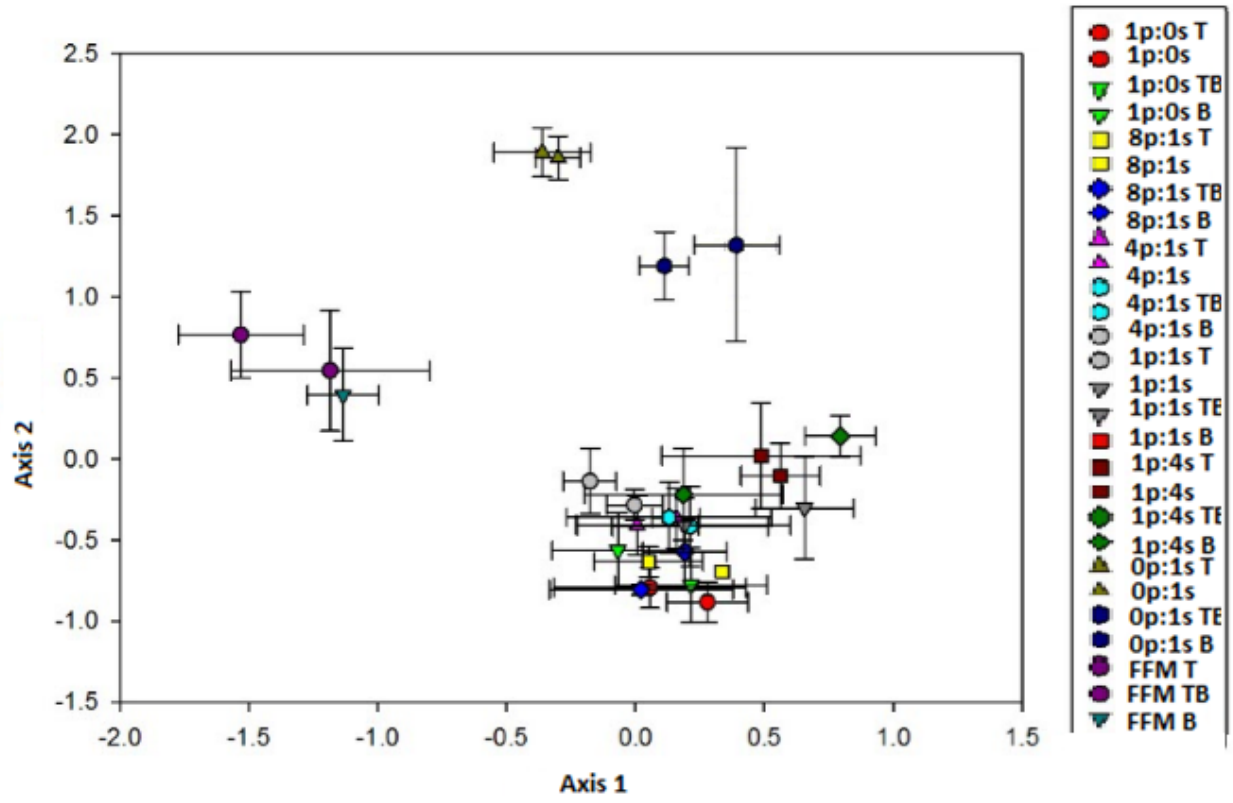

Figure S2: NMS ordination of the Jaccard presence/absence dissimilarity index between treatments ("community membership"). Error bars denote standard error. P indicates the peat content, while s indicates the subsoil content. T indicates the presence of a single *P. tremuloides* sapling, while B indicates the presence of biochar.

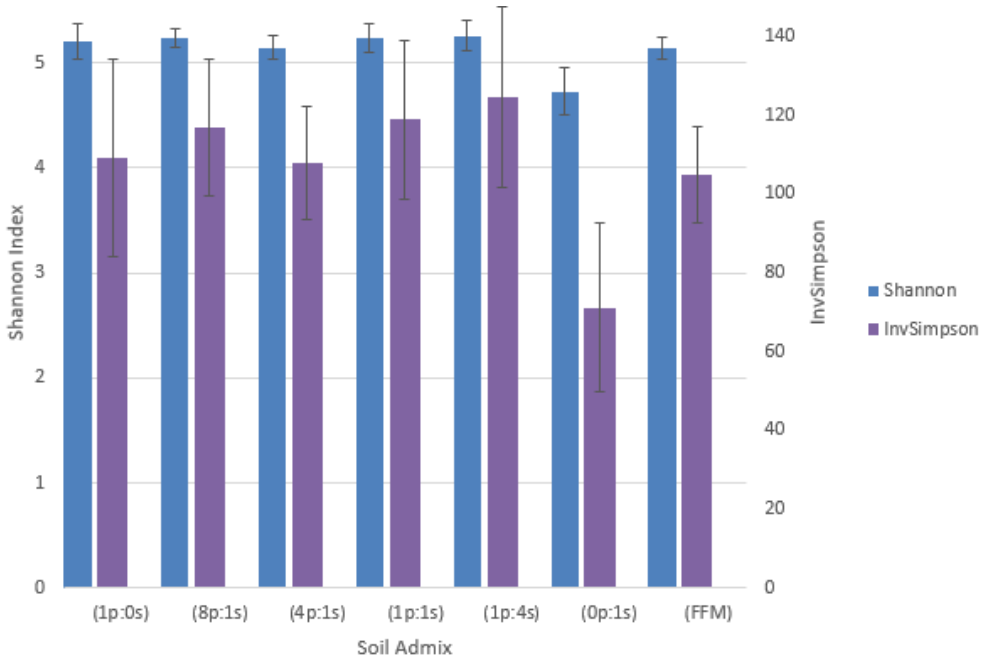

Figure S3: OTU diversity (values from Table 2) of each admixture. Error bars denote standard deviation.

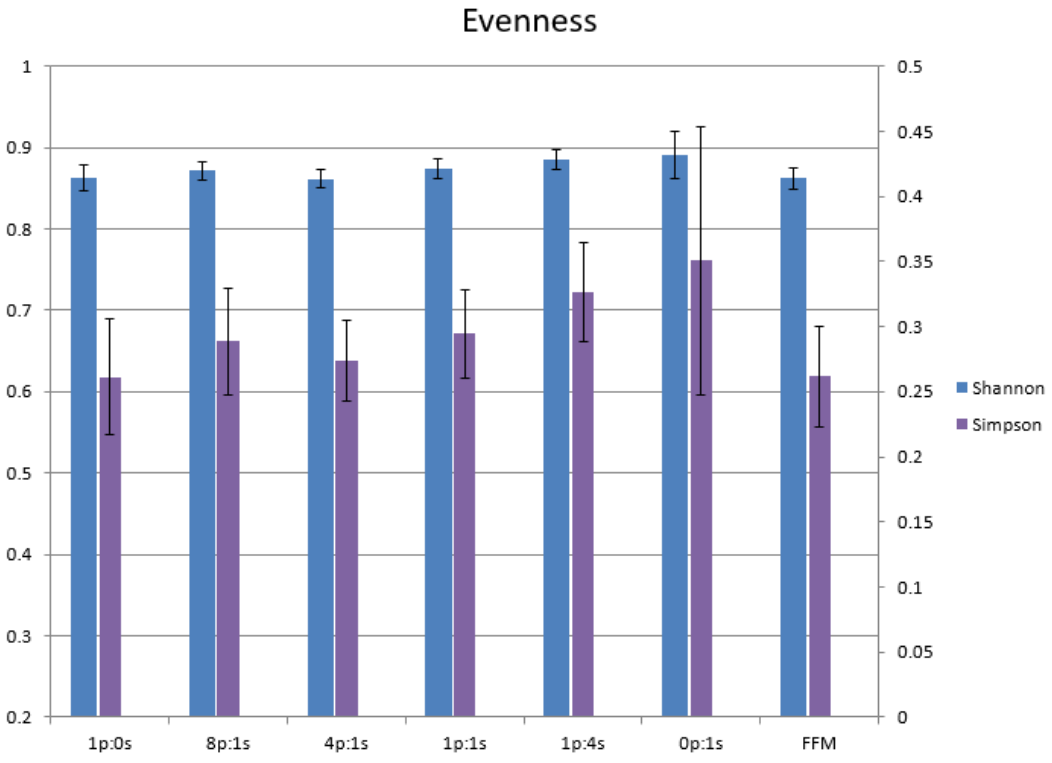

Figure S4: OTU evenness (values from Table 2) of each admixture. Left axis is Shannon evenness, while right axis is Simpson evenness. Error bars denote standard deviation

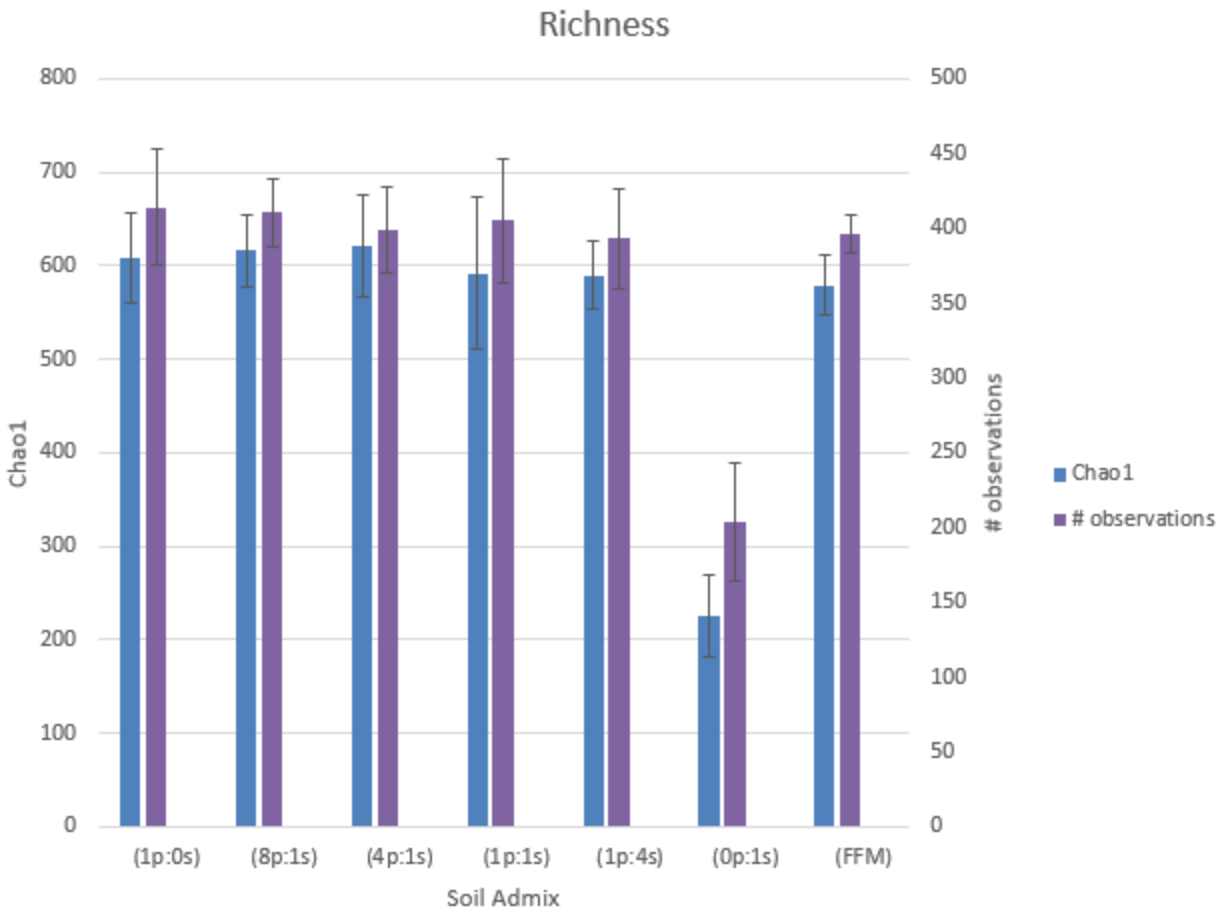

Figure S5: OTU richness (values from Table 2) of each admixture. Error bars denote standard deviation.

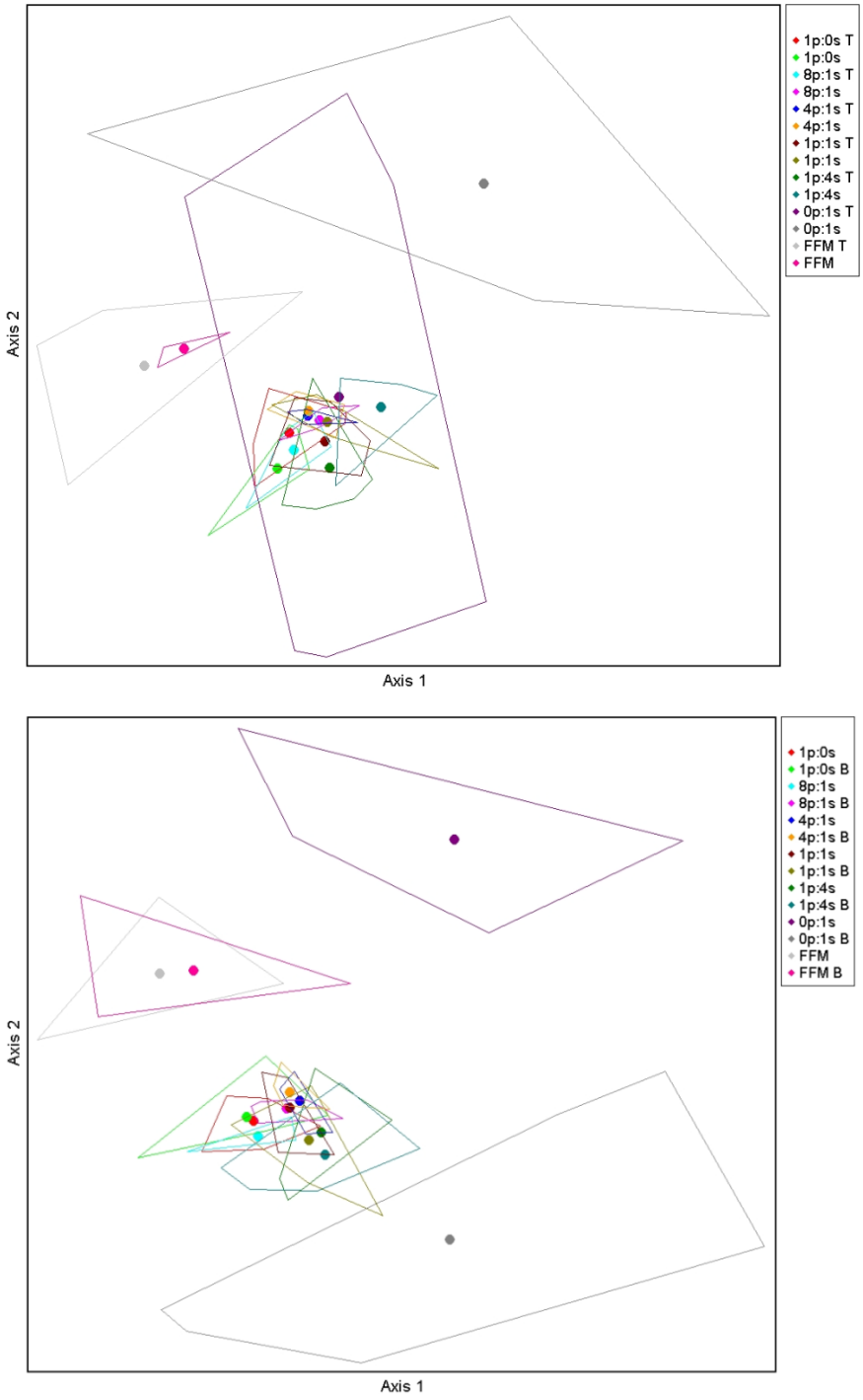

Figure S6: NMS ordination of the θ_{YC} abundance based similarity index between treatments ("community composition"). Error bars denote standard error. P indicates the peat content, while s indicates the subsoil content. T indicates the presence of a single *P. tremuloides* sapling, while B indicates the presence of biochar. Convex hulls indicate variability.

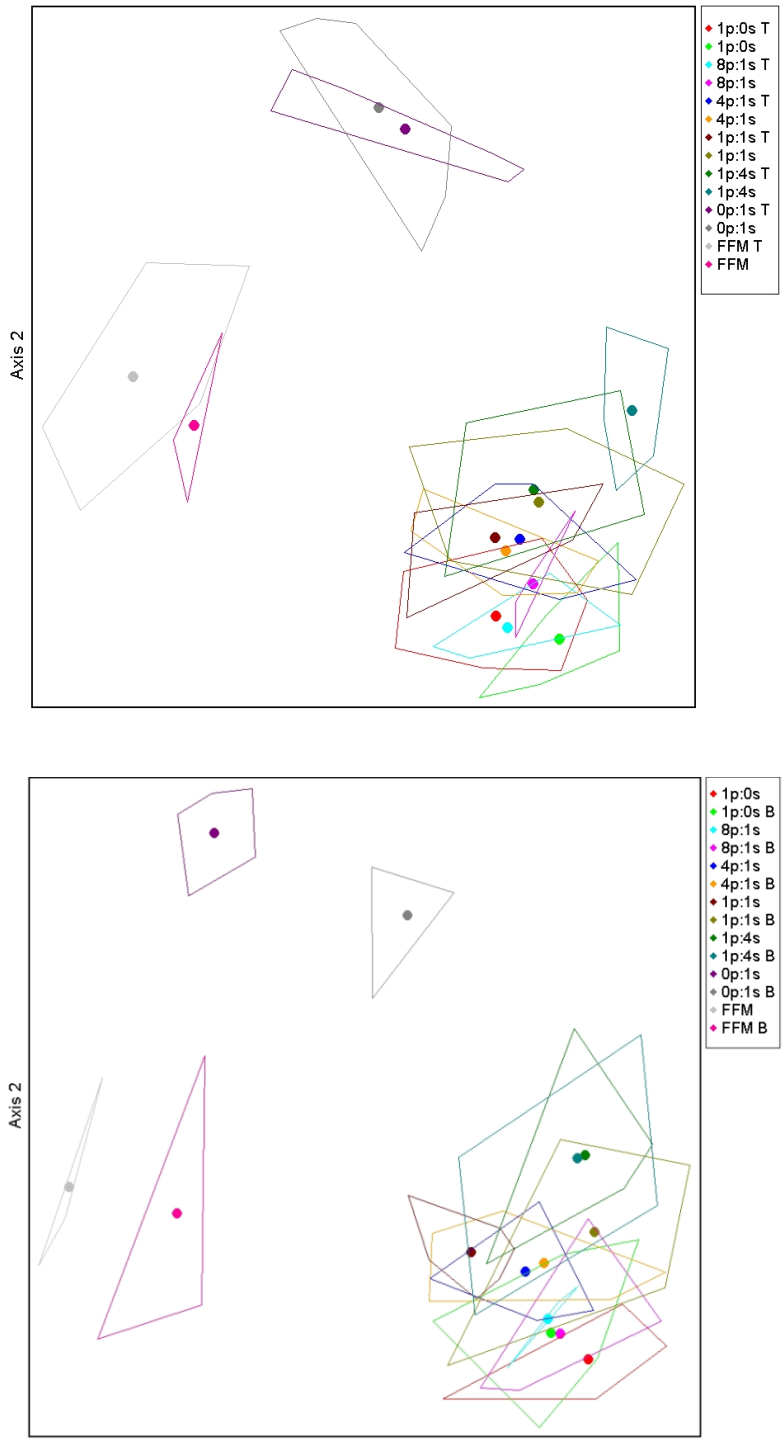

Figure S7: NMDS ordinations of the Jaccard presence/absence dissimilarity index between treatments ("community membership"). Error bars denote standard error. P indicates the peat content, while s indicates the subsoil content. T indicates the presence of a single *P. tremuloides* sapling, while B indicates the presence of biochar. Convex hulls indicate variability.