The Impact of Reclamation and Vegetation Removal on Compositional and Functional Attributes of Soil Microbial Communities in the Athabasca Oil Sands Region

by

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ABSTRACT

Large-scale mining for oil extraction in the boreal forests of Northern Alberta has led to a disturbance footprint of ~ 900 km^2 of land; which, under regulations from the government of Alberta, must be reclaimed to equivalent land capabilities using soil materials salvaged and conserved during land clearing. Microorganisms play pivotal roles in soil nutrient cycling and plant growth during land reclamation and are sensitive to anthropogenic disturbances, making them potential markers of ecosystem health. Thus, the objective of this study was to determine the impact of reclamation and vegetation removal on the composition and function of soil microbial communities in the Athabasca Oil Sands Region (AOSR). While the majority of the cover materials are lowland-derived peat-mineral mix (PMM), the upland-derived forest floor-mineral mix (FFM) is the most suitable as a reclamation substrate; however, FFM is far less abundant. Therefore, as a strategy to maximize the limited supply of forest floor soil, this study also investigated diluting this material with sand. The concept of equivalent land capability is ambiguous; thus, I evaluated bacterial community composition (BCC) via high throughput sequencing of 16S rRNA genes and functional diversity by community-level physiological profiling (CLPP). The ranges of variability for these factors observed in soils with vegetation removed and reclaimed soils were compared to that of undisturbed reference soils. Vegetation removal changed the structure of the soil microbial community with some sites overlapping with the range of natural variability, and increased the overall diversity, within-community interactions, and heterogeneity. Reclamation shifted the microbial community structure to a greater extent, placing it outside the range of natural variability. Different reclamation substrates resulted in distinct microbial communities, with forest floor material (FFM) showing the highest

level of similarity to the range of natural variability and peat mineral mixture (PMM) showing the least. BCC, functional diversity, and soil edaphic parameters all had similar results, but BCC showed the greatest ability to resolve differences between treatments. Altogether, my results suggest both reclamation and vegetation removal alter compositional and functional attributes of the microbial communities of the natural boreal forests soils. Furthermore, BCC provided the greatest information about the impacts of mining practices. Thus, BCC holds promise as a marker of reclamation efficacy and trajectory.

PREFACE

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1. Chapter 1. Introductory chapter

1.2. Surface mining and reclamation

Terrestrial ecosystems offer many services on which human beings depend. Among them, land provides mineral and energy resources, which have increased their demand with the increasing progress of science and technology, economic development, industrial expansion, urbanization and growth of population. Advancement of society thereby increases the needs for mined products as the building blocks for societal growth (Sheoran et al., 2010). However, the extraction or harvesting of most mineral commodities requires at least some level of disruption of the ecosystems that contain the mineral resource. These anthropogenic disturbances can range from localized removal of some portion of habitat to nearly complete ecosystem fragmentation (Lima et al., 2016). In open-pit mining or surface mining, highly mechanized mining processes are used since they represent an efficient means of obtaining high production (Shrestha & Lal, 2011). However, this approach is often linked with large volumes of waste rock and overburden material (Ramani, 2012). During surface mining, soil and rock that vary substantially in physical and chemical properties and overlie ore deposits in depths of 0-200m are physically removed to access the resource and must be transported and stored elsewhere (Ramani, 2012). Therefore, surface mining causes alterations to several components of the ecosystem such as (i) aboveground vegetation, (ii) broader landform and landscape, (iii) belowground communities, (iv) soil structure and fertility, and (v) hydrological regimes (M. S. Li, 2006; Miller & Zégre, 2014; Mummey et al., 2002). Very large-scale mining operations may cause ecosystem fragmentation and affect regional biodiversity (Rooney et al., 2012; Rooney & Bayley, 2012).

It is critical to minimize and mitigate the environmental effects from mining activities in order to maintain functional ecosystems (Prescott et al., 2019). Existing environmental management guidelines and policies refer to the need of restoring the previous ecosystem after the cessation of mining operations (Lima et al., 2016). This goal, however, is rather unrealistic due to thorough perturbations imposed by mining, limit timeframes and costs; therefore, regulators often adopt a different post-mining effort denominated reclamation. Land reclamation aims to restore the pre-mining ecosystem services (i.e. the presence of goods provisioned by soil including biogeochemical and hydrological cycles, biodiversity and climate regulation) within a replacement ecosystem, which implies a repurposing of the landscape (Foley, 2005; Powter et al., 2012). In a mining context, reclamation has components of regrading to fill holes and remove hazardous cliffs or high walls, replacement or rebuilding of minimal surface soils, and re-vegetation (Lima et al., 2016; Munro, 2006), although not necessarily with the original species (Munro, 2006).The ultimate purpose of reclamation is ensuring the continued beneficial use of land resources (Foley, 2005; Powter et al., 2012). Reclamation thus involves returning the productivity of the land with some measures of biotic function and sustainability.

To achieve these end-point goals, reclamation requires the establishment of stable nutrient cycles, plant growth, and the accompanying ecosystem services (Sheoran et al., 2010). Soil is the basis for these biological functions and as such its composition and structure plays a crucial role ensuring the future stability of the reconstructed ecosystem (Dominati et al., 2010). During surface mining practices, soil properties and structure are drastically altered, such that the rearranged soil developed from the disrupted soil mixed with fragmented rock

and earthy material no longer resembles the source material and can be defined as minesoil or Technosol (Ahirwal & Maiti, 2018). These soils can vary depending on local conditions (e.g. geology, climate, land-use), which largely influences the future orientation of reclamation. Reclamation strategies must consequently address important indicators of functionality such as soil structure, soil fertility, microbial communities, and nutrient cycling in order to ensure a self-sustaining ecosystem (Feng et al., 2019; Sheoran et al., 2010).

1.3. The Athabasca Oil Sands Region

1.3.1. Regional overview

The Athabasca oil sands region (AOSR) covers an area of approximately 142,200 km^2 in northeastern Alberta and is located in the boreal forest ecosystem of Canada (Alberta Energy, 2017). The boreal forests of Alberta constitute about 58% of the total land area, mostly situated in the northern Alberta and some southerly extensions as far as Calgary (Bliss et al., 2015; Downing & Pettapiece, 2006). The relatively undulated landscape allows for the development of upland forests and lowland wetlands (Beckingham & Archibald, 1996), which are considered two different ecotypes. In particular, the uplands in the AOSR are (i) mixed-wood forests dominated by either trembling aspen (*Populus tremuloides)* or a combination of trembling aspen and white spruce (*Picea glauca*), or (ii) Jack pine (*Pinus banskiana)* forest stands. Lowland areas with poor drainage, develop as peatlands that support black spruce (*Picea mariana*) fens and bogs (Beckingham & Archibald, 1996).The main component of peatlands is peat, which is derived from partially decomposed plant matter accumulated in the water-saturated anaerobic environment (Warner & Asada, 2006). In bogs, the partially decomposed organic matter is characterized mostly by *Sphagnum*

mosses with woody remains of the ericaceous shrubs; whereas fens are characterized by cyperaceous and brown moss peats (Warner & Asada, 2006). The anaerobic conditions of the water-saturated peat slow the microbial decomposition of the existing organic matter, allowing the accumulation of new organic matter (Waddington & Roulet, 2000). As a result, the long-term water-saturation of the peatlands is a key factor regulating the physico-chemical characteristics of these sites, and thus the microbiological structure. The slow rate of organic matter decomposition results in peat having different soil chemistry than upland boreal forest soils.

1.3.2. Climate

The AOSR is relatively dry and found in the mid-boreal. The mean annual temperatures are -2°C to +1°C. The long, cold winters average -18°C to -14°C, while the short summers are somewhat warmer, averaging +13°C to +15°C (Johnson & Miyanishi, 2008). During June and July there is an extended period of daylight of over 17 hours (Fung & Macyk, 2000) and a frost-free period ranging from 95 to 140 days depending on the topographic location (Macyk et al., 1998). Average annual precipitation is 300 mm to 600 mm with about 70% as wet precipitation. Average annual rainfall is 305 mm and average snowfall is 150 cm. Potential evapotranspiration is a relatively constant 450 to 500 mm, while precipitation fluctuates from year to year (Johnson & Miyanishi, 2008). Given that peak precipitation happens between June and August when the vegetation is actively growing and transpiring, there is little saturated overland flow (Devito et al., 2005).

1.3.3. Soils

Mineral soils in the Boreal Plain are classified as Luvisolic (Boralf) soils. These soils develop under mixed deciduous and coniferous vegetation. Their parent material is typically well supplied with base cations, such as calcium and magnesium, and have loamy or clay dominated soil textures in the eluvial (Ae) and textural (Bt) horizons (Fung & Macyk, 2000; Turchenek & Lindsay, 1982). Among these soils, those developed in sandy deposits are classified as Brunisolic (Cryochrept and Drystrochrept) with a pH between 5.5 and 6.5. Gleysolic (Aquents, Aquepts, Argiaquolls) soils are poorly drained mineral soils which develop due to the influence of waterlogging for extended periods. Soils found in lowland regions at the AOSR include: Regosolic (Entisols) soils which have very weak or no profile development; organic (Histosol) soils derived predominantly from decomposition of vegetation; and Fibrisol (Fibrist), Mesisol (Hemist), and Humisol (Saprist) soils with a undecomposed, intermediate decomposed and advanced decomposed fibric organic material, respectively. In addition, there are Cryosolic (Pergelic) soils derived from organic deposits overlaying permafrost (Fung & Macyk, 2000; Turchenek & Lindsay, 1982).

1.3.4. Vegetation

The landscape in northern Alberta includes a wide range of age classes and stand types resulting from the combination of frequent natural disturbances and edaphic factors (Dhar et al., 2018). In the AOSR, the average age of upland forests is variable due to the frequent wildfires, which is the primary natural disturbance in this system. Insect attacks, wildlife browsing, root and stem diseases, forest harvesting and land clearing for agriculture can also be potential disturbances and thus, influence the plant community composition in this

region (Bergeron et al., 2014; Chávez & Macdonald, 2010; Hart & Chen, 2008). Following wildfires, the re-establishment of plant species is facilitated by (i) the arrival of plant propagules from adjacent undisturbed areas, (ii) seeds or rhizomes of many species (e.g. *Comptonia peregrine*, *Prunus pensylvanica*, *Pteridium aquilinum*, *Rubus idaeus*) that remain dormant for up to 100 years (Whittle *et al.,* 1997) and grow rapidly in response to increased availability of resources following fire, and (iii) the regeneration from vegetative materials (Rydgren et al., 1998). Fast-growing, shade intolerant species (e.g., *Populus tremuloides. Betula papyrifera,* and *Pinus banksiana*) are found following wildfire disturbances (Bergeron et al., 2014) . The understory vegetation is characterized mostly by shade intolerant (e.g. *Chamaenerion angustifolium, Rubus idaeus, Vaccinium myrtilloides*, *Salix* spp.*)* and somewhat shade tolerant species (e.g. *Solidago* spp, *Pteridium aquilinum*, *Calamagrostis canadensis).* . Plant diversity shows a peak during the first 30 to 40 years after disturbance and declines thereafter (Chipman & Johnson, 2002; Rees & Juday, 2002). Nevertheless, communities dominated by *P. tremuloides* and *Pinus* spp. show higher diversity and cover of understory vegetation when canopy gaps develop (Chávez & Macdonald, 2010; Cumming et al., 2000).

In addition to the natural processes of succession, the climate and hydrology in the AOSR also shape the plant community composition (Johnson & Miyanishi, 2008). Generally, hillslopes (upland) that are part of the landscape are affected by the factors of contributing area, slope angle, and substrate transmissivity creating a moisture gradient in which vegetation establishes based on tolerance to moisture (Johnson & Miyanishi, 2008). Thus, on nutrient-rich hillslopes, dry hilltops have a mixture of trembling aspen (*P. tremuloides)*

and white spruce (*P. glauca*), midslopes have white spruce and balsam fir (*Abies balsamea)*, and wet basal slopes have black spruce (*P. mariana*). In contrast, nutrient-poor and better-drained hillslopes exhibit dry hilltops dominated by Jack pine (*P. banskiana*), whereas the basal slopes are dominated by black spruce. However, actual patterns are often more heterogeneous given the influence of past disturbances, variable substrate and groundwater flow patterns (Hart & Chen, 2006). Lowland is poorly drained and normally covered by peat (*Sphagnum spp.*). Shrub fens are dominated by willows (*Salix spp.)* and sedges (*Carex spp.)*, and forest fens by tamarack (*Larix laricina)* and black spruce. Bogs usually occurring as islands in large fens or small potholes, are dominated by short black spruce and moss (*Sphagnum*) (Johnson & Miyanishi, 2008). ~64% of the AOSR is covered by peatlands (Rooney et al., 2012).

1.3.5. Microbiota

There have been few studies of the native microbiota in the AOSR. Most microbial studies have focused on the tailing ponds resulting from the processes of oil exploitation (Harner et al., 2011). The few studies focused on soils have shown Proteobacteria, Actinobacteria, Bacteroidetes and Acidobacteria are the most abundant bacterial phyla in soil and the rhizosphere of plants growing in this ecosystem (Masse et al., 2017; Stefani et al., 2018). Furthermore, members of the phyla Verrucomicrobia, Planctomycetes and Firmicutes have been identified in these soils (Masse et al., 2017; Stefani et al., 2018). The proportion of bacterial orders belonging to the most abundant phyla has indicated an overrepresentation of Rhizobiales, with most of the identified members belonging to the family Hyphomicrobiaceae (Masse et al., 2017). Members of this family are considered important players in the N cycle since they can utilize N_2, NO_3^-, or NH_3 under anoxic conditions

(Anderson et al., 2011; Masse et al., 2017). In addition to Rhizobiales, the orders Planctomycetales, Acidobacteriales, the subgroup 3 from Acidobacteria, and Rhodospirillales have been identified as the most abundant orders; however, their potential ecological roles remain undetermined (Masse et al., 2017).

Bacteria in these soils have been associated with an oligotrophic lifestyle, and their diversity is influenced mainly by certain species of plants such as mosses (e.g. *Polytrichum juniperinum, Lycopodium obscurum)*, deciduous trees and shrubs (e.g. *Pinus banskiana*, *Salix bebbiana*), highlighting the importance of the aboveground-belowground relationships in these soils (Masse et al., 2017). Another study showed that bacteria that thrive inside plants (i.e. endosphere) as well as bacteria in soil associated with plant roots (i.e. rhizosphere) vary considerably across the AOSR. This variability occurs because of the different rhizo-compartments (rhizosphere and endosphere) and host plants (Mitter et al., 2017). Moreover, the same study indicated that other phyla such as Gemmatimonadetes and Acidobacteria are present and restricted to the rhizosphere, whereas Proteobacteria dominates the endosphere.

The taxonomic profile of fungi in AOSR soils indicates dominance of the phyla Agaricomycetes and Leomycetes in both soils and roots of plants in the AOSR. However, fungal members from the phyla Zygomycetes, Pezizomycetes, Euromycetes, Sordariomycetes and Dothideomycetes have been observed in these soils too (Stefani et al., 2018). Moreover, a survey of fungi revealed a presumed dominance of ectomycorrhizal sequence types in natural forest stands, with preliminary indications that *Piloderma sp*., and

perhaps some saprotrophs like *Hydnellum sp*., might be abundant (Dimitriu et al., 2010).The ecological functions predicted for the fungal communities based on ITS gene have demonstrated that the majority of fungal sequences in soils and the rhizosphere at the AOSR are ectomycorrhizal fungi. However, saprotrophs and root-associated fungi are also dominant in the rhizosphere; and yeasts and molds are dominant only in soils (Stefani et al., 2018).

The functional profile of the microbial communities in the AOSR indicates high rates of activities related to N cycling (McMillan et al., 2007). Specifically, N mineralization rates are within the range of 5.3 to 25 mg N $m^{-2}d^{-1}$, while the relatively high rates of ammonification and nitrification are within the range of 3.5 to 13 mg N $m^{-2}d^{-1}$ (McMillan et al., 2007). The most abundant Archaea in the AOSR soils are members of the terrestrial group of the phylum Thaumarchaeota (Masse et al., 2017). Further, the archaeal phylum Euryarchaeota was identified in AOSR soils previously subjected to wildfire (Masse et al., 2017).

Together, these studies indicate a general microbial profile of the AOSR, however, it is important to consider that several factors may be constantly shaping the microbial community structure of these soils. In particular, it has been observed that boreal forests with similar vegetation harbor microbial communities that display spatial dependency at regional (<350 km) (Bach et al., 2009) and within-plot (<1 km) (Bach et al., 2008) scales. Also, environmental factors such as pH and presence of woody debris influence the AOSR soil microbial community composition (Dimitriu et al., 2010).

1.3.6. Oil-sands deposits

The AOSR is located in the Boreal Plain of western Canada, a relatively flat (400–800 m asl) region that was covered by the Laurentide ice sheet 10,000 to 12,000 years ago (Johnson & Miyanishi, 2008). The surficial glacial deposits are deep (30 – 200 m), formed primarily by loam and gravel–sand, and originating from glaciofluvial and lacustrine deposits. These deposits overlie Mesozoic and Cenozoic age sedimentary rocks (largely carbonate) that comprise most of the bedrock and contain the oil deposits (Johnson & Miyanishi, 2008). These oil deposits are distributed in the boreal forests of Alberta in three major reserves: the Athabasca, Cold Lake and Peace River reserves cover about 142,200 square kilometers of northwest Alberta or 23% of the Province (Alberta Energy, 2017). These three deposits contain an estimated economically viable proven reserve of about 1.7trillion barrels (1 barrel = 159 liters) of crude bitumen: a sticky, tar-like form of crude oil (Alberta Energy, 2017; Harner et al., 2011). The largest of the three, the Athabasca deposit, contains 700 billion barrels of bitumen in-place and it is the only deposit in the province where the deposit is sufficiently close to the surface to allow recovery through surface mining (Fung & Macyk, 2000). The other deposits are exploited through *in situ* extraction methods, such as steam-assisted gravity drainage (SAGD; (Johnson & Miyanishi, 2008).

1.4. Mining operations in the AOSR

1.4.1. Surface mining

Within the AOSR, 4750 km^2 of land have been leased for oil sands surface mining and as of 2017, active operations have led to a disturbance footprint of more than 895 km^2 within this region (Alberta Energy, 2017), which corresponds to an average land-use intensity of one

hectare per 100,000 barrels (Grant et al., 2013). Unlike relatively rocky or unproductive hilly areas where surface mining is more common and soil development is much more limited, mining operations in the AOSR require the removal of boreal forest ecosystems, which consist of upland mixed woods and lowland bogs and fens (Downing & Pettapiece, 2006). Generally, when the deposit thickness is less than 45 m below the forest soil or peat bog and overburden (surface material) layers —such as the Athabasca deposit—the oil sands are recovered through surface mining (Fung & Macyk, 2000; Johnson & Miyanishi, 2008). However, deposits situated deeper than 45 m are considered uneconomical for open-pit mining and hence are extracted *in situ* by methods such as steam assisted gravity drainage (SAGD) and cyclic steam stimulation (CSS) (Johnson & Miyanishi, 2008).

The process of surface mining involves the physical removal of large volumes of soil during open-pit mining (Munro, 2006). During excavation, all aboveground vegetation from the topsoil is removed ahead of the mining path and where possible, the topsoil itself and the underlying organic layers are stripped for later use in reclamation (Audet et al., 2015; Fung & Macyk, 2000). After removing and either storing or reusing the topsoil, the next up to 50 m of overburden is removed to allow mining of the underlying crude bitumen sands, which are then transported to the processing plants (Audet et al., 2015; Fung & Macyk, 2000). When deposits are deeper than 45 m, *in situ* oil recovery technology is used. In both SAGD and CSS, steam is injected into the reservoir, causing the bitumen to heat up and liquefy, and then the bitumen emulsion is pumped to the surface and sent to separation plant, where the water is removed from the bitumen (Johnson & Miyanishi, 2008; Oil Sands Magazine, 2017). 12 tons of oil sands are required to produce 1 m^3 of bitumen.

Further, for mining and SAGD methods, the process requires approximately 2 - 4.5 m^3 and 0.2 m^3 of water and 125 m^3 and 214 m^3 of natural gas, respectively (Alberta Energy Regulator, 2017; National Energy Board, 2006). Less water is used for *in situ* methods than for surface mining because 90-95% of steam water can be recycled (Grant et al., 2013; Johnson & Miyanishi, 2008). However, the open-pit mining method in the AOSR currently represents the major source of bitumen from the oil sands (Audet et al., 2015).

The bitumen extracted in both mining and *in situ* methods is a naturally occurring viscous combination of hydrocarbons contained within the oil sands and the most referenced "oil" from Alberta. Mostly, it is found in the intergranular spaces of a mixture of sand grains with silt, clay and water (Fung & Macyk, 2000); and in its natural state is not recoverable at a commercial rate through a well (Oil Sands Magazine, 2017). This form of petroleum is heavier than water (i.e. API gravity <10), hence it cannot be refined into the normal petroleum fractions (Oil Sands Magazine, 2017). However, crude oil blends derived from the oil sands can be produced by removing carbon and sulfur and adding hydrogen to obtain "synthetic crude oil" that can be sold to conventional refineries (Fung & Macyk, 2000; Oil Sands Magazine, 2017). SO_2, NOx, and volatile organics are released as a consequence of the chemical processes for converting bitumen to synthetic crude oil (Alberta Energy Regulator, 2017; Johnson & Miyanishi, 2008). Bitumen concentrations in the sands can vary within a deposit, reaching a maximum of 18% by weight. Anything more than 10% bitumen is considered rich oil sand, from 6 to 8% marginal, and less than 6% is rejected material and is not mined (Fung & Macyk, 2000). The overburden layers as well as the low bituminous content sands contain mostly sand, in addition to shale, silt, and

clay; therefore, they are frequently used as construction materials in roads, embankments and tailing dykes when they are not utilized to reconstruct the surrounding land (Macyk et al., 1998; Oil Sands Magazine, 2017).

During the oil sands extraction process, the bitumen is combined with water at 79-83 °C and caustic soda to separate the oil from other components including clay, sand, dissolved metals, and organic compounds such as polyaromatic hydrocarbons (PAHs), and naphthenic acids (NAs) (Giesy et al., 2010). The oil sands extraction process requires large volumes of water for extracting the bitumen from the oil sands (Grant et al., 2013; National Energy Board, 2006); the resulting oil sands process water is stored in on-site tailings ponds (Giesy et al., 2010). These ponds contain the potentially toxic by-products of the oil extraction, currently cover 176 km^2 of the landscape and contain 830 million m^3 of the tailings waste (Grant et al., 2013). In addition to this legacy, the oil sands extraction processes are a major source of greenhouse gases (GHGs) due to the large consumption of energy derived from natural gas, diesel fuel and coal-based power. The production of oil from bitumen emits higher amounts of GHGs than conventional oil production and is the fastest growing source of climate change pollution in Canada (Grant et al., 2013; National Energy Board, 2006). Similarly, oil sands extraction is a major source of SO_2, NOx, and volatile organics (Alberta Energy Regulator, 2017; Grant et al., 2013). While there have been some efforts in minimizing the production of these gases per produced barrel, the estimated absolute values from the growing industry will still impact the air quality for communities living in this region (Grant et al., 2013; National Energy Board, 2006).

1.4.2. Land reclamation

The *Conservation and Reclamation Regulation* of Alberta outlines the requirements regarding land reclamation that oil companies must achieve following surface mining (Government of Alberta, 1993). Accordingly, land reclamation in Alberta must be based on the concept of equivalent land capability (ELC), which requires that the disturbed site be returned to conditions that support various land uses similar to pre-disturbance conditions (Government of Alberta, 1993; Oil Sands Research And Information Network, 2011; Powter et al., 2012) though not necessarily identical (Government of Alberta, 1993). Final assessments of the physical, chemical and biological characteristics of the land, including drainage, topography, hydrology, soils and vegetation should be conducted to determine whether the land reflects pre-existing conditions (Government of Alberta, 1993). In practical terms, ELC in the oil sands mines of northern Alberta refers to establishing a safe, stable, and non-polluted forest for commercial forestry and associated land uses, and for conditions that support the wildlife habitat found in the ecosites of central mixedwood forests (Oil Sands Vegetation Reclamation Comittee, 1998). While guidelines based on ELC have been developed to facilitate the establishment of a starting point and the evaluation of land capabilities encompassing similar attributes of the pre-disturbance environment (Cumulative Environmental Management Association, 2006; Oil Sands Vegetation Reclamation Comittee, 1998), reliably determining whether the reclaimed ecosystem is moving toward the "natural" analogues can be challenging (Audet et al., 2015). Similarly, deciding whether sites require additional intervention based on the trajectory can also be ambiguous and can represent additional costs (Jackson & Hobbs, 2009). Thus, ELC continues to be a source of significant debate when discussing reclamation and alternate land use options (Powter et al., 2012).

To date, only 0.1% of land disturbed by oil sands mining have been "certified reclaimed" and returned as public land, with another 10% being "under reclamation" and the remaining 90% being disturbed directly by or ahead of mining activities (Alberta Energy, 2017). The differences in soil organic matter composition, soil available N, and microbial communities between reconstructed oil sand soils and natural boreal forest soils of northern Alberta (Dimitriu et al., 2010; Masse et al., 2017; Rowland et al., 2009; Stefani et al., 2018), suggest land-reclamation and conservation frameworks that necessarily target the return of the post disturbance landscape to its predisturbance condition may not always be practical or even possible (Doley et al., 2012; Doley & Audet, 2013). It is therefore likely that novel soil ecosystems will arise from the reconstruction efforts (Chazdon, 2008; Hobbs et al., 2006).

While the post-mining ecosystems may not fully comply with the current legislative framework, the ecosystem services provided by these new landscapes can be both valuable and desirable. Soil reclamation efforts aimed at reestablishing soil functions, rather than simply trying to replicate structural qualities of the previous soil ecosystem, are now seen as key to ensure the long-term sustainability of reclaimed boreal forest landscapes (Audet et al., 2015; Quideau et al., 2013). By reinstating ecological functions, reclamation strategies bring about an enhancement in soil quality, the development of pedogenic processes, and the restitution of soil organic C (SOC) pools, all of which ultimately support revegetation and the ecological resilience for future environmental changing conditions (Dimitriu et al., 2010). The rapid expansion of oil sands exploitation leads to significant modifications to

the natural landscape; the relatively minor progress achieved on land reclamation to date highlights the importance of finding frameworks for assessing reclamation success and improving the outcomes of these practices.

1.4.3. Soil materials used in reclamation

One aspect of reclamation regulation in Alberta is that soil materials must be salvaged and conserved during land clearing for reclamation (Oil Sands Research And Information Network, 2011). Depending on reclamation objectives, these conserved soil materials, soil amendments, vegetation planting programs, and follow-up adaptive management can be employed to accelerate the reclamation process (Alberta Environment and Sustainable Resource Development, 2013).

Ongoing reclamation efforts to reconstruct forest land first involve reconfiguring landforms from overburden or tailing materials. These landforms are then covered with cover soils rich in organic matter from either upland or lowland origin (Audet et al., 2015). Most reclamation in the post mining areas has been completed with two main soil types: peat-mineral mix (PMM), which originates from lowland peat soils, and forest floor-mineral mix (FFM), which originates from forest floor soils(Errington & Pinno, 2015). These soil types have very different physical and chemical properties (see below). In some cases, strategies utilizing combinations of PMM, FFM, and other organic amendments have also been tested (Dietrich et al., 2017; Mackenzie et al., 2014; Pinno et al., 2016).

1.4.3.1. Peat-Mineral Mix

Over half of the area in the mineable sites at the AOSR is lowland peat bogs and fens (Rooney et al., 2012). Therefore, the peat salvaged from lowland sites is abundant and

available for reclamation practices. As a result, it has been utilized as a capping material for the majority of reclamation operations at both lowland and upland sites, and presumably will be the most commonly used reclamation soil in the future at most mine sites (Pinno et al., 2016; Rooney et al., 2012). During reclamation operations, the salvaged surface peat is mixed with underlying mineral soil material having a loam or coarser texture to produce a cover soil, denominated peat-mineral mix (PMM), which is then distributed as the top layer of the post-mining refilled landscapes.

PMM has high organic matter content, high water holding capacity, and a greater potential for natural seedling establishment when compared to the boreal forest soil (Archibald, 2014; Errington & Pinno, 2015), which in turn has led to the establishment of weedy plant species not normally found in the upland boreal forests (Archibald, 2014). Low plant species richness and diversity have been recorded in this type of soil cover. As well, PMM tends to have more bare ground, grasses, and forbs, but less moss, lichen, shrubs, trees, or woody debris than natural forests (Mackenzie & Naeth, 2010; Rowland et al., 2009). The nutrient profile of PMM has indicated a high availability of nitrate, calcium, magnesium and sulfur, while ammonium, P, K and Mn availability is low, relative to natural boreal forest soils (Errington & Pinno, 2015; Rowland et al., 2009).

Microbial activity is relatively low in PMM relative to sites reclaimed with FFM or natural sites (McMillan et al., 2007). Sites reclaimed with PMM have lower microbial biomass carbon, microbial biomass nitrogen, net ammonification, and net organic matter mineralization rates than natural forest sites (McMillan et al., 2007). Microbial extracellular

enzyme activities involved in C, N, and P cycling processes are lower overall in reclaimed sites capped with PMM (Dimitriu et al., 2010). The microbial community structure in PMM differs from that of natural sites, with Gram-negative bacteria dominating in PMM sites and fungi more abundant in natural sites. There is significant regional-scale spatial structuring within reclaimed sites with PMM (Dimitriu et al., 2010), including differences in overall diversity (i.e. α-diversity) and between site diversity (i.e. β-diversity). Fungal-to-bacterial-biomass ratio (Dimitriu et al., 2010; MacKenzie & Quideau, 2010) and total microbial biomass (MacKenzie & Quideau, 2010) largely explains most of the community structure variability. The bacterial communities in PMM are significantly less diverse than FFM and natural sites; and the bacteria in PMM are more associated with a copiotrophic lifestyle (Masse et al., 2017; Stefani et al., 2018). Fungal communities are different between sites reclaimed with PMM and natural sites; specifically, mycorrhizal taxa are poorly represented in PMM (Stefani et al., 2018). However, the fungal taxon richness is comparable to natural sites (Stefani et al., 2018). Moreover, microbial community composition correlates with fluctuations in nitrogen and boron availability, suggesting linkages between microbial communities function and soil nutrient availability (MacKenzie & Quideau, 2010).

Overall, the lowland-derived PMM is a capping material characterized by high content of organic material and high water holding capacity, which promotes the establishment of weedy species. The microbial communities are less diverse than those in natural boreal forest soils and their activity related to C, N, and P is also relatively low.

1.4.3.2. Forest Floor-Mineral mix

Salvage operations strip the forest floor layer from pre-mined upland areas, mix it with underlying mineral soil and the resulting forest floor-mineral mix (FFM) is utilized in reclamation practices (MacKenzie et al., 2012). FFM contains a high density of viable propagules and contains numerous plant species ecologically suited to growth in upland forests (Mackenzie & Naeth, 2010). Soils reclaimed with FFM have a higher overall plant cover, higher plant species richness, higher cover of native plant species, and higher woody plant density than soils reclaimed with PMM (Archibald, 2014). Furthermore, FFM has a low carbon:nitrogen ratio, which may indicate a high potential for mineralizable nitrogen, as well as high levels of available phosphorus and soluble potassium. This higher level of nutrient availability may ultimately reduce the dependency on fertilization relative to reclamation with PMM (Mackenzie & Naeth, 2010). The water holding capacity of FFM is lower than PMM. FFM supports higher levels of initial biodiversity and biomass in the plant community than PMM (Archibald, 2014; Leckie et al., 2004).

FFM has higher gross nitrification rates than natural forests and PMM sites, but comparable levels of microbial respiration, microbial biomass carbon, and microbial biomass nitrogen relative to PMM and natural sites (McMillan et al., 2007). In contrast to PMM, there are few studies of FFM microbial community composition. Bacteria α-diversity in FFM is comparable to natural sites, but β-diversity differs between FFM and natural sites. The community profile of FFM resembles PMM more than the natural sites profile, yet still differs from PMM (Stefani et al., 2018). On the other hand, fungal FFM richness is higher, and the fungal taxa that dominate FFM are significantly different from, either the natural or

PMM sites, ultimately showing that fungal communities in FFM are different from those in PMM and natural sites (Stefani *et al.,* 2018).

As FFM contains a higher number of plant propagules and microorganisms than salvaged peat material (Mackenzie & Naeth, 2010), in addition to a community profile that resembles more the natural sites profile, when possible, the use of FFM is preferred as soil cover in reclamation operations. However, its availability is limited; only approximately 35% of the land can be reclaimed with this soil type under current practices (MacKenzie et al., 2012).

1.4.3.3. Combinations FFM-PMM

Reclamation soils are usually redistributed along relatively large, homogenous areas designated for a single soil type. However, these large areas may not optimize the usage of FFM considering its paucity (Pinno et al., 2016). Consequently, new strategies for maximizing the limited supply of FFM have been developed. For instance, (Béasse et al., 2015) investigated the outcome of mixing equal parts PMM and FFM for use as a reclamation substrate, which resulted in a soil microbial community more similar to that of forest floor material than to peat. Thus, based on soil microbial community composition, using a FFM:PMM mixture instead of pure FFM could increase the volume of material that is more analogous to an upland forest community. A ratio of 1:1 FFM:PMM was the optimum admixture for tree growth, foliar nutrients and available nutrients to approximate natural forest soils (Dietrich et al., 2017).

In addition to combinations of soil covers, a spatial approach has been tested (Pinno et al., 2016). In this study, the reclamation design established islands or patches of differing sizes and shapes of FFM within a matrix of the more abundant PMM. This spatial pattern revealed patch sizes of at least 671 – 960 m^2 better allowed the establishment of native plant species, and in particular woody species. However, smaller patches favored non-native weedy species. The overall results suggest this strategy might be applicable in the AOSR, but further analysis of other characteristics of the land such as microbial communities and soil properties remain to be determined.

1.4.3.4. Amendments

Current reclamation practices often include the addition of fertilizers or other materials to improve the soil quality (i.e. amendments) to place the reclamation on a faster trajectory to ecosystem recovery (Hahn & Quideau, 2013). For example, a controlled release fertilizer promotes a greater tree growth performance than immediate release fertilizer on a PMM capping material (Sloan & Jacobs, 2013). *Populus tremuloides* (trembling aspen) performs better in reclamation soils amended with organic mineral material that slowly releases nutrients when compared with direct fertilizer amendments (Pinno et al., 2012). The fertilizers utilized in reclamation soils generally consist of a mixture of N, P and K and are produced commercially by third parties outside the oil sands mining operations.

Charcoal that is produced by pyrolysis of biomass (i.e. biochar) influences the biogeochemistry of the soils and is found in the boreal forest settings where fire is the primary natural disturbance regime (DeLuca et al., 2015; Hicke et al., 2003). Several

studies have delved into the potential uses and benefits of biochar for use in mine reclamation (Dietrich et al., 2017; Kirby, 2017; Mackenzie et al., 2014). The microbial diversity and richness in FFM and PMM increases following biochar amendments, while only the bacterial community structure in FFM responded (Mackenzie et al., 2014). Biochar additions also improved similarity of PMM to FFM in terms of tree growth, foliar nutrients, and available nutrients (Dietrich et al., 2017). However, the addition of biochar does not shift the PMM microbial community structure or function to be more similar to FFM (Kirby, 2017). It is important to note that the positive effects of biochar or other black carbon produced from the incomplete combustion of organic matter (i.e. pyrogenic carbons) remain to be tested in field trials before recommendations for reclamation protocols can be done.

1.4.3.5.Reference soils—Natural boreal forest soils

In the AOSR, where land-use ELC has to be reinstated, reclamation frameworks are intended to ensure ecosystem processes will support the production of forest goods and services of equal quality and quantity to those prior disturbance (Audet et al., 2015; Government of Alberta, 1993). Soils ultimately support these processes, thus, an approach often employed is to have undisturbed boreal forest soils (NS) as a benchmark comparison to assess the outcome of reclamation (Audet et al., 2015; Quideau et al., 2013; Rowland et al., 2009). The P and K levels in NS are higher than those in reclamation soils, whereas the S level is lower (Mackenzie & Naeth, 2010). The total nitrogen is comparable in NS to both FFM and PMM, though the organic carbon is only comparable to FFM. Furthermore, both soil pH and moisture content of NS is lower than those in both PMM and FFM (Errington & Pinno, 2015; Masse et al., 2017). The NS plant community consists of coniferous and

deciduous trees, shrubs, moss and lichens; and in less abundance by bare ground, grasses and forbs, thus an abundance of woody debris is present (Archibald, 2014; Errington & Pinno, 2015).

NS have higher levels of microbial biomass carbon, and higher rates of net mineralization and net ammonification, than those of the reclaimed sites (McMillan et al., 2007). Further, natural sites have a different microbial community structure than reclaimed sites. These differences are largely due to the higher nitrogen and woody debris availability in native boreal forest soils, in addition to the distinct soil pH. Furthermore, these communities are associated with an oligotrophic lifestyle. The fungal component of the natural site microbiota is dominated by ectomycorrhizae (Dimitriu et al., 2010). Similar to bacteria, the fungal communities do not resemble the communities found in capping soils, although the fungal community in FFM more closely resembles that in NS than does PMM (Masse et al., 2017; Stefani et al., 2018; H. Sun et al., 2014).

1.5. Impacts of reclamation following oil sands exploitation

Surface mining in the AOSR (i) disturbs the landscape, (ii) alters the quality of water required to sustain freshwater ecosystems and the life that depends on it (i.e. environmental flows), (iii) alters the capability of the habitat to support and maintain a balanced ecosystem comparable to state before disruption (i.e. habitat integrity) and, (iv) alters the capacity of ecological processes to regulate the fluxes of energy, nutrients and organic matter through an environment (i.e. ecosystem function) (Rooney et al., 2012). Increasing evidence suggests that post-disturbance landscapes contain new and possibly even irreversibly different aspects of the abiotic and biotic components of the ecosystem (Audet et al., 2015;

Perring et al., 2014). While current reclamation practices focus on restoring sites to closely resemble the surrounding undisturbed ecosystem, reconstruction efforts likely lead to the emergence of novel ecosystems (Dhar et al., 2018; Dimitriu et al., 2010; Hahn & Quideau, 2013; Mummey et al., 2002; Quideau et al., 2013; Stefani et al., 2018). These novel soils do not appear to provide the full range of ecosystem services (e.g. biogeochemistry cycles, diversity) provided by the pre-disturbance condition. Both abiotic and biotic services of the reclaimed soils differ and are less extensive than for undisturbed boreal forest soils (Quideau et al., 2013).

1.5.1. Effects of reclamation on the soil abiotic properties

The SOM quality from reconstructed soils differs significantly from the range of natural variability (Quideau et al., 2013; Turcotte et al., 2009). A post-mining landscape is expected to support less than 35% of the peatlands present before disturbance (an area of 12,414 ha in current mining operations) (Rooney et al., 2012). Replacement of these peatlands with reclaimed soils will result in the loss of 4.8 – 19.9 million tons of stored carbon (Rooney *et al.,* 2012). If scaled up to the whole minable area, the loss of stored carbon would be 11.4 - 47.3 million metric tons, which converted to CO_2 equivalents, would result in the release of 41.8 and 173.4 metric tons of CO_2. Additionally, the replacement of peatland under the current approved area will result in the loss 2,408 – 3,041 metric tons of annual carbon sequestration potential, which scaled up equates to 5,734 – 7,241 metric tons C/y (21,025 – 26,550 t CO_2/y) carbon sequestration potential lost. Taken together, the reclaimed landscape will release carbon as much as 7 years' worth of mining and upgrading emissions at 2010 production levels, and sequester carbon at a much lower rate (Rooney et al., 2012).

Nitrate, calcium, magnesium, and sulfur in reclamation soils are generally higher than in the natural forest ecotypes, while ammonium, P, K, and Mn are generally lower; implying that sites reclaimed with PMM should be fertilized with P, K and Mn to provide an early boost to ecosystem development (Rowland et al., 2009). Without these amendments, these soils are not likely to be capable of supporting ecosystem processes that mimic those of natural boreal forest (Rowland et al., 2009). Nevertheless, even when fertilizers were added to the reclamation cover soils, available nutrient pools in soil solution and in foliar tissue are significantly different between novel reclaimed sites and NS (Hogberg et al., 2020). Reclamation soils have lower levels of essential nutrients such as total C and N, and lower pH, clay content and bulk density than undisturbed reference soils (Masse et al., 2017; McMillan et al., 2007; Ngugi et al., 2018). These differences between reclaimed and undisturbed sites have been attributed to the extraction and stockpiling of soil materials and their replacement on the landscape during reclamation (McMillan et al., 2007).

1.5.2. Effects of reclamation on the soil biotic properties

In soils 20 years after reclamation, total microbial biomass, bacterial biomarkers, fungal biomarkers, and microbial biomass C averaged only 20, 16, 28, and 44% of amounts found in undisturbed soils, respectively (Mummey et al., 2002). These findings indicate that surface mining is highly detrimental to microbial populations and that microbial biomass remains significantly reduced in the long-term. In addition, there is localized enrichment of bacterial and fungal biomass near plant bases in reclaimed soils, suggesting relatively poor soil exploration by roots and microorganisms compared to natural ecosystems (Mummey et al., 2002). Furthermore, gross nitrification and ammonification rates are lower in

reclamation soils than natural forest sites. Reclaimed soils also have lower total microbial biomass, microbial biomass C and N, and litter decomposition rates (MacKenzie & Quideau, 2010; McMillan et al., 2007; Rowland et al., 2009). Exoenzyme (i.e. enzymes released into soil by microorganisms) activity is decreased in reclaimed soils, including of β-glucosidase, β-xylosidase, phenoloxidase, peroxidase, phosphatase, chitinase, and urease (Dimitriu et al., 2010). However, reclamation with FFM exhibited a functional profile more similar to natural soils than reclamation with PMM (Howell & MacKenzie, 2017). Taken together, these findings indicate reclamation leads to limited microbial activity caused by soil reconfiguration.

Bacterial community composition (BCC) and fungal-to-bacteria ratios differ in reclamation soils relative to undisturbed native sites, with these shifts correlating with changes in the nutrient profile (Dimitriu et al., 2010; Howell & MacKenzie, 2017; MacKenzie & Quideau, 2010; Quideau et al., 2013). It appears that the fluctuations in nutrient availability caused by soil reconfiguration affect the BCC (MacKenzie & Quideau, 2010). Further, reclamation soil prescriptions can determine microbial community structure. For example, reconstructed soils overlying tailings sand have higher levels of Gram-negative organisms and reduced levels of important mycorrhizal communities (Dimitriu et al., 2010).

The rhizosphere bacterial community structure is significantly affected by overburden, tailing sands and PMM (Ma et al., 2017; Mitter et al., 2017; Stefani et al., 2018). There is low richness and high evenness of bacterial operational taxonomic units (OTUs) in overburden materials and PMM compared to natural sites (Ma et al., 2017; Mitter et al.,

2017; Stefani et al., 2018). Overburden depletes bacterial classes such as Alphaproteobacteria and Thermoleophilia. PMM causes shifts in major taxa, including a reduction in Proteobacteria, Acidobacteria, and Bacteriodetes and increases in Actinobacteria. Notably, these changes in community structure are associated with changes in aboveground communities (Ma et al., 2017; Masse et al., 2017; Mitter et al., 2017) and changes in nutrient supply (Ma et al., 2017; MacKenzie & Quideau, 2010). Therefore, the limited plant root activities and the differences in vegetation composition in these reclamation soils affect the rhizosphere microbial community and may have implications for ecosystem reestablishment in the disturbed oil sands landscape.

Added to these conclusions, the predicted functional profiles of identified taxa have shown significant differences in bacterial community function among reclaimed soils with different capping materials (layer of clean material placed between contaminated oil sands and the cover soil) in both the rhizosphere and the bulk soil. In PMM over tailing sands, pathways for nutrients transportation and metabolism are enriched and this is more pronounced in the rhizosphere, which indicates a strong effect of the rhizosphere in tailing sands shaping the function of the communities (Ma et al., 2017). In contrast, no differences are found between the bulk soil and rhizosphere of PMM over overburden and without capping material, which indicates a reduced rhizosphere effect. In both PMM with and without capping material, the bulk soil was enriched in pathways related to environmental adaptation, replication and repair, and cell motility, potentially indicating unfavorable soil properties (Ma et al., 2017).

Surface mining has significant impacts on bacterial communities in bulk soil. PMM is even more detrimental to the bacterial diversity in soils than in the rhizosphere in relatively new reclamation sites (Stefani et al., 2018). Conversely, relatively old reclaimed sites have shown greater species richness and evenness as compared with undisturbed soils (Masse *et al.,* 2017; Ngugi *et al.,* 2017); however, these communities have been associated with an copiotrophic lifestyle dependent on constant nutrients amendments (Masse et al., 2017). PMM is depleted in Acidobacteria and Verrucomicrobia (Masse et al., 2017; Stefani et al., 2018) and also has a relatively low abundance of bacterial genera that can establish symbiotic relationships important for nutrient cycling such as *Rhizobium* and *Bradyrhizobium* (Stefani et al., 2018).

Changes in the fungal community composition attributed to cover soils have been assessed using high-throughput sequencing (Pec et al., 2019; Stefani et al., 2018). In contrast to bacterial communities, PMM covers exhibit fungal taxon richness and diversity comparable to natural soils and, surprisingly, the soil fungal community in FFM covers had higher taxon richness and diversity than natural sites (Stefani et al., 2018). The taxonomic profile of fungal communities both in bulk soil and the rhizosphere indicated gradual changes in dominance of fungal phyla such as Agaricomycetes and Leotiomycetes. These changes indicate an underrepresentation of ectomycorrhizal fungi in reclamation soils, which highlights that soil reconfiguration could be detrimental for fungal species that contribute to N cycling (Dimitriu et al., 2010). In comparison with natural sites disturbed by either removal of trees or removal of trees and forest floor, soils reclaimed with either PMM, FFM or sandy-subsoil show a greater loss of fungal OTUs related to ectomycorrhizal fungi;

and this is more prominent in sequences belonging to Agaricales, Atheliales and Russulales(Pec et al., 2019). Nevertheless, it is noteworthy that ectomycorrhizal communities are influenced more strongly by host identity and time since disturbance than by cover material used in reclamation (Hankin et al., 2015; Pec et al., 2019). Furthermore, relatively low values of ectomycorrhizal colonization detected in field and growth chamber assays, indicate that the inoculum in reclamation cover soils has less potential than sites disturbed by vegetation removal or mature sites (Hankin et al., 2015); and this does not vary when either FFM or PMM is used as cover soil (Gaster et al., 2015).

1.5.3. Effects on aboveground vegetation

Plant communities that develop following reclamation differ from those prior to disturbance (Latifovic & Pouliot, 2014); however, common patterns can be observed regarding vegetation responses to oil sands. PMM results in decreased overall plant cover and richness of native species and increased colonization of non-native species compared to natural stands (Archibald, 2014; Mackenzie & Naeth, 2010; Naeth et al., 2013). Further, in the long-term (20 years after reclamation), species richness and diversity in PMM differs significantly from the natural forest; FFM has greater similarity to natural forest than PMM, but the species richness and diversity did not stabilize (Pinno & Hawkes, 2015). While the cover and richness of non-native species in PMM shows a declining trend with time, analysis of the species co-occurrence shows a random plant community assembly; which indicates the plant communities remain unstructured, thereby inhibiting the establishment of certain species (Dhar et al., 2018; Pinno & Hawkes, 2015). Reclamation using FFM has shown fewer impacts to the resultant post-mining landscape; however, there is still a significant detriment observed when using this cover type. FFM stockpiling has resulted in

a significant and rapid (for as little as 10 months) decline in seed viability (up to 100%) (Mackenzie et al., 2012). Furthermore, despite the favorable cover and richness characteristics, FFM remains strongly associated with a large proportion of introduced weed species (predominantly *S. arvensis*) and grasses (deBortoli, 2017). At an early reclamation stage, the plant community composition in both FFM and PMM cover soils is different from natural reference sites, even when fertilizers were applied to the reconfigured soil (Errington & Pinno, 2015). Stockpiling of cover soil materials affects the species richness, composition and diversity, for instance, reducing species richness and diversity compared with directly placed cover soil (Buss et al., 2020; Macdonald et al., 2015; Naeth et al., 2013).

1.6. Impacts of vegetation removal on soils prior to excavation

As part of the chain of events occurring during surface mining operations, aboveground vegetation is cleared ahead of the mining path and the soil and underlying layers are stripped for use in reclamation (Audet et al., 2015). However, the timeframe between vegetation removal (a.k.a. clearcutting) and extraction of the cover soils can extend to several years; causing alterations to the forest soils attributable to the vegetation removal.

The more surficial disturbance during vegetation removal causes a less severe impact to the ecosystem than land disturbance linked to surface mining (Hannam et al., 2006; Johnson & Miyanishi, 2008). However, after clear-cutting of the boreal forests of Alberta, the microbial communities are significantly altered (Hannam et al., 2006; Hynes & Germida, 2013; Smith et al., 2008). The microbial community composition in LFH and Ae horizons of soil is immediately changed by vegetation removal (Hynes & Germida, 2013; Smith et

al., 2008), with Gammaproteobacteria and Alphaproteobacteria augmented in response to this disturbance (Smith et al., 2008). Similarly, the total microbial biomass, microbial biomass carbon, and microbial biomass nitrogen decrease immediately and remain lower two years after vegetation removal (Hynes & Germida, 2013; Smith et al., 2008). However, it remains to be established whether the soil microbial community functions are affected by vegetation removal.

Vegetation removal also has a significant impact on soil physical and chemical properties of the LFH soil horizon and, to a lesser extent, the mineral horizons (Hynes & Germida, 2013; Schmidt et al., 1996). Several nutrients including N, P, K, Ca, S and Mn decreased significantly after vegetation removal, indicating a poorer substrate quality (Schmidt et al., 1996). This depletion is likely the result of an initial post-vegetation removal 'flush' of N, P and K attributed to input of woody material and the cessation of plant nutrient uptake (Hynes & Germida, 2013). Overall, the changes in biotic and abiotic properties of soils after vegetation removal have been attributed to the (i) interruption of plant nutrient uptake, (ii) input of clear-cut slash residues and root remnants, (iii) loss of substances released by the plant community, and (iv) mixing of forest floor and mineral soil induced by logging machinery. These shifts have clear and immediate consequences for microbial community structure and function (Attiwill & Adams, 1993; Chanasyk et al., 2003; Grigal, 2000; Hodge & Fitter, 2013).

1.7. Microbial communities as markers of ecosystem recovery/soil quality

The main objectives, tools, and approaches for soil quality assessment have changed significantly over time. The original definition of soil quality relied mostly on the

productivity of the soil in an agricultural context (Bünemann et al., 2018). Currently, the definition of soil quality includes components of (i) the multiple soil processes that underpin the delivery of ecosystem services (i.e. multi-functionality), (ii) the presence of these goods provisioned by soil including biogeochemical and hydrological cycles, biodiversity and climate regulation (i.e. ecosystem services), (iv) the capacity of the soil to remain unchanged when subject to disturbance (i.e. resistance) and (v) the ability of soil to return to a pre-disturbed state after disturbance (i.e. resilience) (Bünemann et al., 2018). Reclamation aims to reestablish key ecosystem services and biogeochemical processes by reinstating ecosystem functions. Thus, to achieve these end-point goals, reclamation strategies should bring about an enhancement in soil quality (Dimitriu et al., 2010).

Microbial communities are potential markers of soil quality as they are crucial in soil functioning (Barrios, 2007). The plethora of functions soil microbes perform include a central role in nutrient and carbon cycling, plant community dynamics, and eco-evolutionary responses of ecosystems to global change (Bardgett & van der Putten, 2014). Nevertheless, biological indicators are still underrepresented in soil quality assessments and mostly limited to broad measurements such as microbial biomass and soil respiration (Harris, 2003; Mummey et al., 2002; Muñoz-Rojas et al., 2016; Waterhouse et al., 2014). These measurements are often interpreted along with abiotic parameters such as pH, nutrients, and cations to establish connections between changes in soil function with changes in the chemical and physical profile of soils, and potential aboveground vegetation performance (Lehman et al., 2015).

Recent advancements in soil biology have promoted the viability of using new technologies as indicators for soil quality assessment (Hartmann et al., 2015; Kumari et al., 2017). The high resolution, accessibility, and rapidity of molecular methods focusing on DNA and RNA have shown a greater potential for integration of microbial community structure and function into soil quality assessment (Bouchez et al., 2016; Thiele-Bruhn et al., 2019). These technologies can be applied in conjunction with or substitute for existing biological and biochemical soil quality indicators in monitoring programs (Hartmann et al., 2015; Hermans et al., 2017). For instance, in a study monitoring soils across Europe, novel molecular methods, including high-throughput sequencing, functional gene measurements and multiple enzyme assays were the top soil quality indicators, with bacterial and archaeal diversity, measured by molecular methods, as the top indicator (Stone et al., 2016). Likewise, in chronosequence studies monitoring the trajectory of reclaimed soils after open-pit mining, high-throughput sequencing methods along with broad abiotic measurements provided useful information regarding the relative progression (Ngugi et al., 2018; Sun et al., 2017). In this case, the compositional changes and the taxonomic transition accompanying chronosequence age, the potential within-community interactions, and the soil factors involved in the transition were all useful factors obtained in the study. Together, these data provide robust information establishing connections between abiotic factors and microbial community structure, which ultimately helps to describe the ecosystem services that are disrupted by disturbance, thereby providing information about the ecosystem integrity.

Soil microbial communities seem to be powerful markers of soil quality and ecosystem restoration (Harris, 2003; Mummey et al., 2002). Microbial communities are good soil quality markers because they are: (i) important for ecosystem function and usually relevant to the objectives of the assessment program, (ii) sensitive to environmental change, (iii) provide a response that can be differentiated from natural variation, (iv) have a low variability in response, and (v) can be aggregated to provide an assessment of the entire system (i.e. integrative) (Andreasen et al., 2001; Dale & Beyeler, 2001; Harris, 2003). Microbial communities can provide a more detailed assessment of soil processes (Bünemann et al., 2018), which can contribute to building knowledge of soil spatial and temporal variability and provide tools to precisely monitor soil quality in a variety of impacted environments (Bouchez et al., 2016; Hermans et al., 2017). However, the applicability of these measurements by site managers can be hampered by the complex nature of the data. The lack of standard operating procedures and accepted threshold values, especially for molecular methods, make comparison and interpretation of results challenging (Harris, 2003). To address this concern, (Harris, 2003) recommends the use of multidimensional ordination, the development of simple indices, and the presentation of a minimum number of incisive measurements; all of which should be set within the context of well-characterized reference target systems. For land reclamation systems, the reference may be represented by a range of variability observed in natural undisturbed forests (Rowland et al., 2009). The distance from the range of natural variability, with variability referring to diversity, vegetation structure, and ecological structure can be used to determine (i) whether a reclaimed system is moving towards or away from the range of natural variability (i.e reclamation trajectory), (ii) how quickly a reclaimed system is

moving towards or away from the range of natural variability (i.e. rate of recovery) and, (iii) the time required for a reclaimed site to closely resemble the range of natural variability (i.e. time of recovery).

1.8. Biodiversity and ecosystem multifunctionality

Biodiversity is a major determinant of community and ecosystem dynamics and functioning (Tilman et al., 2014). While biodiversity is sometimes used to describe species richness (i.e. the number of different species in a system), its definition can also include concepts such as evenness (the relative abundance of species in a system), morphological, functional, and phylogenetic diversity (Purvis & Hector, 2000). However, regardless of the definition in use, biodiversity loss can affect ecosystem functions and services (Bell et al., 2005; Delgado-Baquerizo et al., 2016; Loreau et al., 2001; Yachi & Loreau, 1999). Three principal mechanisms underlie the current understanding of how biodiversity affects ecosystem functioning. First, different species use slightly different resources; so that species-rich communities are more productive because more of the overall resource is used i.e. resource partitioning (Bell et al., 2005; Loreau & Hector, 2001). Second, there is variation in the magnitude of individual species' effects on ecosystem functioning. Thus, species-rich communities are more productive because they have an increased likelihood of containing species with a large effect on ecosystem functioning, i.e. keystone species (Bell et al., 2005; Schulze & Mooney, 1994). Third, multiple species share the same focal functions within the ecosystem. Therefore, more diverse communities are more likely to maintain functional ecosystems given environmental fluctuations, i.e. the insurance hypothesis (Yachi & Loreau, 1999).

Microcosm experiments have shown that reduction in bacterial species, or more specifically functional groups, results in the decrease of bacterial respiration and biomass (Bell et al., 2005; Naeem & Li, 1997) or metabolic activity (Langenheder et al., 2010). Models based on both global and regional-scale surveys assessing the effects of species richness on the expected temporal mean and variance of ecosystem processes have shown: (i) a buffering effect, i.e., a reduction in temporal variance of functionality (or more stability of the functionality through time), (ii) a performance enhancing effect, i.e., an increase in the temporal mean of functionality (or increase in function over time), and (iii) an overall significant contribution of microbial diversity to ecosystem multifunctionality when species richness is higher (Bell et al., 2005; Delgado-Baquerizo et al., 2016; Yachi & Loreau, 1999). The coexistence of multiple distinct taxa or genomes capable of performing the same focal biochemical function is important to provide an "insurance" or a buffer, against environmental fluctuations (Louca et al., 2018). Different species respond differently to disturbances, thus, under changing conditions, the coexistence of multiple distinct taxa or genomes capable of performing the same function (i.e. functional redundancy) is critical for maintaining ecosystem processes (Loreau et al., 2001; Louca et al., 2018). In the boreal region of Alberta, where the most frequent disturbances are wildfires (Audet et al., 2015), the coexistence of multiple different taxa is important to provide a buffering effect and thus, maintain the ecosystem processes after this disturbance (Masse et al., 2017). However, drastic changes in biodiversity imposed by open-pit mining limit the buffering capacity of biodiversity. Human activities will likely reduce the rates at which ecosystem services such as climate regulation and soil fertility are being maintained due to the rapid degradation of these services over time (Delgado-Baquerizo et al., 2016). A good approach

for monitoring reclamation practices in the AOSR is therefore to examine the microbial communities that ultimately are linked to the overall ecosystem processes (Schimel & Schaeffer, 2012).

1.9. Research project

1.9.1. The emergence of novel soil ecosystems

Large-scale mining for oil extraction in the boreal forests of Northern Alberta has led to a disturbance footprint of ~ 900 km^2 of land; which, under regulations from the government of Alberta, must be reclaimed to equivalent land capabilities using soil materials salvaged and conserved during land clearing. Previous research suggests reconstructed soils using these materials results in novel soil ecosystems that do not mirror undisturbed boreal forest soils. However, the frameworks used for assessing ecosystem recovery have focused primarily on soil nutrients, plant community composition, or broad measurements of microbial communities. Microbial communities underlie many ecosystem processes and play pivotal roles in nutrient cycling and plant growth. Thus, this thesis examined the impact of reclamation and vegetation removal on compositional and functional attributes of soil microbial communities in the AOSR.

1.9.2. Research question and hypotheses

Measurements of catabolic carbon activity such as community-level physiological profiling (CLPP) provide a picture of soil biological activity and potential carbon source utilization. Similarly, high resolution methods, such as high throughput sequencing of 16S rRNA genes enable the examination of microbial community structure in depth. Together, these methods hold promise to allow more thorough understanding and use of microbes as markers of

reclamation efficacy and trajectory. I addressed the following question: How are soil microbial communities, biological activity, and chemistry affected by disturbance (i.e. vegetation removal) and reclamation? The ambiguity of the concept of equivalent land capability continues to be a source of significant debate when discussing reclamation; to address this question I evaluated the different attributes of microbial communities by comparing the range of variability observed in reclaimed and clear-cut soils to that of undisturbed reference soils.

I hypothesized that while removal of vegetation alters microbial communities and activity in soils, it does so to a lesser extent than in reclaimed soils. Thus, the microbial community in the soils with vegetation removed is more similar to the range of natural variability than those in reclaimed soils. Further, I hypothesized that the source in reclamation substrates influences the level of similarity to the reference site; such that microbial community and function in FFM is more similar to the range of natural variability than in PMM.

1.9.3. Aims and objectives

I aimed to assess how soils are affected by vegetation removal prior surface mining and by reclamation at an early stage. To achieve this, I first characterized the microbial community composition and functional profile of disturbed (vegetation removed) and reclaimed sites, in addition to their similarity to the range of natural variability. Second, I determined the microbial soil chemical properties of disturbed (vegetation removed) and reclaimed sites, in addition to their similarity to the range of natural variability. Third, I determined the influence of soil chemical and physical characteristics on microbial community composition.

1.9.4. Experimental design

Soils collected from reclaimed sites at an early stage and soils from sites with vegetation removed on the Canadian Natural Resources Horizon Mine site in the AOSR were used to determine the impact of these anthropogenic disturbances on the microbial community composition and soil biological function. The composition was measured through high-throughput DNA sequencing of the PCR-amplified 16S rRNA genes. Functional attributes were measured via CLPP; however, functional attributes were not measured for sites with vegetation removed. Soil physico-chemical parameters were used to study the influence on microbial community composition and ultimately, to understand the belowground dynamics in reclamation and disturbed systems. Multivariate analyses such as multidimensional ordination were the main tool used to establish differences between reclaimed, vegetation removed and reference soils (i.e. natural boreal forest soils).

2. Chapter 2. The impact of reclamation and vegetation removal on compositional and functional attributes of soil microbial communities in the Athabasca Oil Sands Region

Author Contributions:

Juan Camilo Santana-Martinez, M. Derek MacKenzie, and Brian Lanoil conceived and designed the experiments; Juan C. Santana-Martinez and Angelica M. Aguirre-Monroy performed the experiments; Juan C. Santana-Martinez, Angelica M. Aguirre-Monroy, and Brian Lanoil analyzed the data, Juan C. Santana-Martinez, M. Derek MacKenzie, and Brian Lanoil contributed reagents/materials/analysis tools; Juan C. Santana-Martinez wrote the paper.

2.2.Introduction

Open-pit mining of oil sands is an extraction process that has severe and long-term impact on ecosystems (Audet et al., 2015; Lima et al., 2016). In the Athabasca Oil Sands Regions (AOSR), located in the boreal forests of northern Alberta, open-pit mining has led to a disturbance footprint of ~ 900 km^2 of land (Alberta Energy, 2017). During mining activities, vegetation, soil and other overburden are removed to a depth of 80 m, altering the broader landform, hydrology and biogeochemistry of the area (Audet et al., 2015; Lima et al., 2016). It is therefore crucial to reclaim the post-mining land to reestablish a self-sustaining and functioning ecosystem. Existing environmental management guidelines and policies often require full reclamation of mining sites after closure as a prerequisite for approval permits and land leases. For example, the Government of Alberta mandates that land disturbed by surface mining must be reclaimed to an 'equivalent land capability', and that soil materials must be salvaged and conserved for this purpose during land clearing (Government of Alberta, 1993; Powter et al., 2012). Therefore, once the extraction of the oil sands is completed, the spent mine pit is backfilled and capped with cover soils that are removed during surface-mining, intending to return the site to a natural state.

Cover soils are rich in organic matter and are made of either upland-derived forest floor-mineral mix (FFM) or a lowland-derived peat-mineral mix (PMM). When possible, salvage operations strip the forest floor layer from upland areas prior to mining. During harvesting, this type of soil is mixed with underlying mineral soil; the resulting soil, termed forest floor material (FFM), is utilized in reclamation practices as the most recommended surface soil (MacKenzie et al., 2012). FFM is preferentially used as soil cover in reclamation because the microbial biomass, plant propagules, and nutrient composition more closely resembles

that of the upland boreal forests of the area (MacKenzie et al., 2012; Mackenzie & Naeth, 2010). However, the availability of FFM is limited; only ~35% of the land will be reclaimed with this soil type under current practices. Conversely, over half of the existing landscape in the mineable sites at the AOSR is lowland peat bogs and fens (Rooney et al., 2012). Therefore, the peat salvaged from lowland, which is mixed with mineral soils during harvesting and is termed peat mineral mix (PMM), is abundant and available for reclamation. However, its high organic matter content, high water holding capacity and a greater potential for natural seedling establishment (Archibald, 2014; Errington & Pinno, 2015) has led to the establishment of non-native plant species in long-term reclamation studies (Archibald, 2014).

Currently, it is unclear which soil features indicate a successful reclamation trajectory in oil sands reclamation sites. While the *a priori* goal of reclamation would seem to be to restore these sites to closely resemble the surrounding undisturbed upland ecosystem, differences in soil organic matter composition, soil available nitrogen, and microbial communities between reconstructed oil sand soils and natural boreal forest soils of northern Alberta indicate that these are novel soil ecosystems (Dimitriu et al., 2010; Hemsley, 2012; Stefani et al., 2018; Turcotte et al., 2009). These novel soil ecosystems do not provide the full range of ecosystem functions available prior to disturbance (Audet et al., 2015). Therefore, soil reclamation efforts aimed at reestablishing soil functions, rather than simply trying to replicate structural qualities of the original soil ecosystem, are now seen as key to ensure the long-term sustainability of reclaimed boreal forest landscapes (Quideau et al., 2013). However, reliably determining whether long-term ecological trajectories are bringing about soil functions analogous to the predisturbed condition can be challenging.

One approach to assess reclamation success is to compare several different markers of ecosystem function in reclaimed soils to the variability of those parameters within the natural ecosystem (Rowland et al., 2009). In the AOSR, different markers that have been used to assess ecosystem recovery of reclaimed landforms include soil nutrients, plant community composition and microbial communities (Dimitriu et al., 2010; Errington & Pinno, 2015; Hahn & Quideau, 2013; Hogberg et al., 2020). Bacterial communities play pivotal roles in soil nutrient cycling and plant growth, are sensitive to anthropogenic disturbances, provide a response that can be differentiated from natural variation, and can be aggregated to provide an assessment of the entire system (i.e. integrative) (Harris, 2003; Mummey et al., 2002). Nevertheless, the bacterial measurements that have been used on oil sands reclamation sites to date either focused on broad estimates of microbial activity and biomass or examined the bacterial communities using low resolution methods such as phospholipid fatty acid (PLFA) and denaturing gradient gel electrophoresis (DGGE) profiling (Béasse et al., 2015; Dimitriu et al., 2010; Hahn & Quideau, 2013). Higher resolution methods, such as high throughput sequencing of 16S rRNA genes, have allowed for in-depth (species-level) characterization of microbial communities, thereby demonstrating a more thorough use of bacteria as markers of reclamation efficacy and trajectory in few studies conducted in the AOSR (Ma et al., 2017; Masse et al., 2017; Stefani et al., 2018) as well as in other mining sites (Ngugi et al., 2018; Sun et al., 2017).

Considering the many roles of microorganisms in soil functioning, the objective of this study was to evaluate how soil BCC and function are affected by clearcutting prior to surface mining and in reclaimed sites at an early stage. In addition, I assessed the interactions between soil physico-chemical characteristics and microbial communities in

the AOSR. These objectives were addressed by comparing the range of variability of clear-cut soils and different reclamation cover soils (PMM, FFM and a sand-FFM mixture) to the range of variability observed in undisturbed reference soils, i.e. boreal forest soils. I hypothesized that while removal of vegetation alters the microbial communities and activity in soils, it does so to a lesser extent than in reclaimed soils. Furthermore, I hypothesized that the source in reclamation substrates influences the level of similarity to the reference sites; such that BCC and function in FFM is more similar to the range of natural variability than in PMM.

2.3. Materials and methods

2.3.1. Site description and sampling sites

The study area is located in the Athabasca Oil Sands Region (AOSR) in northern Alberta, Canada. This area has a mean annual temperature of 1°C and a mean annual rainfall of 418.6 mm, with an average of 342 mm occurring as rainfall during the growing season (June-August). The mean monthly temperatures range from 17.1°C in July to -17.4°C in January, and there are an average of 97 frost-free days per year (Environment and Climate Change Canada, 2011). The AOSR is situated within the central mixed-wood region of the Canadian boreal forest, with mesic upland sites dominated by tree canopy species such as trembling aspen (*Populus tremuloides*), white spruce (*Picea glauca*), and Jack pine (*Pinus banksiana*). Medium- to fine-textured Gray Luvisols and Brunisols are the typical soils of the area. Organic soils are found in wetland areas (Fung & Macyk, 2000).

Sample sites were located on an oil sands mine lease in North-eastern Alberta in the regional municipality of Wood Buffalo within a 12 km radius of the mine site where the study took place (57°20'N, 111°54'W, Fig. S1 in the Appendix). Samples were collected from reference natural undisturbed soil sites (NS, n=29), sites with vegetation removed (i.e. clear cut sites) (VR, n=56), and sites reclaimed with forest floor material (FFM, n=54), a FFM-sand mix (Sand-FFM, n=42), or a peat-mineral mix (PMM, n=54) as the soil substrate.

NS were randomly selected from areas classified as “d-ecosites” using the Alberta Vegetation Inventory (Alberta Sustainable Resource Development., 2005) in proximity to the study site that had not experienced any visible physical impacts from the mining

process. The d-ecosites are the target for reclamation on oil sands sites where this study was located, are characterized by low-bush cranberry, a mesic moisture regime, with a medium nutrient regime (Beckingham & Archibald, 1996). The sites with vegetation removed were randomly selected from sites that had experienced removal of vegetation and heavy equipment traffic between 2012 and 2017. The reclaimed sites were established during the winter of 2017 by placing approximately 0.2 m of cover material over an overburden structure consisting of mainly marine shales and a 1.0 meter upper layer of overburden. Cover materials were differentiated based on the origin of the organic matter. FFM was made by combining salvaged materials from pre-mined upland areas with the underlying mineral soil. PMM was made of salvaged materials from lowland peat bogs and fens areas mixed with underlying mineral soil. FFM and PMM soil covers differ in organic matter content, volumetric water holding capacity and chemical properties, as described by (Errington & Pinno, 2015). FFM contains a higher number of plant propagules and microorganisms than PMM (MacKenzie et al., 2012; Mackenzie & Naeth, 2010). In addition, a FFM-sand mix, composed of FFM mixed with sandy material from the lease site was also tested as a cover soil as a means to overcome the limited availability of FFM. Sand-FFM was treated as a different reconstructed soil substrate during our analyses. At each reclaimed site, one sample was taken from each of three cardinal directions (North, East, and West) to create a robust representation of variability within the reclamation soils being tested (US Environmental Protection Agency (USEPA), 2002). Sampling locations within the reclaimed sites were selected randomly from pre-established sampling points assigned by the operator.

2.3.2. Sampling protocol

For the characterization of microbial communities and chemical data, samples were collected from the sample sites identified above (Fig. S1 in the appendix) during October to November 2016 (VR samples), June 2017 (NS samples), or July 2018 (PMM, FFM and FFM-sand samples). Soil samples were collected as 4.5 cm diameter cores, roughly 10 cm in depth, using a custom-made tube extractor. Soil core samples were placed in plastic bags and put on ice for transport. In the laboratory, samples were passed through a 4.00 mm sieve and homogenized, and a subsample of approximately 20g was taken from each sample and frozen at -80°C until microbial DNA analysis was performed. The remaining soil was stored at 4°C for microbial activity and soil physicochemical analysis.

2.3.3. Soil chemical and physical analysis

A commercial analysis facility (ALS Labs, Edmonton, Canada) used standard methods to measure the chemical and physical parameters of soil in this study (United States Environmental Protection Agency (US EPA), 2003) . Physical and chemical parameters measured in this study included: soil pH, electrical conductivity (EC), sodium adsorption ratio (SAR), total organic carbon (TOC), soil texture (sand (2.0-0.05 mm), silt (0.05 mm – 2 mm) and clay (< 2 mm) ratios), available nitrogen (NO_3^-), available sulfur (SO_4^{2-}), available phosphorus (PO_4^{3-}), available potassium (K^+) , cations in soil (Ca^+ , K^+, Mg^+, Na^+), moisture content, and percentage of water saturation.

2.3.4. Soil functional analysis

A community-level physiological profiling (CLPP) approach to measure the microbial activity in soil was conducted (Campbell et al., 2003; Howell & MacKenzie, 2017). Briefly,

100 g of soil was brought to 60% field capacity with deionized water and pre-incubated for two weeks at 25°C. After this incubation period, the analysis was performed utilizing a custom multiple substrate induced respiration method based on the MicroResp system (Macaulay Scientific Consulting Ltd., Aberdeen, Scotland). Carbon substrates evaluated included carboxylic acids, amino acids and carbohydrates differing in chemical structure and functional groups (Table 1 in Campbell *et al*., 2003). Soil samples were evenly distributed with substrates in deep 48-well modified MicroResp plates and incubated at 25°C for 6h. The CO_2 produced from substrate respiration generated a colorimetric reaction with the indicator gel in an attached microplate, which were read at 570 nm wavelength using a Synergy HT Multi-Mode Microplate Reader (BioTek Instruments Inc., Winooski, VT, USA). The amount of CO_2 was modeled based on the equations provided by MicroResp and calibrated with infrared gas analysis. Due to loss of samples resulting from field handling issues, CLPP analysis of VR samples was not completed; thus, they are not included in this analysis.

2.3.5. DNA extraction and sequencing

DNA was extracted in triplicate from 0.25 g of soil using the DNeasy PowerSoil Kit (Qiagen, USA) according to the manufacturer's protocol. A DNA extraction blank was included for each sample batch of roughly 9 samples. Triplicate extractions were pooled and the DNA concentration of each pooled sample and the DNA extraction blanks were measured with a Qubit dsDNA HS assay kit according to the manufacturer's protocol (Thermo Fisher Scientific, Canada). A mock community was constructed with DNA from 12 different bacterial species as a positive control for sequencing. Pooled DNA extracts, DNA extraction blanks, and the mock community were sent for 16S rRNA gene sequencing

to Microbiome Insights (Vancouver, Canada). The V4 region of the 16S rRNA gene was PCR-amplified using primers 515F and 806R, based on the protocol recommended by the Earth Microbiome Project (EMP). Amplicons generated from the soil samples, DNA extractions blanks and the constructed mock community were sequenced with an Illumina MiSeq platform using the 250-bp paired-end kit (V2 500-cycle PE Chemistry, Illumina, USA). The total DNA concentration was also used as a proxy for soil microbial biomass.

2.3.6. Bioinformatics analyses

A total of 7,046,683 paired-end Illumina sequences were obtained from the 259 samples, 30 DNA extraction blanks and the mock community. DNA paired-end reads were merged individually using USEARCH v11.0.667 (Edgar, 2010), resulting in a total of 6,598,309 reads. These reads were merged into a single FastQ file using Mothur v.1.41.3. (Schloss et al., 2009). 6,547,711 reads passed quality checks using USEARCH and were checked for singletons and uniques. Chimeric sequences (24,889 total) were removed and sequences were clustered at 97% similarity using USEARCH, generating 11,323 OTUs. Reads were mapped to the OTU sequences to generate an OTU table with the read counts per sample using USEARCH. Contaminant OTUs were identified with the *decontam* package (Davis et al., 2018) using R Studio V. 1.3.959 (RStudio Team, 2020). As a result, 233 of 11,323 OTUs were identified as potential contaminants and were removed for further analysis.

The OTU table was normalized by rarefying to the lowest number of reads possible in the samples without eliminating more than the 10% of the samples, using the 'total group' algorithm in Mothur. As a result, all the blanks and 24 samples from the VR sites were eliminated from the dataset. A rarefaction curve (Fig. S2 in the appendix) was generated

using Mothur. Taxonomic identification was performed using the Silva database v.132 in Mothur and the OTUs identified as mitochondria and Archaea were removed from the OTU table using the R package *phyloseq* (McMurdie & Holmes, 2013) in RStudio V. 1.3.959 (RStudio Team, 2020).

2.3.7. Statistical analyses

All statistical analyses were performed using RStudio v. 1.3.959 (RStudio Team, 2020). Physical, chemical, and biological characteristics of the soils were visualized using a scaled principal component analysis (PCA) in the *vegan* R package (Oksanen et al., 2019). To test for differences between sites, pairwise permutational multivariate analyses of variance (PERMANOVAs) using 999 permutations were performed on the Euclidean matrix (Martinez, 2020). Pairwise Wilcoxon rank tests with Benjamini & Hochberg adjustments using the base functions in Rstudio (RStudio Team, 2020) were utilized to determine significant differences between each soil parameter per treatment.

Microbial diversity at each site (α-diversity) was calculated using the mean number of OTUs, the Chao1 index, inverse Simpson index, Shannon diversity index, and Pielou's evenness index with the *phyloseq* and *microbiome* R packages (Lahti & Shetty, 2017; McMurdie & Holmes, 2013). The significance of differences in α-diversity metrics between sites was calculated by pairwise Wilcoxon rank tests with Benjamini & Hochberg adjustments (RStudio Team, 2020). Similarly, the differences in the taxonomic composition across sample types were determined by Wilcoxon rank sum tests with Benjamini & Hochberg adjustments using the *Metacoder* package in R (p-value <0.05) (Foster et al., 2017).

BCC composition data was Hellinger-transformed before the between-site (β-diversity) analysis. BCC and CLPP data were visualized with principal coordinate analysis (PCoA) and non-metric multidimensional scaling (NMDS), respectively, using Bray-Curtis dissimilarity with the *vegan* R package (Oksanen et al., 2019). To test for differences between sites, pairwise PERMANOVAs using 999 permutations were performed on the Bray-Curtis matrices (Martinez, 2020). To examine heterogeneity of the sites and similarity with respect to the natural sites, a subset of comparisons within site and a subset of comparisons with respect to the natural sites were conducted on the Bray-Curtis dissimilarity matrix. The mean dissimilarity of each sample was calculated to obtain a single value per sample and the differences between sites were evaluated using a Kruskal-Wallis test, followed by a pairwise Wilcoxon rank test with a Benjamini & Hochberg adjustment. To test for differences in community composition between sites, an analysis of similarity (ANOSIM) was performed on the Bray-Curtis matrix using the *vegan* package in R (Oksanen et al., 2019). ANOSIM calculates the *R* statistic which ranges from 0 (no community separation) to 1 (dissimilarity between communities). The taxon profile of the different sites was examined at the genus and family level by finding the 50 most abundant OTUs using the R package *Fantaxtic* (Teunisse, 2020). To further understand the grouping behavior and trends of the soil sites seen in the ordination plots, a hierarchical cluster using Bray-Curtis distance with Ward linkage was performed on the Hellinger-transformed data (Maechler et al., 2019).

Potential within-community interactions between organisms were studied by constructing co-occurrence networks using the *igraph* R package (Csardi & Nepusz, 2006). The OTUs representing >1% of total reads were used to build individual networks for each sample

type. Spearman's rank correlation (SRC) coefficients for all possible pairs of OTUs in each sample type and their significance level were computed using the *Hmisc* R package (Harrell Jr, 2019). Edges in the networks were built utilizing statistically significant SRCs (False discovery rate (FDR) < 0.05) with absolute SRC coefficients of > 0.75. Nodes in the networks represent individual OTUs that are significantly correlated with at least one other OTU. Numbers of nodes, edges, average degree and clustering coefficient were calculated using the *igraph* R package (Csardi & Nepusz, 2006). In order to identify overlapping nodes and edges, the individual networks of reclaimed and VR soils were intersected with the NS network using the 'intersection' function in the *igraph* R package (Csardi & Nepusz, 2006).

To examine the influence of physicochemical parameters on BCC composition and site variability, a canonical redundancy analysis (RDA) was conducted using the *vegan* package in R (Oksanen et al., 2019). The Hellinger-transformed BCC composition data was constrained by the matrix of physicochemical data in order to identify variables that significantly explained the distribution of microbial communities in the studied soils. Significant variables were selected using the forward selection algorithm implemented by the *ordiR2step* function in *vegan.* The RDA model, axis, and explanatory variables were tested using a permutation test with 999 permutations. This approach was also performed at the phylum and class level by merging OTUs with the same taxonomy at the phylum and class taxonomic ranks using the tax_glom function in *phyloseq* (McMurdie & Holmes, 2013).

2.4. Results

2.4.1. Natural boreal forest soils have a small range of variability

Natural reference soils had a relatively narrow range of variability in all factors assessed. The soil physical and chemical characteristics and microbial biomass exhibited a small range of variability in NS sites that was significantly different from the other sample types ($p<0.001$), despite overlapping with VR sites (Fig. S3 in the Appendix). Some individual edaphic parameters were significantly lower in NS sites (pH, EC, available PO_4^{3-}, Mg^+, Na^+, Ca^+) compared to the other sample types, while the microbial biomass was the highest.

The overall diversity of soil bacterial communities (Fig. 1) showed the lowest variability in NS sites as indicated by the Shannon Diversity Index and Inverse Simpson Diversity Index, suggesting that the overall diversity of these communities is homogeneous across samples. I also measured taxon evenness in two ways: Pielou's Evenness Index and the fraction of the BCC represented by the top 50 OTUs. Both showed low variability across NS samples. Furthermore, the BCC of NS sites was significantly different from the rest of the sample types ($p<0.001$), and exhibited a small range of heterogeneity comparable to that of FFM sites and overlapping with VR sites (Fig. 2A, 2B). The BCC in NS sites was correlated with high K^+ and with low pH ($p<0.001$) at both OTU and class level, suggesting these factors may influence the abundance of particular taxonomic groups in the NS (Fig. 3; Fig. S10 in the Appendix). For instance, the classes Alphaproteobacteria from the phylum Proteobacteria and Acidobateriia from the phylum Acidobacteria were significantly more abundant in NS sites and were also correlated with these two parameters (Table S3, Fig. S10 in the Appendix).

Community level physiological profiles (CLPP) from NS sites clustered separately from the reclaimed sites (Fig. 4; $p<0.001$) and the range of variability in NS samples was significantly smaller than the rest of the sites (Figs. 4A, 4B). NS soils showed increased respiration following the addition of monosaccharide and disaccharide carbohydrates (glucose, trehalose, arabinose, galactose N-acetyl glucosamine and to a lesser extent fructose) and several amino acids (γ-aminobutyric acid, alanine, lysine, and arginine), but not carboxylic acids (oxalic acid, citric acid, malic acid, and keto-glutaric acid). However, the total soil potential respiration (Fig. 4D) was not significantly different from the rest of the sites.

2.4.2. Removal of vegetation alters the edaphic parameters and BCC of boreal forest soils

Vegetation removal led to increased variation of the soil physical and chemical parameters and differed significantly ($p < 0.001$) from the range of natural variability, although VR overlapped to some extent with the range of natural variability (i.e. NS sites, Fig. S3 in the Appendix). Some individual edaphic parameters were significantly higher in NS soils (i.e. silt and microbial biomass) than in VR sites, whereas most parameters were higher in VR sites (i.e. pH, TOC, cation concentration, EC, available nitrate, and available sulfate). However, VR sites showed a wider range of variation in most parameters measured (i.e. cation concentrations, available nutrients, pH, EC, and TOC; Table S1 in the Appendix).

Disturbance caused by vegetation removal increased the overall diversity of the community (Fig. 1), which was also reflected in the observed taxa profile at the OTU level (Fig. S4 in the Appendix). Across VR samples, the top 50 OTUs represented up to 30% of the community; whereas the top 50 OTUs in NS samples represented up to 50%, corroborating

the observed increase in the Pielou's evenness index of the NS community. Vegetation removal substantially increased the heterogeneity of BCC (Fig 2A, 2B), with the range of variability of VR soils only partially overlapping the range of NS samples (Fig. 2A). The centroids of the NS and VR soil microbial communities were significantly different in ordination space ($p<0.001$). Hierarchical clustering analysis of these samples (Fig. 2D) showed NS and VR samples tended to cluster with each other, suggesting the communities in these soils shared more similarities when compared with the rest of soil types. This clustering was supported by the ANOSIM R value (R=0.124, P=0.001), although not by the dissimilarity metric (Fig. 2C). The community compositions of VR and FFM sites were equally dissimilar to the range of NS samples, although this measurement exhibited a high range of variation in VR sites (Fig 2C). The high heterogeneity of VR sites suggested their bacterial communities were influenced by all the parameters included in the RDA model, except for Na^+ and clay content at the OTU-level (Fig. 3), as well as at the class and phylum level (Fig. S10 and S11 in the Appendix). The classes Alphaproteobacteria and Acidobacteriia, which correlated with NS samples, were also correlated with some overlapping VR samples. However, the remaining highly dispersed VR sites were correlated with several other bacterial classes, including Verrucomicrobiae from the phylum Verrucomicrobia, Thermoleophilia from Actinobacteria, Planctomycetacia from Planctomycetes, Deltaproteobacteria from Proteobacteria, and Blastocatellia and subgroup 6 from Acidobacteria (Fig. S10 and S11 in the Appendix), indicating that more taxonomic groups are associated with edaphic parameters in these samples than in NS.

The taxonomic composition at the phylum level indicated that the relative abundance of 12 (out of 17) dominant phyla (each representing >1% reads) significantly differed between

VR and NS samples (Table S2, Fig. S5 in the Appendix). A similar distinction in taxonomic composition was observed at the class level (Table S3, Fig. S6 in the Appendix); however, it was evident that relative abundances of taxa in VR samples were more evenly distributed than in NS sites. At the genus level, taxonomic differences between VR and NS were the most marked (Table S4; Fig. S7 in the Appendix). In NS samples, the top 15 genera across all sample types represented 19.3% of the community, they represented only 8.7% of the community in VR soils.

After disturbance, ecosystem functionality is affected by changes in within-community interactions even before the richness of the community is altered (Moreno-Mateos et al., 2020), implying that other features of bacterial communities, such as interactions between community members, may be important for ecosystem function. Therefore, to determine differences in the level of interactions among microbial community members between sample types, I generated separate co-occurrence networks for each treatment group (Table 1, Fig. S8 in the Appendix). The number of interacting OTUs remained relatively stable in VR when compared to NS; however, vegetation removal increased the number of potential interactions between OTUs, and the connectance (the fraction of all possible links present in a network) of the community (Table 1). Co-occurrence networks of each treatment group were intersected with the NS network to find overlapping edges and nodes (Table 1, Fig. S9 in the Appendix). The VR network showed the highest portion of overlapping edges (66%) and nodes (16%) with the NS network. Taken together, these results indicate vegetation removal alters to a lesser extent the existing potential within-community interactions of the communities in the boreal forest soils than reclamation; however, vegetation removal increases the overall potential within-community interactions and connectance.

2.4.3. Edaphic parameters, BCC, and soil function of boreal forest soils are distinctively affected by different reclamation substrates

Physical and chemical parameters in reclaimed soils differed significantly ($p < 0.001$) from both NS and VR sites and did not overlap at all with NS sites. The ranges of variability of the reclaimed soils generally overlapped with each other (Fig S3 in the Appendix). FFM and sand-FFM sites clustered closer to NS than PMM sites and overlapped more with each other ($p > 0.05$) than with the PMM sites, indicating they have more similar edaphic properties to NS when compared to the PMM sites. Some individual edaphic parameters were higher in NS soils (i.e. silt content, microbial biomass, moisture content, and available K^+ and PO_4^{3-}) than in reclaimed soils while other parameters were higher in reclaimed soils (i.e. pH, sand content, cation concentration, EC, and available sulfate; Table S1 in the Appendix). Overall, soil parameters differed between NS and all reclaimed soils, but did not differentiate between FFM and sand-FFM cover soils.

Among the reclaimed sites, PMM samples had significantly lower overall diversity than other treatments (Fig. 1). The microbial community in PMM was dominated by a few OTUs, so that in some samples the 50 most abundant OTUs represented up to 75% of the community (Fig. S4 in the Appendix). In contrast, reclamation with FMM and sand-FFM substrates did not seem to decrease the overall diversity of the community (Fig. 1). In fact, both FFM substrates increased the overall diversity of the community when compared with the NS samples according to the mean number of OTUs, the Chao1 richness and Shannon diversity indices.

BCC in reclaimed soils were separate from the range of NS and VR soils, but their ranges of variability overlapped with each other (Fig. 2A). However, despite overlap, the centroids

of the different reclamation substrates were significantly different amongst each other ($p<0.001$), as well as different from the communities in NS and VR samples ($p<0.001$). Microbial communities in PMM soils were the least heterogeneous from all sample types and they formed a major cluster with few samples from other treatments in the hierarchical clustering analysis (Figs 2A, 2D). Further, the PMM soils BCC was the most dissimilar to NS, according to both the dissimilarity metric (Fig. 2C) and the ANOSIM R value (R=0.995, P=0.001). FFM and NS soil BCC showed similar levels of heterogeneity, while sand-FFM BCC showed the highest level of heterogeneity among reclaimed soils (Fig 2A, B). FFM soils tended to cluster distinctively from the rest of the sample types; however, a few samples grouped within the major PMM cluster. On the other hand, 35% of Sand-FFM samples (Group 1) clustered together while the remaining 65% (Group 2) was distributed within PMM, FFM and VR clusters (Fig. 2D), suggesting Sand-FFM samples were not cohesive and the source material may be fundamentally different across samples. The BCC in FFM samples and Sand-FFM samples were equally dissimilar to NS according to the dissimilarity metric (Fig. 2C); however, the ANOSIM R values showed the Sand-FFM communities were more similar to the NS samples (R=0.383, P=0.001), than the FFM communities were (R=0.775, P=0.001). These results indicate diluting with sand decreases the distance to the range of natural variability; however, it is unclear which sand features are contributing for this.

PMM bacterial communities were positively correlated with sand content, available NO_3^-, available Ca^{2+}, and EC, but negatively correlated with silt content, available PO_4^{3-}, MC, and saturation (Fig. 3). The correlation with these edaphic parameters was also observed with the classes Bacteroidia of the phylum Bacteroidetes, Gammaproteobacteria of the phylum

Proteobacteria, and Actinobacteria from the phylum Actinobacteria, which were positively correlated with PMM sites (Fig. 10S in the Appendix). Most FFM bacterial communities were positively correlated with pH, Na^+, SAR, and clay content; however, some samples were instead correlated to EC, available NO_3^-, available PO_4^{3-}, saturation and moisture content. The same correlation was observed at the class and phylum level, with the classes Gemmatimonadetes from the phylum Gemmatimonadetes, and the candidate classes KD4-96 and MB-A2-108 from the phyla Chloroflexi and Actinobacteria, respectively, associated with FFM samples (Fig. 10S and 11S in the Appendix). The distribution of sand-FFM sites indicated samples in Group 1 were negatively correlated with Na^+ and clay content, while Group 2 was a highly dispersed group with samples influenced by the same variables influencing PMM and FFM sites (Fig. 3). At the class and phylum level, Group 1 Sand-FFM soils did not show any positive correlation with individual bacterial groups, however; Group 2 Sand-FFM samples showed the same associations between taxonomic groups and edaphic parameters observed in both PMM and FFM sites (Fig. 10S and 11S in the Appendix). Taken together, distinct edaphic parameters explain the differences in BCC across reclamation substrates and can influence major taxonomic groups associated with different cover soils.

The relative abundance of dominant phyla (>1% reads) in reclaimed sites differed from the NS sites and among sample types (Table S2, Fig. S5 in the Appendix). Relative to NS sites, the abundances of 10, 8, and 10 of dominant phyla (out of 17) were significantly different in FFM, Sand-FFM, and PMM samples, respectively (Table S2 in the Appendix). At the class level, differences in the relative abundances of the dominant groups were also observed between all three reclamation substrates (i.e. FFM, Sand-FFM and PMM) and NS

sites (Table S3, Fig. S6 in the Appendix); however, it was evident that the taxonomic composition among reclamation soils was more similar between each other than with the NS sites (Fig. S6 in the Appendix). The similarities between reclamation substrates were more pronounced in the top 15 genera, which represented 34.1%, 38.6% and 56.5% of the community in FFM, Sand-FFM and PMM, respectively, but only 19.3% in NS sites (Table S4; Fig. S7 in the Appendix).

In addition to the changes in the taxonomic composition, there were changes in the potential within-community interactions of the microbial communities in reclaimed sites (Table 1, Fig. S8 and S9 in the Appendix). Overall, the number of interacting OTUs and potential interactions remained relatively stable in FFM samples when compared to NS sites; however, there was an increase in the number of interacting OTUs and the number of interactions between OTUs in soils reclaimed with Sand-FFM relative to NS, whereas reclamation with PMM decreased both factors. As well, reclamation promoted positive interactions between OTUs (PMM>FFM>Sand-FFM). Reclamation with FFM and PMM did not alter the connectance of OTUs; however, the connectance of the community increased in Sand-FFM. When intersected with the NS network, the sand-FFM network had the highest number of overlapping nodes and edges; however, these overlapping nodes represented 47% of the total and just 7% of the edges in the sand-FFM network. The lowest number of overlapping nodes and edges were found in the PMM network. Similarly, the percentage they represented in the original networks also decreased from FFM to PMM. Together, these results indicate soils disturbed by vegetation removal share a higher proportion of OTUs and interacting OTUs with the NS soils when compared with those of reclaimed soils.

Reclamation substrates showed overlapping functional profiles (i.e. CLPP), although distinct from the functional profile of NS sites. PMM sites, however, clustered the farthest away from the NS sites (Fig. 4A). The most variable reclaimed sites were sand-FFM, followed by FFM, with PMM the least variable (Fig. 4B). Furthermore, when compared with the NS sites, PMM sites were significantly less dissimilar than sand-FFM sites; whereas the FFM sites were equally dissimilar to both sand-FFM and PMM sites (Fig. 4C). FFM and Sand-FFM samples responded to the same carbohydrates and amino acids that stimulated the NS samples; however, some of the FFM and Sand-FFM samples also responded to the amino acid cysteine and, to a lesser extent, to various carboxylic acids. PMM soil respiration was negatively impacted by most of the carbohydrates and amino acids; respiration in these soils increased only in response to the addition of cysteine and to a lesser extent by the carboxylic acids. The CLPP for all treatment groups were significantly different between each other ($p<0.05$). Overall soil functional activity (Fig. 4D) in FFM soils was not significantly different from the NS soils; however, sand-FFM and PMM soils had significantly lower overall activity. In sum, reclamation with sand-FFM and PMM decreased the utilization of carbohydrates and the basal respiration of the soils, whereas reclamation with FFM increased the utilization of amino acids and carboxylic acids, relative to NS (Fig. S12 in the Appendix). Altogether, the differences in the functional profile directly demonstrates the impact of reclamation on ecosystem function, validating the links between BCC and edaphic parameters, in which similar patterns were observed.

Figure 1. Alpha diversity metrics for bacterial communities. Metrics include the (A) observed number of OTUs, (B) the Chao1 non-parametric richness estimator, (C) Shannon diversity index, (D) inverse Simpson diversity index, and (E) Pielou's evenness index. NS – Natural soil, VR = Vegetation removed, FFM = Forest floor mineral mix, Sand-FFM = Sand-forest floor mineral mix, PMM = Peat mineral mix. Similar letters indicate no significant difference between sample types according to pairwise Wilcoxon Rank sum tests with a Benjamini-Hochberg adjustment.

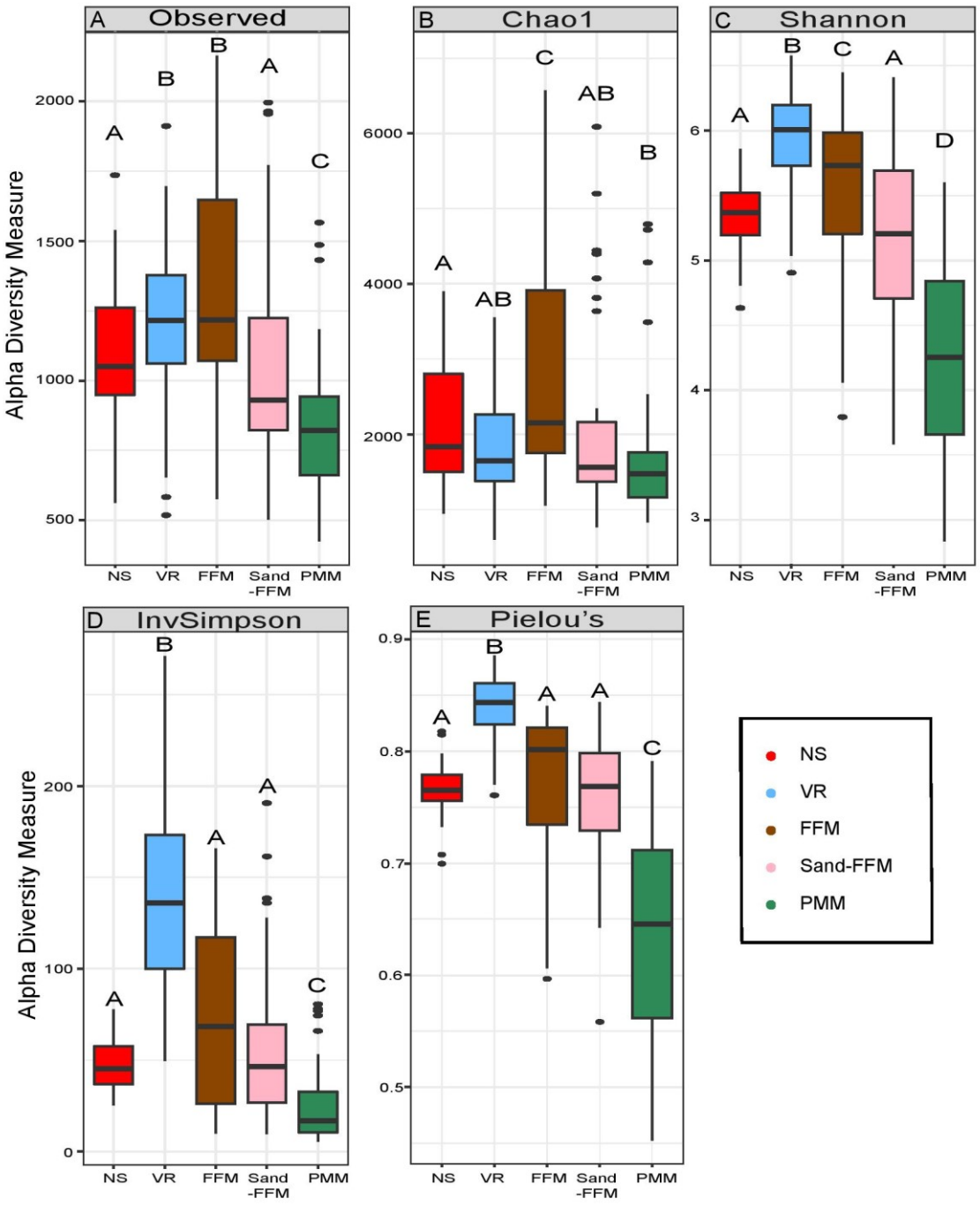

Figure 2. (A) Principal coordinate analysis and (D) hierarchical clustering of the bacterial communities in the different soil types. Bray-Curtis dissimilarity of samples (B) within sample type and (C) relative to the NS reference samples based on bacterial community composition. Similar letters on box-and-whisker plots indicate no significant difference between sample types according to pairwise Wilcoxon Rank sum tests with a Benjamini-Hochberg adjustment. Site type abbreviations are the same as in the legend to figure 1.

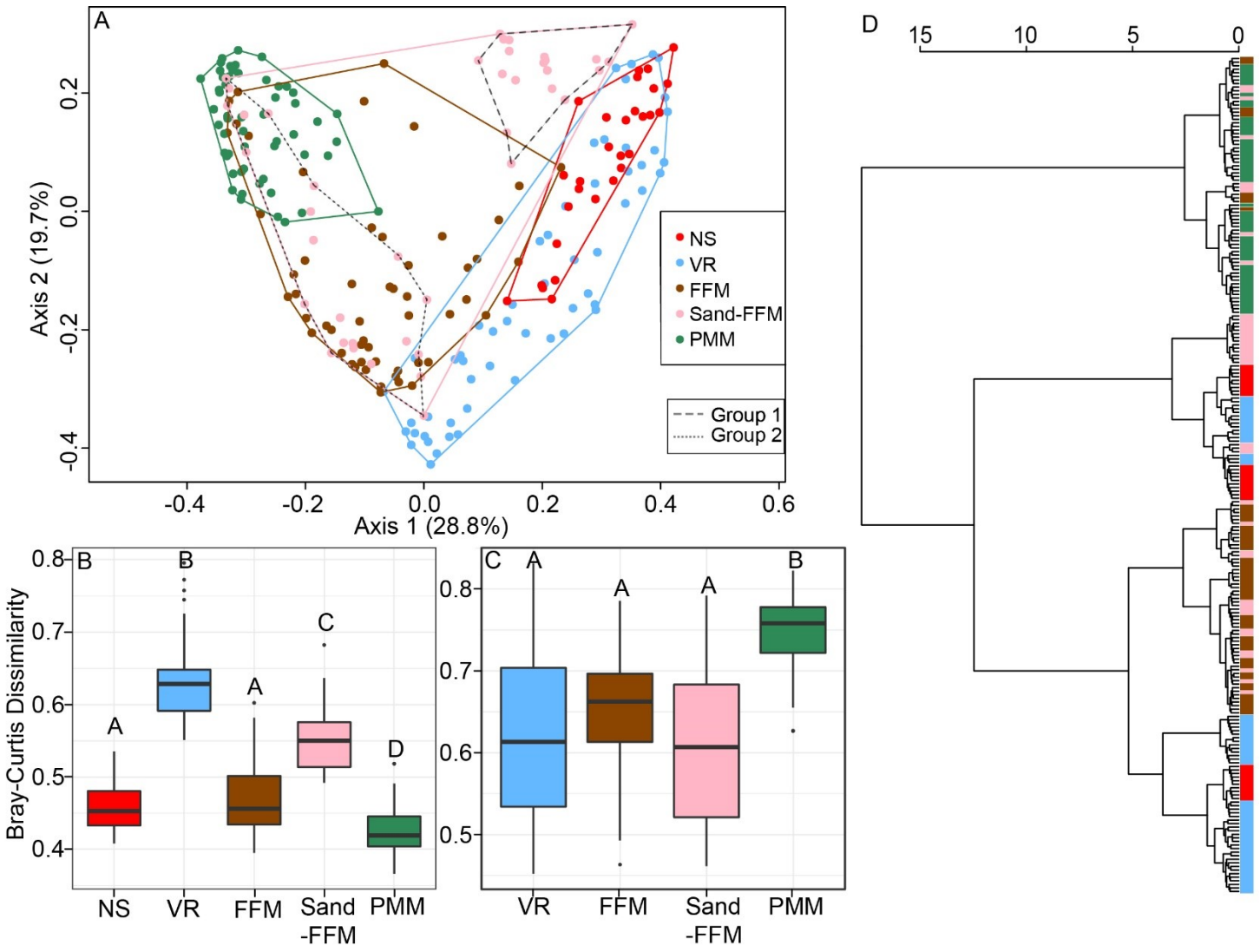

Figure 3. Canonical redundancy analysis of the bacterial communities performed at the OTU level showing relations between site and soil characteristics in the different sample types. (***, p<0.001). Site type abbreviations are the same as in the legend to figure 1.

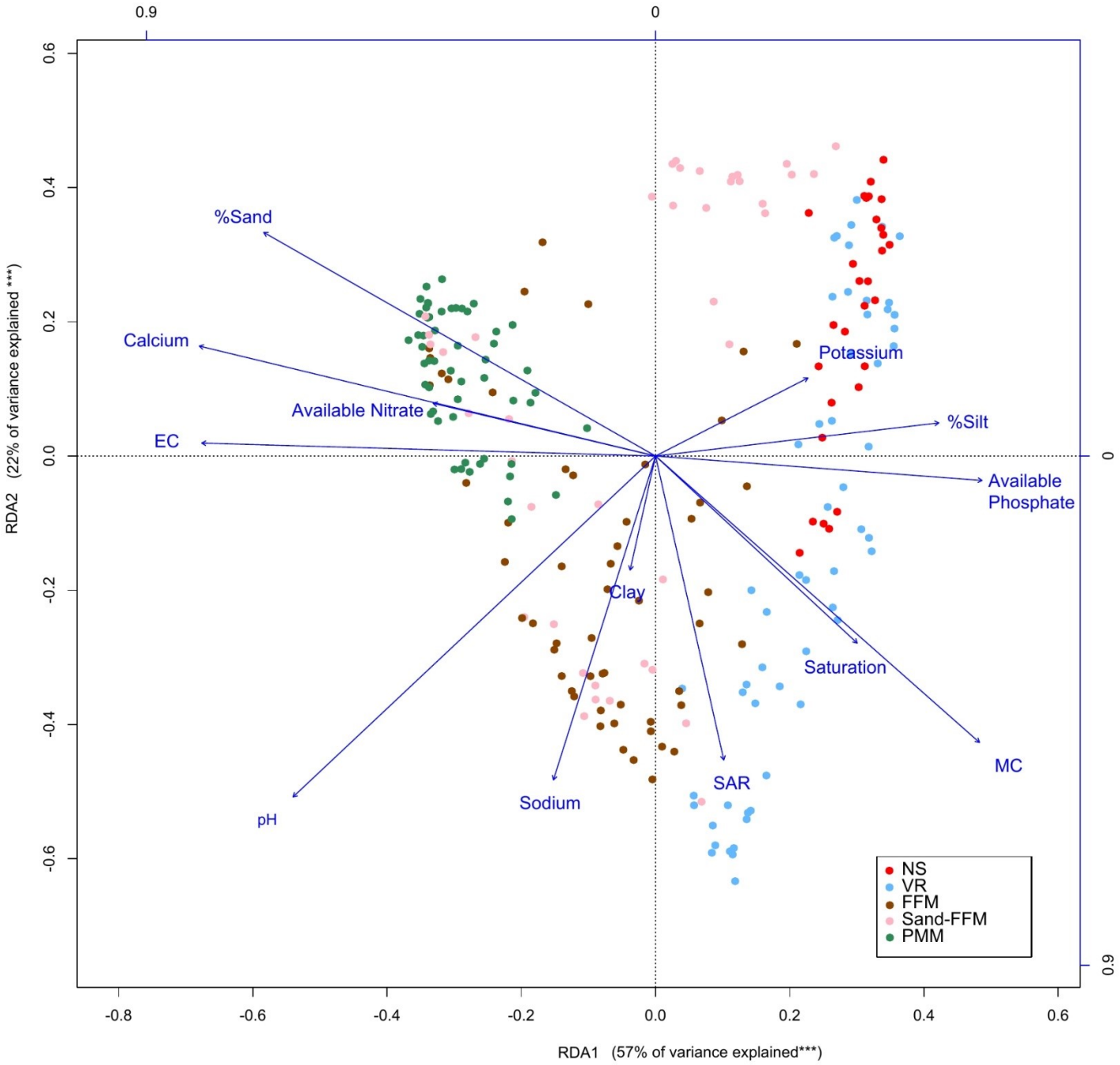

Figure 4. (A) Non-metric multidimensional scaling ordination of CLPP in the different sample types (stress = 0.108). Length of the vectors indicates the influence of the parameter in the ordination of data. Bray-Curtis dissimilarity of samples (B) within sample type and (C) relative to the NS reference samples based on CLPP data. (D) Total CO_2 rate production in the different sample types based on CLPP data. Similar letters indicate no significant difference between sample types according to pairwise Wilcoxon Rank sum tests with a Benjamini-Hochberg adjustment. Site type abbreviations are the same as in the legend to figure 1.

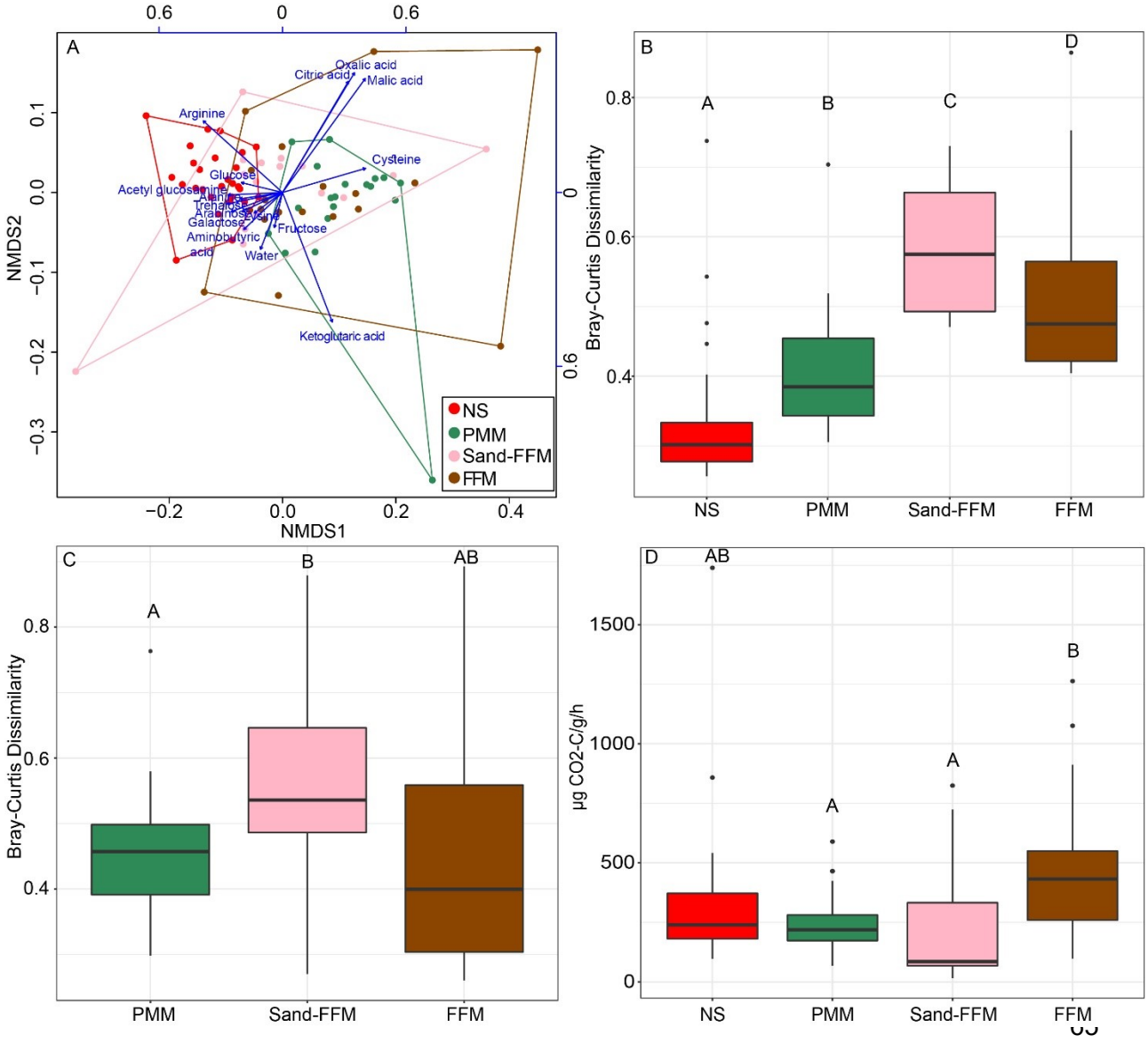

Table 1. Bacterial network properties of different sample types and shared properties when intersected with the NS network. NS = Natural soil, VR = Vegetation removed, FFM = Forest floor mineral mix, Sand-FFM = Sand-forest floor mineral mix, PMM = Peat mineral mix.

	NS	VR	FFM	Sand-FMM	PMM
No. Nodes	123	122	139	194	98
No. Edges	647	1128	604	3732	314
No. pos/neg edges	447/200	740/388	542/62	2981/841	287/27
Avg degree	10.52a	18.49b	8.69a	38.47c	6.41d
Global Clustering coefficient	0.52	0.57	0.51	0.68	0.55
Local Clustering coefficient	0.58a	0.65b	0.51a	0.74c	0.53a
Shared nodes with links	NA	80(0.66)	45(0.32)	92(0.47)	21(0.21)
Shared edges	NA	176(0.16)	88(0.15)	253(0.07)	38(0.12)

Numbers in parentheses indicate the percentage calculated based on the total of a given property in the original network. Means within a row followed by the same letter are not significantly different according to a pairwise-Wilcoxon rank sum test with a Benjamini-Hochberg adjustment.

2.5. Discussion

BCC, overall diversity, soil function, and soil physico-chemical parameters differed between undisturbed boreal forests soils, reclaimed sites, and vegetation removed sites. These findings are consistent with previous studies showing that boreal forest soils are altered by reclamation (Dimitriu et al., 2010; Quideau et al., 2013; Stefani et al., 2018) and by vegetation removal (Hynes & Germida, 2013; Smenderovac et al., 2017). However, the present study simultaneously assessed the impact of clearcutting and reclamation, which allowed me to compare the severity of these anthropogenic disturbances related to surface mining. Altogether, the distance from the range of natural variability showed the changes caused by vegetation removal were less drastic than the ones caused by reclamation. All attributes measured showed reclaimed soils did not approach the range of natural variability at this early stage of recovery. Within reclaimed soils, PMM differed the most with the range of natural variability. Overall, these findings also indicate factors measured in this study can be used to assess the level of disturbance as a way to assess reclamation trajectory.

Natural reference soils had a relatively narrow range of variability in all factors. Although the NS samples in this study were randomly selected from ecosites targeted for reclamation on oil sands and did not show physical impacts from the mining process (Alberta Sustainable Resource Development., 2005), it is noteworthy that they do not represent the whole spectrum of natural boreal forest soils. The landscape in northern Alberta includes a wide range of age classes and stand types resulting from the combination of frequent natural disturbances and edaphic factors (Dhar et al., 2018). The variable frequency of wildfire outbreaks (approximately every 60-90 years) imposes considerable variations in

post-disturbance stand development, which in turn produces a wide variety of mixedwood forest conditions existing as a mosaic in time and space (Bergeron et al., 2014; Dhar et al., 2018). In sites that had recently experienced a wildfire, microbial communities were more similar to those at reclaimed sites and differed from those in mature forest (Stefani et al., 2018). Similarly, key biogeochemical attributes in soil were different in three different ecosites of the northern Boreal forest soils, associated with differing plant cover (Quideau et al., 2013). Thus, the overall range of natural variability may be higher than I observed in this study. Nevertheless, the NS sites were representative of mature boreal forest of the d-ecotype commonly found in this area. Future studies aimed at more thoroughly characterizing the baseline range of natural variability in boreal forest soils may provide a more complete picture of the full range of natural variability. Including other ecotypes, sites disturbed by different levels of fire and at different moments in time, in addition to rangelands (if locally desirable), could aid to establish the range of ecosystems target for reclamation. As well, future studies could compare to ranges of variability established by local end-point goals based on anticipated post-disturbance land-uses (e.g. conservation sites, agricultural land; Audet et al., 2015).

As I hypothesized, loss of vegetation caused fewer alterations to the boreal forest soil when compared to reclamation; however, it still represented a significant disturbance to the soils. The impact of vegetation removal was evident in the alteration of the edaphic factors, overall diversity and BCC. Consistent with our findings, after vegetation removal, a flush of nutrients (including C, N, P, S and K) has been observed in other studies (Grigal, 2000; Hynes & Germida, 2013; Shrestha & Chen, 2010; Simard et al., 2001). Furthermore, the physical structure of the soil changes, with significant increases in the microaggregate size

fraction as a result of the destabilization of soil macroaggregates. This change can consequently lead to an exposure of originally inaccessible sites within aggregates to soil microorganisms (Siebers & Kruse, 2019). Physical changes, along with the observed flush of nutrients, may have introduced new ecological niches that can be exploited by novel microbial populations. Correspondingly, there was a significant increase in the overall diversity (evenness and richness) of the VR samples when compared to the NS samples. A similar increase in overall diversity and enzymatic activity of soil communities has been observed previously following vegetation removal (Chanasyk et al., 2003; Danielson et al., 2017; Martin, 2019; Siebers & Kruse, 2019). Increased diversity has been linked to an increase in the existence of multiple soil processes that underpin the delivery of ecosystem services (i.e. multi-functionality) (Delgado-Baquerizo et al., 2016; Louca et al., 2018). There was also an increased co-occurrence network complexity in vegetation removal sites, with more links between co-occurring OTUs, and a higher connectance. Previous studies have shown that in response to disturbance, loss of interactions in the community occur even before species disappearance, thus affecting functionality and ecosystem services (Moreno-Mateos et al., 2020; Valiente-Banuet et al., 2015). Therefore, the increase in co-occurrence could be linked to resistance and resilience of the community to perturbations (Karimi et al., 2017).

While the BCC in VR sites overlapped with the range of natural variability, there was a significant change in response to vegetation removal. Previous studies using phospholipid fatty acids (PLFA), T-RFLP, and 16S rRNA analyses have shown a shift in the BCC immediately post-harvest (Danielson et al., 2017; Hynes & Germida, 2013; Smenderovac et al., 2017), however, to my knowledge, increased heterogeneity in response of vegetation

removal has not been recorded previously. The changes in BCC can in part be attributed to changes in soil parameters. Most edaphic factors were correlated with BCC in VR sites, and several nutrients in soils such as N, C, and P, were increased in response to vegetation removal. Previous studies have seen the simultaneous change in the BCC and soil parameters (N, P, K, and Ca availability and C/N ratio) after vegetation removal (Hynes & Germida, 2012a, 2012b, 2013). Alternatively, the removal of vegetation established a new set of environmental conditions that selected for different soil organisms (and changed the soil parameters) than those that were successful in intact conditions. For instance, taxonomic shifts were observed in VR soils including dominant bacterial groups such as Acidobacteria, Actinobacteria, Alphaproteobacteria, Betaproteobacteria, and Verrucomicrobia, which have been also recorded in previous studies (Hartmann et al., 2012; Martin, 2019). Alterations that might lead to the new ecosystem characteristics include the compaction of the soil due to the use of heavy equipment to remove the vegetation (Grigal, 2000), increased short-term litterfall and associated organic matter input into the soils, the decreased quality of organic input due to high levels of woody debris during harvesting (Attiwill & Adams, 1993), the sudden cessation of substances released by plant roots (Hodge & Fitter, 2013), and the shift in the water balance and temperature of the soils due to erosion (Chanasyk et al., 2003). These alterations occur heterogeneously in soil, which might explain the increased heterogeneity in the BCC and edaphic parameters of VR samples. By removing vegetation, nutrient release zones and root gaps are created. As well, the plant litter is redistributed and further mixed with the mineral soil, thereby increasing patchiness in the litter layer and soil, which in turn increases the spatial variability in soil moisture and temperature, and influences nutrient mineralization and

transport (Chanasyk et al., 2003; Gundale et al., 2006; Guo et al., 2002; Mitchell et al., 2004; Robertson et al., 1993).

The differences in edaphic parameters, overall diversity, taxonomic composition, BCC, functional diversity, and microbial biomass were more marked in sites reclaimed with PMM. This divergence from reference sites has been observed previously, and it persists in the longer term (Hahn & Quideau, 2013; Masse et al., 2017; Stefani et al., 2018). As evidenced by correlation analysis, the BCC was related to high salinity, and low P availability and moisture content in PMM soils. During reclamation, the overburden layers, characterized by elevated salinity, are mixed with salvaged reclamation materials (Fung & Macyk, 2000), potentially resulting in high salinity soil profiles that may influence the microbial communities, as well as raise concerns about the limitation of tree growth (Duan et al., 2015). In addition, the low availability of P may also hamper the growth of certain plant species in PMM soils and facilitate the invasion of weedy species (MacKenzie & Quideau, 2012; Pinno et al., 2012), which in turn could be driving the BCC due to alteration in the belowground-aboveground relationships. Low soil moisture in PMM can be another important factor shaping the microbial communities. Soil moisture is a crucial determinant of C and N availability and plays a pivotal role in structuring microbial communities and activities in soil (Banerjee et al., 2016). Correspondingly, our data showed decreased basal respiration and activity related to carbohydrates in PMM soils with respect to NS sites, which is consistent with previous observations (Béasse et al., 2015). Furthermore, in our study, the β-diversity, microbial biomass, and the abundance of Alphaproteobacteria and Actinobacteria varied in soils with different levels of moisture. This variation is consistent with previous findings in boreal forest soils (Dimitriu &

Grayston, 2010; Swallow & Quideau, 2013), and using microcosms (Banerjee et al., 2016) that have shown the largest effects of soil moisture on the abundances, as measured by qPCR, of Alphaproteobacteria and Actinobacteria, the β-diversity, and overall microbial abundance . I suggest the BCC in PMM are initially different from those in the reference sites, since they are sourced from wetlands while the reference sites are upland boreal forests. The composition of organic matter as well as the physical characteristics in the PMM are fundamentally different from those in the reference soils due to their lowland peat origin (Turcotte et al., 2009), which in turn could also drive the BCC.

Among the reclaimed substrates, PMM sites exhibited the narrowest range of variability for BCC, functional profile, and edaphic parameters. Interestingly, this homogeneity has been reported previously only for bioavailable nutrients. The level of resource homogeneity might be important for quantifying successful reclamation trajectory as this is reflected in belowground function (Dietrich & MacKenzie, 2018). Also, spatial heterogeneity of soil nutrients is an essential factor in recovering forests, as resource homogeneity might support the establishment of only the species that can cope with high levels of competition; or weaken species that need unique regeneration niches for successful reestablishment (Fraterrigo et al., 2005; Gundale et al., 2006). Therefore, the low resource heterogeneity may be reflected in the low heterogeneity of the BCC and functional diversity, and in turn may hamper the establishment of plants. It is likely that the combination of multiple factors in PMM sites may have selected microbial communities with a very homogenous profile distinct from the communities in natural boreal forest soils. Namely, (i) the salvaged peat materials from PMM were all sourced from a unique spot or were homogenized prior to use as reclamation substrate, (ii) the coevolution of vegetation in peatlands with specific

belowground microorganisms formed homogeneous communities adapted to the characteristic conditions of these environments, and do not differentiate geographically (Andersen et al., 2013), or (iii) only selected microorganisms can survive the transition from a lowland peat environment to an upland PMM, given the specialized nature of the community (Andersen et al., 2013). Under the assumptions (ii) and (iii), organisms in PMM either have cooperated and adapted to similar ecological niches in peatlands or have adapted during the transition to new niches in upland PMM; those without the ability to cope with these specific conditions were filtered out (Morriën et al., 2017; Sun et al., 2017; Zelezniak et al., 2015). The observed decrease in the overall diversity (richness and evenness) of the community as well as the decreased network complexity in PMM support these two hypotheses.

Reclamation with either FFM or Sand-FFM approached the range of natural variability more closely than PMM; indicating fewer alterations to the boreal forest soils. Prior studies indicate that FFM recreates soil conditions more similar to natural boreal forests soils than PMM, in terms of BCC (Hahn & Quideau, 2013; Stefani et al., 2018), edaphic parameters (Hogberg et al., 2020), and soil functional profile (Howell & MacKenzie, 2017). In my study, the BCC in FFM was correlated with SAR, pH and sodium; however, the dispersion of the samples in the ordination space correlated with multiple edaphic parameters. This dispersion indicates that these soils are physically and chemically variable, which ultimately drives heterogeneous microbial communities across samples. The forest floor naturally exhibits a degree of patchiness which might drive the heterogeneity in the soil biota (Mitchell et al., 2004). In FFM cover soils, this variability might be exacerbated by the processes of mixing with the mineral layers and by the direct effects of vegetation

removal prior to the stripping of the forest floor. Spatial heterogeneity of nutrient bioavailability on FFM reclaimed sites is likely a factor that positively affects reestablishment of vegetation and could also be associated with the establishment of a diverse BCC across sites (Dietrich & MacKenzie, 2018).

FFM diluted with sand showed an unclear pattern of increased heterogeneity and sub-clustering with no clear associations with individual edaphic parameters. In Sand-FFM Group 2, samples were highly dispersed and were similar to both PMM and FFM communities; whereas in Sand-FFM group 1 samples were the most similar to the range of natural variability and did not overlap with the rest of the reclamation or VR samples. Furthermore, the Sand-FFM network analysis did not provide a clear understanding of the potential intra community interactions, likely due to habitat filtering, i.e. samples were drawn from very different environments (Berry & Widder, 2014). In the context of reclamation, this could represent an unpredictable trajectory of ecosystem recovery; I suggest that this could be an area of future study. Investigating what mixture and what type of sand will provide the best outcome (i.e. the most similar to either NS and/or FFM) and the most predictable outcome (i.e. the least variability beyond the range of FFM), can provide a strategy to maximize the limited supply of forest floor.

Of the parameters I examined, BCC provided the most information about the impacts of mining practices. The high resolution of BCC as measured by high throughput sequencing can distinguish between different reclamation substrates and disturbed sites and can potentially be used to assess optimal approaches for land reclamation. As FFM has been identified as the best substrate here, and in other studies (Hogberg et al., 2020; Howell & MacKenzie, 2017; Stefani et al., 2018), BCC could be used as a tool to provide more

information about the similarity of particular approaches to using this substrate and, over time, may give information on the trajectory of reclamation success. For instance, BCC could be used to assess combinations of PMM-FFM, FFM-sand or when using amendments. Edaphic parameters might be difficult to interpret given their high variability in sites; however, if combined with BCC by correlation methods such as redundancy analysis, they could be important predictors to understand the belowground dynamics in reclamation systems. On the other hand, the CLPP method I utilized to study the functional profile did not provide high resolution results such as the ones observed using high-throughput sequencing; however, this method provided a more direct reflection of the functional diversity of the community in soil. While 16S rRNA in combination with edaphic parameters only allows to make inferences about the soil functions, CLPP remains relevant to provide direct evidence of how these soil functions are varying across samples. Therefore, a combination of high-throughput sequencing and CLPP can be utilized as an integrated perspective for assessing ecosystem recovery. Functional genes assessed by real time qPCR could be a complementary approach to assess soil functionality considering that this method is sensitive and can target specific functions in soil that may be relevant in reclamation (Thiele-Bruhn et al., 2019); however, the usefulness of this approach remains to be determined.

Rowland et al. (2009) suggests a goal of bringing reclaimed ecosystems to within the range of natural variability, with variability referring to diversity, vegetation structure, and ecological processes. The implementation of this approach could quantitatively determine the distance a disturbed system is from the range of natural variability and how that distance may increase or decrease with time, i.e. determine the trajectory of reclamation.

While the early revegetation objectives in the AOSR were to establish native or introduced grass and shrub species to control erosion; modern mine operators revegetate using native trees and understory plant species, with the intention of promoting the reestablishment of a boreal forest community (Masse et al., 2017). Results from studies comparing peat- and FFM amended plots suggest that the forest floor puts both the microbial and plant communities of the reclaimed sites on a faster trajectory toward a natural forest than peat does (Béasse et al., 2015; Hahn & Quideau, 2013). The long-term monitoring of the dynamics of above- and belowground diversity thus is extremely important to ensure that reclaimed plots are on the right ecological trajectory toward the range of natural variability, and ultimately will provide similar ecosystem services to the pre-disturbed condition (Stefani et al., 2018).

With further time-series studies, I propose that an index can be developed to monitor reclamation trajectory. This quantitative approach can be used to determine whether a reclaimed system is moving towards or away from the range of natural variability, the rate of this trajectory and the time to achieve reclamation success (Niu et al., 2018; Zhu et al., 2019). Previous quantitative indexes that have been proposed for assessing soil reclamation in the AOSR rely on the soil nutrient profile or the plant community composition, disregarding the crucial role of soil microbes (Hogberg et al., 2020; Raab & Bayley, 2012). Further research in this area may establish baseline information to develop a useful tool comprising biotic factors included in this study for quantitatively assessing reclamation success. A numerical framework for monitoring and managing the condition of disturbance can classify the reclamation as successful or ecologically sustainable (Mukhopadhyay et al., 2014), which in the AOSR has not been established yet (Audet et al., 2015).

2.6. Conclusion

The sites on this study were sampled two to five years after vegetation removal and two years after reclamation soil placement; thus, only the initial response of the microbial communities and edaphic parameters is presented here. However, differences between reclamation soils, vegetation removed soils and natural stands are clearly discernible. In light of my results, reclamation imposes a more drastic disturbance to the boreal forest than the removal of vegetation as a part of the chain of events in a mining extraction process. Among reclamation substrates, FFM diluted with sand approached closest to the range of natural variability, but this was observed only for a subcluster of the samples. Thus, sand material could be further studied to better identify features that ensure a trajectory towards the range of natural variability. The high salinity, low moisture and low P availability of PMM influencing microbial communities may hamper the success of ecosystem recovery, by limiting the tree growth. Therefore, evaluating approaches that buffer the effect of salinity in this material are of special importance in reclamation protocols. Bacterial community composition provided the highest resolution of all the factors assessed; therefore, it could be used as a suitable tool to assess the trajectory of reclamation processes. Since the soil functional diversity in reclaimed soils seemed to correlate well with the patterns observed in BCC; assessing these two factors together can provide a more integrated perspective of ecosystem recovery.

3. Chapter 3. Conclusion

3.1. Primary Findings

My results show that the bacterial community composition (BCC) and functional profile of boreal forests in the Alberta Oil Sands Region (AOSR) are significantly altered by anthropogenic disturbances related to open-pit mining. Although other studies have shown similar findings in soils impacted by reclamation and vegetation removal (Dimitriu et al., 2010; Hahn & Quideau, 2013; Hynes & Germida, 2013), this study utilized high-throughput sequencing and physiological profiling, which allowed for a more in-depth characterization of the impacts. Furthermore, this study simultaneously assessed the impact of vegetation removal and reclamation, which provided a comparison of the severity of these anthropogenic disturbances. Overall, the distance from the range of natural variability indicated changes caused by vegetation removal were less dramatic than the ones caused by reclamation. Within reclamation soils, peat-mineral mix (PMM) differed the most from the range of natural variability.

While previous studies have shown the microbial communities shift in response to vegetation removal (Danielson et al., 2017; Hynes & Germida, 2013; Smenderovac et al., 2017), my results showed that there is a significant increase in the heterogeneity and overall diversity of the communities. Also in response to vegetation removal, several nutrients increased in soil and were significantly correlated with the bacterial community composition. Thus, I suggest a flush of nutrients that occurs immediately following vegetation removal and could be linked to the interruption of plant uptake and input of slash residues and root remnants (Hynes & Germida, 2013; B. M. Shrestha & Chen, 2010) may have introduced new

ecological niches exploited by the microbial communities. Supporting this hypothesis, my results showed an increase in the overall diversity and in the network complexity of VR samples. In response to disturbance, loss of interactions in the community occur even before species disappearance, thus affecting functionality and ecosystem services (Moreno-Mateos et al., 2020; Valiente-Banuet et al., 2015). Therefore, the increase in co-occurrence could be linked to increased functionality in these soils (Karimi et al., 2017).

BBC, overall diversity, and functional profiles differed between reclaimed and natural boreal forest soils, indicating that reclaimed soils do not approach the range of natural variability at an early stage of recovery. While this finding agrees with previous studies (Howell & MacKenzie, 2017; Stefani et al., 2018), my findings also demonstrated that microbial communities in reclamation cover soils have different levels of heterogeneity and overall diversity based on the type of soil being used. Soil reclaimed with PMM had a significantly lower heterogeneity and overall diversity when compared to the natural range of variability; and similar to previous observations, it was the least similar to the range of natural variability (Howell & MacKenzie, 2017; Stefani et al., 2018). In contrast, soils reclaimed with forest floor mineral mix (FFM) had a BCC closer to the range of natural variability, and this similarity to native soils (NS) was more pronounced for one subcluster of the material diluted with sand. The increased heterogeneity and sub clustering patterns observed for FFM mixed with sand (Sand-FFM) suggests the starting material was highly variable, yielding heterogeneous microbial communities across samples.

High salinity, low P availability, and low moisture content were related to PMM soils, which indicates these edaphic factors play a significant role shaping bacterial communities in PMM soils and could potentially influence the establishment of plant species (Duan et al., 2015). Furthermore, I hypothesized the homogeneity of edaphic parameters in PMM could be reflected in the homogeneity of BCC and the functional profile, since low resource heterogeneity can lead to more homogeneous belowground functionality (Dietrich & MacKenzie, 2018). This homogeneity was also evidenced by the reduced network complexity in PMM soils compared to NS. In FFM samples, multiple edaphic parameters were associated with the BCC, with the dispersion indicating these soils are physically and chemically variable, thereby driving heterogeneous microbial communities across samples. The variation in BCC was exacerbated in Sand-FFM samples, suggesting that mixing with sand materials leads to more variation in the associated edaphic parameters.

3.2. Contributions to the Field

Large-scale mining for oil extraction in the boreal forests of Northern Alberta has produced a disturbance footprint of ~ 900 km^2 of land; which, under regulations from the government of Alberta, must be reclaimed to equivalent land capabilities using soil materials salvaged and conserved during land clearing (Audet et al., 2015). Currently, it is unclear which soil features indicate a successful reclamation trajectory; however, one approach to measure ecosystem recovery is to compare several different markers of ecosystem function in reclaimed soils to the variability of those factors within the natural ecosystem (Rowland et al., 2009). Therefore, in this study, I utilized compositional and functional attributes of microbial communities as markers of ecosystem function, considering that microbial communities underlie most soil biogeochemical processes and ecosystem services that land

reclamation practices attempt to reconstruct and support (Harris, 2003; Mummey et al., 2002). Overall, the findings in this study indicate microbial communities are useful tools for estimating the distance of reclaimed soils to the range of natural variability in undisturbed natural soils, and ultimately, are a reasonable measure of recovery. In particular, BCC was the one factor that was able to clearly differentiate all of the different soil types. The resolution provided by BCC, therefore, could be more readily used as a marker for optimizing reclamation protocols than the other factors measured here. This high level of resolution was achievable due to advantages offered by high-throughput sequencing. Other studies have used microbial communities as markers of ecosystem function (Dimitriu et al., 2010; Hahn & Quideau, 2013); however, they have relied on lower resolution methods such as phospholipid fatty acids (PFLA) and denaturing gradient gel electrophoresis (DGGE). Edaphic parameters alone can be difficult to interpret given their high variability; however, if combined with microbial community composition, they could be important predictors to understand the belowground dynamics in reclamation systems. Such an approach can be accomplished through correlation methods like redundancy analysis. High throughput sequencing of 16S rRNA genes to examine microbial community structure thus holds promise to allow more thorough use of microbes as markers of reclamation efficacy and trajectory, and ultimately in ecosystem monitoring projects.

As suggested by this and previous studies, FFM is preferred as soil cover in reclamation operations because the seedbank, chemistry, and microbiology of the soil more closely resembles the upland ecosystems (MacKenzie et al., 2012; Mackenzie & Naeth, 2010); however, FFM availability is limited such that only approximately 35% of the land can be

reclaimed with this soil type under current practices (MacKenzie et al., 2012). One approach to increase the availability of FFM is to mix it with a (theoretically) neutral material such as sand. My results with the Sand-FFM samples showed an unclear pattern of increased heterogeneity and sub-clustering of the microbial communities. These findings suggest the sand starting material was not neutral and is variable. Thus, Sand-FFM may be of unpredictable trajectory in the context of ecosystem recovery without further characterization of the sand. However, some sand-FFM samples closely approached the range of natural variability. The edaphic parameters and microbial communities of the sands used to compose the sand-FFM sites were not characterized in my study. Sandy materials are readily available in mining sites, but may vary significantly. Therefore, I suggest that a thorough characterization may indicate which materials are most appropriate for FFM dilution and that not all sand is equal.

3.3. Improvements to the study and future directions

My thesis analyzed the soil biological function by measuring the catabolic diversity of microbial communities via community-level physiological profiling (CLPP) (Campbell et al., 2003). The results showed microbial community function correlated well with the BCC; however, CLPP did not provide sufficient resolution to establish a statistical support for this correlation. CLPP is a measure of microbial functional diversity in soils as it provides more information than broad microbial measurements widely used to assess function (Gomez et al., 2006). However, the technique does have several shortcomings that might have contributed to the lack of resolution; specifically, this technique is based on the utilization of select carbon sources by organisms that are exclusively heterotrophic (Thiele-Bruhn et al., 2019). As a consequence, CLPP does not reflect the full spectrum of microbial functions in

a soil community. Thus, to study the soil microbial function, I recommend supplementing the CLPP by the analysis of functional genes by qPCR, which are indicative of specific transformation processes or soil functions. In the context of soils impacted by anthropogenic disturbances related to surface mining, qPCR analysis of gene sequences related to plant growth promotion (e.g. mtDNA of arbuscular mycorrhizal fungi) and nutrient cycling (e.g. *amoA* and *nifH* for nitrogen cycle or *phoN* and *phoD* for P cycling) can be used as proxies of known microbial processes in soil (Bergkemper et al., 2016; Hirsch et al., 2010). Furthermore, a major advantage of using qPCR assays is that they are more sensitive and reproducible in addition to being designed for high-throughput analysis which might allow for analysis in conjunction with high-throughput compositional data (Thiele-Bruhn et al., 2019). However, the qPCR method involves more complexity and an increased cost when compared to the CLPP (Lladó & Baldrian, 2017). Alternatively, the assessment of soil microbial function can be complemented through metagenomics. Metagenomics can be used to identify and quantify all the metabolic pathways involved in a microbial community and thus, provide a wider picture of the functions in soils while simultaneously characterizing the microbial community structure (Garris et al., 2016). Nevertheless, the microbial function evaluated via metagenomics only predicts potential soil functionality, and does not provide information about functions performed by active members in a community, as CLPP does (Jansson & Hofmockel, 2018). As a result, each of these methods (i.e. CLPP, qPCR, and metagenomics) have benefits and drawbacks; however, together they could provide more detailed information about the function of soils, linking BCC to ecosystem health more directly.

While I determined BCC across samples, I did not assess other biological markers such as fungal, archaeal or meso faunal communities. In forest ecosystems, the development and health of trees rely on their interactions with the entire soil microbiome. Along with bacteria, the colonization of short roots by mycorrhizal fungi in boreal forests facilitates carbon transport and respiration. As well, fungi are important drivers of ecosystem multifunctionality, including organic matter mineralization, climate regulation and nutrient cycling (J. Li et al., 2019; Read et al., 2004). The archeal communities play roles in nutrient cycling as some of the groups present in boreal forests are ammonia oxidizers (Masse et al., 2017). Mesofaunal communities can contribute to the stabilization of the physical structure of soil and can play significant roles in C-cycling by decomposing and redistributing organic matter (Coleman et al., 2018). Considering the goal of reclamation is to re-establish soil functions as key to ensure the long-term sustainability of the land, fungal, archaeal and mesofaunal communities should also be assessed as potential markers of ecosystem recovery. I recommend the analyses of ITS genes for fungi, 16S rRNA genes for archaea, and 18S rRNA genes for mesofauna via high-throughput sequencing in future studies; such analyses along with the analysis of bacterial 16S rRNA profiles may provide a better perspective of the overall impacts of anthropogenic disturbances in belowground communities. While mesofaunal communities are typically characterized via microscopy, amplification of the 18S gene could be used to accurately identify a large range of invertebrate taxa (Horton et al., 2017).

Ecological recovery after disturbance may require decades (Ngugi et al., 2018; S. Sun et al., 2017). As seen in other studies, reconstructed ecosystems have a different organic matter

composition, soil available nitrogen, and microbial community composition when compared with natural boreal forest soils; thus indicating the emergence of new ecosystems (Dimitriu et al., 2010; Turcotte et al., 2009). My thesis only presented the short-term response of microbial communities after disturbance; therefore, a question remains as to whether over time the different reclamation treatments and vegetation removed soils approach or diverge from the range of natural variability and the rate at which this occurs. To address these questions, the different compositional and functional attributes should be monitored at different temporal scales (i.e. seasonally and interannually) for at least several years to reveal microbial community shifts.

A common approach to assess ecosystem health after disturbances and to monitor reclamation practices is the development of quantitative indices that integrate several variables representing the majority of the variation in the ecosystem, and comprising the ecological, functional and/or structural aspects of an ecosystem (Raab & Bayley, 2012; Rebecca C. Rooney & Bayley, 2012). These systematic methods are established using values determined locally. For example, specific measures for biotic or abiotic properties in a particular region or even site can be used to provide a score that either allows users to compare a disturbed site to an undisturbed condition of the ecosystem (Hogberg et al., 2020; Mukhopadhyay et al., 2014) or to classify the disturbance along a gradient (Beck & Hatch, 2009). Ideally, the interpretation of index scores provide information regarding the state of an ecosystem, with notable advantages over different approaches such as multivariate analysis and surveys of specific indicators (Beck & Hatch, 2009; Mukhopadhyay et al., 2014). Indexes, despite their derivation from multivariate statistical analyses, do not require

complex statistical procedures for their use. Hence, their application and interpretation is relatively easy (Beck & Hatch, 2009). Furthermore, indexes provide a numerical framework for monitoring and managing the condition of disturbances and can ultimately classify the reclamation as successful or ecologically sustainable (Mukhopadhyay et al., 2014). A soil quality index developed for reclamation in the AOSR has been developed using foliar and soil nutrients (Hogberg et al., 2020).. Biological integrity indices have been developed based on submersed and floating vegetation in wetlands (Rooney & Bayley, 2012), and plant communities (Raab & Bayley, 2012). Both soil quality and biological metrics proved to be valuable tools for assessing reclamation success. Vegetation can be a useful bioindicator due to community attributes such as immobility (therefore exposure to local stressors), relatively high growth rate, well-documented life history and tolerances, and relative ease of sampling (Teels & Adamus, 2002). However, considering that reclamation substrates directly affect the soil community, and that plant communities respond to the belowground functionality, biological indices that include microbial biodiversity and physicochemical parameters in soil can provide more reliable conclusions about the reclamation success.

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Appendix: Supplementary Information for Chapter 2

Figure S1. Map of study sampling locations at the Mine site and surrounding areas. (Image: Google Earth, 2020). NS – Natural soil, VR = Vegetation removed, FFM = Forest floor mineral mix, Sand-FFM = Sand-forest floor mineral mix, PMM = Peat mineral mix.

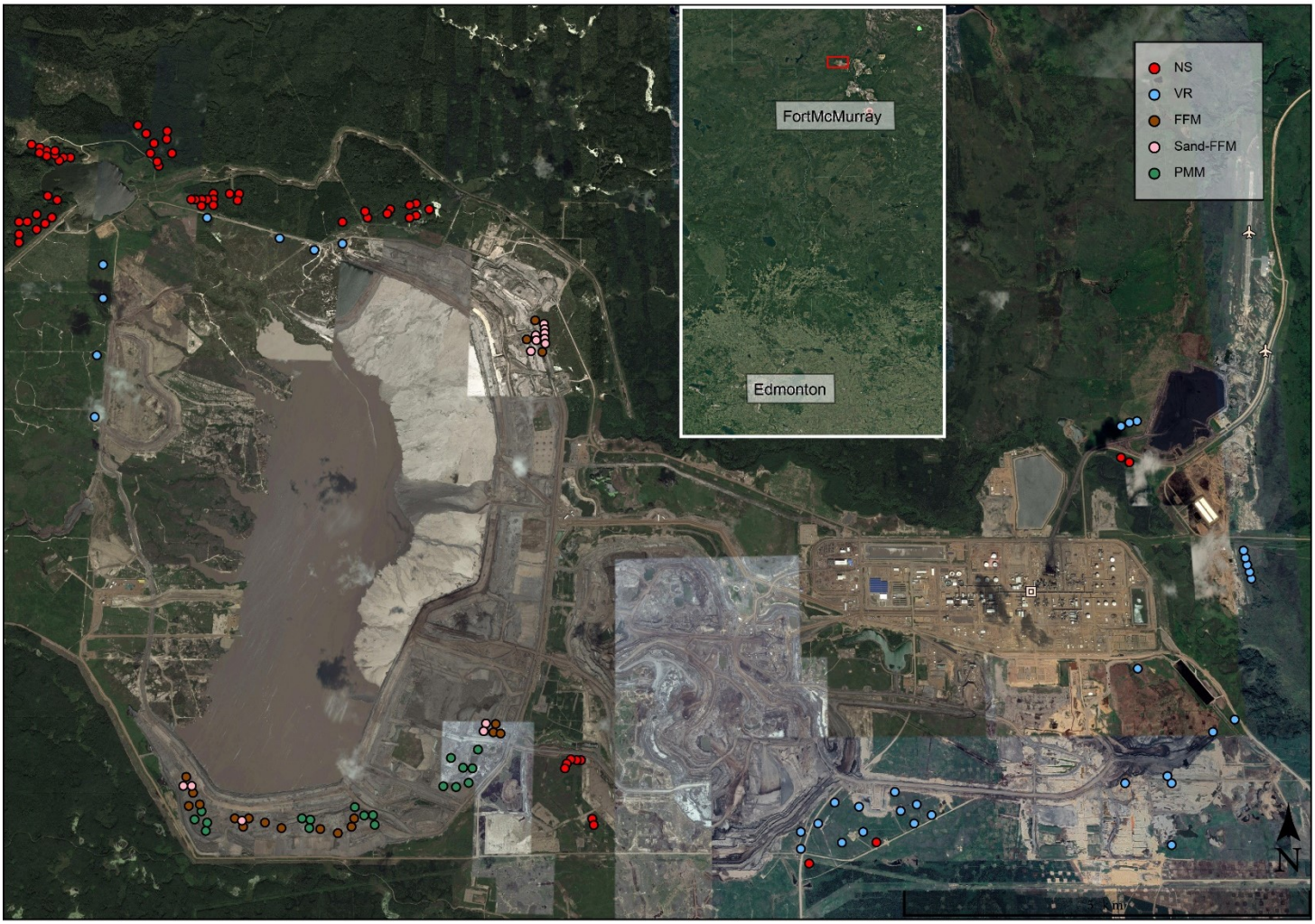

Figure S2. Rarefaction curves for all samples. Each curve represents the subsampled richness level at each level of sequencing intensity from 0 to 10,000 sequences. Site type abbreviations are the same as in the legend to figure S1.

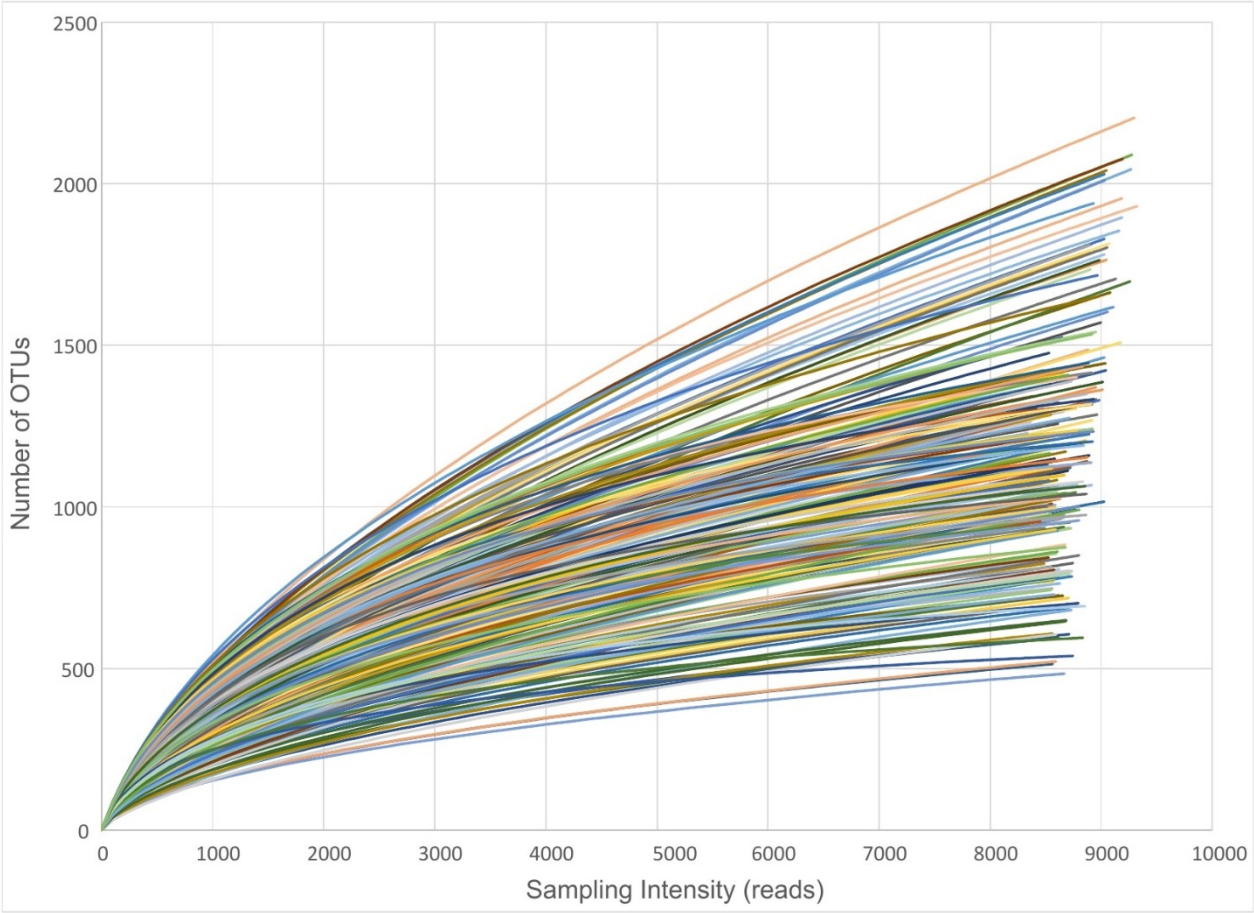

Figure S3. Principal components analysis of the soil chemical and physical parameters, and microbial biomass in the different sample types. Length of the vectors indicates the influence of the parameter in the ordination of data. Site type abbreviations are the same as in the legend to figure S1.

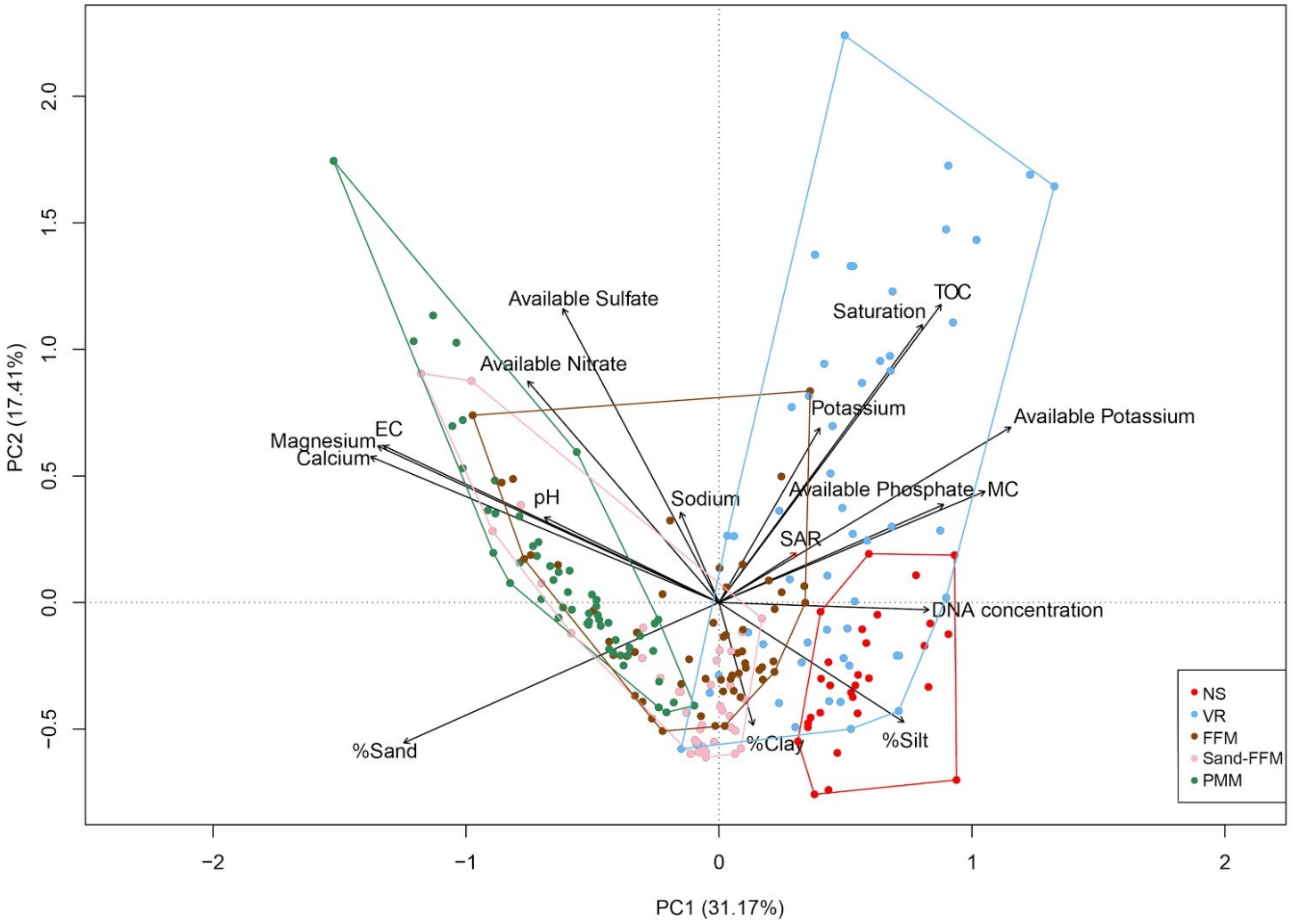

Figure S4. Relative abundance plots of top 50 OTUs in each sample grouped by sample type. OTUs with different shades of the same color belong to the same bacterial Class. Site type abbreviations are the same as in the legend to figure S1.

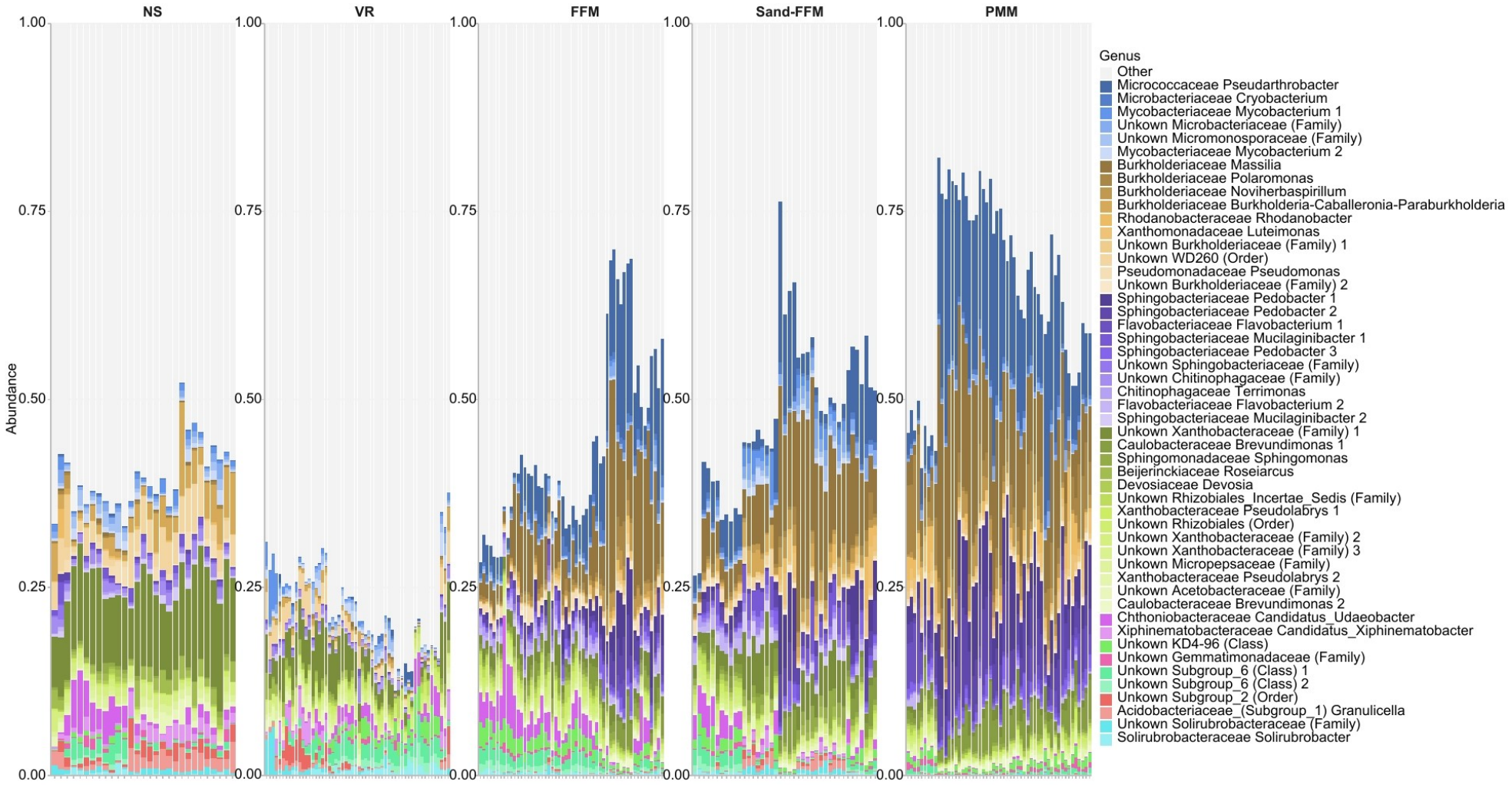

Figure S5. The relative abundance of bacterial communities at the phylum level. Phyla <1% across samples were grouped into "others" alongside unassigned taxa. Site type abbreviations are the same as in the legend to figure S1.

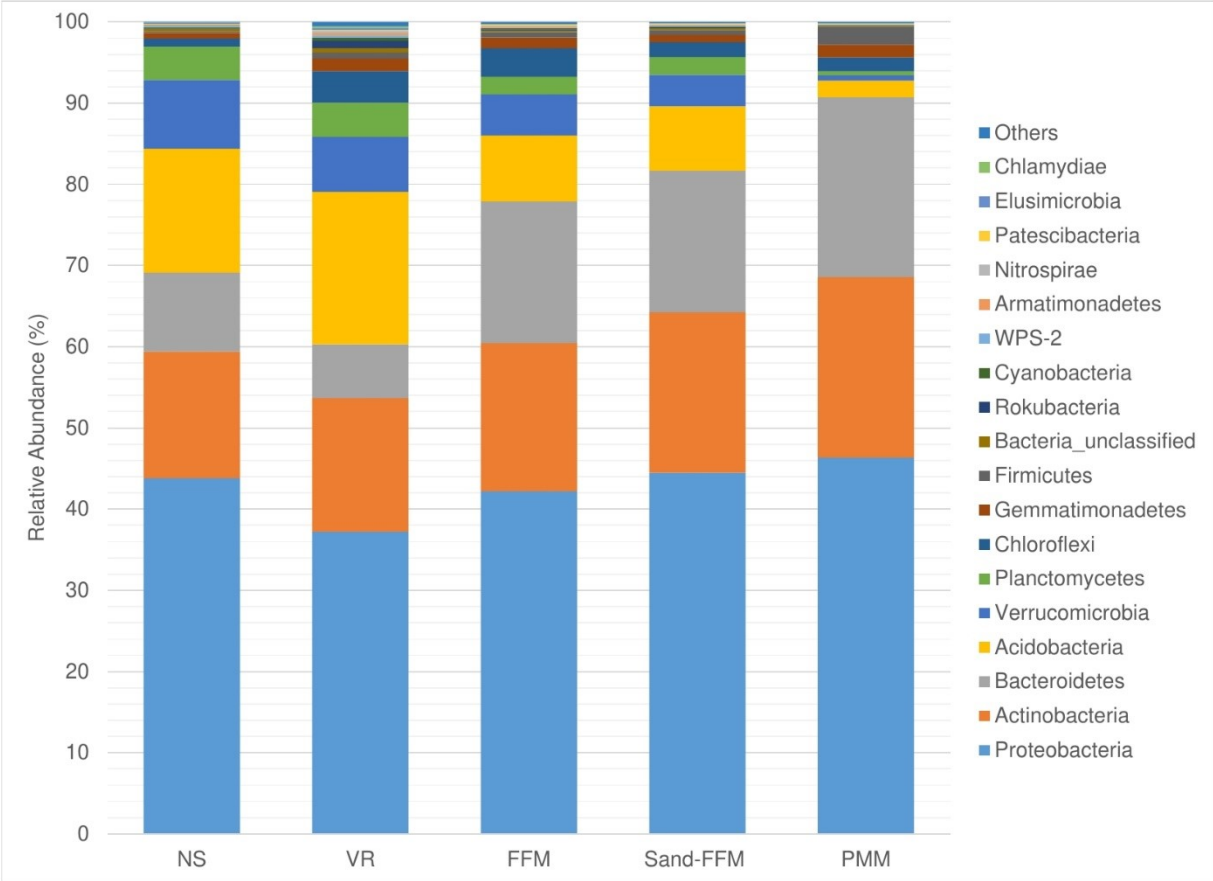

Figure S6. The relative abundance of bacterial communities at the class level. Classes <1% across samples were grouped into "others" alongside unassigned taxa. Site type abbreviations are the same as in the legend to figure S1.

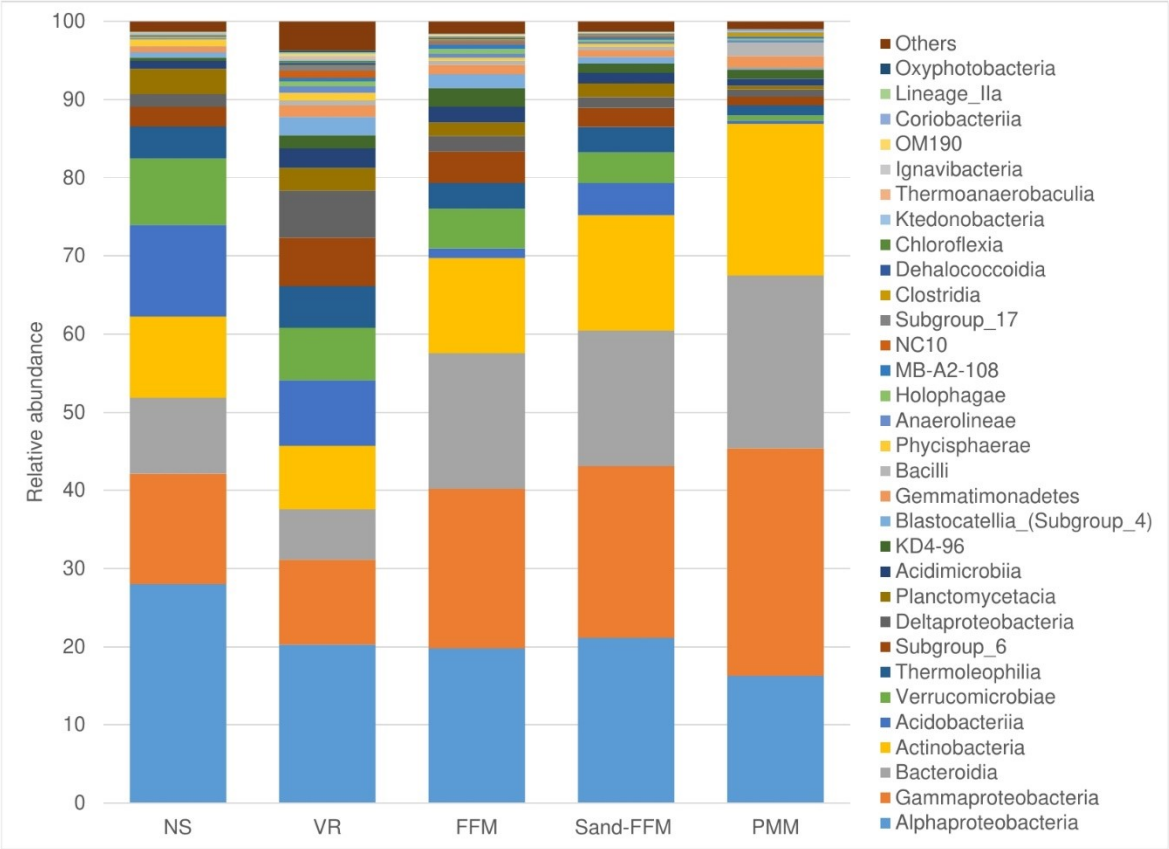

Figure S7. The relative abundance of the top 15 most abundant genera across samples. "Others" represent genera outside the top15 alongside unassigned taxa**.** Site type abbreviations are the same as in the legend to figure S1.

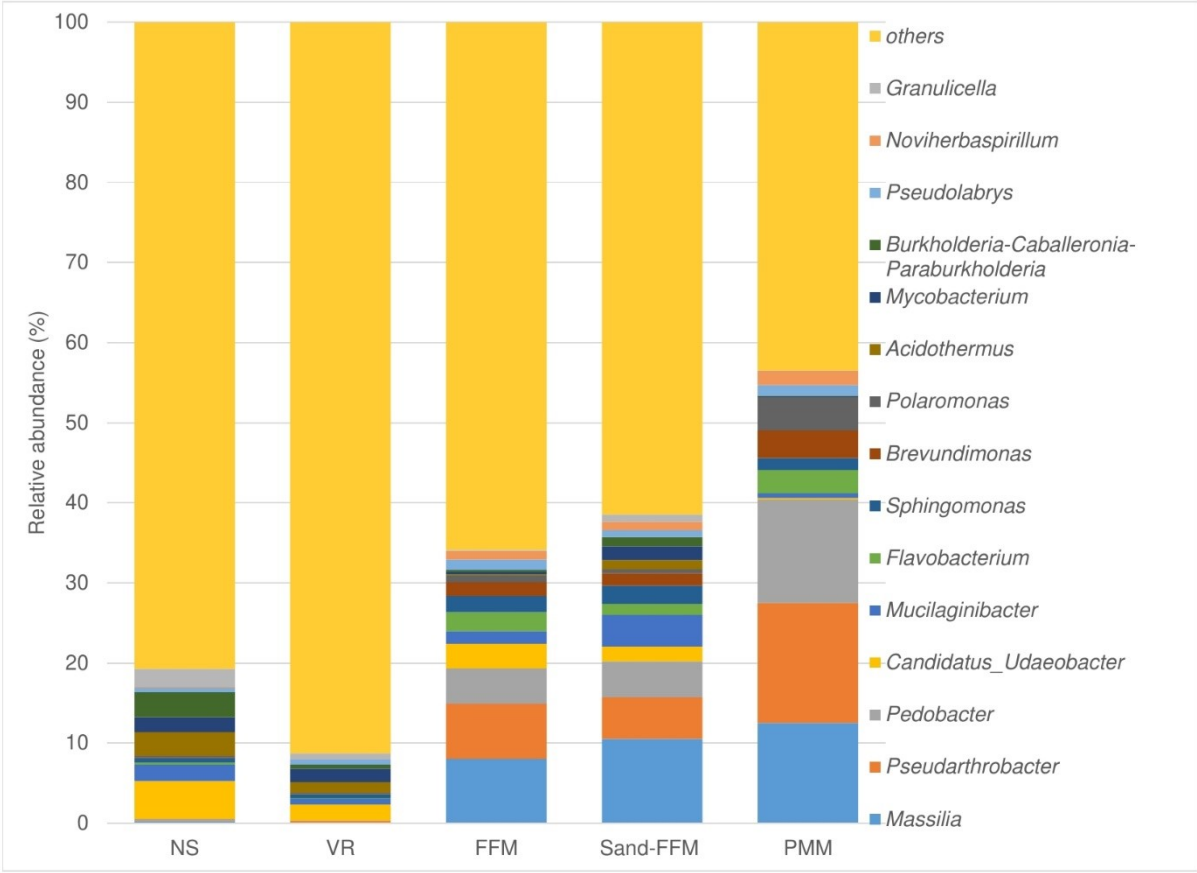

Figure S8. Co-occurrence network analysis of bacterial communities in the different sample types. Each node represents a bacterial OTU, and an edge represents a Spearman correlation with a correlation coefficient of more than 0.75 (blue) or less than -0.75 (red) and statistically significant (FDR<0.05). The size of each node is proportional to the Log2 of its degree. The colors of nodes represent their classifcation at the class level. Site type abbreviations are the same as in the legend to figure S1.

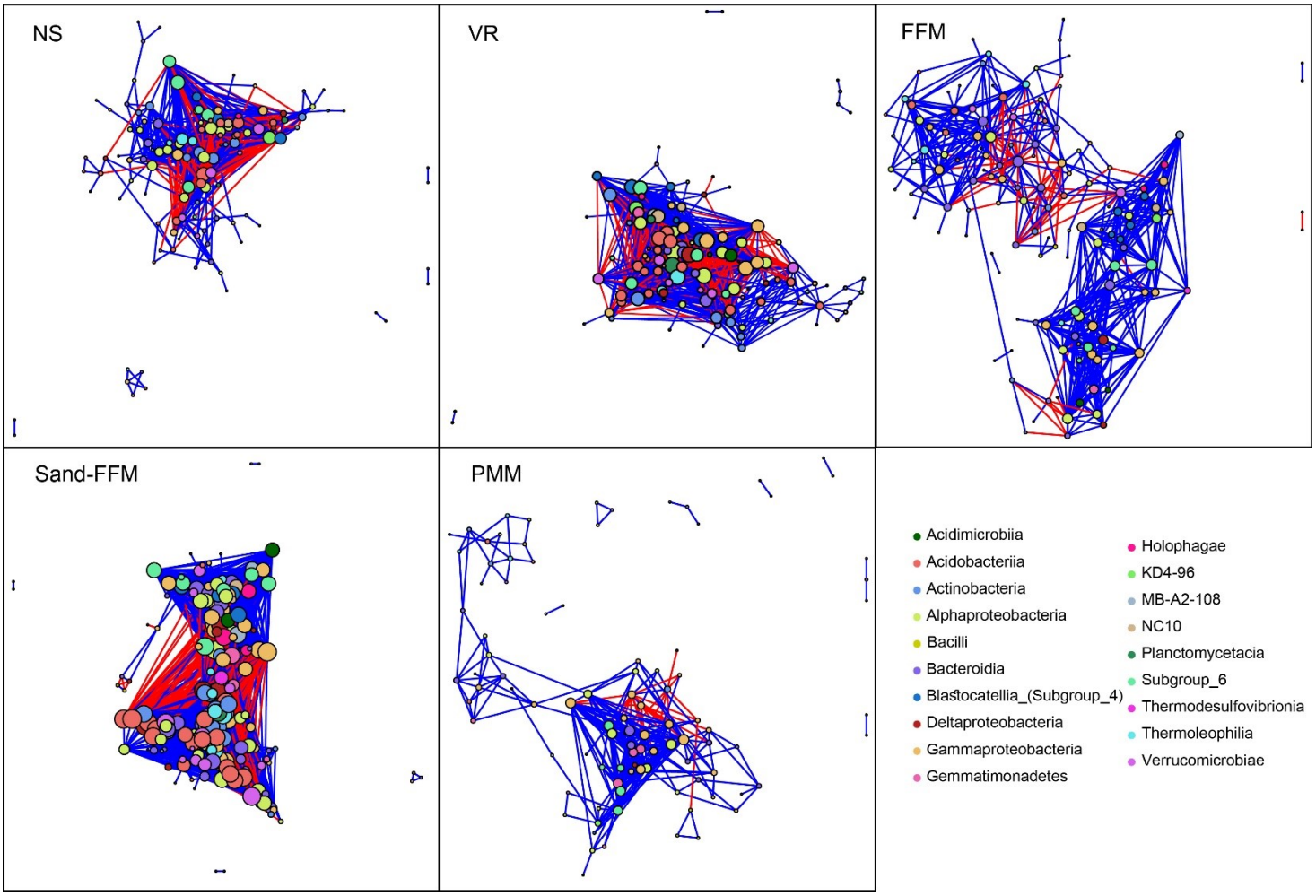

Figure S9. Co-occurrence networks of bacterial communities in the different sample types when intersected with the NS network. Each node represents a bacterial OTU, and an edge represents a Spearman correlation with a correlation coefficient of more than 0.75 (blue) or less than -0.75 (red) and statistically significant (FDR<0.05). The size of each node is proportional to the Log2 of its degree. The colors of nodes represent their classification at the class level. Site type abbreviations are the same as in the legend to figure S1.

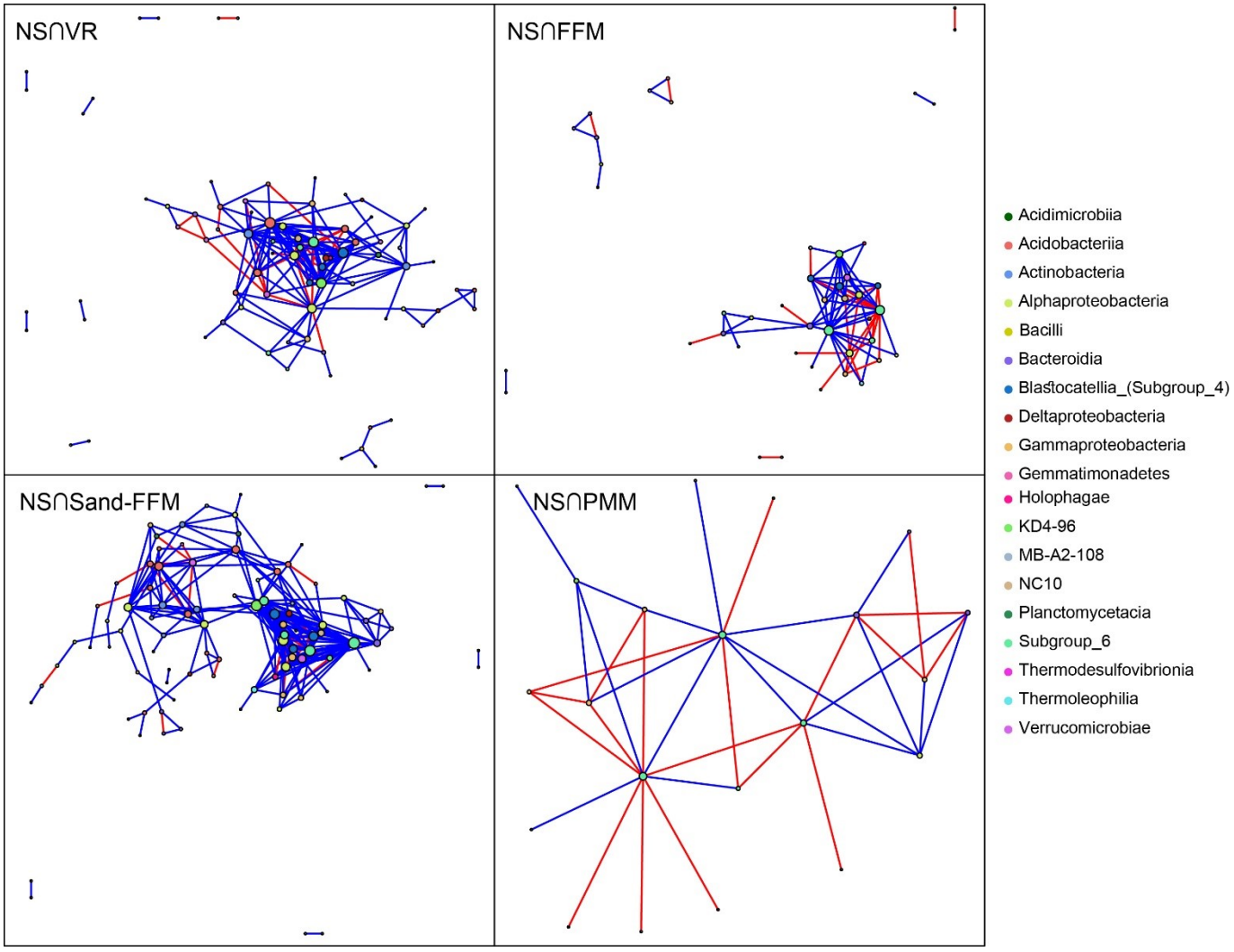

Figure S10. Canonical redundancy analysis of the bacterial communities performed at the class level showing relations between site and soil characteristics and bacterial classes in the different sample types (***, p<0.001) . Site type abbreviations are the same as in the legend to figure S1.

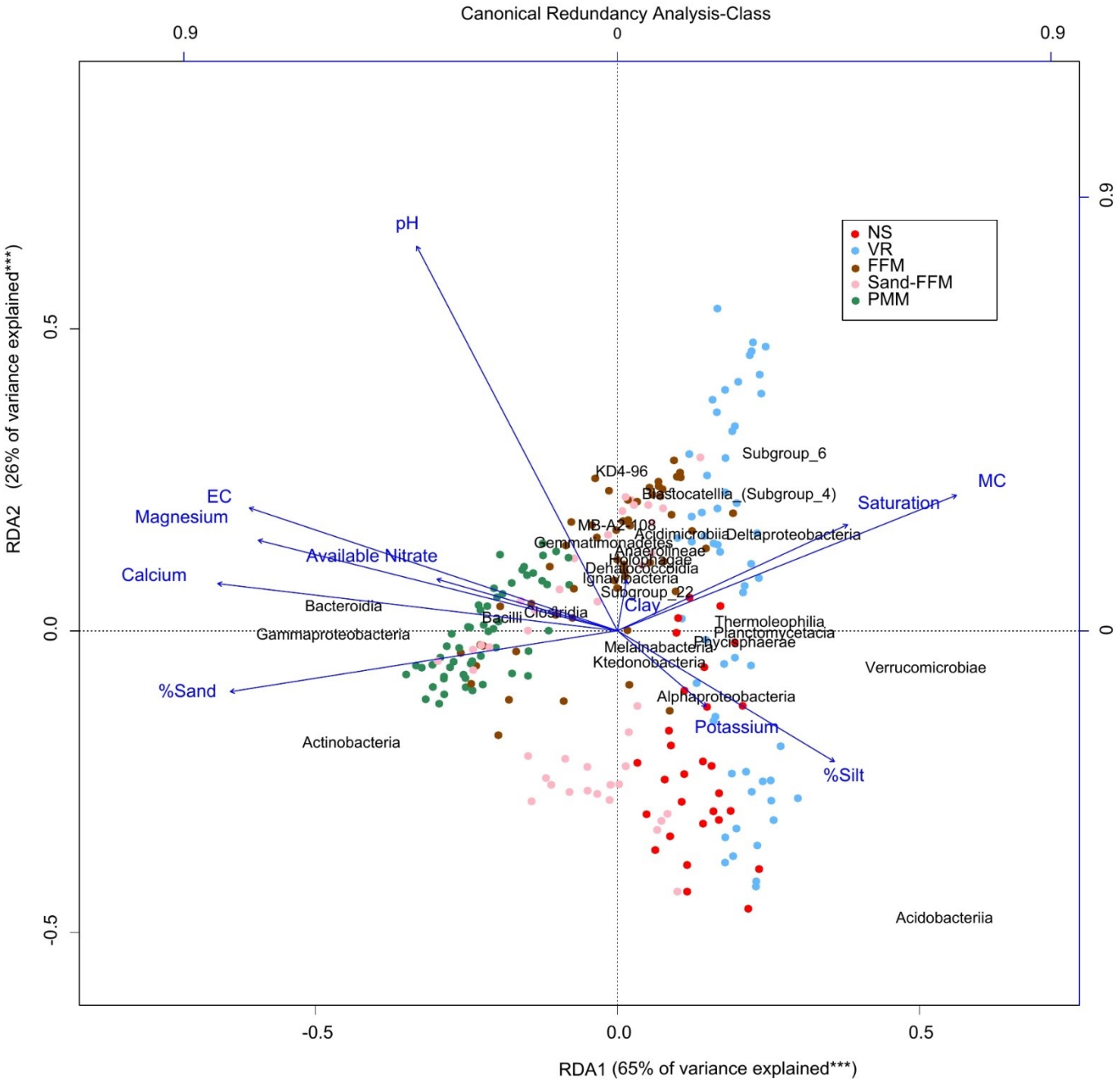

Figure S11. Canonical redundancy analysis of the bacterial communities performed at the phylum level showing relations between site and soil characteristics and bacterial phyla in the different sample types (***, p<0.001; **, p<0.01). Site type abbreviations are the same as in the legend to figure S1.

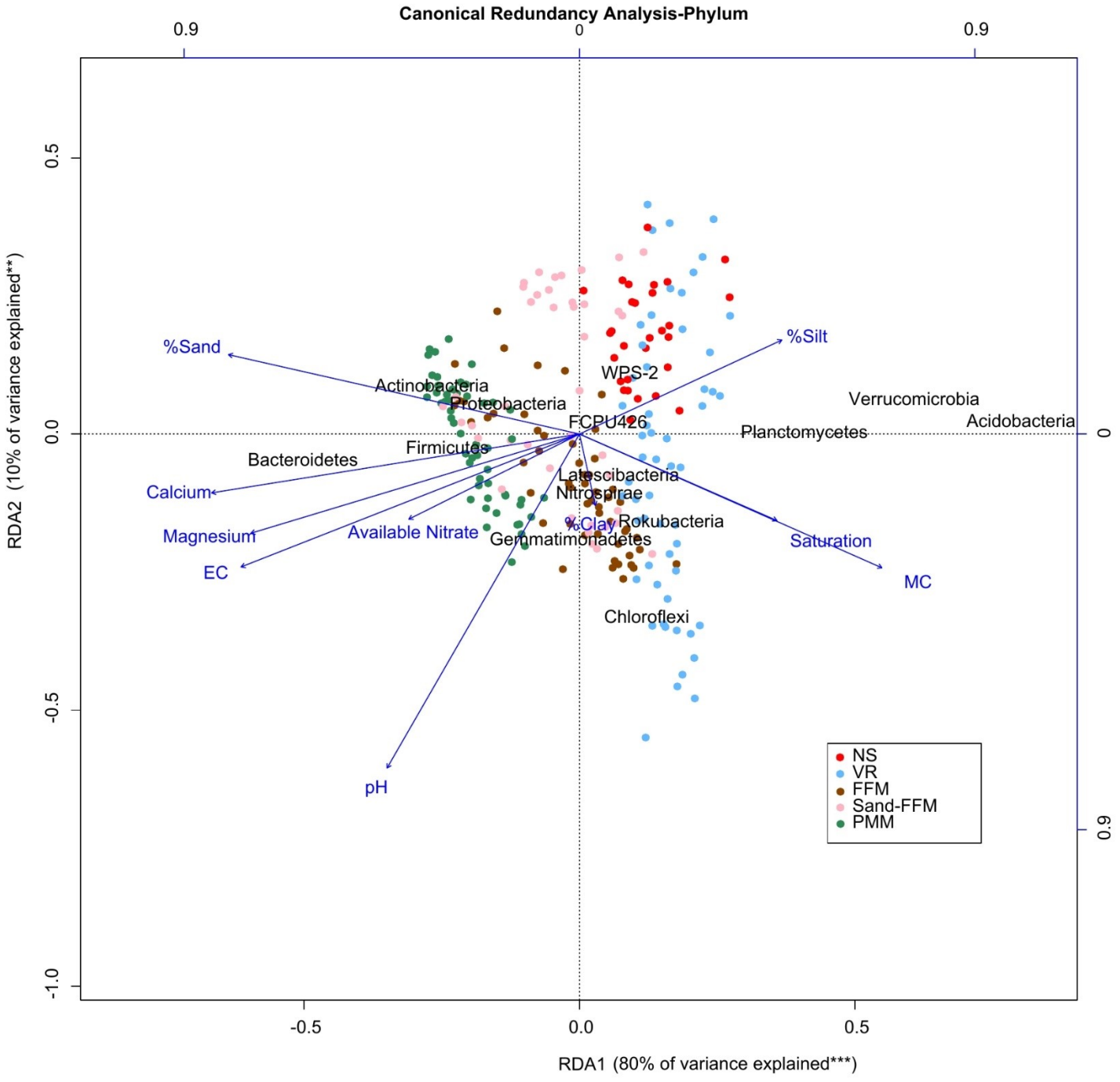

Figure S12. Microbial communities CO_2 rate production in the different sample types when stimulated with different types of carbon substrates. Similar letters indicate no significant difference between sample types according to pairwise Wilcoxon Rank sum tests with a Benjamini-Hochberg adjustment. Site type abbreviations are the same as in the legend to figure S1.

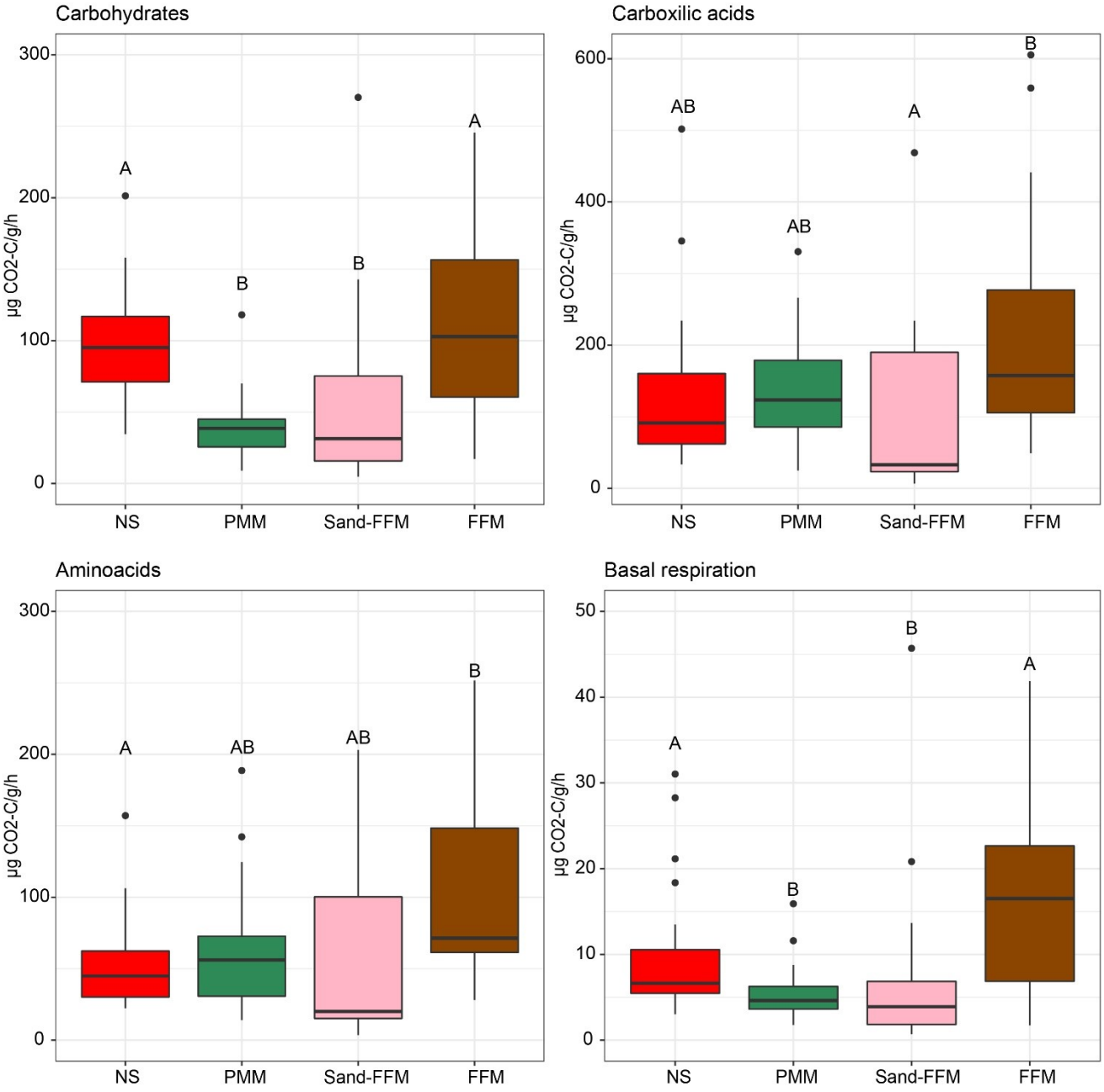

Table S1. Comparison of mean (±SD) physical and chemical soil parameters, and soil microbial biomass among different sample types. NS = Natural soil, VR = Vegetation removed, FFM = Forest floor mineral mix, Sand-FFM = Sand-forest floor mineral mix, PMM = Peat mineral mix.

Treatment	pH	Electro Conductivity (dS m^{-1})	Soil Moisture (%)	Biomass (mg DNA/ g Soil)	Total Organic Carbon (%)	Sand 0.55mm-2.0mm (%)	Silt 2µm-0.05mm (%)	Clay (%)	Available Nitrate (mg/kg)
PMM	6.72±0.32^{a}	2000.18±743.58^{a}	11.18±9.23^{a}	1.63±0.75^{a}	3.73±1.16^{a}	77.25±7.58^{a}	14.95±3.15^{a}	7.80±4.97	18.88±20.60^{a}
Sand-FFM	6.2±0.86^{b}	901.19±843.55^{b}	17.70±21.00^{a}	4.01±4.16^{b}	3.49±2.42^{b}	73.68±16.28^{a}	17.72±9.28^{a}	8.59±7.24	8.09±15.04^{b}
FFM	6.77±0.51^{a}	1185.18±847.78^{c}	31.48±19.65^{b}	6.28±4.07^{c}	7.55±5.00^{c}	64.14±16.46^{b}	24.75±11.08^{b}	11.10±6.61	3.53±3.47^{b}
VR	6.06±1.06^{b}	561.25±263.17^{b}	43.51±18.36^{c}	13.50±7.22^{d}	19.00±14.39^{d}	37.73±29.15^{c}	50.78±22.62^{c}	11.51±10.21	7.78±5.29^{c}
NS	5.14±0.40^{c}	308.46±63.98^{d}	50.25±24.60^{c}	26.66±33.07^{e}	7.82±4.19^{c}	31.74±17.15^{c}	58.11±14.87^{c}	10.14±4.82	2.31±0.93^{b}

Treatment	Available Sulfate (mg/kg)	Available Phosphate (mg/kg)	Available Potassium (mg/kg)	Calcium (mg/L)	Magnesium (mg/L)	Potassium (mg/L)	Sodium (mg/L)	SAR	Saturation (%)
PMM	138.33±117.78^{a}	3.32±0.93^{a}	38.94±11.87^{a}	466.09±229.23^{a}	74.72±37.72^{a}	13.79±5.08^{a}	25.21±22.22^{a}	0.31±0.34^{a}	47.02±14.15^{a}
Sand-FFM	44.37±55.56^{b}	13.43±9.65^{b}	50.98±21.75^{b}	172.02±218.21^{b}	32.75±29.98^{b}	11.52±5.76^{b}	29.86±29.68^{b}	0.67±0.65^{b}	49.81±23.90^{a}
FFM	75.20±79.99^{c}	8.03±5.21^{c}	80.59±60.84^{c}	188.73±183.08^{c}	40.10±29.30^{c}	10.95±7.03^{b}	64.21±53.35^{c}	1.25±0.89^{c}	98.79±75.36^{b}
VR	87.81±118.56^{c}	33.95±31.83^{d}	267.09±199.00^{d}	63.50±39.27^{d}	18.14±9.61^{d}	19.76±14.99^{ac}	30.56±31.35^{ab}	0.95±1.10^{c}	385.80±419.22^{c}
NS	15.37±6.01^{d}	30.65±23.08^{d}	233.69±107.51^{d}	41.78±13.75^{e}	12.13±3.47^{e}	19.20±11.99^{c}	8.76±6.33^{d}	0.34±0.30^{a}	124.35±59.45^{d}

Means within a column block followed by the same letter are not significantly different according to a pairwise-Wilcoxon test.

Table S2. The relative abundances of bacterial communities at the phylum level. Phyla <1% across samples are not displayed. Site type abbreviations are the same as in the legend to table S1.

Phylum	NS	VR	FFM	Sand-FFM	PMM
Proteobacteria	43.82ab	37.22c	42.19a	44.49ab	46.33b
Actinobacteria	15.57a	16.45a	18.24ab	19.74b	22.24b
Bacteroidetes	9.75a	6.62b	17.48c	17.43c	22.16d
Acidobacteria	15.25a	18.79b	8.09c	7.95c	2.02d
Verrucomicrobia	8.49a	6.74a	5.08b	3.90b	0.66c
Planctomycetes	4.10a	4.24a	2.18b	2.17b	0.52c
Chloroflexi	0.98a	3.88b	3.49b	1.84ac	1.70c
Gemmatimonadetes	0.70a	1.57b	1.30b	0.90a	1.58b
Firmicutes	0.16a	0.72b	0.67b	0.59b	2.22c
Rokubacteria	0.07a	0.89b	0.19b	0.09a	0.02c
Cyanobacteria	0.12a	0.38b	0.13a	0.09a	0.05a
WPS-2	0.25a	0.27ab	0.03c	0.14b	0.02c
Armatimonadetes	0.08a	0.27b	0.11a	0.10a	0.04a
Nitrospirae	0.00a	0.28bc	0.17b	0.06c	0.04a
Patescibacteria	0.10abc	0.10b	0.12abc	0.07c	0.08abc
Elusimicrobia	0.09a	0.29b	0.04a	0.02a	0.00a
Chlamydiae	0.04a	0.18b	0.04a	0.03a	0.02a

Means within a row followed by the same letter are not significantly different according to a pairwise-Wilcoxon rank sum test with a Benjamini-Hochberg adjustment.

Table S3. The relative abundances of bacterial communities at the class level. Classes <1% across samples are not displayed. Site type abbreviations are the same as in the legend to table S1.

Class	NS	VR	FFM	Sand-FFM	PMM
Alphaproteobacteria	27.98a	20.26b	19.78b	21.12b	16.26c
Gammaproteobacteria	14.14a	10.88b	20.41c	21.97c	29.12d
Bacteroidia	9.73a	6.44b	17.37c	17.37c	22.1d
Actinobacteria	10.4a	8.13a	12.16ab	14.73b	19.44b
Acidobacteriia	11.72a	8.37b	1.25c	4.17d	0.41e
Verrucomicrobiae	8.49a	6.74a	5.08b	3.90b	0.66c
Thermoleophilia	4.09a	5.31b	3.30c	3.26c	1.30d
Subgroup_6	2.53a	6.21b	4.02c	2.44a	1.07d
Deltaproteobacteria	1.66ab	6.04c	1.98a	1.36bd	0.93d
Planctomycetacia	3.21a	2.89b	1.73c	1.78c	0.48d
Acidimicrobiia	1.03ab	2.48c	2.01d	1.37a	0.91b
KD4-96	0.42a	1.7b	2.38a	1.18a	1.17a
Blastocatellia_(Subgroup_4)	0.67a	2.35b	1.77b	0.84ac	0.21c
Gemmatimonadetes	0.69a	1.48b	1.25b	0.85a	1.52b
Bacilli	0.11a	0.61b	0.52b	0.48b	1.74c
Phycisphaerae	0.84a	0.99b	0.35c	0.34c	0.03d
Anaerolineae	0.19a	0.85b	0.53c	0.28a	0.27a
Holophagae	0.12a	0.62b	0.57b	0.23a	0.2a
MB-A2-108	0.03a	0.46b	0.65c	0.29b	0.21b
NC10	0.07a	0.89b	0.19b	0.09a	0.02c
Subgroup_17	0.09a	0.65b	0.29c	0.16a	0.05a
Clostridia	0.04a	0.09a	0.13a	0.09a	0.44b
Dehalococcoidia	0.03a	0.30b	0.17b	0.09a	0.06a
Chloroflexia	0.05ab	0.25c	0.15a	0.10ab	0.04b
Ktedonobacteria	0.17ab	0.23a	0.02c	0.04c	0.03bc
Thermoanaerobaculia	0.06a	0.21b	0.08ac	0.06ac	0.02c
Ignavibacteria	0.01a	0.17b	0.11a	0.06a	0.06a
OM190	0.04a	0.24b	0.07a	0.04a	0.00a
Coriobacteriia	0.00a	0.02a	0.05a	0.03a	0.25b
Lineage_IIa	0.07a	0.23b	0.03ac	0.01c	0.00c
Oxyphotobacteria	0.03a	0.22b	0.03a	0.03a	0.01a

Means within a row followed by the same letter are not significantly different according to a pairwise-Wilcoxon rank sum test with a Benjamini-Hochberg adjustment.

Table S4. The relative abundances of the top 15 genera across sample types. Site type abbreviations are the same as in the legend to table S1.

Genus	NS	VR	FFM	Sand-FFM	PMM
Massilia	0.19a	0.08b	8.04c	10.53cd	12.54d
Pseudarthrobacter	0.08a	0.19a	6.88b	5.2b	14.96d
Pedobacter	0.27a	0.04b	4.41c	4.48c	12.9d
Candidatus_Udaeobacter	4.72a	2.00bc	3.08ab	1.83c	0.21d
Mucilaginibacter	2.05a	0.74b	1.55c	3.98a	0.58bc
Flavobacterium	0.27a	0.08b	2.4c	1.36d	2.92c
Sphingomonas	0.59a	0.49a	1.99b	2.29b	1.48c
Brevundimonas	0.05a	0.02a	1.73b	1.54b	3.47c
Polaromonas	0.19a	0.12b	0.8c	0.53d	4.13e
Acidothermus	2.95a	1.38b	0.15c	1.11b	0.04c
Mycobacterium	1.84a	1.64a	0.32b	1.7a	0.1b
Burkholderia-Caballeronia-Paraburkholderia	3.17a	0.53bc	0.26b	1.17c	0.04d
Pseudolabrys	0.53a	0.68a	1.32b	0.87a	1.32b
Noviherbaspirillum	0.11a	0.03b	1.07c	1.04c	1.81d
Granulicella	2.27a	0.68b	0.11c	0.92b	0.05c

Means within a row followed by the same letter are not significantly different according to a pairwise-Wilcoxon rank sum test with a Benjamini-Hochberg adjustment.