RESPONSE OF SOIL GEOCHEMICAL PROPERTIES AND MICROBIAL COMMUNITIES TO LONG-TERM STORAGE IN TWO MINE OPERATIONS IN THE INTERIOR OF BRITISH COLUMBIA: IMPLICATIONS FOR RESTORATION PRACTICES

by

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ABSTRACT

Mining activities are often severely disruptive to the landscape and a major barrier to reclamation after mining is lack of quality topsoil. This project addresses knowledge gaps in the industry by exploring the compositional nature of topsoil stockpiles and their ability to facilitate post-mining revegetation after long-term storage. To do this, we conducted an extensive profile characterization of two topsoil stockpiles in the interior of British Columbia, where soil geochemical properties and microbial communities with high-throughput sequencing were investigated. Both stockpiles show depleted soil quality and significant changes compared to reference soils. Importantly, there were declines in microbial diversity, major shifts in community structure, and a reduction in soil nutrients with increasing stockpile depths in one of the stockpiles. These results highlight the important influence of topsoil-stockpile height on geochemical properties and microbial communities in the soil, which ultimately influences the success of restoration. This research can help industry to optimize restoration and expediate recovery in their mine-closure practices and provides insights into the general structure of the microbiome existing across a gradient in severely disturbed mining soils.

Keywords: Mine reclamation, soil microbial ecology, ecosystem restoration, soil response to disturbance, high-throughput sequencing

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1.0 GENERAL INTRODUCTION

1.1 Overview of Ecosystem Restoration

Widespread environmental degradation through human activities is leading to biodiversity loss and declines in ecosystem services (Cardinale et al., 2012; Hooper et al., 2005). Ecosystem services including carbon storage, climate regulation, nutrient cycling, soil fertility, provision of medicines and cultural association are intrinsically linked to human livelihood (Catovsky et al., 2002; Douglas, 2017). For instance, it has been suggested that up to 60% of the world's arable land is nutrient deficient or polluted causing major hindrances in food production (Fageria et al., 2008). These losses to the natural environment can be mitigated by ecosystem restoration efforts, which focuses on expediting the recovery of damaged ecosystems to a pre-disturbance state (Bullock et al., 2011). Alternatively, the system can recover on its own through natural processes without human intervention much more slowly (Suding et al., 2016).

Restoration efforts are often designed to expedite natural succession so that the desired endpoint is reached quicker (Palmer et al., 2017). For instance, tree planting in tropical systems can accelerate soil recovery by promoting microbial biomass and carbon inputs (Moreno-Mateos et al. 2015). The Society for Ecological Restoration (SER) defines ecological restoration as the "process of assisting the recovery of an ecosystem that has been degraded, damaged or destroyed" (SER International Science & Policy Working Group, 2004) . In general, the goal of a fully restored ecosystem is to be self-sustaining and resilient. The significance of restoration efforts is highlighted in a meta-analysis of 89 restoration projects around the world showing a substantial increase of biodiversity and ecosystem services in restored sites compared to non-restored sites (Rey Benayas et al., 2009). Additionally, the 2020 Aichi Biodiversity Targets released by the Convention of Biological Diversity (CBD) aim for the restoration of ecosystems that provide essential services, as well as the restoration of at least 15% of degraded ecosystems (CBD, 2017). However, the restoration of a site greatly depends on the practitioner and their specific goals.

The outcomes and goals for restoration are highly variable. Goals typically include reaching certain diversity indices, vegetation structures, and/or ecological processes (Ruiz-Jaen & Aide, 2005). However, restoration outcomes and timescale depend on disturbance level, fragmentation,

and landscape conditions. Additionally, restoration goals and targets are largely shaped by cultural values, indigenous knowledge, economics, and policy that are specific to the landscape of interest. Understanding restoration and ecosystem ecology is increasingly important during the growing prevalence of anthropogenic disturbances and climate crises (Food and Agriculture Organization of the United Nations, 2020).

The practice and theory of ecological restoration has developed rapidly over the past few decades as well as the integration of ecological concepts to restoration projects (Suding et al., 2016), particularly community assembly and succession theory (Weiher et al., 1998; Weiher and Keddy 1999; Wainwright et al., 2018). Community assembly builds off of succession theory by emphasizing the random chance that results in very different outcomes of community succession (Weiher and Keddy 1999). Community assembly theory aims to describe how species composition change over time and suggests that the first species to establish in an ecosystem has priority, and this priority effect can determine which species are successful later on. The order in which species establish is important because they can have positive, neutral, or negative effects on each other (Suding et al., 2016). For instance, Young et al. (2014) and Ploughe et al. (2020) found that an early introduction of native grasses significantly reduced the negative effects to the plant community from invasive exotics. Additionally, it is common practice in disturbed areas to introduce legumes first as a nurse plant to create favourable soil conditions for other species to establish (Suding et al., 2016). The theory particularly focuses on the importance of three filters in determining local community composition: dispersal, physical environment, and biotic interactions. Therefore, we can use community assembly principles to reach restoration goals by purposefully introducing certain species into a disturbed landscape. Succession theory assumes the continuous and gradual recovery after a discrete disturbance and landscapes are believed to return to their historical state or a known trajectory to a point of equilibrium (Suding et al., 2016). For example, seeding of later-successional species can expedite restoration to forests in degraded pastures (Cole et al., 2011). This suggests that human interventions post-disturbance can improve restoration by accelerating the rate of succession towards a later stage by introducing components of a later successional stage (Mcclain et al., 2011). Additionally, succession models assume that ecosystem development will occur through natural processes over time (Christensen, 2014), suggesting restoration without human intervention can occur, but the

process is slower. These frameworks and others are used to understand the natural world around us and are particularly useful tools in restoration projects (Heneghan et al., 2008).

1.2 Soil and Restoration

1.2.1 Soil Health and Function

Soil is a complex ecosystem comprising of abiotic components (organic matter, water, air, minerals) and biotic components (bacteria, fungi, protists, and animals). Soil health or functionality can be defined as the capacity of a soil to sustain certain ecosystem functions such as plant productivity, water and air quality, and provide habitats for biodiversity (Brussard, 1997; Fitter et al., 2005). Traditional landscape restoration science has put the emphasis on aboveground communities, such as plants and insects, but recently the importance of belowground processes and biota have become increasingly recognized (Heneghan et al., 2008; Kardol & Wardle, 2010). For instance, soil nutrient manipulations have been increasingly applied to manage invasive species in restoration (Davis et al., 2000; Knauf et al., 2021).

Soils are the major terrestrial supply of nutrients supporting plants and other organisms. Soil communities are highly complex and diverse ranging from larger organisms, such as earthworms through to microscopic organisms, such as bacteria and fungi; however, the majority of this diversity comes from the unseen microbial communities which are not well understood (Delgado-Baquerizo, 2019; Maron et al., 2018). Bacterial and fungal communities are essential to vital terrestrial processes including nutrient cycling, climate regulation and pollution degradation (Bardgett & van der Putten, 2014). Additionally, soil microbes shape terrestrial ecosystem dynamics through their close relationships with plants (Bauer et al., 2015; Kardol & Wardle, 2010; Kulmatiski et al., 2008). Despite this, there is a lack of data on soil biodiversity and soil physical and chemical indicators and their interconnectedness with overall ecosystem components is poorly understood (Food and Agriculture Organization of the United Nations, 2020). Consequently, there has been an increasing focus in restoration and soil research aiming to understand how belowground geochemical properties and microbial communities influence plant dynamics and overall restoration success (Garris et al., 2016; Harris et al., 1989; Heneghan et al., 2008; van der Heijden et al., 2008; Wagg et al., 2014).

1.2.2 Role of Key Soil Geochemical Properties

The geochemical properties play a large role in the success of plant establishment and other high level ecosystem components including water retention and microbial functionality. Most plants take up nutrients needed for their metabolism through their roots within the soil medium. Essential plant nutrients are categorized as macronutrients (primary nutrients and secondary nutrients) and micronutrients. Key primary plant nutrients are nitrogen (N) mainly in the form of ammonium (NH_4^+) and nitrate (NO_3^-), phosphorus (P) in the form of phosphates (HPO_4^{-2}, $H_2PO_4^-$, and PO_4^{-3}), and potassium (K^+). Primary nutrients are required in a relatively large amount for plant metabolism and growth and are frequently applied to soils in fertilizers. Secondary nutrients are calcium (Ca^{2+}), magnesium (Mg^{2+}), and sulfur (S) in the form of sulphate (SO_4^{2-}). Secondary nutrients are typically present in soils and are not often required to be applied artificially. Micronutrients include iron (Fe) in the form of iron (III) oxide-hydroxide ($Fe(OH)_3$) precipitate, boron (B) in the form of H_3BO_3, zinc (Zn^{2+}), copper (Cu^{2+}), manganese (Mn^{2+}) and molybdenum (Mo) in the form of (MoO_4^{-2}, $HMoO_4^-$, and H_2MoO_4), chloride (Cl^-), and nickel (Ni^{2+}). Very small amounts of micronutrients are required for plant growth, however they become toxic beyond a threshold concentration (Naeem et al., 2017)

In addition to the nutrient composition of soil, soil properties including pH, electrical conductivity (EC), and organic matter (OM) are responsible for driving ecosystem functions and are sensitive to disturbances (Baer & Birgé, 2018; Brevik et al., 2015). Soil pH is a measure of the activity of hydrogen ions (H^+) and affects plant growth through its regulation of nutrient solubility and metal toxicity. Most plant nutrients are available between pH 6.0 and 7.5 and the optimum range for microbes is pH 5.0 to 8.0 (Smith & Doran, 1996). Acidic soils (low pH) are common in the mining industry due to waste materials from heavy metal extractions (Costigan et al., 1981). EC is an important soil quality indicator as a proxy of salinity, which influences toxicity and nutrient availability in soils (Jurinak et al., 1987; Muñoz-Rojas, 2018). Salinity typically reduces the availability of essential nutrients N, P, and K due to the competition from high amounts of Ca, Na, Cl, and S ions (Jurinak et al., 1987; Naeem et al., 2017). OM is an important source of nutrients and improves soil structure. Importantly, OM slowly releases nutrients through decomposition by soil organisms, providing a reservoir of plant nutrients. Low levels of organic matter are typical in degraded soils, which can result in poor nutrient supply to plants (Baer, 2016).(Baer, 2016).

Nutrient deficiency is one of the most common factors limiting plant growth and establishment of sites impacted by mining (Baer, 2016; Sheoran et al., 2008, 2010) causing a hinderance to restoration success (Chen et al., 1998). Soil degradation post-mining may be amended with the addition of soil nutrients in the form of organic inputs or fertilizer. For example, Silva et al. (2013, 2015) demonstrated a single application of nutrients and organic matter in the form of biosolids led to the rapid and spontaneous revegetation of abandoned mines in central Brazil that had been disturbed for several decades. In addition to improving plant establishment in disturbed lands, soil geochemical properties can shape the plant community trajectory during restoration. For instance, two studies demonstrated that the manipulation of N levels in soil optimal for native species, led to an increase in native grass diversity (Baer et al., 2004; Perry et al., 2010). Thus, the characterization of key geochemical properties in the soil profile is critical in post-mining reclamation planning (Baer, 2016).

1.2.1 Role of Soil Fungal and Bacterial Communities

Plants heavily depend on soil microbes for various provisions, including the bioavailability of nutrients in the soil and for the delivery of these nutrients. The root surface area often does not adequately provide proper uptake of all needed nutrients in the soil, therefore, associations with bacteria and fungi are essential to establishment and nutrient uptake, especially in nutrient poor environments (Miransari & Omidi, 2011). In addition to this, it is well documented that soil bacteria and fungi directly influence plant productivity and health through various mechanisms such as suppression of disease and altered biotic interactions between plants and other organisms (Bever et al., 2010; van der Heijden et al., 2008; Wang, 2017). Furthermore, it has been estimated that 80-90% of terrestrial plants form symbiotic arbuscular mycorrhizal fungi (AMF) associations (Smith & Read, 2008) and plants in mine-impacted sites often reach a higher percentage of mycorrhizal associations, indicating their importance to plant establishment in disturbed ecosystems (Wang, 2017). Although recent sequencing technologies have allowed a substantial improvement in the understanding of soil microbial composition and diversity, the ecological role and identity of microbial taxa are still not well understood (Delgado-Baquerizo, 2019).

Soil microbes can assist plants in nutrient uptake partly because they are major drivers in geochemical fluxes. For instance, symbiotic and free-living nitrogen-fixing bacteria convert N

from the atmosphere to bioavailable NO^{3-}, promoting plant productivity. Furthermore, bacteria and fungi decompose dead OM such as leaf litter, releasing bioavailable nutrients for plants. Importantly, mycorrhizal fungi and nitrogen-fixing bacteria are responsible for providing 5-20% of all N and up to 75% of P in grasslands and savannahs (van der Heijden et al., 2008). Another way soil bacteria and fungi can increase nutrient uptake is through upregulation of genes during nutrient deficiencies (Bisis & Kumar, 2016). Additionally, fungi can modify resources through fungal symbionts, called common mycorrhizal networks (CMNs), where nutrients are captured and transferred between plants (Bever et al., 2010).

Because of this resource partitioning, and other mechanisms such as disease suppression and shifting biotic interactions, manipulations of soil microbes can significantly promote plant growth and alter plant community structure (Bardgett & van der Putten, 2014; Bauer et al., 2015; Bever et al., 2010; Reynolds et al., 2003; van der Heijden et al., 2008). For example, the addition of AMF can promote ecological restoration of mine sites by improving nutrient uptake and tolerance of plants, improving soil structure, and quality thereby helping maintain ecosystem functions (Wang, 2017). Consequently, integrating soil ecological knowledge including soil microorganisms and chemical properties is an important area of research and is essential to the restoration of natural systems in mine disturbed landscapes (Birnbaum et al., 2017; Callaham et al., 2008; Sheoran et al., 2010; van der Heijden et al., 2008; Wall, 2012).

1.3 Overview of Disturbance from Mining Activities

1.3.1 Environmental Impacts

Mining operations often require the complete removal of soil from a site. Heavy equipment is used to clear vegetation and soils from landscapes, reducing biodiversity, soil nutrients, and soil structure. These processes are severely disruptive to landscapes, however necessary to meet growing energy and material demands. As of 2017, Canada produces 60 minerals and metals at 200 active mines and 7 000 pits and quarries. Canada's mining industry is a key contributor to the economy; in 2017 the industry contributed 5% to Canada's Gross Domestic Product. It has provided over 634 000 jobs and is the industry with the highest Indigenous representation after fishing in the private sector (Mines Canada, 2019). Left to natural processes, these disturbed ecosystems take decades to restore (Bradshaw, 1997). While necessary, mining activities are

destructive to the environment (Cooke & Johnson, 2002). New scientific knowledge and technologies are needed to inform and guide reclamation policies and practices that will enhance reclamation, such as wildlife habitat, commercial forestry, or indigenous forage grounds.

Gold along with copper and silver were the first metals known to be used by humans in the Stone Ages, typically in the form of nuggets found in riverbeds. Significant natural gold deposits can be found in quartz veins in rock formations, alluvial deposits, and within copper and lead deposits (Gasparrini, 1993). Gold can be mined using surface mining including quarries, open pits, and mounting top removal or using underground mining. The activities associated with gold mining and other types of mining affect most components of the environmental – atmosphere, hydrosphere, pedosphere, biosphere, and lithosphere (Matschullat & Gutzmer, 2012; Miao & Marrs, 2000). Arsenic minerals associated with gold are dispersed in the environment. Gold like many other mined products generate a large amount to mine waste such as overburden, barren rocks, tailings, heap leach, and mine water causing environmental damage. Sulphide tailings from gold ore processing can be a major source of acid mine drainage. In addition to pollution and waste by-products from processing, large-scale land degradation is a consequence of gold mining and most other types of mining (Gasparrini, 1993; Miao & Marrs, 2000). In open-cast mining, the area must be completely stripped of vegetation to remove the overburden covering the mineral deposits. Underground mining can cause 2-11 times less land disturbance compared to surface mining (Miao & Marrs, 2000), however still causes significant soil disturbances.

1.3.2 Regulatory Framework in British Columbia

The Mines Act (1996) and the Health, Safety and Reclamation Code for Mines in British Columbia (2017) require mining operations to conduct an environmental protection and reclamation program to ensure that land, watercourses, and cultural heritage resources will be returned to a safe and environmentally sound state and to an appropriate land use. Under the BC Mines Act, mining companies are required to conduct the reclamation of lands prior to mine closure (1996). Furthermore, Part 10 of the Code (Mine Plan and Reclamation Program Information), focuses on reclamation and closure (2017). For example, Section 10.7.6 (Long-term Stability) states that “Land, watercourses and access roads shall be left in a manner that ensures long-term stability” and Section 10.7.8 (Growth Medium) states that “all surficial soil materials removed for mining purposes shall be saved for use in reclamation programs…”.

Although somewhat vague, these frameworks in BC highlight the specific need for proper soil management and successful soil restoration on mine affected lands.

1.3.3 Salvaging and Stockpiling Topsoil

Soil destruction, particularly topsoil, is one of the most important environmental impacts of mining activities. Topsoil is defined as the surface "A" (organo-mineral) horizons of the soil profile and lies above the subsoil or "B" horizon containing the upper portion of parent rock material (Alberta Soil Advisory Committee). Healthy topsoil is a critical component in severely disturbed landscapes such as mine sites because it supports faster plant establishment, higher plant survival rates, and protects against invasive species by providing a strong native seed bank. Stockpiling topsoil is a common restoration strategy in mining that includes the removal and storage of topsoil, which is re-spread at the time of mine closure for reclamation. When topsoil is re-applied to the disturbed area, it creates similar pre-disturbance conditions for plants to establish in (Strohmayer, 1999). However, storage practices can alter and damage the soils physio-chemical properties and biota hindering its ability to support ecosystem restoration (Mummey et al., 2002b).

The process of topsoil deterioration often begins with stripping and relocation with heavy machinery. Although not well understood, the severity of the loss in soil quality has been shown to impacted by the length of time and the depth of the stockpile (Abdul-Kareem & McRae, 1984; Birnbaum et al., 2017; Ghose & Kundu, 2004; Golos et al., 2016; Mummey et al., 2002b; Sheoran et al., 2010). The storage period for stockpiled soil can range from months to decades and stockpiles are often meters deep (Strohmayer 1999). There is limited research on long-term topsoil storage, but existing literature has shown a decrease in soil nutrients, soil structure, and microbial biomass (Ghose & Kundu, 2004; Mummey et al., 2002a). For instance, (Harris & Birch, 1989) found a dramatic reduction in the presence of bacteria below 2 meters of a topsoil stockpile. As the duration and size of storage increase stockpiles can become stratified in content, particularly when below 1-3 meters (Boyer et al., 2011; Mackenzie, 2013), resulting in a reduction in seed viability and an increase in soil compaction and anaerobic conditions (Abdul-Kareem & McRae, 1984; Harris et al., 1989). However, properly managed stockpiles have the potential to minimize soil loss, preserve quality, and improve plant establishment upon respreading.

It is often recommended in soil best management practices that stockpiles remain under 1 meter and less than 1 year old to maintain topsoil quality (The City of Calgary Parks, 2018). Furthermore, mixing topsoil and subsoils will typically degrade topsoil quality and should be stored in separate piles onsite, or at least placed in the same order that they were removed (BC Ministry of Energy & Mines, 2002). Stockpiles should be vegetated within 30 days with native seed suited to the area (BC Ministry of Energy & Mines, 2002; Mackenzie & Renkema, 2013), reducing the potential for surface erosion and promoting stability (The City of Calgary Parks, 2018). Once created, stockpiles need to be monitored for stability, surface erosion, vegetation establishment, and presence of invasive species (BC Ministry of Energy & Mines, 2002).

1.4 Research Objectives

In the context of restoration ecology, it is key to understand how the management of long-term topsoil stockpiling alters soil chemical and microbial composition, which heavily influences plant establishment and community dynamics. There is very known about how topsoil quality is impacted by management practices during storage, particularly from storage height. This project explores the chemical and biological compositional nature of topsoil stockpiles to better understand their viability to support post-mining restoration after storage. I predicted that the topsoil stockpiling practices at New Afton and QR mill in British Columbia (B.C.) have had adverse effects on soil quality and therefore, restoration viability. To address this hypothesis, I conducted a two-part study; Chapter 2.0 investigates how soil geochemical properties are impacted by topsoil storage and Chapter 3.0 investigates how soil microbial community composition is impacted by topsoil storage. Both chapters set out to investigate the topsoil stockpile restoration suitability from two gold operations in the interior of B.C. and examine the impacts from stockpile height on soil quality. Chapter 4.0 contains general conclusions of this research including key findings, management implications, limitations, and future research. Lastly, Appendix A shows findings from a supplementary experiment assessing the impacts of soil depth on plant establishment in a greenhouse trial. The results from this project contribute knowledge regarding soils response to topsoil stockpiling and will be useful to restoration ecology practitioners and researchers. Broadly, it provides new information on the approaches of ecological restoration post-mining with an emphasis on aboveground-belowground linkages and helps to better understand how soil responds to severe ecological disturbance.

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2.0 INVESTIGATING IMPACTS FROM TOPSOIL STOCKPILE HEIGHT ON SOIL GEOCHEMICAL PROPERTIES

2.1 Introduction

In British Columbia (B.C.), disturbed landscapes from mining activities are required by law to reclaim land to a self-sustaining state. British Columbia was one of the first provinces in Canada to enact that mining companies must reclaim disturbed land caused by mining activities through the Mines Act (Mines Act, 1996). Additionally, mining companies are required to produce annual reclamation reports outlining a summary of all mining activities as well as all research and monitoring for the Environmental Protection Reclamation Program. There are a wide variety of potential restoration targets for any given mining operation depending on various factors such as historical use, culture, physical and biological characteristics, and mine type (Mborah et al., 2015), but ultimately the goal is to create a self-sustaining ecosystem that is resilient and requires no further human interventions (SER International Science & Policy Working Group, 2004).

Under the provincial regulations, salvaged topsoil is stored and re-spread post-mining to help expedite ecological succession and return disturbed sites to a historic state. Native topsoil is a critical source of seeds and propagules and provides beneficial physical, chemical, and microbial properties for restoration and plant establishment. Successful plant community recovery has been reported from re-spreading topsoil stockpiles post-mining. For example, Hall et al. (2010) found approximately 66% plant species recovery for a forest ecosystem in the Appalachian Mountains and Holmes (2001) found that plant species recovered by 66% in a shrubland in South Africa.

The deterioration of topsoil quality due to disturbance is one of the greatest hindrances to restoration success during mining operations. Topsoil salvage, storage, and replacement during mining operations can have adverse effects on soil quality (Abdul-Kareem & McRae, 1984; Ghose & Kundu, 2004; Golos et al., 2016; Harris et al., 1989; Stahl et al., 2002; Thurber Consultants Ltd. et al., 1990; Wick et al., 2009), resulting in long-term consequences for restoration (Mummey et al., 2002b). Topsoil buried deep in a storage pile may become anaerobic, which alters physical, chemical, and biological components of the soil. Additionally, stripping and relocating topsoil often results in severe compaction from heavy machinery.

Moreover, the equipment often causes admixing topsoil with lower quality subsoil and parent materials during stripping, further degrading topsoil quality in stockpiles. Severely compacted soils are known to have lower oxygen levels, restricted root growth, poor drainage, and nitrogen loss from denitrification (Abdul-Kareem & McRae, 1984; Boyer et al., 2011; Buresh & Patrick, 1978). For example, Birnbaum et al. (2017) found that 10-year-old stockpiles resulted in significantly lower plant biomass compared to plants grown on younger stockpiles.

Nutrient availability and suitable geochemical conditions (including salinity, electrical conductivity (EC) and pH) are critical for the establishment and sustainment of plant communities. Macronutrients (nitrogen (N), phosphorus (P), potassium (K), sulphur (S), magnesium (Mg), and calcium (Ca)) and micronutrients (iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), boron (B)) in soil are required for plant health and growth, and the concentration of these major nutrients are commonly manipulated to achieve restoration goals. For example, organic amendments such as manure, biosolids, and wood chips are widely used to provide nutrients and to improve soil quality for restoration (Larney & Angers, 2012). Additionally, Gardner et al. (2012a) found that the application of biosolids on copper mine tailings significantly improved plant establishment by increasing nutrient availability. Because soil geochemical properties and nutrient levels impact restoration outcomes (Baer, 2016; Knauf et al., 2021), understanding the content of salvaged topsoil for restoration purposes is critical.

This chapter investigated the geochemical changes occurring within stockpiles at two mines in British Columbia in an effort to understand how management impacts soil stockpile viability for reclamation. The primary value of this work is assisting industrial operators to optimize topsoil stockpile assets, and for the development of tools for assessing soil health that are directly relevant to reclamation practices.

2.2 Methods

2.2.1 Study Sites

2.2.1.1 New Afton Mine

New Gold's New Afton copper-gold mine was located approximately 10 kilometers west of Kamloops in British Columbia's Southern Interior. It is situated within the historic Afton Mine (Figure 2.1), formerly owned by Afton operating Corp. New Afton began commercial production

in July 2012 and is the largest underground hardrock mine in Canada. It comprises of underground workings, historic support facilities, a historic open pit, a concentrator, and a tailings facility. The end land use objective is to return the ecosystem to native grasslands that support wildlife and traditional hunting opportunities by First Nations (New Gold, 2017). New Afton is located within the traditional territories of the Tk'emlúps and Skeetchestn Bands. These bands are part of the larger cultural group known as the Secwépemc or Shuswap First Nation. Additionally, New Afton is in the Bunchgrass (BGxw1) biogeoclimatic zone at approximately 700 m in elevation. The Biogeoclimatic Ecosystem Classification (BEC) system in British Columbia incorporates information on climate, soils, and vegetation to provide a framework for management practices (Meidinger & Pojar, 1991). The BGxw1, commonly known as the "middle grasslands" is dominated by bluebunch wheatgrass, Junegrass, big sagebrush and rabbit brush (Lloyd et al., 1990). The primary soil orders within the mine site include Tranquille, Trapp Lake, Godey, and Timber. These soils are moderately saline and calcareous and are dominated by Orthic Brown and Dark Brown Chernozems with one occurrence of an Eluviated Eutric Brunisol soil (Government of British Columbia, 2018). The tailings in the New Afton mine are alkaline (pH >8.5) and are high in copper and molybdenum.

2.2.1.2 QR Mill

Barkerville Gold Mines Ltd. (BGM) (formally International Wayside Gold Mines) is a Canadian company headquartered in Toronto, Ontario. Barkerville Gold Mines Ltd. BGM has various gold mines around the Barkerville-Wells area. Barkerville Gold Mines' Quesnel River (QR) mill in Cariboo is located approximately 80 km east of the city of Quesnel in the southern interior of B.C. Ore, concentrate, and waste rock from the Bonanza Ledge Mine and Cariboo Gold Mine are transported, stored, and processed in the QR mill. The current reclamation goals set for QR mill are to restore the landscape so that it does not require further human intervention (Barkerville Gold Mines Ltd., 2019). QR mill is situated in the traditional territories of the Secwépemc or Shuswap First Nation and lies within the moist, warm Sub-Boreal Spruce (SBSmw) BEC zone based on the BC provincial BEC system. The SBSmw is part of the Canadian Boreal Forest Region and Quesnel Highland (mean annual precipitation ranges from 440-900 mm) and is dominated by Douglas-fir, red-stemmed feathermoss, knights plume, hybrid white spruce, subalpine fir, and electrified cat's-tail moss (Annas & Coupe, 1979). The QR mill

and surrounding area is dominated by Bedenesti, Deserters, and Dominion soil orders. These soils consist primarily of Brunisolic Gray Luvisols and Luvisolic Humo-Ferric Podzols that were deposited by glacial ice (Government of British Columbia, 2018).

2.2.2 Soil Sampling

2.2.2.1 New Afton Mine

New Afton has a 6-year-old, 25-meter-deep topsoil stockpile (50.654442, -120.509320) with approximately 250 600 m^3 of topsoil materials. Because additional topsoil materials have been added throughout the mine life, the oldest soil is at the bottom of the stockpile and the youngest soil is at the surface. Four soil cores were extracted via solid stem auger drilling by Geotech Drilling Ltd provided by New Afton. during September 26th and 27th of 2018, with each core being approximately 3 meters apart. The first 1.53 m was sampled in 0.3 m increments, then once every 0.3 m until the bottom at 13.7 m. Thus sampling depths were at 0.3 m, 0.6 m, 0.9 m, 1.2 m, 1.5 m, 3.0 m, 4.6 m, 6.1 m, 7.6 m, 9.1 m, 10.7 m, 12.2 m, and 13.7 m. The outer 1 cm of the soil core was dis-carded to ensure that collected soil was not contaminated by upper layers. Soil was placed into two 1 L Whirl-Pak® bags and Falcon® tubes as they were pulled up soil from the stockpile. Post-collection, samples were combined by depth intervals of the stockpile are as follows; 0.0-0.6 m, 0.6-1.5 m, 1.5-6.1.0 m, and 6.1-13.7 m. The soil stockpile at the time of sampling was sparsely vegetated with grasses and weedy species, likely from natural regeneration. A nearby grassland site was sampled as a reference site, where approximately 6 kg of soil from the top 10 cm was collected using a trowel. The Whirl-Pak® samples were stored in a -20ºC freezer at the Research Greenhouse and the Falcon® tube samples were stored in a -80ºC freezer in the TRUGen laboratory at TRU until analysis.

2.2.2.2 QR Mill

QR mill has a 20-year-old, 6-meter-deep topsoil stockpile (52.670306, -121.783556). It is a combination of organic soil and general till soil stripped form the surface layers. The stockpile was intended to cover and re-contour during post-mining reclamation. Sampling at the QR mill of Barkerville Gold Mines Ltd. was completed in May 2019. Three soil pits approximately 100 m apart were dug using an excavator in May 2019 to access various layers of the stockpile from the surface to the bottom at 575 cm. In the field, soil was placed into two 1 L Whirl-Pak® bags

and Falcon® tubes as soil was removed from the stockpile. The stockpile at the time of sampling had vegetation cover, including cottonwood stands in the northwest corner. An adjacent undisturbed forested site was sampled as a reference site, where approximately 16 kg of soil from the top 10 cm was collected The Whirl-Pak® samples were stored in a -20^{o}C freezer at the Research Greenhouse and the Falcon® tube samples were stored in a -80^{o}C freezer in the TRUGen laboratory at TRU until analysis.

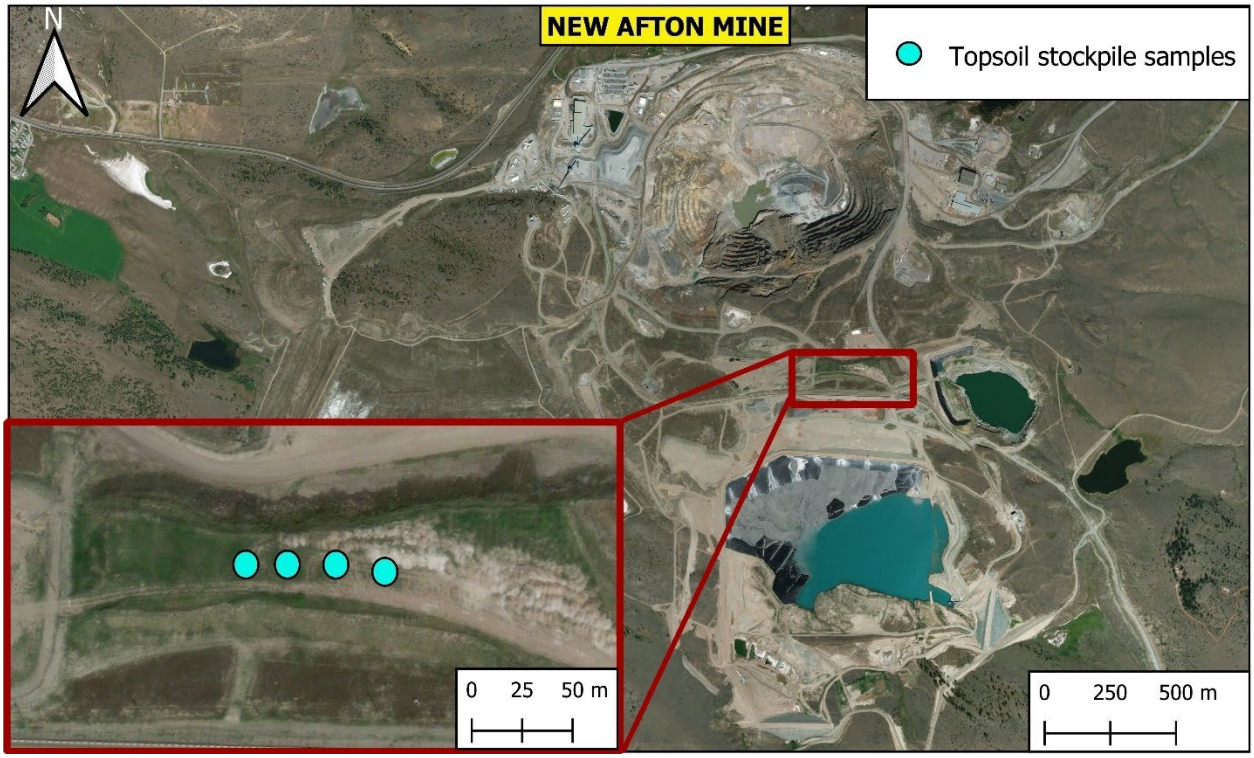

Figure 2.1 Aerial image of the New Afton mine site, including a close-up of the topsoil stockpile of interest that shows the locations of four soil core samples. Map generated in QGIS® with Bing VirtualEarth background.

a) b)

Figure 2.2 Photos showing a) sampling of the topsoil stockpile in New Afton via auger driller and b) soil collection set up where soil was placed into Whirl Pak© bags and Microcentrifuge tubes.

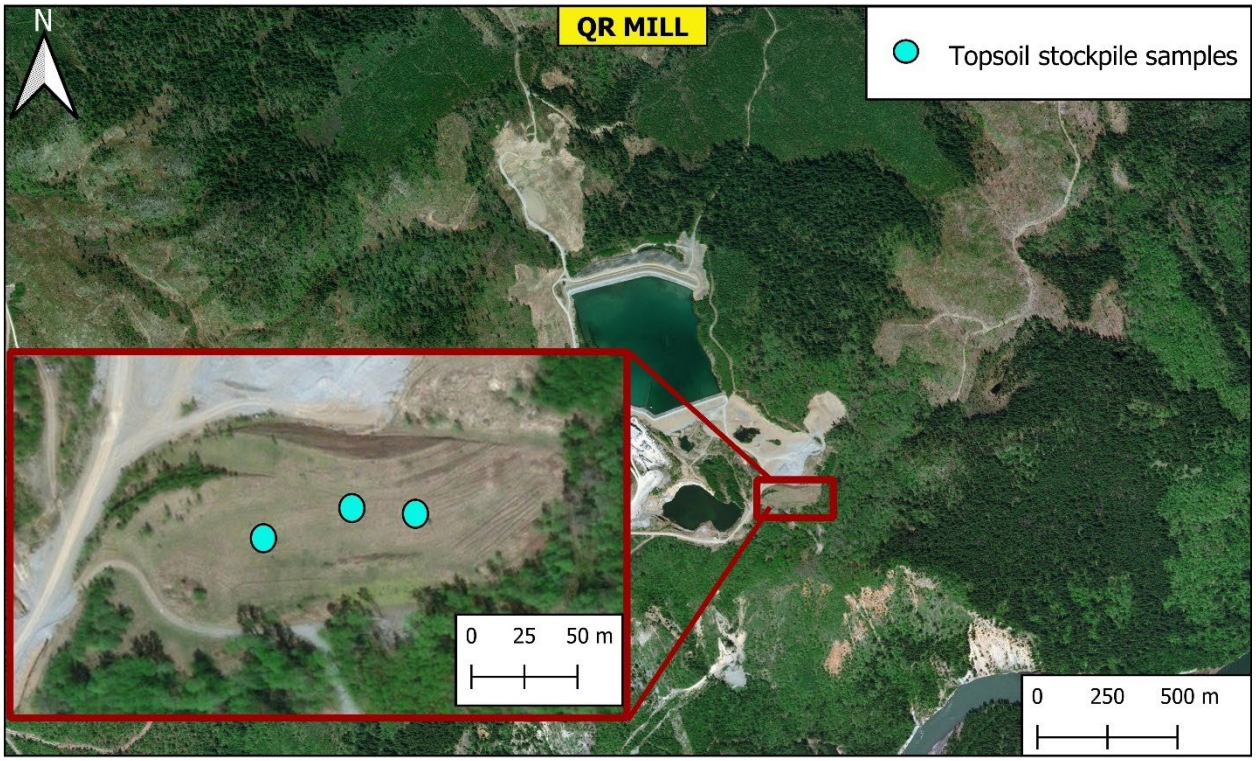

Figure 2.4 Aerial image of the QR mill, including a close-up of the topsoil stockpile of interest that shows the locations of three soil pit samples. Map generated in QGIS® with Bing VirtualEarth background.

a) b)

Figure 2.3 Pictures showing a) sampling of the topsoil stockpile in QR mill via mechanical digger and b) a sample hole exposing part of the topsoil profile

2.2.3 Experimental Design and Statistical Analysis

For analyzing stockpile depth effects on soil geochemical properties, a linear mixed effects regression was used that included depth as a fixed factor and soil cores or soil pits as a random factor (lme4 and lmertest in R). The reference soil characteristics were included in figures and analysis primarily as a benchmark and were not included in statistical testing. Residual plots of geochemical variables were used to determine if a log(x+1) transformation was necessary. Al, Cu, Fe, Mg, Mn, Na, P, NH4, and H for the New Afton dataset, and Cu, S, Zn, NH_4, NO_3, OM, and C/N variables for the QR mill dataset, were log(x+1) transformed. Multicollinearities between variables were tested using Spearman's rank correlation in 'varclus' function in the nmle R package. Highly correlated (Spearman's $p^2>0.7$) data were excluded, specifically soil Ca in the QR mill dataset (Land, 2015). The composition of all measured soil properties with depth were summarized using principal component analysis (PCA) on scaled geochemical variables (ggfortify in R). Values of variables below detection limits were set to have the value of the detection limit.

After sampling New Afton soil samples were combined to meet the minimum requirement of sample amount for testing. Smaller soil sample intervals were purposely formed near the surface of the stockpile because the most changes and activity were likely to occur at the surface level. However, the exact depths sampled were a function of the sampling ability of the auger drill used. Given the large depth range in each pooled sample used for geochemical analysis, stockpile depth was treated as categorical variable in the New Afton data analysis. Conversely, QR mill soil samples were collected at a point depth for each soil pit. More soil samples were purposely taken near the surface of the stockpile, however, the exact depths sampled were largely driven by the sampling ability of the excavator. Because samples more closely represented a single depth, stockpile depth was treated as a numerical variable in the QR mill data analysis.

The elemental composition of the soil samples was measured at the Analytical Laboratory at the Ministry of Environment and Climate Change Strategy in Victoria, B.C. The samples were prepared by heating soil samples at 70ºC for 24 hours, followed by sieving through a 1 mm pan. Analyses composed of a profile of major elements; total Al, B, Ca, Cu, Fe, Mg, Mn, P, K, S, and Zn via acid, microwave digestion followed by inductively coupled plasma–optical emission spectrometry (Sandroni et al., 2003) , available P via Bray P-1 extraction ultraviolet analysis (Bray & Kurtz, 1945), and available NH_4-N and NO_3-N via potassium chloride extraction

(Kachurina et al., 2000). Organic matter and moisture content were measured in-house via Loss-on-ignition (LacCore, 2013), and soil-water pH (H_2O) and EC were determined using a Palintest® 800 meter. Loss-on-ignition was calculated by weighing approximately 1.5 g of soil into pre-weighed tins and then heating in succession at 105ºC then 500ºC for 12 and 5 hours respectively, until constant weights were achieved. After each succession, the dried soil was weighed to calculate water content and organic content of the soil. Total C, S, and N amounts was measured with a ThermoScientific CHNS Elemental Analyzer. These samples were prepared by drying in an oven at 70ºC for 24 hours followed by sieving through a 1 mm pan and grinding with a mortar and pestle.

2.3 Results

2.3.1 General Trends

In order to explore the effect of soil stockpile depth on geochemical properties, PCA plots were drawn (Figure 1 and Figure 2), illustrating that stockpile depth led to significant changes in soil properties at both New Afton ($R^2 = 0.15$, $P < 0.01$) and QR mill ($R^2 = 0.28$, $P < 0.01$). In New Afton, PC1 explains 34.7% of variation and PC2 explains 19.7% of variation observed between samples (Figure 2.5). In QR mill, PC1 explains 32.9% and PC2 explains 18.5% (Figure 2.6). The PCA clearly showed variations among different stockpile depths at QR mill, but not New Afton. Additionally, PCA clearly showed geochemical variations among the soil samples from stockpiles and reference soils at both sites. The corresponding reference soil properties from both sites were included on the PCA plots as a benchmark, and not included in the principal component calculations (Figure 2.5; Figure 2.6).

2.3.2 Macronutrients (N, P, K, S, Mg, Ca)

Soil NH_4-N showed a notable increase below the 152-610 cm depth interval in the New Afton stockpile (Figure 2.7, $P = 0.08$) from an average of 0.27 mg/kg to 2.5 mg/kg at the bottom 610-1372 cm interval. Ammonium also increased significantly with depth in the QR mill topsoil stockpile (Figure 2.8, $R^2 = 0.18$, $P = 0.05$) and ranged from 3.6 mg/kg to 18.8 mg/kg.

Soil NO_3-N decreased with depth in the New Afton stockpile (Figure 2.7; $P = 0.04$), from an average of 27 mg/kg and 30.5 mg/kg in the top 152 cm to 13.5 mg/kg at the bottom 610-1372

cm. The corresponding reference soil had a lower soil NO_3-N content at 5.6 mg/kg. Conversely, at QR mill there were no significant differences in soil NO_3-N with depth (Figure 2.8, $R^2 = 0.02$, $P = 0.38$), and the average range was between 7.5 mg/kg and 1.4 mg/kg. The reference soil for QR mill had a much higher level of soil NO_3-N at 29.1 mg/kg.

There was no evidence that the carbon/nitrogen (C/N) ratio changed significantly with stockpile depth in New Afton (Figure 2.7, $P = 0.11$) ranging from 6.6 to 10.6, and similar to the reference soil. There was evidence that C/N increased with stockpile depth in the QR mill topsoil stockpile (Figure 2.8, $R^2 = 0.35$, $P < 0.01$), ranging from 7.8 to 23.4. Generally, the reference soil (average C/N = 15.2) was most like the bottom half of the topsoil stockpile at the QR mill site.

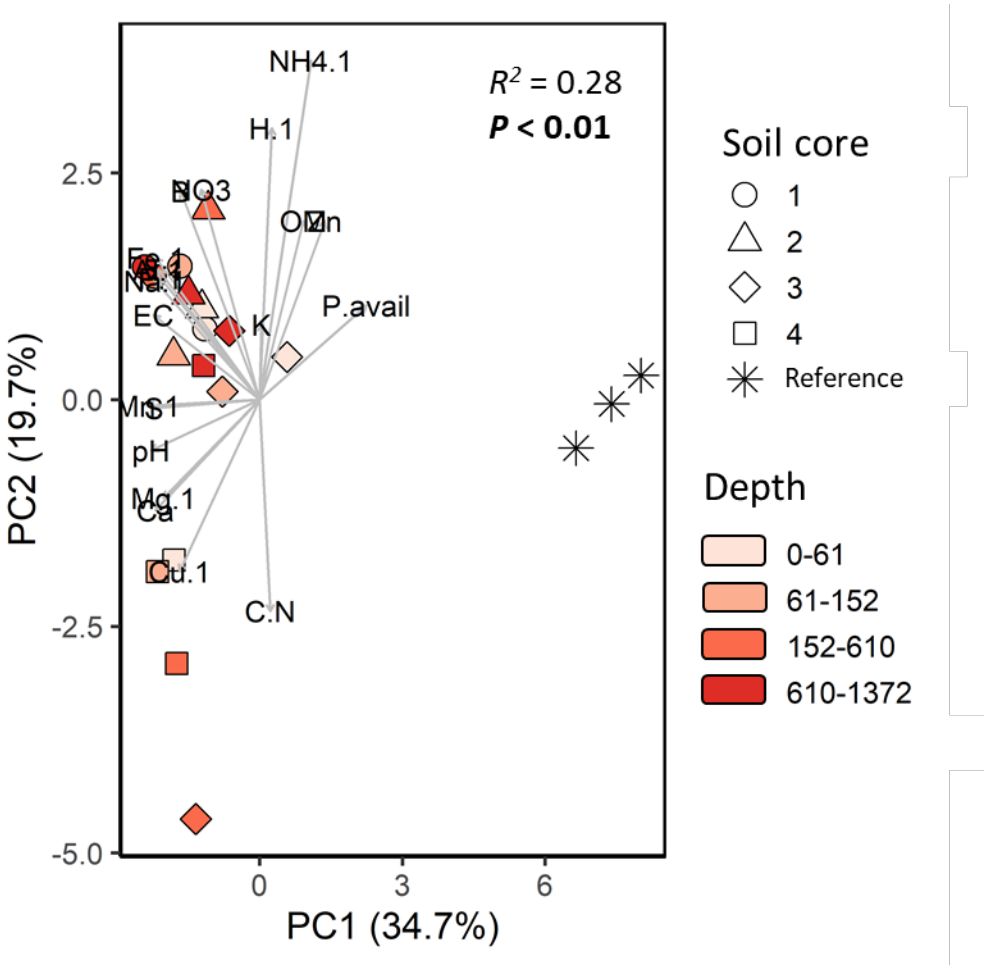

Figure 2.5 PCA plots showing differences in soil chemical properties at New Afton with changing stockpile depth. PC1 accounts for 34.7% and PC2 accounts for 19.7% of variation observed between soil samples.

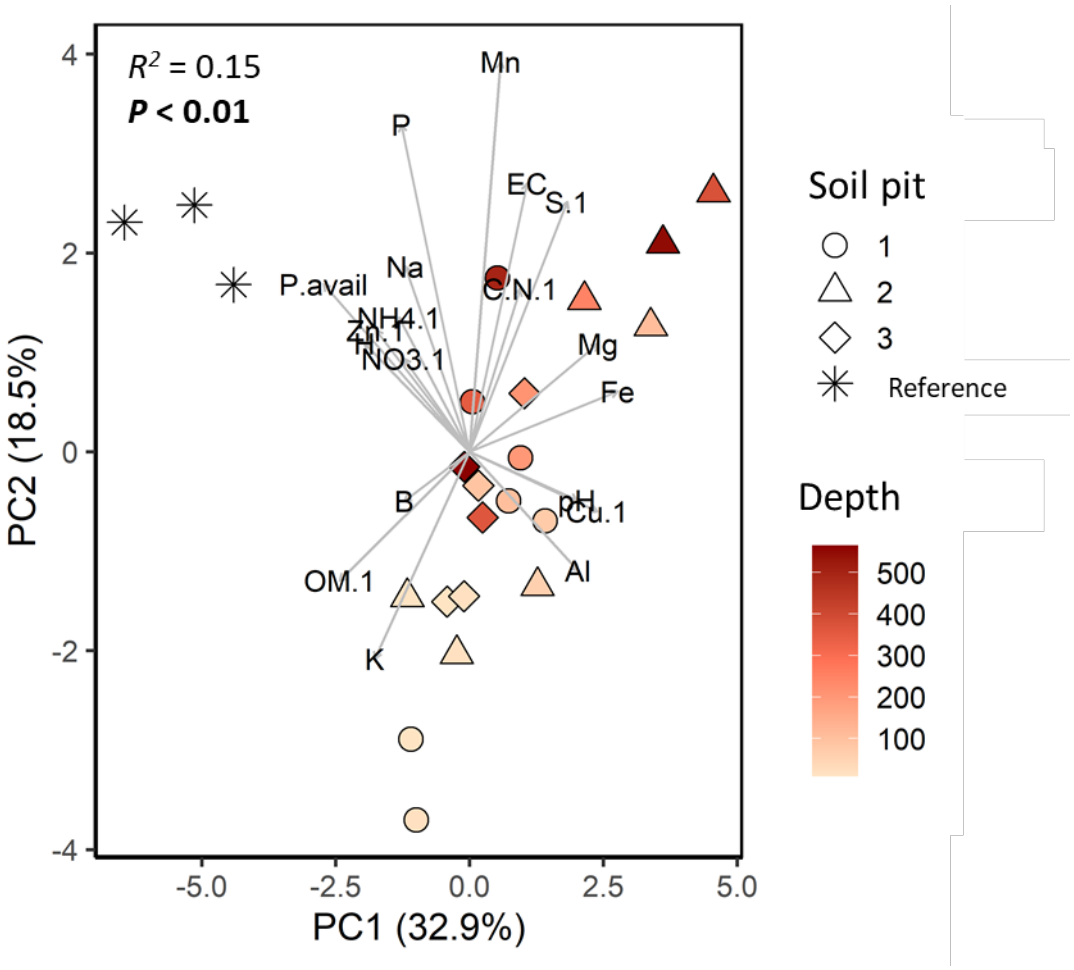

Figure 2.6 PCA plots showing differences in soil chemical properties at QR mill with changing stockpile depth. PC1 accounts for 32.9% and PC2 accounts for 18.5% of variation observed between soil samples.

There was no evidence that total P content or available P content changed with stockpile depth in New Afton (Figure 2.7, $P = 0.63$, $P = 0.82$, respectively). Total soil P content ranged between 0.1 % and 0.13 % and available P content range from 1.1 mg/kg to 10 mg/kg in the New Afton stockpile. The corresponding reference soil for New Afton had an average total P content of 0.08% and available P content of 27.7 mg/kg. There was no evidence that total P content changes significantly with depth (Figure 2.7, $R^2 = 0.076$, $P = 0.24$), however available P decreased steadily with increased stockpile depth (Figure 2.8, $R^2 = 0.37$, $P = 0.01$). QR mill total soil P content range between 0.07% and 0.12% and available P content range from 0.41 mg/kg to 18 mg/kg. The corresponding reference soil for New Afton had an average total soil P content of 0.13% and an average available P content of 145 mg/kg (not shown in figure).

There was no evidence that K levels changed significantly with stockpile depth in the New Afton (Figure 2.7, $P = 0.39$). There was some evidence that K levels changes significantly with depth in the QR mill topsoil stockpile (Figure 2.8, $R^2 = 0.16$, $P = 0.08$). Neither stockpile appeared to be notably different than their reference soils.

There was some evidence that soil S levels increased with depth in the New Afton stockpile (Figure 2.7, $P = 0.06$). After 0-61 cm depth, S increased by approximately 45% in the upper depth intervals. Soil S was notably higher in the New Afton stockpile compared to the reference soil. There was also some evidence that S increased with depth in the QR mill stockpile (Figure, $R^2 = 0.16$, $P = 0.07$). The QR mill stockpile generally has similar S levels to the reference soil.

There was no evidence that Mg levels changed significantly with stockpile depth in the New Afton (Figure 2.7, $P = 0.13$) and QR mill topsoil stockpile (Figure 2.8, $R^2 = 0.16$, $P = 0.1$). Both stockpiles showed elevated levels of Mg compared to their respective reference soil.

There was no evidence that calcium (Ca) levels changed significantly with stockpile depth in the New Afton (Figure 2.7, $P = 0.41$) and appeared to have elevated levels of Ca compared to the reference soil.

2.3.3 Micronutrients (Fe, Mn, Zn, Cu, B)

There was some evidence that Fe levels increased significantly with depth in New Afton (Figure 2.7, $P = 0.07$), where average Fe levels increased by 7% from the top of the stockpile (0-61 cm) to the bottom (610-1372 cm). Soil Fe was notably higher in the stockpile soil (ranging from 39 000 mg/kg to 44 000 mg/kg) compared to the reference soil (average = 30 666 mg/kg) in

New Afton. There was little to no evidence that Fe levels increased significantly with depth in QR mill samples (Figure 2.7, $R^2 = 0.11$, $P = 0.14$), ranging from 40 000 mg/kg to 63 000 mg/kg. Soil Fe was higher in the stockpile soil compared to the reference soil (average = 33 000 mg/kg) for QR mill.

There was very little to no evidence that soil Mn changed significantly with stockpile depth in New Afton (Figure 2.7, $P = 0.29$). Soil Mn ranged from 870 mg/kg to 1000 mg/kg and the corresponding reference soil for New Afton had an average Mn content of 660 mg/kg. There was evidence that soil Mn increased steadily with stockpile depth in QR mill (Figure 2.8, $R^2 = 0.34$, $P < 0.01$) despite one outlier with 840 mg/kg Mn at 565 cm. Mn values ranged from 680 mg/kg to 1400 mg/ kg and the corresponding reference soil for QR mill had an average Mn content of 1133 mg/kg.

There was evidence that soil Zn changed significantly with stockpile depth in New Afton (Figure 2.7, $P < 0.01$), increasing from an average of 65.3 mg/kg at the surface to 71.0 mg/kg at the bottom of the stockpile, with a range from 58 mg/kg to 80.0 mg/kg. The corresponding reference soil for New Afton had an average Zn content of 79.7 mg/kg. There was also possible evidence that Zn increased with stockpile depth in QR mill (Figure 2.7, $R^2 = 0.18$, $P = 0.05$). Soil Zn ranged from 58.0 mg/kg to 140.0 mg/kg and the corresponding reference soil for QR mill had an average Zn content of 154 mg/kg.

There was a spike of copper (Cu) at the 152-610 cm sample interval with an average of 495 mg/kg (up to 840 mg/kg was detected) in the New Afton stockpile compared to the rest of the stockpile (ranging from 120.0 mg/kg to 160.0 mg/kg, Figure 2.7, $P < 0.01$). There was little to no evidence Cu levels changed significantly with QR mill stockpile depth (Figure, $R^2 < 0.01$, $P = 0.9$) and Cu content ranged from 83 mg/kg to 250 mg/kg.

There was very little to no evidence that boron (B) changed significantly with stockpile depth in New Afton (Figure 2.7, $P = 0.27$). B ranged from 12.0 g/kg to 27.0 mg/kg and the corresponding reference soil for New Afton had an average B content of 12.3 mg/kg. There was very little to no evidence that soil Mn changed significantly with stockpile depth in QR mill (Figure 2.8, $R^2 = 0.04$, $P = 0.38$), ranging from 7.0 mg/kg to 13.0 mg/kg and the corresponding reference soil for QR mill having an average of 11.6 mg/kg.

2.3.4 Salt Content

After a 16% decrease in soil EC at the surface to 61-152 cm to 1190.6 µm/S, EC increased to an average of 1445.8 µm/S (Figure 2.7, $P = 0.01$) in the New Afton stockpile. The New Afton stockpile had notably higher EC levels than the reference soil (average 67.3 µS/ cm). There was evidence that soil EC increased significantly with stockpile depth at QR mill (Figure 2.8, $R^2 = 0.23$, $P = 0.03$), ranging from 39.6 µS/ cm to 217.0 µS/ cm. The QR mill stockpile had similar EC content to the reference soil (average 119.4 µS/ cm). Based on the reclamation suitability ratings for EC, the New Afton stockpile was rated as fair at all depths and the QR mill stockpile was rated as good at all depths (Table 1)

Despite a 20% decrease at the 152-610 cm depth interval, Na appeared to increase steadily with depth, from 2200 mg/kg average at the surface (0-60 cm) to 3025 mg/kg average at the bottom (610-1372 cm) interval (Figure 2.7, $P = 0.01$) for the New Afton Site. Its corresponding reference soil had lower Na content at an average of 766.7 mg/kg. There were no significant changes in soil Na observed in the QR mill stockpile with values ranging from 570 mg/kg to 810 mg/kg and the reference soil had an average soil Na of 840 mg/kg (Figure 2.8, $P = 0.13$). Based on the reclamation suitability ratings for SAR, the New Afton stockpile was rated as poor or unsuitable at all depths and the QR mill stockpile was rated as fair at all depths (Table 1).

Table 1 Reclamation suitability ratings for the topsoil stockpiles in grassland (New Afton) and forested (QR mill) ecosystems. G = good; F = fair; P = poor; U = unsuitable.
Source: (Macyk et al., 2004)

Depth (cm)	pH	EC	SAR[a]
New Afton: Grassland			
0-61	F	G	P/ U[b]
61-152	F	G	P/ U[b]
152-610	F	G	P/ U[b]
610-1372	F	G	P/ U[b]
Reference	G	G	F
QR Mill: Forest			
0-10	F	G	F
10-20	G	G	F
60-120	F	G	F
200-260	G	G	F
350-390	G	G	F
500-575	F	G	F
Reference	G	G	F

EC = Electrical Conductivity
[a] = Sodium Absorption Ratio; see Section 2.4.4 and Appendix B. for more information.
[b] = May be characterized as poor or unsuitable based on soil texture and moisture content.

2.3.5 pH

There was evidence of significant differences in pH with stockpile depth at New Afton (Figure 2.7, $P = 0.03$). The topsoil stockpile at New Afton was slightly alkaline, with pH values ranging from 8.0 to 8.3, the corresponding reference soil had an average pH of 7.3. Average pH was lowest at the bottom of the stockpile (pH 8) and highest in the 152-610 cm depth interval (pH 8.18). The QR mill stockpile soil was acidic to neutral and ranged from pH 5.4 to 7.3, with no evidence of significant changes in pH with soil depth (Figure, $R^2 = 0.02$, $P = 0.52$), the corresponding reference soil had an average pH of 5.63. Based on the reclamation suitability ratings for pH, the New Afton stockpile was rated as fair at all depths and the QR mill stockpile was rated as good or fair at varying depths (Table 1).

2.3.6 Organic Matter

There was no evidence of significant changes in soil organic matter (OM) content with depth at the New Afton stockpile (Figure 2.7, $P = 0.43$). Soil OM ranged from 1.6% to 4.0% and the corresponding reference soil had an average soil OM at 3.3%. Conversely, there was an immediate decrease in soil OM moving away from the surface of the QR mill stockpile after 20 cm in the (Figure 2.8, $R^2 = 0.28$, $P = 0.02$). Soil OM values ranged between 2.7% and 11.1% and the reference soil had an average soil OM content of 10.2%.

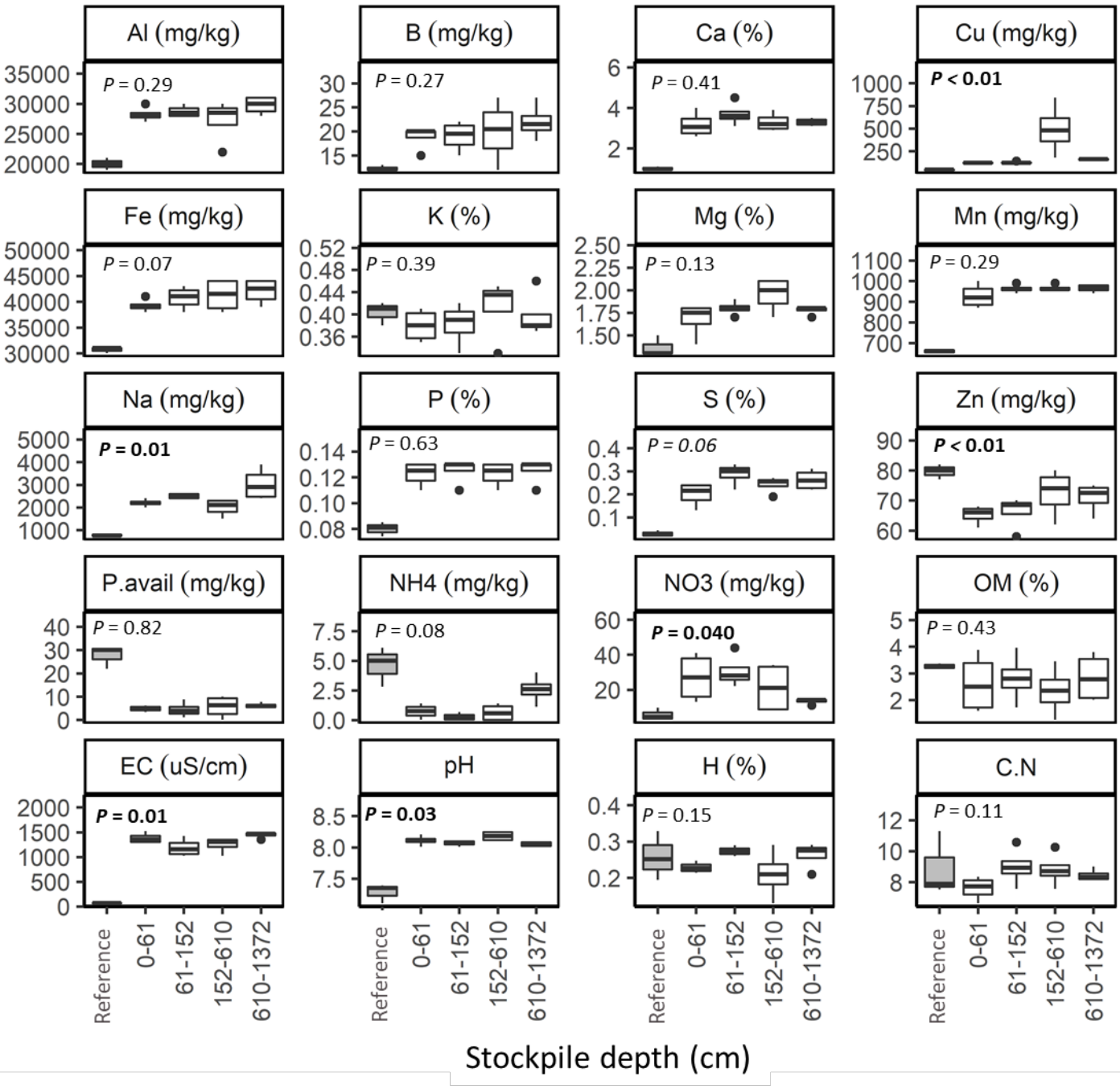

Figure 2.7 Boxplots showing differences in geochemical variables with stockpile depth at the New Afton site.

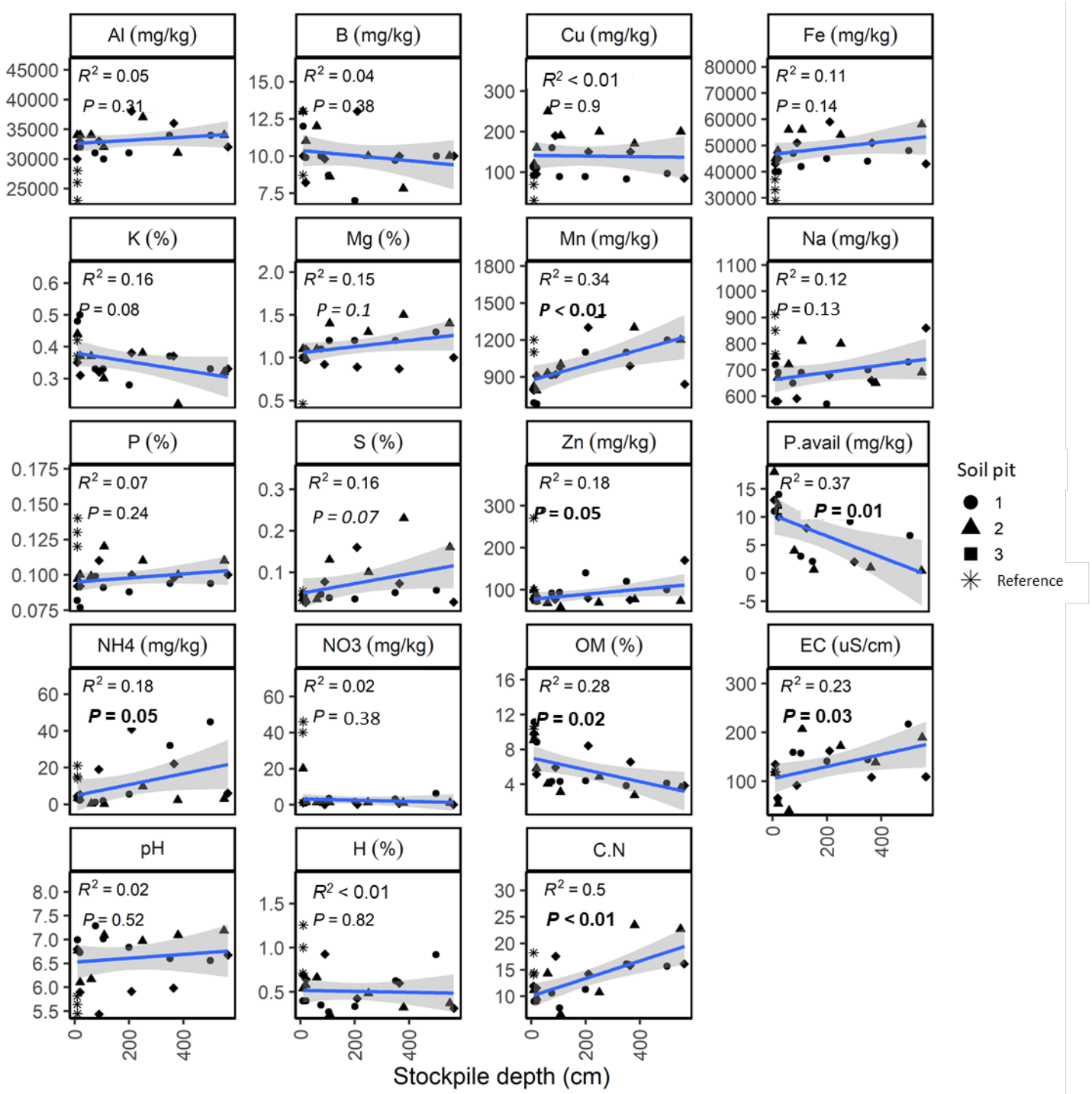

Figure 2.8 Linear regression plots showing changes in geochemical variables with stockpile depth at the QR mill site. The blue lines represent a linear model and the shaded area in grey represents the 95% confidence intervals.

Note: the reference samples are not shown in the available P plot.

2.4 Discussion

To assess the effect of stockpile storage height on topsoil quality, macronutrients, micronutrients, salt content, pH, and organic matter were measured at varying depths from the topsoil stockpiles at New Afton and QR mill

2.4.1 Macronutrients (N, P, K, S, Mg, Ca)

Nitrogen is one of the most limiting nutrients for plant growth and productivity. Plants acquire nitrogen from the soil, mainly in the form of NH_4-N and NO_3-N, although NO_3-N is the preferable form for take-up by plants. Microbial decomposition converts organic nitrogen into bioavailable NH_4-N (mineralization) and, furthermore, through a two-step process, nitrifying bacteria can then oxidize NH_4-N into bioavailable NO_3^- (nitrification). Nitrate often dominates in aerobic soils, while NH_4-N tends to be more prevalent in acidic and anaerobic soils (Hachiya & Sakakibara, 2017). The soil N results in this study are generally consistent with other research; for example, two soil restoration studies at coal mine sites (Harris and Birch, 1989; Williamson and Johnson, 1990) found that when soil was stockpiled in piles that were more than 100 cm deep, there was a large accumulation of NH_4-N (up to 70 mg/ kg) in the topsoil. Conversely Abdul-Kareem 1984 saw little variation in the amount of NO_3-N and NH_4-N. Our results showed some evidence of NH_4-N accumulation in both the New Afton (at and below 610 cm) and QR mill (at and below 100 cm) topsoil stockpiles, which may indicate anoxic conditions at these depths. The increasing C/N ratio in the QR mill stockpile supports this as this indicates lower rates of microbial decomposition (Ghose & Kundu, 2004). Because of the high pH levels in the New Afton stockpile, the accumulation of NH_4-N from anaerobic conditions may have been lessened and resulted in retention of NO_3-N. In contrast NH_4-N was dominant in QR mill topsoil stockpile, especially below 100 cm. This difference in stockpiles may be a function of site age; the QR mill stockpile was approximately 14 years older than the New Afton stockpile, allowing more time for denitrification of NO_3-N to NH_4-N. Alternatively, the differences may be a result of site factors including soil pH, climate, and geological history. In general, NO_3-N content for New Afton were quite high (often higher than 10 mg/kg) compared to the reference levels, whereas NH_4-N content was mostly below reference levels.

Phosphorus is an essential nutrient for plant structures and is necessary for various biochemical reactions and is typically taken up as $H_2PO_4^-$. Only the QR mill topsoil stockpile showed a decreasing trend in available P with depth, perhaps due to stockpile age (20 years). This was likely a result of immobilization by soil microbes in anoxic conditions within the stockpile, corresponding with the decrease in OM. This finding was consistent with other research; for example, one study found that soil N and P decreased with depth in claypan soils (Hsiao et al., 2018). According to the Interpretations for Soil Test Phosphorus and Potassium Guidelines for Southern British Columbia (BC Ministry of Agriculture, 2010) document, both topsoil stockpiles characterised here are classified to having low amounts of available P concentrations (5-19 ppm). The New Afton reference soil had a medium (20-39 ppm) concentration, and the QR mill reference soil had high (>100 ppm) levels of available P. Because P availability is pH-dependent, P reduction in the New Afton topsoil may be linked to the alkaline soil conditions. In alkaline soils, (pH > 7), P precipitates with Ca, reducing availability. Deficiency in soil P generally results in stunted plant growth and poor establishment.

The majority of S in soil is found in OM and was released through microbial mineralization processes. Our results indicated that total S likely increased with stockpile depth in both stockpiles. For example, S reached 0.29% at 61-152 cm depth, increasing 45% from the top of the stockpile (0-61 cm) in New Afton. The slight accumulation with stockpile depth may be a result of S leaching throughout the piles (Baer, 2016). Both stockpiles had elevated levels of total S (up to 0.29%) above the recommended guideline (0.05%) for agricultural land (CCME, 1999) and higher than the corresponding reference soils; however, the ranges present in the stockpiles are considered typical for organic soils (K. A. Brown, 1982). It is possible that anaerobic conditions allowed sulphate-reducing bacteria to increase the amount of hydrogen sulfide acid (H_2S) and decrease the amount of H_2SO_4.

Secondary macronutrients and primary cations, such as Ca, K and Mg are critical for photosynthesis, signal transduction and structure in plants (Yan & Hou, 2018). These nutrients do not change throughout the stockpile profiles examined here. In general, these nutrients are likely sufficient in both stockpiles for revegetation upon re-spreading. Although, Ca levels in the New Afton stockpile samples (up to 4.5%) were higher than the reference soil (up to 1.1%), the stockpile may benefit from additional Ca inputs due to the high Na levels (Table 1; Figure 3) (Naeem et al., 2017).

2.4.2 Micronutrients (Fe, Mn, Zn, Cu, B)

Necessary micronutrients (e.g., Cu, Fe, Mn, and Zn) are typically found in sufficient levels in soil (Horneck et al. 2011). Mn tended to increase with depth in QR mill stockpile only and Fe increased with depth in New Afton only, whereas Zn increased with depth in both stockpiles. The accumulation of Fe and Zn compared to reference soils may indicate anaerobic conditions within the piles. In general, there was an accumulation of Fe throughout both stockpiles compared to their reference soils. There was a spike in Cu concentration at the New Afton stockpile at 152-610 cm depth, reaching up to 840 mg/kg, so this section of the stockpile should be avoided when respreading. Additionally, the QR mill stockpile suffered from elevated Cu levels at all depths. The Cu may be present at high amounts at this depth due to contamination from natural sources while salvaging. The majority of samples from both stockpiles had levels above the recommended concentration for agricultural and residential lands (63 mg/kg) and commercial and industrial (91 mg/kg) (CCME, 1999). Additionally, some samples in both piles had Cu levels above the thresholds for livestock/ plant/ invertebrate toxicity (150 mg/kg) and microbial impairment (350 mg/kg). Impacts from local mineralogy or admixing with Cu-containing bedrock during topsoil stripping could explain the high levels of Cu in these stockpiles.

Soil B is an important nutrient for cell wall structure. Soils typically have between 10 and 80 mg/kg of elemental B, although most of which is not available to plants (Naeem et al., 2017). The results show that soil B does not change within the pile profiles at either site and will be sufficient to support plant growth (ranging between 15 to 27 mg/kg in New Afton and 7 to 13 in QR mill).

2.4.3 Salt Content

Electrical conductivity (EC) is a measure of cations or anions of a solution and was associated with soil salinity and soluble nutrients (Smith & Doran, 1996). Because of this, EC is important for understanding soil quality. The statistically significant p-values for pH and EC measured with the Palintest® 800 meter in the New Afton stockpile are unlikely to have biological or environmental importance (mean pH and EC ranging from 8.1 to 8.2 and 1191 µS/cm to 1446 µS/cm, respectively). Low p-values were likely due to the minimal variation

(maximum standard error for pH was 0.04 and EC was 90.5) seen between soil samples (Figure 2.5). This is supported by Table 1 where sustainability ratings for EC and pH are classified with the same ratings throughout varying stockpile depths. Soil EC was much higher than the reference soil (average 67.3 µS/cm), which was likely the result of elevated Na content in the stockpile. The EC levels in the QR mill soil tended to increase with stockpile depth, indicating an increase in a soluble salt, perhaps Mg or Na. Nevertheless, both piles are characterized as good in terms of their EC levels for reclamation suitability (Table 1) and are not considered saline (Smith & Doran, 1996).

Soil Na is not necessary for plant growth and high levels of Na can damage soil structure and plant growth. Soil Na content in New Afton increased 38% from the surface of the pile to the bottom of the pile and was approximately 187% higher than the reference soil. While there are no known studies measuring soil Na levels in topsoil stockpiles, the increase in Na within the stockpile depths may have occurred due to leaching of salts down the pile or chemical diffusion which can move salts where there are differences in concentration (Thurber Consultants Ltd. et al., 1990). The general elevated Na levels in the stockpile may be a result of the semi-arid conditions in the Kamloops region, where high temperatures and low precipitation are common. Here, the rates of evaporation may have caused an accumulation of Na in the pile. High salt concentrations can cause an ionic imbalance, reducing K and Ca availability and creating drought conditions for plants by reducing the water potential (Naeem et al., 2017). According to the guidelines from the Soil Quality Criteria Relative to Disturbance and Reclamation, the New Afton stockpile was poor or unsuitable for reclamation at all depths (depending on soil texture), due to the high SAR content (Table 1). Additionally, the SAR and EC levels in the New Afton stockpile indicate the soil as sodic. Sodic soils are often alkaline (typically greater than pH 8.5) as a result from the hydrolysis of sodium carbonate (Na_2CO_3), releasing hydroxyl groups (OH^-) (Kumaragamage et al., 2021).

2.4.4 pH

Soil pH is an important measure of quality for plant growth as it heavily influences microbial community composition and the availability of soil nutrients and toxic elements (Smith & Doran, 1996). The soil pH at New Afton was alkaline, often above pH 8.0 at all depths. Using the guidelines from the Soil Quality Criteria Relative to Disturbance and Reclamation (Macyk et al.,

2004), the New Afton topsoil stockpile was classified as fair (Table 1), having moderate soil limitations due to high soil pH (>8.0). Conversely, the QR mill topsoil stockpile was slightly acidic and had none to slight limitations for revegetation (Macyk et al., 2004; Smith & Doran, 1996).

High pH in soils can negatively impact nutrient availability to plants. A rise in pH can increase mineralization, reducing the presence of N and could indicate the presence of free carbonates. Additionally, the availability of P and K can be significantly reduced (Smith & Doran, 1996). Alkalinization of soil can occur in arid and semi-arid ecosystems when there was minimal rainfall such as in the New Afton site. These effects are likely compounded by the fact that the stockpile was likely highly compacted, creating an impenetrable barrier that would negate any rainfall and allow salts to accumulate beneath the surface. Alternatively, an increase in pH can occur due to admixing with calcareous subsoils (Thurber Consultants Ltd. et al., 1990). In support of this, the parent materials of the dominant soils in the New Afton mine area (Tranquille, Timber, and Trapp Lake) are calcareous and saline. Thus, in typical soil profiles of this area the pH rises considerably in the C-horizon (approximately pH 8.5) compared to the A-horizon (approximately pH 7.1) (Government of Canada, 2019). It is likely that incorporation of the alkaline subsoils and parent materials occurred resulting in the alkaline and sodic conditions in the topsoil stockpile at New Afton.

2.4.5 Organic Matter

Soil OM includes decomposing plant and animal residues and is an important source of plant nutrients and soil structure (Salehi et al., 2011). Organic matter in the New Afton stockpile did not fluctuate with depth, but the stockpile had approximately 15% to 39% less OM content compared to the undisturbed soil. Although considerably higher than New Afton soils, organic matter in the QR mill stockpile decreased sharply after the first 10 cm (OM content was approximately halved). This roughly corresponding to the decline in available P and rise in C/N ratio, indicating microbial decomposition in QR mill. Organic matter is degraded through respiration (mineralization); this process is much slower under anaerobic conditions compared to aerobic (Kumaragamage et al., 2021). Therefore, the relative decline in OM in these piles likely happened relatively quickly during oxygenic conditions and has not changed substantially during the anoxic phase. A drop in OM content with topsoil stripping and storage has been

generally recognized in the few available studies (Ghose & Kundu, 2004). For example, one 3-year study found that OM and soil structure declined with increased storage time (Wick et al., 2009) and another study found that OM declined with stockpile depth (Abdul-Kareem & McRae, 1984). Alternatively, OM content in these piles may have decreased OM content due to mixing with subsoils while stripping. Further, higher levels of OM at the surface of QR mill stockpile may be due to litter and vegetation establishment. Despite, the losses of OM content in these piles, it was likely to be adequate to support plant establishment post-mining.

2.4.6 Conclusions

Our results show that stockpile height was a key factor to some soil geochemical properties, which ultimately impacts restoration success on mine sites. However, the impact of soil depth on nutrient deterioration was not as great as some other studies suggest (Ezeokoli et al., 2019; Harris et al., 1989; Thurber Consultants Ltd. et al., 1990). There was not a substantial decline in nutrients with stockpile depth in the taller, younger pile (New Afton), except for fluxes between NO_3-N and NH_4-N. However, in the shorter, older pile (QR mill), OM and available P appeared to deteriorate with increasing stockpile depths. This may indicate stockpile height was not as influential as stockpile age (Golos et al., 2016). While most nutrient levels of the New Afton soils were within an acceptable range for reclamation, many were depleted compared to the reference soil. Additionally, we found an accumulation of NH_4-N, Mn, and Zn with stockpile depth, suggesting anaerobic conditions. Both stockpiles suffered from copper levels above the recommended CCME concentration for agricultural/ residential and commercial/ industrial land at most depths. Additionally, some samples were above the threshold levels of Cu set by the Contaminated Sites Regulation for toxicity to livestock, invertebrates, plants, and microbial activity. Further, the New Afton stockpile was rated as unsuitable or poor restoration soil due to a high sodium absorption ratio. While some key nutrients do not change with depth and are likely able to sustain revegetation, we found significant deviations in the overall stockpile soil, especially deeper soils, compared to the native undisturbed soil. The stockpile soil conditions suggest there may be challenges for native vegetation establishment during restoration efforts.

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3.0 INVESTIGATING IMPACTS FROM TOPSOIL STOCKPILE HEIGHT ON SOIL MICROBIAL COMMUNITIES

3.1 Introduction

The mining industry in British Columbia is an essential source of resources and is a key contributor to the economy. As of 2020, there were 16 major metal and coal mines, and during 2020, the mining industry produced approximately $7.3 billion dollars and provided more than 30 000 jobs (Ministry of Energy, Mines, and Low Carbon Innovation, 2020). Mining activities typically include mineral exploration, mineral development, mine production, and mineral processing. These disturbance events cause lasting negative environmental impacts such as ecosystem degradation, habitat destruction, pollution, and loss of soil carbon (Tripathi et al., 2016). Federal and provincial regulations ensure that the restoration or reclamation of landscapes are conducted at mine sites to repair disturbances and return the land to a sustainable ecosystem with a historical level of productivity. Mining is particularly damaging to ecosystems because soils are stripped from landscapes and require reconstruction, which take hundreds or thousands of years if left to natural processes (Bradshaw, 1997).

Topsoil is a valuable bioactive substrate hosting a wide variety of organisms including plants, microorganisms, animals, viruses, and protists. Specifically, topsoil is defined as the uppermost part of the soil profile, typically ranging in depth from 7 to 25 cm and contains the highest concentration of organic matter and microorganisms (Soil Science Society of America, 2008). Moreover, topsoil which has been disturbed by human activity such as tillage or mining, is referred to an Ap horizon. To preserve valuable topsoil in mining operations, it is common practice to store stripped topsoil on site as a topsoil stockpile for ecosystem rehabilitation. Stockpiling topsoil can be used post-mining to provide nutrients, structure, seeds, and amend waste materials on site. However, long-term storage of topsoil has shown to deteriorate soil health by altering its geochemical properties and microbial communities (Ghose & Kundu, 2004; Gorzelak et al., 2020; Harris et al., 1989; Mummey et al., 2002b). For example, Boyer et al. (2011) observed that topsoil stockpiles contained compacted and anaerobic soil below one meter, resulting in a low abundance and diversity of earthworms. Moreover, a laboratory study found

that stockpiling topsoil caused a decrease in soil organic matter degradation rate implying diminished microbial activity (Felton & Taraba, 1994).

Soil health is key in restoration success and ecosystem functioning; soil organic matter, soil nutrients and soil microorganisms play major roles in maintaining healthy sustainable ecosystems. Although living soil microorganisms are less than 1% of the total soil volume (Adhikari & Hartemink, 2016), a single gram of soil can contain about 10^4 species (Roesch et al., 2007). Currently, only a small proportion of microbial diversity has been identified (Delgado-Baquerizo, 2019; Delgado-Baquerizo, Oliverio, Brewer, Benavent-González, et al., 2018; Tedersoo et al., 2014). Soil microorganisms, particularly bacteria and fungi provide many ecosystem functions including roles as biological regulators, chemical cyclers, and ecosystem engineers (Saccá et al., 2017), but are extremely sensitive to disturbances and may take decades for recovery (Bastida et al., 2008; Costantini et al., 2016; Mummey et al., 2002b). Therefore, soil microorganisms are a key component to steering the recovery of disturbed ecosystems and research on soil microbial functions and communities in various ecosystems and scales are important.

Special attention should be paid to soil microbial communities in revegetation of mine soil, where severe, large-scale disturbance occurs, particularly because soil microbes are large drivers in organic matter decomposition and nutrient cycling of macro- and micronutrients critical for plant growth and establishment (Gruber, 2015). Furthermore, soil bacteria and fungi form close relationships with plants, providing services such as nutrient acquisition and protection against environmental stress and pathogens. For example, Chen et al. (2007) found that mycorrhizal colonization significantly increased plant growth in copper (Cu) contaminated soils. This was thought to be a result of an increased phosphorus (P) acquisition and decreased Cu concentrations in plant roots. Using their long, root-like hyphae network, arbuscular mycorrhizal fungi (AMF) can acquire P in areas that are difficult to access. Additionally, AM fungi secrete organic acid activating unavailable P sources and can increase expression of phosphate transporter genes in plants (Wang, 2017). Therefore, soil bacteria and fungi are paramount to the restoration process of a landscape as well as maintaining a sustainable and resilient ecosystem. The inclusion of microbial community composition data when monitoring soil quality provides crucial insights and a more accurate understanding of how land management impacts the soil ecosystem.

Microorganisms cultured in the lab for study and characterization has been occurring since 1870s. However, the use of sequencing in the 2000s highlighted that the number of observable, culturable bacteria and fungi are just a tiny fraction of those that are present but unseen (Nilsson et al., 2018). Therefore, molecular techniques including high-throughput DNA sequencing, have been used as an alternative to culturing and characterization of microorganisms. Although this sequencing technology has been around since the early-mid 2000s, the ability for accurate and easy large-scale identification of microbial communities in the environment has not been available until the last decade with improvements to third-generation sequencing (Land et al., 2015). Characterizing soil microbial genomics in mining operations has the potential to aid our ability to restore degraded sites by providing insights and improving our understanding of how land management impacts the soil ecosystem and determining the responses of soils to disturbances and to assess ecosystem sustainability.

Understanding how microbial communities respond to disturbance and how they recover is critical for optimizing restoration practices. Additionally, at a time when high-throughput sequencing technology has provided novel information about microbial taxa, we are able to characterize the microbial communities throughout the topsoil stockpile depths, which will provide new information to the field of ecological restoration. Although, it is well understood that soil microbial communities are important for many ecosystem functions, it is less well known how soil microbial activities and composition are impacted from mining disturbance. Furthermore, very little is known of the impacts to soil microbial communities from depth gradient in soil stockpiles. Examining soil microbial community composition across a known environmental gradient from the top to the bottom of topsoil stockpiles has the potential to provide insights into factors that shape the microbiome in stored soils and address knowledge gaps. This study aims to improve our understanding of how bacterial and fungal communities in topsoil respond to soil disturbance from stripping, piling, and long-term storage on mine sites by providing soil microbial community composition across a depth gradient. Research investigating soil responses over varying disturbance events and across a range of ecosystem types is critical to developing a unifying theory of ecosystem resilience and recovery. This is especially critical at a time when anthropogenic forces including climate change are causing increasingly extreme changes to the environment.

3.2 Methods

3.2.1 Soil Sampling and Study Sites.

Information about the two study sites, New Gold's New Afton and Barkerville Gold Mines' Quesnel River (QR) mill and details regarding soil sampling are covered in Section 2.2.1 and Section 2.2.1. Additionally, information about how the soil geochemical data was analysed is covered in Section 2.2.3.

3.2.2 Metabarcoding Microbial Communities

The soil microbial community composition in each sample was characterised for both fungal and bacterial OTUs in the Applied Genomics Laboratory at TRU. Deoxyribose nucleic acids (DNA) from the soil samples was extracted using the MagAttract PowerSoil DNA Kit (Qiagen Inc.). Extractions were followed by DNA quantification using Qubit™ dsDNA HS Assay Kit (Thermo Scientific). The set of primers: 341R and 806F were used to amplify the 16S rRNA gene and the set of primers: ITS86F and ITS4R were used to amplify the internal transcribed spacer (ITS) region between the 5.86S rRNA gene and the 28S rRNA genes (Vancov & Keen, 2009). Samples were run through a second round of polymerase chain rection (PCR) including the addition of barcoded primers. The thermocycler program for first round bacterial and fungal amplicon generation consisted of: 95°C for 4 min followed by 25 cycles of 95°C for 30 seconds, 53.4°C for 45 seconds and 72°C for 2 minutes, with a final extension at 72°C for 5 minutes. For generating sequencing barcode adapted bacterial and fungal amplicons, the thermocycler program was the same except that 20 cycles were used with an annealing temperature of 65°C.

After the first and second round of PCR, the amplicons were purified using the Inovant and AgenCourt AMPure (Beckman Coulter Inc.) magnetic beads prior to sequencing. Barcoded amplicons were quantified using a Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, Mississauga, ON) and visualized on an agarose gel. This was followed by pooling samples in equimolar amounts and purified following agarose gel electrophoresis using an E.Z.N.A Gel Extraction Kit (Omega Bio-Tek) and on an Ion Torrent PGM (Life Technologies Inc., Carlsbad, CA), where the library dilution factor was determined using an Ion Library Quantitation Kit and sequenced them on an Ion S5XL (ThermoFisher Scientific) (Fantini et al., 2015).

3.2.3 Processing and Statistical Analysis

Data were processed using AMPtk version 1.5.1 (Palmer et al., 2018) for quality filtering, OTU clustering at 97% sequence identity, and to assign taxonomies. Kept reads, above 10 000, were compared for taxonomy-based analysis. For both bacteria and fungi, amplicons were analyzed using the Quantitative Insights Into Microbial Ecology (QIIME) v. 1.9.0 (Caporaso et al., 2010) workflow. Reads were filtered to remove reads below a quality filter (Q20). Chimeras were filtered using "USEARCH" and taxonomy was assigned using the assign_taxonomy.py QIIME script with the GreenGenes (DeSantis et al., 2006) database using "UCLUST". Detection limits will vary between samples due to differences in yields between sequencing runs and unequal representation of samples in pooled sequencing libraries (Hugerth & Andersson, 2017). Thus, to compare data sets efficiently and to avoid bias, the reads were rarified as suggested by McKnight (2019). Rarefaction curves of the observed richness were calculated in R using 1000-fold resampling without replacement using the Vegan package (Appendix D). Read numbers were standardized to 10 000 reads per sample to reduce bias (McKnight, 2019). Operational Taxonomic Units (OTUs) were normalized using the "rarefy_even_depth" function in the Phyloseq package without subsampling. To test for significant differences in the composition and/or relative abundances of bacteria and fungi in samples from different stockpile depths, non-parametric PERMANOVA tests were performed using the "Adonis" function from the Vegan package in R with 999 permutations (Anderson, 2001).

After processing the sequencing data, our analysis classified 11 828 bacterial and 27 074 fungal read counts in New Afton and 10 496 bacterial and 17 273 fungal read counts in QR mill. Additionally, our results identified 7 627 bacterial and 2 856 fungal OTUs in New Afton and 6 894 bacterial and 1 797 fungal OTUs in QR mill. A sampling depth of 10 000 reads per sample appeared to be sufficient OTU richness according to rarefaction curves (Appendix D).

To determine if the mean relative proportion of taxonomic orders varied significantly between stockpile depth, rare OTUs were removed (less than 1% of total abundance per sample) and were then collapsed OTUs at the Phylum level. Mixed effects linear regression model was used to test for significant differences among stockpile depths. PIME was used in conjunction with Phyloseq to find the OTUs in the bacterial and fungal taxa that changed the most over stockpile depths (set to a prevalence level of 70%). PIME builds randomized decision trees, where each tree gives a vote for the prediction of the target variable (Dobbler & Roesch, n.d.;

Roesch et al., 2020). To measure differences in OTU richness in bacterial and fungal communities between stockpile depths, Shannon Diversity Index was calculated for each depth. Because the data violated assumptions of normal distribution even after transformations, Kruskal-Wallis Rank Sum test was used to determine if there were any significant differences between depth intervals. Non-metric multidimensional scaling (NMDS) ordinations ("metaMDS" function in vegan, try = 500) was used to describe patterns in microbial community composition (based on unweighted UniFrac distance measures) between stockpile depths. Permutation-based significance tests (n = 999) with the "envfit" function were used to fit geochemical and microbial variables to the NMDS ordination using soil pits as set blocks.

3.3 Results

3.3.1 Community Composition

Results showed that the topsoil stockpiles were dominated by the following bacterial phyla: Acidobacteria, Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria; and Acidobacteria in New Afton (Figure 3.1) and Actinobacteria, Bacteroidetes, Candidatus_Saccharibacteria, Chloroflexi, Firmicutes, Proteobacteria, Spirobahaetes, and Verrucomicrobiota in QR mill (Figure 3.2). The identified soil fungi were mainly composed of Ascomycota, Basidiomycota, and Mortierellomycota in New Afton (Figure 3.1), and Ascomycota, Basidiomycota, Mortierellomycota, and Rozellomycota phyla in QR mill (Figure 3.2).

Both bacterial and fungal phyla were generally consistent between different stockpile depths in New Afton (Figure 3.1, Appendix E), except for the decrease in Mortierellomycota with depth, whereas several of the bacterial and fungal phyla fluctuated significantly with stockpile depth in QR mill (Figure 3.2, Appendix E). Of the five bacterial phyla in New Afton and nine bacterial phyla in QR mill, all were present in the stockpile soil and reference soil, except for Spirochaetes in QR mill. Of the three fungal phyla in New Afton and four fungal phyla in QR mill, all were present in the stockpile soil and reference soil. The proportion of Firmicutes tended to increase at the bottom of the New Afton stockpile (610-1372 cm) and had much lower presence in the reference soil (Figure 3.1, Appendix E, $P = 0.06$) proportion of Mortierellomycota decreases with stockpile depth (Appendix E, $P = 0.012$). The proportion of Bacteroidetes, Chloroflexi, Firmicutes, and Spirochaetes increased substantially or were present

exclusively in the bottom of the QR mill stockpile below 60-120 cm (Figure 3.2, Appendix E, $P < 0.05$). The proportion of Acidobacteria, Actinobacteria, Proteobacteria, and Verrucomicrobia decreased substantially or were present exclusively in the surface of the QR mill stockpile above 60-120 cm (Figure 3.2, Appendix E, $P < 0.05$). Congruent with the bacteria results, the proportion of Rozellomycota significantly increased at the bottom of the QR mill stockpile after 60-120 cm (Figure 3.2, Appendix E, $P = 0.01$). There was approximately 10-19% unknown bacterial phyla and 8-35% unknown fungal phyla identified in New Afton and 12-15% unknown bacterial and 1-6% unknown fungal phyla identified in QR mill. The relative proportion within a sample also composed of approximately 3-5% bacterial phyla and 1% fungal phyla identified in New Afton and 1-3% bacterial phyla and 1-3% fungal phyla in QR mill that had less than 1% relative proportion within a depth and were combined and categorized in as an 'others' group.

The PIME analysis identified the top ten OTUs that showed significantly different proportion between stockpile depths. Six bacteria genera were identified in the New Afton stockpile: *Fibrobacter, GP4*, *Inquilinus*, *Nocardiodes*, *Pedobacter*, and *Subdivision3 Genera Incertae Sedis* (Appendix F). Seven fungi classes were identified in the New Afton stockpile: *Alternia*, *Cladophialophora*, *Gamsia*, *Geomyces*, *Mortierella*, *Phialemonium*, and *Preussia*. Six bacteria genera were identified in the QR mill stockpile: *Anaeromyxobacter*, *GP1*, *Mycobacterium*, *Rhizobium*, *Roseiarcus*, and *Subdivision3 Genera Incertae Sedis* (Appendix F). Six fungi genera were identified in the QR mill stockpile: *Chaetomium*, *Conicochaeta*, *Exophiala*, *Leohumicola*, *Penicillium*, and *Pseudeurotium*. There was a large proportion of bacteria and fungi that were unclassified at the genus level at both sites.

3.3.2 Alpha Diversity

Our results showed no evidence of significant changes in alpha diversity measures for bacterial or fungal communities (observed and Shannon index) with stockpile depth in New Afton ($P > 0.1$, Figure 3.3). The bacterial and fungal alpha diversity in the New Afton stockpile was more similar to the reference soil than the deep topsoil samples. In contrast, the observed ($R^2 = 0.39$, $P < 0.01$) and Shannon index ($R^2 = 0.34$, $P = 0.02$) diversity for bacterial communities and the observed diversity for fungal ($R^2 = 0.44$, $P < 0.01$) communities in the QR mill stockpile significantly decreased with depth (Figure 3.4). There was no evidence that the Shannon index decreased with depth in fungal communities in the QR mill stockpile (Figure 3.4, $R^2 = 0.08$, $P =$

0.2). The bacterial and fungal alpha diversity of the surface of the QR mill stockpile was most like the reference soil.

3.3.3 Beta Diversity and Geochemical Properties

Operation taxonomic units obtained from NMDS showed a weak, but statistically significant correlation between stockpile depths and bacterial ($R^2 = 0.11$, $P < 0.01$) and fungal ($R^2 = 0.10$, $P < 0.01$) community composition (Figure 3.5) in New Afton. The results may suggest weak spatial structure for fungal communities across the four depth intervals. Additionally, the bacterial and fungal communities in the reference soil was relatively distinct from the stockpile soil in New Afton (Figure 3.5). Operation taxonomic unit communities obtained from NMDS showed a weak, but statistically significant correlation between stockpile depths and bacterial ($R^2 = 0.25$, $P < 0.01$) and fungal ($R^2 = 0.14$, $P < 0.01$) community composition (Figure 3.6) in QR mill. The results suggest spatial structure for bacterial and fungal communities across stockpile depths. Additionally, the bacterial and fungal reference soil was relatively similar to the surface of the stockpile soil and distinct from the bottom depths (Figure 3.6).

Envfit analysis shows that the majority of variation in beta-diversity of New Afton bacterial communities was attributed to available Zn, Fe, and Mn ($P < 0.05$), while fungal communities was attributed to available NO^3-N and C/N (Figure 3.5, $P < 0.05$). Additionally, the majority of variation in beta-diversity of QR mill was attributed to available P, organic matter (OM), potassium (K), ammonium (NH_4-N), manganese (Mn), electrical conductivity (EC), P, sulphur (S), iron (Fe), and magnesium (Mg) ($P < 0.05$) for bacteria and available P, OM, NH_4-N, Mn, P, sodium (Na), and pH ($P < 0.05$) for fungi (Figure 3.6).

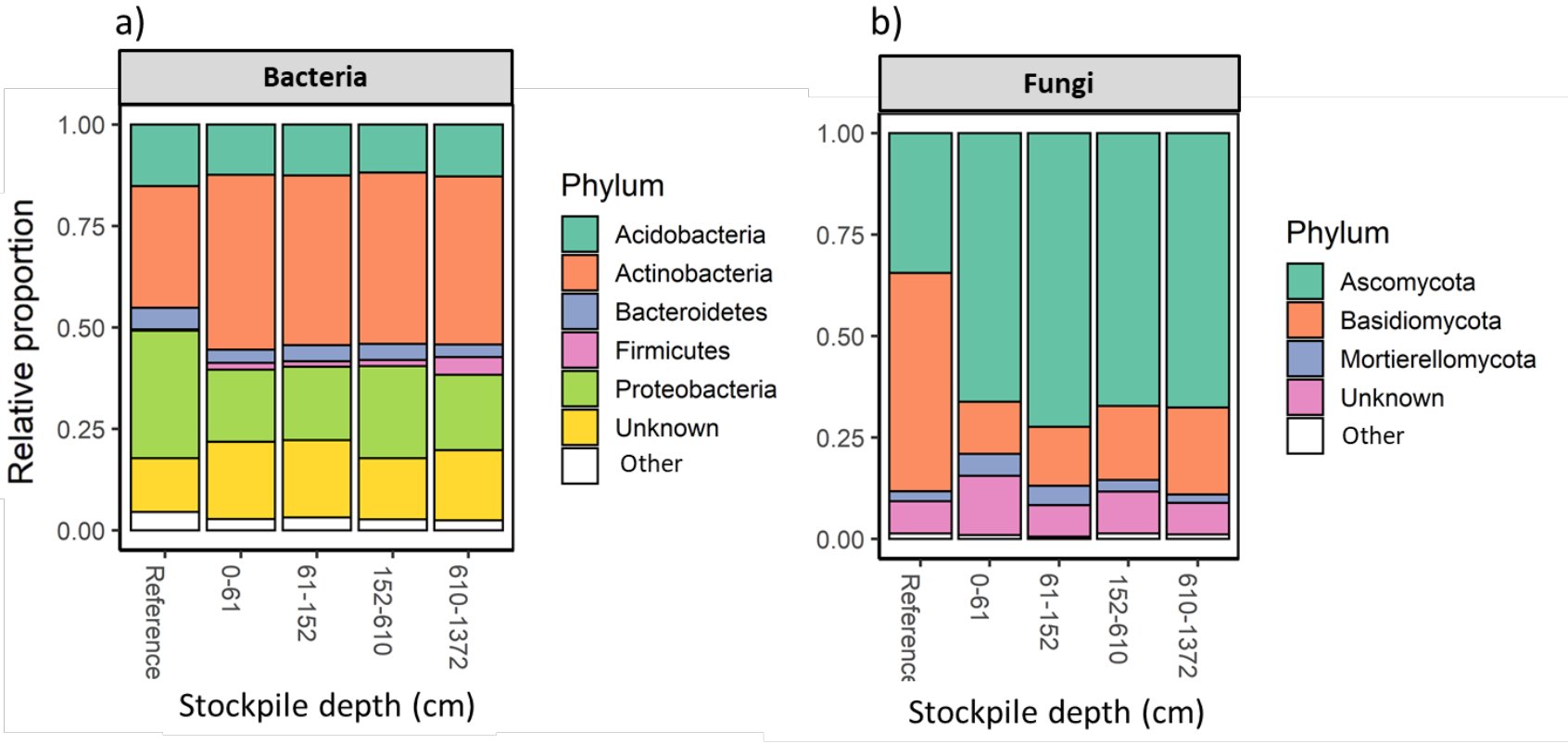

Figure 3.1 Coloured barplots showing relative proportion of a) bacterial and b) fungal phyla by stockpile depth in New Afton. Phyla categorized in the "Other" group are composed of combined phyla with <1% relative abundance.

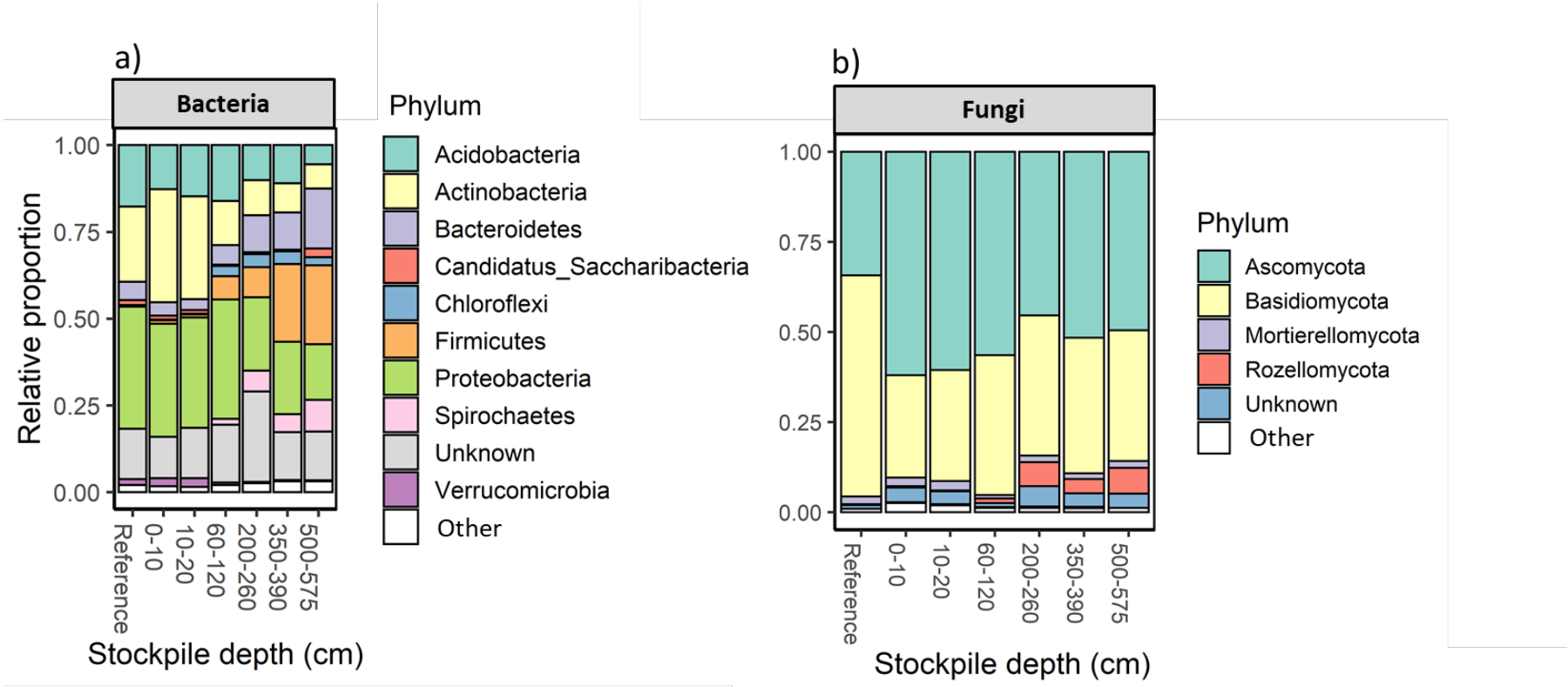

Figure 3.2 Coloured barplots showing relative proportion of the top 99% a) bacterial and b) fungal phyla by stockpile depth in QR mill. Phyla categorized in the "Other" group are composed of combined phyla with <1% relative abundance.

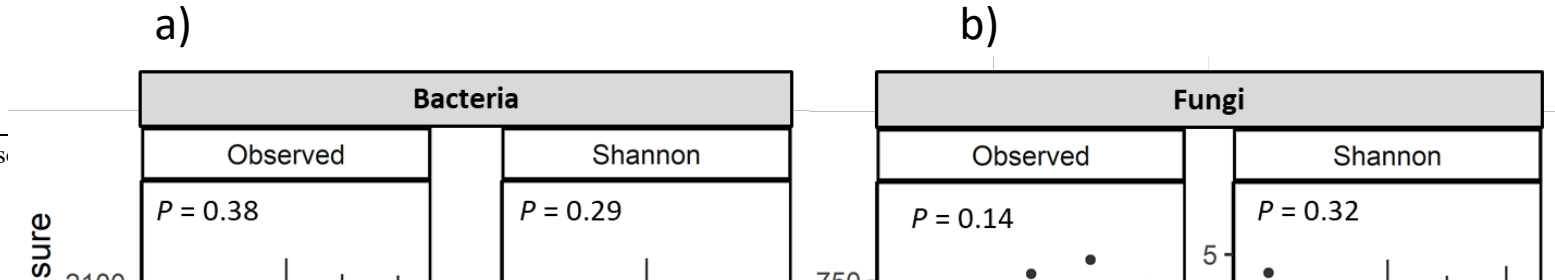

Figure 3.3 Bar plots showing observed and Shannon diversity measures of a) bacterial and b) fungal communities from varying stockpile depth intervals and reference soil from New Afton.

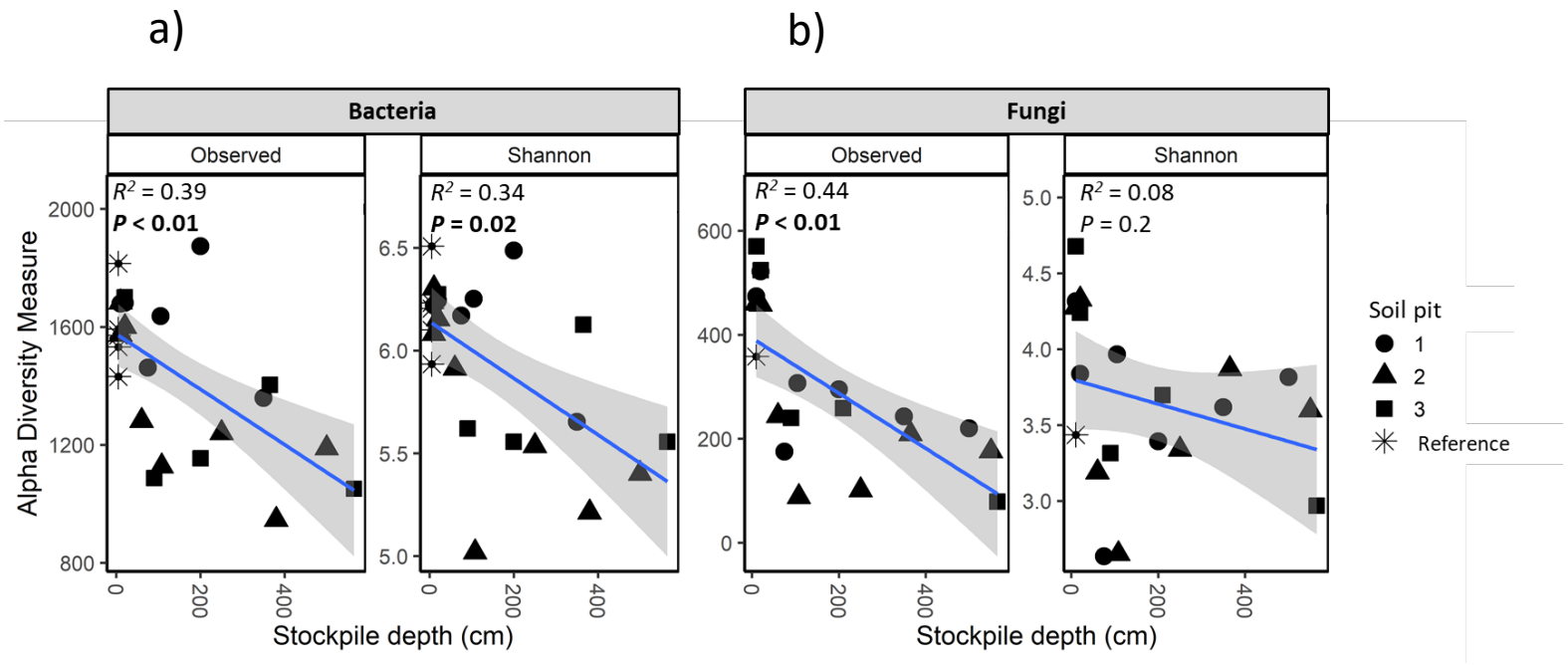

Figure 3.4 Regression plots showing observed and Shannon diversity measures of a) bacterial and b) fungal communities from varying stockpile depth intervals and reference soil from QR mill. The blue lines represent linear models and the shaded area in grey represents a 95% confidence interval.

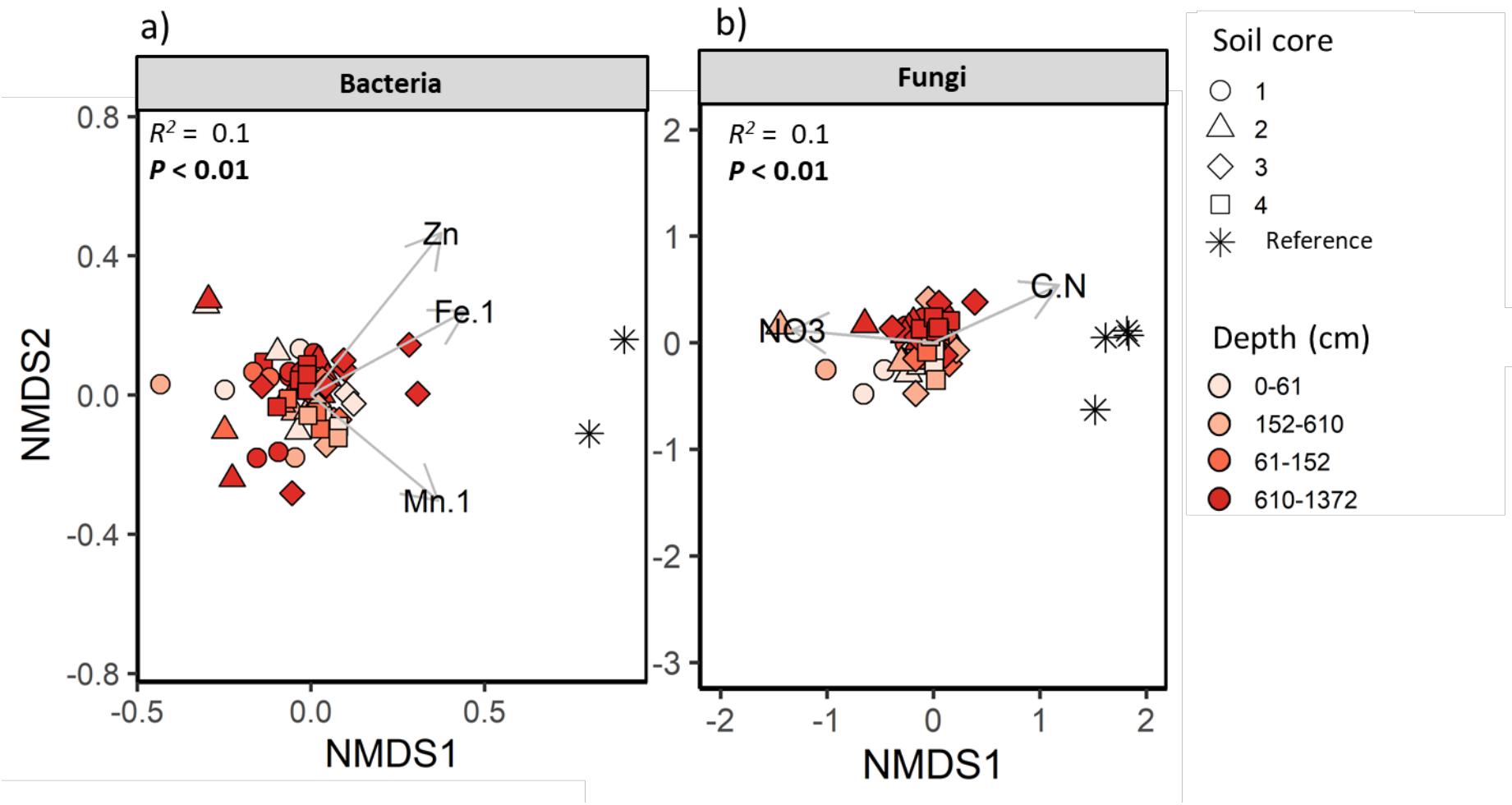

Figure 3.5 Non-metric multidimensional scaling (NMDS) analysis for New Afton. NMDS scatterplot of samples representing the OTU community composition of soil a) bacterial and b) fungal biota from a reference soil and at four depths from four soil core samples from the New Afton stockpile. The distance between points indicated the degree of difference based on Unweighted Unifrac similarities of OTU composition in each sample.

Note: Chemical parameters ending in ".1" have been normalized by log transformation.

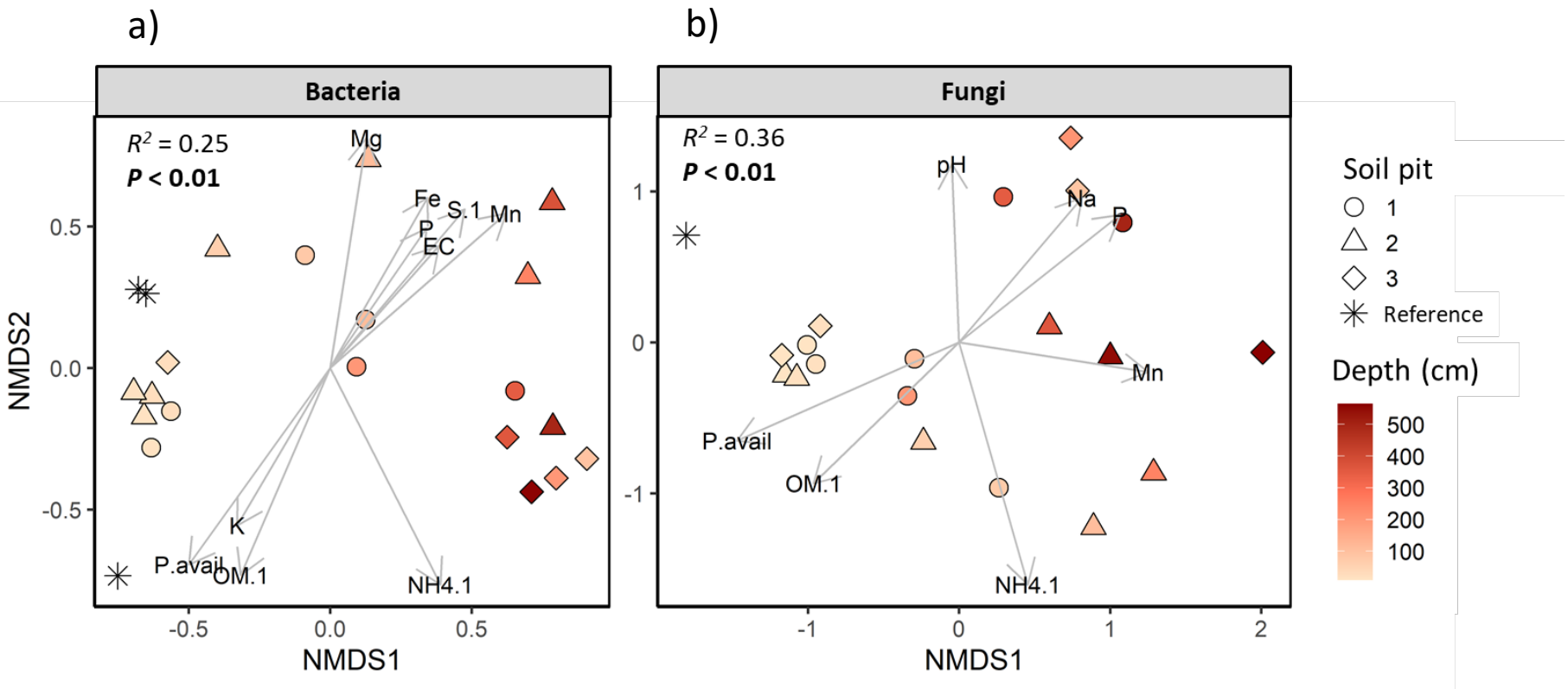

Figure 3.6 Non-metric multidimensional scaling (NMDS) analysis for QR mill. NMDS scatterplot of samples representing the OTU community composition of soil a) bacterial and b) fungal biota from a reference soil and at varying depths from three soil core samples from the QR mill stockpile. The distance between points indicated the degree of difference based on Unweighted Unifrac similarities of OTU composition in each sample. NMDS calculations based on significant soil chemical parameters and the ordination scores on each NMDS axis.

Note: Chemical parameters ending in ".1" have been normalized by log transformation

3.4 Discussion

3.4.1 Community Composition

Through our molecular analysis, we provided an assessment of response of soil microbial communities caused by long term storage of topsoil. The bacterial and fungal community structure at New Afton and QR mill were generally inconsistent with those described by global trends (Figure 3.2) (Delgado-Baquerizo, Oliverio, Brewer, Benavent-gonzález, et al., 2018; Tedersoo et al., 2014). Similar to global trends in soil bacterial communities (Delgado-Baquerizo, Oliverio, Brewer, Benavent-González, et al., 2018), Actinobacteria and Proteobacteria were the dominant bacteria phyla identified throughout the New Afton stockpile and the surface of the QR mill stockpile, however, the proportions were less than expected. Additionally, proportions of Planctomyctes, Verrucomicrobia, and Chloroflexi in New Afton were much lower than projected in this study; they were either not present or have a relative proportion less than 1%; only the presence of Planctomycetes was missing from the QR mill samples. Comparing to global trends and other profiles, the QR mill samples contained a relatively high proportion of rare phyla at the bottom of the stockpile, which started around 60-120 cm, including Spirochaetes, Firmicutes, Candidatus Saccharibacteria, and Rozellomycota (Delgado-Baquerizo, Oliverio, Brewer, Benavent-gonzález, et al., 2018; Eilers et al., 2012; Gorzelak et al., 2020; Hansel et al., 2008; Tedersoo et al., 2014). Our results also differed from the study by Gorzelak et al. (2020) where more than half of the bacterial sequences found in topsoil stockpiles were Chloroflexi. While the soil microbial communities do not typically follow patterns shown in other studies, the New Afton stockpile samples were generally consistent with the reference soil. In contrast, the microbial communities in the QR mill stockpile shifted considerably, and the reference soil most resembles soils in the surface samples.

Despite several large geochemical alterations with stockpile depths in New Afton, such as the large influx of Cu levels in the middle of the stockpile (Figure 2.7), the microbial community remained relatively consistent over depth. This was contrary to the understanding that microbial communities are highly sensitive and reactive to environmental changes. One explanation was that the major factors known to influence soil microbial community structures (for example pH and OM) remained relatively stable through stockpile depths in New Afton (Figure 2.7). The

most pronounced changes found within the topsoil stockpile profiles occurred in QR mill. The change in the relative proportions of Firmicutes, Bacteroides, Spirochaetes, and Candidatus Saccharibacteria along the stockpile depths was apparent.

Coniochaeta, a known plant pathogen (Delgado-Baquerizo et al., 2020) was found throughout the QR mill stockpile depths and had notable increased with depth until 390 cm, where its relative proportion was more than 50%. Additionally, the plant pathogen, *Alternaria* (Delgado-Baquerizo et al., 2020), was found in all stockpile depths in New Afton, but not the reference soil. These pathogens may have negative impacts on plant productivity and health during post-mining reclamation. A higher-than-normal fungal plant pathogen load are found in other topsoil stockpile samples as well, namely *Phoma*, *Fusarium*, and *Alternaria* (Ezeokoli et al., 2019b; Gorzelak et al., 2020).

Anaeromyxobacter was present from 60-120 cm depth to the bottom 500-575 cm depth in QR mill, potentially decreasing with depth. This supports the understanding that anaerobic conditions in topsoil stockpiles generally become apparent around 100-200 cm (Abdul-Kareem & McRae, 1984; Boyer et al., 2011; Harris et al., 1989; Williamson & Johnson, 1990), which was also shown in our geochemical data for QR mill (Figure 2.8). It is possible that anaerobic taxa existed around 100 cm in New Afton, but was not captured by our analysis.

Unexpectedly, we found a higher proportion of *Rhizobium,* a nitrogen-fixing bacteria (Zahran, 1999) in the middle of the QR mill stockpile, from 60-120 down to 350-390 cm (Appendix F). Rhizobia are known to be resilient to stockpile soil disturbance (Jasper, 2007)and may have thrived at these depths due to reduced biota competition along with NH_4-N accumulation at these depths. Similarly, *Leohumicola*, typically heat-tolerant, mycorrhizal soil fungus (Hambleton et al., 2005) increased at the same depths. Birnbaum et al. (2017) found a higher presence of soil arbuscular mycorrhizal fungi, but not *Rhizobia*, in 10-year-old topsoil stockpiles compared to younger stockpiles, suggesting that mycorrhizal communities in stockpiles re-establish over time. However, only the top 10 cm of the stockpiles were sampled in this study. Both *Leohumicola* and *Rhizobia* are highest around 60-120 cm of the QR mill stockpile, likely having a positive impact on plant growth and health during respreading.

The differences in these soil microbes in QR mill corresponded to similar changes in soil OM, C/N, EC, available P, and K changes with stockpile depth (Figure 3.6). Thus, soil resource availability is likely a key factor responsible for the observed changes throughout the profiles.

Additionally, communities from depths below 20 cm or 60 cm were relatively similar compared to the surface samples, likely due to the decreased edaphic factors deep in the stockpile.

3.4.2 Alpha Diversity

Alpha diversity was measured to capture the diversity within samples by estimating the observed OTUs (richness) and Shannon's diversity index. Decreases in microbial diversity occurred in QR mill only, with the most pronounced changes occurring below approximately 60 cm for bacteria and below 20 cm for fungi. A decrease in microbial diversity with depth has been observed in other studies, anticipated to be from depleted C levels (Eilers et al., 2012; Hansel et al., 2008). In support of this theory, the observed trend of microbial diversity depletion with stockpile depth in QR mill generally corresponded to the decrease in OM and increase in C/N, especially when soil was deeper than 20 cm. Additionally, Gorzelak et al. (2020) found that bacterial richness tended to decrease with stockpile age. Overall, this may indicate that less surface microorganisms, typical for topsoil, are not thriving below the surface in large stockpiles stored for long periods. Furthermore, a lower microbial diversity could have negative impacts on plant communities during re-spreading. For instance, Maron et al. (2018) demonstrated that lower microbial diversity negatively affected ecosystem functioning such as nutrient availability. Furthermore, Tedersoo et al. (2014) found that a decrease in fungal richness was correlated with a decrease in plant richness, however, likely a result from shared environmental variables as opposed to direct effects from plant-fungal linkages.

3.4.1 Beta Diversity and Geochemical Properties

Beta diversity measures are estimates of similarity or dissimilarity between populations. The NMDS plots showed that the bacterial and fungal communities present in the stockpile samples are relatively similar and vary greatly compared to the reference soil in QR mill (Figure 3.5). However, the surface bacterial and fungal communities in QR mill were more similar to the reference soil compared to deep samples (Figure 3.6). This indicates that the storage of topsoil can steer communities in the deeper soils were farther away from a historical structure, potentially creating a greater barrier to restoring native ecosystems. For example, Mummey et al. (2002) showed that re-applied stockpiled topsoil with depleted microbial activity had detrimental effects on restoration, even 20 years after seeding of the reclaimed sites. Furthermore, the

significant correlation observed between the soil microbial communities and geochemical properties with varying stockpile depths may indicate a change in ecosystem nutrient cycling as stockpile depth increases.

Current research has revealed pH to be the best predictor of microbial composition and diversity (Xiao et al., 2021; Xue et al., 2018), but not over a depth gradient (Fierer et al., 2003). The results showed soil pH as an important predictor for fungal beta diversity in QR mill, but not bacteria. Additionally, pH has been strongly linked to AMF distribution (Davison et al., 2021), therefore, it is likely that pH may be the cause to the increase in *Leohumicola* fungi in the middle of the stockpile.

3.5 Conclusions

Our results showed that stockpile height is a key factor to soil microbial communities in one of the two sites studied. The change in microbial communities ultimately impacts restoration success on mine sites. Our results have demonstrated that bacterial and fungal communities show comparable responses to stockpile depth in long-term topsoil storage. Relative proportions of dominant phyla, alpha diversity, beta diversity of bacteria and fungi showed little to no changes between stockpile depths in New Afton, whereas they both showed considerable alterations in the QR mill stockpile samples, especially when deeper than approximately 60-120 cm. The altered microbial community structure and diversity observed in the bottom of the QR mill stockpile may have negative impacts for soil biological and chemical processes in the future, such as nutrient cycling and vegetation establishment when re-applied (Gorzelak et al., 2020; Mummey et al., 2002b). The potential positive influence from the influx of the beneficial microorganisms *Rhizobia* and *Leohumicola* found below the surface of the QR mill stockpile may be outweighed by the negative effects from plant pathogens (Gorzelak et al., 2020) *Coniochaeta* and *Alternaria.* Additionally, the alterations observed in microbial communities in both stockpiles compared to the reference soil may hinder post-mining restoration to a native ecosystem. However, it is possible that soil microorganisms will recover over time in soil stockpiles, especially if they are revegetated (Banning et al., 2011; Jasper et al., 1987; Sheoran et al., 2010) . The relatively smaller changes with stockpile depth observed at New Afton compared to QR mill may be a product of various site-specific influences, such as the difference in stockpile age or the timing of additional of fresh topsoil.

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4.0 GENERAL CONCLUSIONS

4.1 Key Findings

Our results contribute to a new perspective for ecosystem restoration practices which emphasizes belowground processes. The literature on soil impacts of stockpiling topsoil is limited, especially those examining samples in large stockpiles below the first meter, thus not accurately representing the whole stockpile. In this study, we captured soil quality changes occurring with stockpile height by sampling and characterizing whole soil profiles. The results confirmed the hypothesis that topsoil storage height has had adverse effects on topsoil quality, but only at one of the sampled profiles, QR mill. However, there was evidence of depleted soil quality and significant geochemical and microbial alterations with depth at both sites. The older, shorter stockpile (QR mill) seemed to experience relatively more nutrient depletion and microbial community shifts with depth compared to the younger, taller stockpile (New Afton). Though, the soil quality at each mine operation will also be strongly influenced by site specific differences that are not accounted for in this study such as stockpile management, subsoil presence, soil texture, climate, and geology. For example, Abdul (1984) found that nutrient fluctuations in stockpile profiles were influenced by soil texture.

At one or both of the sampled topsoil profiles, there was a decline in nutrients and microbial diversity and an accumulation of metals, and beneficial, pathogenic, anaerobic, and rare microbial genera with increasing stockpile depth. The geochemical properties and microbial communities in the stockpiles, especially the deeper soils, varied from reference soils and global trends for soil bacteria and fungi (Delgado-Baquerizo, Oliverio, Brewer, Benavent-González, et al., 2018; Tedersoo et al., 2014), which may negatively affect the ability to restore the sites to a historical state (Baer, 2016; Kumar & Gopal, 2015). The variations observed in bacterial or fungal communities were best explained by pH, electrical conductivity (EC), organic matter (OM), nitrate (NO_3-N), ammonium (NH_4-N), carbon/nitrogen (C/N) ratio, total and available phosphorus (P), potassium (K), sulphur (S), magnesium (Mg), manganese (Mn), zinc (Zn), iron (Fe), and sodium (Na). Many of these changes, including an increase in anaerobic bacteria and an accumulation of metals became apparent when comparing to surface samples with those below approximately 60-120 cm, likely indicating anaerobic conditions, which was generally

consistent with other findings (Abdul-Kareem & McRae, 1984; Boyer et al., 2011; Harris et al., 1989). Most nutrient levels in the stockpiles were likely able to sustain revegetation post-mining. However, the New Afton stockpile suffered from copper levels above the recommended CCME concentration for agricultural/ residential and commercial/ industrial land and all depths were rated unsuitable or poor as a restoration soil due to a high sodium absorption ratio. While some studies show a recovery in soil geochemical properties and microbial communities towards reference conditions over long periods of time (Gasch et al., 2014; Wick et al., 2007), others such as Mummey et al. (2002) showed long-lasting negative implications for the disturbed site.

Given the results detailed in this study, the potential of the topsoil after long-term storage is likely not enough to fully restore a functional and structurally representative native habitat at both mining operations without amendments. These stored soils, especially New Afton, will require enhancement prior to placement for reclamation to achieve historical conditions. Importantly, these results demonstrated the importance of interdependent ecological, geochemical, and biological processes, and the role of belowground processes in determining the success of restoration efforts.

4.2 Topsoil Management Implications

4.2.1 Stockpile Height

Findings from Golos et al. (2016) suggested that plant establishment is worse when grown in stored subsurface topsoil. Similarly, our results showed that topsoil height was a key factor in how topsoil retains its quality components and functions especially when soil depth reached below approximately 60-120 cm depths. Particularly, at these depths, there was a decline in key plant nutrients (available P, NO_3-N, and OM) and microbial diversity and an accumulation of metals (Cu, Fe, and Zn) in one or both of the stockpiles. These results generally aligns with other findings (Abdul-Kareem & McRae, 1984; Boyer et al., 2011; Harris et al., 1989) and with current best practice recommendations to keep topsoil stockpiles for less than 1 year and under 600 cm (Natural Resources Canada, 2017); although, some best management practices recommend stockpiles below 130 cm (The City of Calgary Parks, 2018).

4.2.2 New Afton

Apart from changes in Cu, Fe, Na, Zn, NO_3-N, and Mortierellomycota, the New Afton soil had relatively consistent geochemical properties and microbial communities with stockpile depth. However, there was strong evidence of overall deterioration due to storage compared to the reference soil compared to the reference soil which may hinder restoration efforts. A previous Masters project found that roughening and loosening of the topsoil stockpile at New Afton prior to seeding, improved the success of native plant establishment on the stockpile of interest (Baethke, 2015). Although, the study only looked at the surface of the stockpile and a greenhouse experiment showed poor plant health when grown in stockpile soil at varying depths in New Afton (1Appendix A). Many samples in the stockpile contained copper levels above the recommended CCME concentration for agricultural/ residential and commercial/ industrial land (CCME, 1999) and all depths were alkaline and rated unsuitable or poor as a restoration soil due to a high sodium absorption ratio (SAR) (Macyk et al., 2004). Although soil texture was not measured in this study, there was a high frequency of coarse fragments found in the New Afton stockpile. The high SAR, alkalinity, accumulations of metals, and large percentage of stones and cobbles is likely to have a negative affect on revegetation and will require soil amendments.

Because Na was high and EC was relatively low, calcium (Ca) amendments are recommended in New Afton to improve the sodium absorption ratio. Additionally, S or lime can be added to neutralize pH (BC Ministry of Agriculture, 2015), although organic amendments have been known to reduce pH as well. Organic amendments should be added to improve soil nutrients, and decrease metal availability (Mendez & Maier, 2008; Ohsowski et al., 2012). Biosolids and compost amendments have shown to reduce some metals and increase nutrients, however Gardner et al. (2012) and Sidhu et al. (2016) found no improvement of soil Cu concentrations. The addition of arbuscular mycorrhizal fungus (AMF), *Glomus mossae,* improved plant establishment reduced Cu availability and increased nutrient availability in Cu tailings (Chen et al., 2007). As discussed in Appendix A, recent research has suggested that native soil inoculations can significantly improve soil quality and microbial communities (Carbajo et al., 2011; Emam, 2016; Li et al., 2015; Middleton & Bever, 2012). Although, potentially economically difficult, this would assist in steering restoration to a historical state. Therefore, a combination native donor soil, Ca, S fertilizer or lime, biosolids or compost, and AMF is suggested to ameliorate the topsoil conditions at New Afton.

4.2.3 QR mill

Although the stockpile was rated as fair or good at all depths for reclamation suitability based on salinity and pH (Macyk et al., 2004), many samples in the stockpile contained copper levels above the recommended CCME concentration for agricultural/ residential and commercial/ industrial land (CCME, 1999), which may have negative impacts on revegetation success. Additionally, the stockpile had depleted levels in nutrients OM, N, and P and microbial diversity.

Similar to New Afton, Organic sources such as biosolids or compost should be added to restore soil microbial diversity, decrease metal availability, and improve soil nutrients (Mendez & Maier, 2008; Ohsowski et al., 2012), namely OM, N, and P. Biosolids and compost amendments have shown to reduce some metals and increase nutrients, however Gardner et al. (2012) and Sidhu et al. (2016) found no improvement of soil Cu concentrations. The addition of AMF, *Glomus mossae,* improved plant establishment reduced Cu availability and increased nutrient availability in Cu tailings (Chen et al., 2007). As discussed in Appendix A, recent research has suggested that native soil inoculations can significantly improve soil quality and microbial communities (Carbajo et al., 2011; Emam, 2016; Li et al., 2015; Middleton & Bever, 2012). Although, potentially economically difficult, this would assist in steering restoration to a historical state. Therefore, a combination native donor soil, biosolids or compost, and AMF is suggested to ameliorate the topsoil conditions at QR mill.

4.3 Limitations and Future Research

The sequencing approach in this study allowed the detection of structural changes in microbial community at individual microbial taxa that occurs with varying physical properties. However, a limitation of some 16S and ITS high-throughput sequencing of taxonomic groups is that most strains functionality cannot be predicted (Beiko, 2015). For instance, closely related strains can differ significantly in metabolism (Douillard et al., 2013). Therefore, making conclusions about microbial community functions at the genera level concisely is somewhat challenging. Moreover, biases during polycyclic chain reaction (PCR) can occur, where shorter fragments are preferentially amplified and mistakes such as chimaera formations (Nilsson et al., 2018). Furthermore, rarefying samples to create equal sequencing depths discards some samples resulting in lost information.

This study assessed 20 major geochemical elements in the soil to measure soil quality, however, because the results demonstrated high levels of Cu in both topsoil stockpiles, future topsoil stockpile research should assess other contaminants of concern commonly associated with copper mines including Cd, Co, Cr, Ni, and Pb. Moreover, not all nutrient sources for plants were considered in this study. For example, in accordance with most research, we focused on NO_3-N and NH_4-N nitrogen sources due to their dominance in agricultural soils. However, plants also absorb NO_2^{-2} and organic N sources including urea, amino acids, and peptides (Hachiya & Sakakibara, 2017). Although, there may have been sources of nutrients that were not calculated as part of our study; we believe it would not significantly change our analysis in this study.

Limitations of soil quality that may impact restoration success were uncovered during this study. However, it is unknown how restoration and plant establishment may unfold once soil is respread on site. A field or greenhouse study investigating how plants establish and respond to soil from varying stockpile depths would provide critical information regarding restoration success.

Results of this study are likely weakened from compounding factors of the potential of subsoils and parent materials mixed with topsoil in the piles during salvage. This can unevenly dilute the topsoil quality and cause differences within the stockpile and cannot be accounted for. Future operational studies on topsoil stockpiles should try and obtain as detailed information as possible to account for these variables. Additionally, this study did not assess soil texture content which has major impacts on plant establishment and overall soil quality. Future research in this area should include soil texture analysis (percent sand, silt, and clay) to improve the quality of results as it is closely related to soil geochemistry and biological functions.

Additional analyses can be explored using the geochemical and molecular data collected in this study. For example, further processing of the sequencing data can reveal and identify important soil taxa occurring with stockpile depth and geochemical properties. This study sampled two stockpiles at two sites; more samples at other Cu-Ag operations including multiple stockpiles at a single site are needed to be able to observe key patterns in topsoil quality changes from stockpile height and reduce influences from site specific factors (i.e., stockpile age, ecosystem, climate, geology). The next phase should be to study more stockpiles and incorporate field or greenhouse studies to test how native vegetation establishes at varying depths and over

time. Researching the response of soil properties to disturbance will increase our knowledge in the effects of stockpile storage on ecosystem restoration success.

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APPENDIX A. GROWTH RESPONSE TO TOPSOIL STOCKPILE DEPTH AND NATIVE SOIL INOCULATIONS

Introduction

To alleviate soil chemical deficiencies and altered microbial activities, thus enhancing ecosystem recovery of disturbed sites, it is common practice to build degraded soils with the addition soil amendments. Amendments can improve organic matter, nutrient content, microbial activity, and soil structure. The addition of amendments such as manures, biosolids, manure plant litter, biochar, and arbuscular mycorrhizal fungi (AMF) have demonstrated to successfully increase health and productivity in degraded soils allowing for effective revegetation (S. Brown et al., 2007; Gardner et al., 2012a; Larney & Angers, 2012; Ohsowski et al., 2012). However, care must be taken to consider site-specific conditions and avoid promoting invasive or non-target species (Laungani et al., 2016). Additionally, there was question as to whether the cost applying amendments on a landscape level was economically viable. Therefore, research investigating ecologically, and economically viable restoration practices was critical to optimize mine-closure closure activities and accelerate ecological succession.

There has been a growing interest in research investigating local inoculum sources as soil amendments to reduce the ecological impact of introducing foreign communities (Ohsowski et al., 2012) as native field soil can be an important source of nutrients and local microorganisms. Congruent with this suggestion, several studies have demonstrated that native AMF strains are more effective than commercial strains and proposed that whole soil transfers exceed individual strains in promoting restoration (Emam, 2016; Paluch et al., 2013b; Rowe et al., 2007). This has been supported by recent studies investigating the use of local field soil additions to help restore native vegetation. For example, Middleton and Bever (2012) demonstrated that 9% inoculations with field soil significantly increased mid to late successional plant species. Another study found that donor soil from local sites significantly increased plant community biomass in abandoned arable soil in both 20% and 50% inoculations (Carbajo et al., 2011). Moreover, studies have found that soil inocula not only significantly promoted ecosystem restoration, but also aided in

steering plant community composition and development; where additions from late-successional systems can enhance the growth of late-successional plant species (Bauer et al., 2015; Carbajo et al., 2011; Kardol et al., 2006). This previous work and others (Emam, 2016; Li et al., 2015) have indicated a potential for using local soil inoculations to expedite succession in restoration; demonstrating that native soil can expedite the recovery of native plant re-establishment. A study aiming to optimize topsoil in restoration further supports this phenomenon in finding that the mixing of waste rock with healthy topsoil did not compromise the ability of topsoil to support plant establishment (Merino-Martín et al., 2017). Because microorganisms are prolific and ubiquitous, only small amounts of local soil addition may be required to observe positive results, thus providing an ecological and economic sustainable option for native restoration.

The aim of this greenhouse study was two-fold. The first objective was to test the potential soil property changes with stockpile depth on plant growth by growing plants in soil of increasing stockpile depths. Secondly, this study aims to test the usage of undisturbed, native field soil additions (5% and 10% by volume) in stockpiled topsoil to enhance plant growth. *Pseudoroegneria spicata* (bluebunch wheatgrass) is a drought-tolerant species, native to B.C.'s interior (Klinkenberg, 2020). Changes in soil properties occurring from stockpile depth and native field soil additions was expected to cause differences in plant growth of bluebunch wheatgrass. Important soil components that could influence plant growth are soil structure, nutrients, or microbial activities. Assuming that long-term storage and stockpile height has caused some type of degradation of the topsoil, we hypothesize that plants grown in deeper soil intervals will have significantly lower plant growth; additionally, we hypothesize that the addition of undisturbed local soil at 5% and 10% inoculations will enhance plant growth.

Methods

Soil Sampling and Preparation

Soil profiles were collected from two topsoil stockpiles, one 7 years old and 15 meters deep from New Gold's New Afton in September 2018, and the other 20 years old and 6 meters deep from Barkerville Gold Mine's Quesnel River (QR) mill in May 2019. The soil depth intervals were grouped into roughly four and six depth categories for New Afton and QR mill, respectively. Using a trowel, three replicates of reference/ native soil samples (30x30 cm) were

collected from the upper 20 cm layer from a nearby undisturbed site for each mine operation. Additional site and sampling information was outlined in Chapter 2.

Soil samples were stored in -20ºC chest freezer at the Thompson Rivers university Research Greenhouse and thawed at room temperature twelve hours before the greenhouse experiment began. The sampling replicates (four cores in New Afton and three holes in QR mill) were combined and thoroughly homogenized in large paper bags. Large rocks, roots, and other materials were removed by hand prior to potting soil.

Germination Test

The germination rate of seeds of bluebunch wheatgrass were tested prior to experiment set up to ensure seed viability. Four petri dishes (33 mm diameter x 18 mm deep) were lined with filter paper. Each dish received 35 seeds of bluebunch wheatgrass. Petri dishes were placed in the greenhouse under controlled conditions (temperature set to 20ºC, and natural and artificial light, day/night 16 hours/ 8 hours). The filter paper was kept saturated with tap water. The number of germinated seeds was recorded at 2 and 4 days. Results of the germination test showed that the germination rate of bluebunch wheatgrass seeds used were approximately 96%.

Experimental Design

In this greenhouse study, we aim to test the effects of increasing stockpile depth and the addition of native soil inoculations on plant growth. A total of 100 mL of soil was added to 250 mL plastic pots using spoons and trowels. The trowels and spoons were sterilized with 75% ethanol between treatments, but not between replicates Five bluebunch wheatgrass seeds were placed in 100 mL of varying topsoil stockpile depth intervals with a plug of native soil at 0%, 5%, 10%, and 100% (by volume) depending on the treatment (Table 3.1). The native soil treatments (5% and 10%) were compared to each other and the negative (0%) and positive (100%) control.

If stockpile depth and/ or native soil inoculations influence plant growth, then there will be an observed increase in shoot length, shoot weight, root length, and/or root weight. Each treatment at each depth interval was replicated ten times. A random number generator and trays were used to set up non-replicated randomized blocks in the greenhouse where each treatment was present once in every tray (Figure A-1). Pots were placed in the greenhouse under controlled

conditions (temperature set to maintain 21-25ºC, natural and HID lighting, day/night 16 hours/ 8 hours, and 40-45% relative humidity). Once plants were established, they were pruned to two plants per pot.

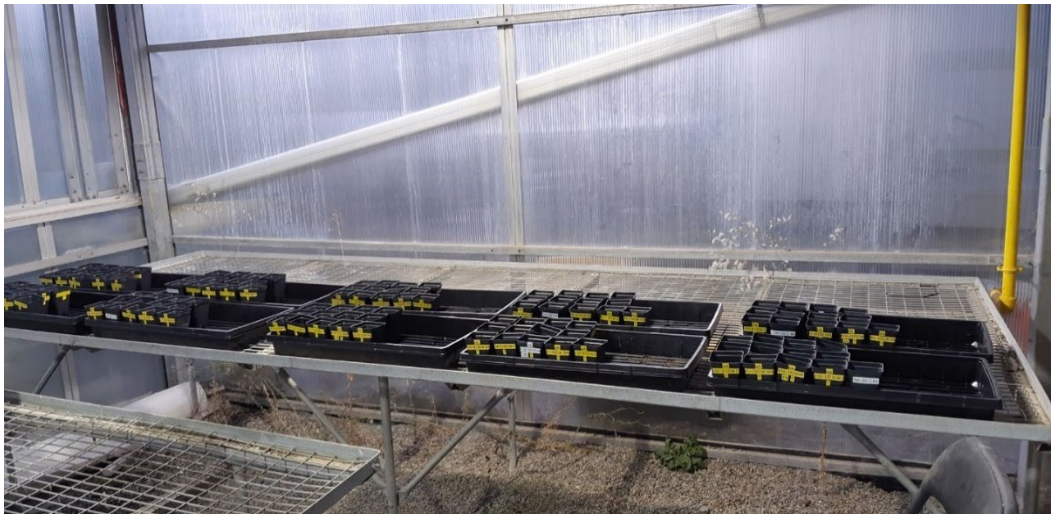

Figure A-1 View of QR mill pots set up in a randomized complete block design used for the greenhouse growth experiment at the TRU Research Greenhouse.

Table A-1 List of soil depth intervals sampled from New Afton and QR mill sites with varying native soil inoculation percentages for the greenhouse study.

Treatment	New Afton stockpile depth interval	QR mill stockpile depth interval	Reference soil/ native soil	Stockpile soil
1	0-61 cm	0-10 cm	0%	100%
2	61-152 cm	10-20 cm	0%	100%
3	152-610 cm	60-120 cm	0%	100%
4	610-1372 cm	200-260 cm	0%	100%
5	-	350-390 cm	0%	100%
6	-	500-575 cm	0%	100%
7	0-61 cm	0-10 cm	5%	95%
8	61-152 cm	10-20 cm	5%	95%
9	152-610 cm	60-120 cm	5%	95%
10	610-1372 cm	200-260 cm	5%	95%
11	-	350-390 cm	5%	95%
12	-	500-575 cm	5%	95%
13	0-61 cm	0-10 cm	10%	90%
14	61-152 cm	10-20 cm	10%	90%
15	152-610 cm	60-120 cm	10%	90%
16	610-1372 cm	200-260 cm	10%	90%
17	-	350-390 cm	10%	90%
18	-	500-575 cm	10%	90%
19	New Afton native soil	QR native soil	100%	0%
20	New Afton native soil	QR native soil	100%	0%
21	New Afton native soil	QR native soil	100%	0%

Data Collection and Analysis

Unfortunately, during this study, technical issues with the research greenhouse may have impacted results. There was an instance where misters were constantly running for more than 24 hours, flooding several trays due to dysfunctional humidity sensors. Additionally, during a power outage, the greenhouse was unable to regulate conditions and inside temperatures reached up to 40^{o}C. These events may have contributed to the poor plant survival overall. However, due to the blocking design of the greenhouse experiment, it was likely that these events effected all the pots within a tray/ block equally, thus not altering effects between depths or treatments.

After four months, bluebunch wheatgrass were removed from pots and underwent three rounds of rinsing in warm tap water to gently remove soil from the roots. The roots and shoots

were separated then wet weight and length were measured. Shoot dry mass was measured and recorded after drying for 48 hours at 60ºC.

The experiment was set up as a two-factorial non-replicated randomized block design. We were interested independently in the fixed effects from stockpile depth and the fixed effects from the inoculation treatments. Random effects from blocks in the greenhouse were also considered. Examining residual plots showed substantial violations of the assumption of homoscedasticity and normality for linear regressions; furthermore, using Levene's test for normality of variance showed that the plant measurement variables had significant P-values ($P < 0.05$). The distribution and residuals of the data were not improved with transformations. Therefore, we tested differences in means with the non-parametric Friedman rank sum test to test mean differences in the plant growth parameters between stockpile depths or inoculation treatments with random effects from blocks. The Friedman rank sum test was followed by a pairwise comparison Conover's test for a two-way balanced complete block design with holm's p-adjustment (PMCMR package in R).

Results

In the New Afton stockpile treatments, there was some evidence of differences in plant growth between stockpile depths ($P = 0.1$). Specifically, it was observed that plants grown in the bottom depth (610-1372 cm) had significantly higher shoot weight than those grown in the above 0-61 cm and the 152-610 cm depth intervals (Table A-2). All other New Afton treatments, including the reference soil plants, were not significantly different than each other when comparing between stockpile height ($P > 0.05$).

In the QR mill stockpile treatments, there was evidence that there were significant differences in root length ($P = 0.0012$), root weight ($P < 0.001$), and shoot weight ($P = 0.014$) between stockpile depths in the lowest soil depth interval (500-575 cm) for the 0% treatment. Specifically, we observed that there were significantly lower root length, root weight, and shoot weight in the bottom soil depth interval than those grown in some of the higher soil depth intervals (Table A-3). In addition to the 0% treatment, there were significant differences observed from the 5% treatment in root length ($P<0.001$), root weight ($P < 0.01$), and shoot weight ($P < 0.01$). In this case however, plants grown in the bottom interval were significantly higher than some of those in the higher depth intervals (Table A-3).

There were no other significant differences in the QR mill treatments when comparing between stockpile height ($P > 0.05$). However, plants grown in the reference soil showed to have significantly larger root length, shoot length, root weight, and shoot weight compared to those grown in most of the stockpile soil depths for all treatments ($P < 0.05$, Table A-3). For instance, the reference soil plants showed to have an average of 69%, 49%, 90%, and 87% larger root length, shoot length, root weight, and shoot weight compared to the largest of those grown in varying topsoil stockpile depths at the 0% treatment.

In the New Afton stockpile treatments, there was little to no evidence that there were any significant effects of 0%, 5%, 10%, or 100% native soil additions on plant growth for all stockpile depths (Table A-4).

In the QR mill stockpile treatments, there were significant differences between native soil additions for root length ($P = 0.028$), shoot length ($P = 0.023$), root weight ($P = 0.019$), and shoot weight ($P = 0.032$) in the bottom depth interval (500-575 cm). Specifically, results show that in the bottom depth interval, plants grown in 0% treatment had significantly lower root length, shoot length, root weight, and shoot weight than those grown in the 5% and 100% native soil addition treatments, but not 10% (Table A-5). Additionally, the 10% native soil addition treatments had significantly lower root length, shoot length, root weight, and shoot weight than the 100% treatments, but not 5%.

Table A-2 Growth response of topsoil stockpile depth from the New Afton site (n=10, df=6).

		Native soil treatment		
	Stockpile depth (cm)	0%	5%	10%
Root length (mm)	0-61	0.94 (5.9)[a]	0.32 (0.32)[a]	3.91 (1.48)[a]
	61-152	1.47 (0.617)[a]	1.64 (1.11)[a]	0.99 (0.99)[a]
	152-610	1.07 (0.621)[a]	2.14 (1.62)[a]	0.36 (0.36)[a]
	610-1372	3.37 (0.96)[a]	4.36 (1.72)[a]	2.89 (2.44)[a]
	Reference	4.93 (2.11)[a]	4.93 (2.11)[a]	4.93 (2.11)[a]
	P value	0.632	0.171	0.134
	Friedman chi-squared	4.02	6.41	7.03
Shoot length (mm)	0-61	7.61 (2.42)[a]	4.42 (1.92)[a]	7.62 (2.33)[a]
	61-152	21.5 (14.1)[a]	5.13 (2.66)[a]	4.39 (2.24)[a]
	152-610	5.06 (1.89)[a]	4.61 (2.42)[a]	2.74 (1.9)[a]
	610-1372	11.9 (2.53)[a]	8.52 (2.48)[a]	5.4 (2.85)[a]
	Reference	7.44 (2.53)[a]	7.44 (2.53)[a]	7.44 (2.53)[a]
	P value	0.374	0.71	0.632
	Friedman chi-squared	4.24	2.14	2.57
Root weight (mg)	0-61	0.113 (0.0721)[a]	0.1 (0.1)[a]	1 (0.476)[a]
	61-152	0.144 (0.112)[a]	0.417 (3.10)[a]	0.453 (0.453)[a]
	152-610	0.002 (0.002)[a]	1.10 (1.06)[a]	0.069 (0.069)[a]
	610-1372	0.65 (0.397)[a]	0.928 (0.463)[a]	0.121(0.0808)[a]
	Reference	0.85 (0.611)[a]	0.914 (0.459)[a]	0.914 (0.459)[a]
	P value	0.409	0.120	0.149
	Friedman chi-squared	3.98	7.32	6.77
Shoot weight (mg)	0-61	0.288 (0.112)[b]	0.375 (0.178)[a]	0.9 (0.402)[a]
	61-152	0.477 (0.168)[ab]	0.59 (0.387)[a]	0.406 (0.22)[a]
	152-610	0.188 (0.0755)[b]	0.194 (0.129)[a]	0.288 (0.212)[a]
	610-1372	1.06 (0.259)[a]	1.21 (0.66)[a]	0.406 (0.22)[a]
	Reference	1.34 (0.554)[ab]	1.34 (0.554)[a]	1.34 (0.554)[a]
	P value	0.103	0.626	0.632
	Friedman chi-squared	7.71	2.61	2.57

Note: Columns followed by similar letters are not significantly different at the 5% probability level. Values in brackets are standard errors. Bolded values indicate statistical significance in at least one of the groups at the 5% probability level.

Table A-3 Impact of topsoil stockpile depth on plant growth measurements from the QR mill site (n=10,

		Native soil treatment		
	Stockpile depth (cm)	0%	5%	10%
Root length (mm)				
	0-10	2.62 (1.39)^{a}	0.490 (0.490)^{ab}	1.77 (1.20)^{a}
	10-20	0.430 (0.298)^{a}	0.00 (0.00)^{a}	1.53 (1.53)^{a}
	60-120	3.58 (1.85)^{a}	1.69 (1.69)^{ab}	1.95 (1.95)^{a}
	200-260	0.850 (0.850)^{ab}	0.00 (0.00)^{a}	2.79 (1.94)^{ab}
	350-390	0.330 (0.330)^{a}	0.00 (0.00)^{a}	0.00 (0.00)^{a}
	500-575	0.00 (0.00)^{a}	5.06 (2.11)^{bc}	2.11 (1.53)^{a}
	Reference	8.43 (2.02)^{ab}	8.43 (2.02)^{c}	8.43 (2.02)^{b}
	P value	0.00119	0.000150	0.0253
	Friedman chi-squared	22.0	26.9	14.4
Shoot length (mm)				
	0-10	7.47 (2.55)^{ab}	3.73 (1.93)^{a}	4.11 (2.85)^{a}
	10-20	4.22 (2.20)^{ac}	0.00 (0.00)^{a}	3.01 (2.01)^{a}
	60-120	3.95 (2.08)^{ac}	2.90 (2.00)^{a}	1.61 (1.61)^{a}
	200-260	3.17 (2.13)^{ac}	0.00 (0.00)^{a}	3.33 (2.26)^{a}
	350-390	4.15 (2.23)^{ac}	0.00 (0.00)^{a}	3.17 (2.12)^{a}
	500-575	0.00 (0.00)^{c}	7.55 (3.18)^{ab}	4.25 (2.43)^{ab}
	Reference	14.6 (3.37)^{b}	14.6 (3.37)^{b}	14.6 (3.37)^{b}
	P value	0.00413	0.000619	0.0387
	Friedman chi-squared	19.0	23.6	13.3
Root weight (mg)				
	0-10	1.40 (1.30)^{ab}	0.057 (0.057)^{ab}	3.32 (2.88)^{a}
	10-20	0.036 (0.0318)^{ac}	0.00 (0.00)^{a}	0.130 (0.130)^{a}
	60-120	1.38 (1.02)^{ac}	1.02 (1.02)^{ab}	0.124 (0.124)^{a}
	200-260	0.206 (0.206)^{ac}	0.00 (0.00)^{a}	0.956 (0.664)^{a}
	350-390	0.083 (0.0766)^{ac}	0.00 (0.00)^{a}	0.00 (0.00)^{a}
	500-575	0.00 (0.00)^{c}	5.76 (2.36)^{bc}	0.547 (0.505)^{a}
	Reference	14.0 (4.95)^{b}	14.0 (4.95)^{c}	14.0 (4.95)^{b}
	P value	0.000671	0.0000936	0.00529
	Friedman chi-squared	23.4	28.0	18.4
Shoot weight (mg)				
	0-10	1.20 (0.622)^{a}	0.309 (0.167)^{ab}	0.711 (0.523)^{ab}
	10-20	0.258 (0.134)^{ab}	0.00 (0.00)^{a}	0.382 (0.345)^{ab}
	60-120	0.421 (0.219)^{ab}	0.194 (0.156)^{ab}	0.132 (0.132)^{a}
	200-260	0.336 (0.227)^{ab}	0.00 (0.00)^{a}	1.04 (0.741)^{ab}
	350-390	0.347 (0.275)^{ab}	0.00 (0.00)^{a}	0.332 (0.266)^{ab}
	500-575	0.00 (0.00)^{b}	2.94 (1.24)^{bc}	0.539 (0.382)^{ab}
	Reference	11.6 (8.69)^{a}	11.6 (8.69)^{c}	11.6 (8.69)^{b}
	P value	0.0141	0.000653	0.155
	Friedman chi-squared	15.9	23.5	9.35

Note: Columns followed by similar letters are not significantly different at the 5% probability level. Values in brackets are standard errors. Bolded values indicate statistical significance in at least one of the groups at the 5% probability level.

Table A-4. Impact of native soil inoculations on plant growth measurement for the new Afton site (n=10, df=3).

		Stockpile depth (cm)			
	Native soil treatment	0-61	61-152	152-610	610-1372
Root length (mm)	0%	0.94 (0.590)[a]	1.47 (0.617)[a]	1.07 (0.621)[a]	3.37 (0.96)[a]
	5%	0.32 (0.32)[a]	1.64 (1.11)[a]	2.14 (1.62)[a]	4.36 (1.72)[a]
	10%	3.91 (1.48)[a]	0.99 (0.99)[a]	0.36 (0.36)[a]	2.89 (2.44)[a]
	100%	4.93 (2.11)[a]	4.93 (2.11)[a]	4.93 (2.11)[b]	4.93 (2.11)[a]
	P value	0.1771	0.246	0.133	0.486
	Friedman chi-squared	4.93	4.15	5.60	2.44
Shoot length (mm)	0%	7.61 (2.42)[a]	21.5 (14.1)[a]	5.06 (1.89)[a]	11.9 (2.17)[a]
	5%	4.24 (1.92)[a]	5.13 (2.66)[a]	4.61 (2.42)[a]	8.52 (2.48)[a]
	10%	7.62 (2.33)[a]	4.39 (2.24)[a]	2.74 (1.9)[a]	5.4 (2.85)[a]
	100%	7.44 (2.53)[a]	7.44 (2.53)[a]	7.44 (2.53)[a]	7.44 (2.53)[a]
	P value	0.7801	0.35560	0.6312	0.45990
	Friedman chi-squared	1.0875	3.2432	1.726	2.5862
Root weight (mg)	0%	0.104 (0.0443)[a]	0.246 (0.119)[a]	0.007 (0.00496)[a]	0.784 (0.352)[a]
	5%	0.1 (0.1)[a]	0.417 (0.31)[a]	1.1 (1.06)[a]	0.928 (0.463)[a]
	10%	1 (0.476)[a]	0.453 (0.453)[a]	0.069 (0.069)[a]	0.121 (0.0808)[a]
	100%	0.914 (0.459)[a]	0.914 (0.459)[a]	0.914 (0.459)[a]	0.914 (0.459)[a]
	P value	0.3245	0.32730	0.26350	0.3131
	Friedman chi-squared	3.4714	3.45	3.98	3.6
Shoot weight (mg)	0%	0.288 (0.112)[a]	0.477 (0.168)[a]	0.188 (0.0755)[a]	1.06 (0.259)[a]
	5%	0.375 (0.178)[a]	0.59 (0.387)[a]	0.194 (0.129)[a]	1.21 (0.66)[a]
	10%	0.9 (0.402)[a]	0.406 (0.22)[a]	0.288 (0.212)[a]	1.06 (0.751)[a]
	100%	1.34 (0.554)[a]	1.34 (0.554)[a]	1.34 (0.554)[a]	1.34 (0.554)[a]
	P value	0.5007	0.76860	0.5282	0.4836
	Friedman chi-squared	2.36	1.1	2.2	2.45

Note: Columns followed by similar letters are not significantly different at the 5% probability level. Values in brackets are standard errors. Bolded values indicate statistical significance in at least one of the groups at the 5% probability level.

Table A-5 Impact of native soil inoculations on plant growth measurement for the QR mill site (n=10, df=3).

		Stockpile depth (cm)					
	Native soil treatment	0-10	10-20	60-120	200-260	350-390	500-575
Root length (mm)							
	0%	2.62 (1.39)[a]	0.43 (0.30)[a]	3.58 (1.85)[a]	0.850 (0.850)[a]	0.330 (0.330)[a]	0.00 (0.00)[a]
	5%	0.490 (0.490)[a]	0.00 (0.00)[a]	0.00 (0.00)[a]	0.00 (0.00)[a]	0.00 (0.00)[a]	5.06 (2.11)[bc]
	10%	1.77 (1.20)[a]	1.53 (1.53)[a]	1.53 (1.53)[a]	2.79 (1.94)[a]	0.00 (0.00)[a]	2.11 (1.53)[ab]
	100%	8.43 (2.02)[b]	8.43 (2.02)[b]	8.43 (2.02)[b]	8.43 (2.02)[b]	8.43 (2.02)[b]	8.43 (2.02)[c]
	P value	**0.0132**	**0.00528**	**0.0254**	**0.00683**	**0.000707**	**0.0275**
	Friedman chi-squared	10.7	12.7	9.31	12.2	17.0	9.14
Shoot length (mm)							
	0%	7.47 (2.55)[a]	4.22 (2.20)[a]	3.95 (2.08)[a]	3.17 (2.13)[a]	4.15 (2.23)[a]	0.00 (0.00)[a]
	5%	3.73 (1.93)[a]	0.00 (0.00)[a]	2.90 (2.00)[a]	0.00 (0.00)[a]	0.00 (0.00)[a]	7.55 (3.18)[bc]
	10%	4.11 (2.85)[a]	3.01 (2.01)[a]	1.61 (1.61)[a]	3.33 (2.26)[a]	3.17 (2.12)[a]	4.25 (2.43)[ab]
	100%	14.6 (3.37)[b]	14.6 (3.37)[b]	14.6 (3.37)[b]	14.6 (3.37)[b]	14.6 (3.37)[b]	14.6 (3.37)[c]
	P value	*0.0522*	**0.00186**	**0.0119**	**0.00354**	**0.00530**	**0.0234**
	Friedman chi-squared	7.72	14.9	11.0	13.6	12.7	9.49
Root weight (mg)							
	0%	1.40 (1.30)[a]	0.036 (0.0318)[a]	1.38 (1.02)[a]	0.206 (0.206)[a]	0.083 (0.0766)[a]	0.00 (0.00)[a]
	5%	0.0570 (0.0570)[a]	0.00 (0.00)[a]	1.02 (1.02)[a]	0.00 (0.00)[a]	0.00 (0.00)[a]	5.76 (2.36)[bc]
	10%	3.32 (2.88)[a]	0.130 (0.130)[a]	0.124 (0.124)[a]	0.956 (0.664)[a]	0.00 (0.00)[a]	0.547 (0.505)[ab]
	100%	14.0 (4.95)[b]	14.0 (4.95)[b]	14.0 (4.95)[b]	14.0 (4.95)[b]	14.0 (4.95)[b]	14.0(4.95)[c]
	P value	**0.0148**	**0.00144**	**0.00553**	**0.00255**	**0.000835**	**0.0187**
	Friedman chi-squared	10.5	15.5	12.62	14.3	16.6	10.0
Shoot weight (mg)							
	0%	1.2 (0.622)[ab]	0.258 (0.134)[a]	0.421 (0.219)[a]	0.336 (0.227)[a]	0.347 (0.275)[a]	0.00 (0.00)[a]
	5%	0.309 (0.167)[a]	0.00 (0.00)[a]	0.194 (0.156)[a]	0.00 (0.00)[a]	0.00 (0.00)[a]	2.94 (1.24)[bc]
	10%	0.711 (0.523)[a]	0.382 (0.345)[a]	0.132 (0.132)[a]	1.04 (0.741)[a]	0.332 (0.266)[a]	0.539 (0.382)[ab]
	100%	11.6 (8.69)[b]	11.6 (8.69)[b]	11.6 (8.69)[b]	11.6 (8.69)[b]	11.6 (8.69)[b]	11.6 (8.69)[c]
	P value	*0.0711*	**0.00515**	**0.0171**	**0.0248**	**0.0140**	**0.0324**
	Friedman chi-squared	7.03	12.8	10.2	9.37	10.6	8.78

Note: Columns followed by similar letters are not significantly different at the 5% probability level. Values in brackets are standard errors.

Bolded values indicate statistical significance in at least one of the groups at the 5% probability level.

Furthermore, there were no other significant differences in the QR mill treatments when comparing between native soil addition treatments (P>0.05). However, plants grown in the reference soil showed to have significantly larger root length, shoot length, root weight, and shoot weight compared to those grown in most of the stockpile soil depths for all treatments ($P < 0.05$, Table A-5). For instance, the reference soil plants showed to have an average of 68.9%, 48.8%, 76.3%, and 89.7% larger root length, shoot length, root weight, and shoot weight compared to the largest of those grown in varying native soil treatments at the 0-10 cm depth interval.

Discussion

Growth Response to Topsoil Stockpile Height

While there was significant response in shoot weight to stockpile depth at the 152-610 cm depth, there were no other observed differences with stockpile depth in the New Afton stockpile. The similar of growth response to stockpile depth in the New Afton samples are expected given the microbial and chemical results; where ordination analyses demonstrated a very high similarity between all depths of the New Afton Stockpile. The similarity between samples throughout depth was likely due to the mixing of the stockpile in New Afton mine. While there were more changes in plant growth observed with stockpile depth compared to New Afton, there was still little evidence of consistent significant changes in plant growth in response to stockpile depth. Here, there was evidence that plants grown in the bottom layer did worse in the 0% treatment than those grown in the topsoil depth; however, plants grown in bottom layer were also observed to do significantly better than those grown in some of the upper depths in the 5% treatment. It was possible, but unlikely that the native soil additions caused positive results in the bottom layer at 5% because the same improvement was not observed in the 10% treatment at the same depth. Interestingly, plants grown in the reference soil from QR mill did much better than those grown in the QR mill stockpile soil, for most of the treatments, however, this was not the case for New Afton. Soil content as result of site-specific factors could explain the differences observed between the soils sampled from the two reference sites. Although both sites are within the southern interior of British Columbia, in the Montane Cordillera Ecozone (Marshall et al. 1999), New Afton was in a semi-arid grassland and QR mill was located 350 km north in a forest ecosystem. We observed large differences in the nutrient content of these soils in Chapter 2. For

example, the QR mill reference soil had an average of 68%, 72%, 81%, and 65% more OM (%), NH_4-N (mg/kg), NO_3-N (mg/kg), and C (%) than New Afton's reference soil, likely driving the large difference in plant growth for the two sites in the greenhouse experiment.

Growth Response from Native Soil Addition

While there was no evidence of native soil treatments having a significant impact on plant growth in New Afton stockpile, there was some evidence that it improved plant growth in the QR mill stockpile soil at the bottom depth interval (500-575 cm). Plant growth was observed to improve in QR mill stockpile soil with the 5% native soil addition, compared to those grown in just stockpile soil (0% native soil addition). However, there was not an increase in plant growth seen in the 10% treatment, thus indicating that it was unlikely that the significant increase seen in plant growth in the 5% treatment was a result of the soil addition.

There was little to no evidence that there were any further significant differences in plant growth between native soil treatments in the QR mill soil or the New Afton soil. A possible reason for this it that the addition was too low to have a substantial impact plant growth. Previous studies have shown native soil additions to have positive impacts on plant growth in concentrations as low as 9% in a field study (Middleton & Bever, 2012) and positive impacts on soil health with additions as low as 12.5% and as high as 50% (Li et al., 2015). This would likely be the case for the QR mill plants, where reference soil plants (100% native soil addition) were significantly larger than plants grown in QR mill stockpile soil treatments. However, reference soil plants and stockpile soil plants in the New Afton samples were not significantly different from each other.

Another reason that the native soil additions may not have performed well in this study, was because the additions on were likely on partially degraded soil instead of completely degraded soils. Various studies with positive results have looked at adding native soils to highly contaminated or deficient materials such as mineral soils, sterile medium, tailings, and waste rock (Emam, 2016; Kardol & Wardle, 2010; Li et al., 2015). Thus, it was possible that the topsoil stockpiles, while having some deficiencies, are not degraded so severely that small additions of healthy native field soil causes a significant growth response.

Furthermore, the lack of positive impacts observed from native soil additions could be limited by the length of this experiment. There may be long term impacts that were not captured in this study; for example, Wubs et al. (2016) showed an ability to steer and promote plant

community structure with soil additions in a 6-year field study. Additionally, soil microorganisms and nutrient status might have been significantly altered by the native soil additions, but this was not measured in our analyses. Moreover, soil microorganisms may not have had long enough to proliferate in the pots. Here, we attempted to observe immediate growth responses from native soil additions, however, over time, effects of soil biota may have become more impactful.

We created a plug (hot spot) around the seeds to test the approach of introducing the native material locally to plants so to increase feasibility of field applications. This approach was applied with success by Middleton and Bever (2012). However, it may not have been effective in this study due to some of the reasons listed in the previous paragraphs, or due to competition from biota in the stockpile samples.

Conclusions

The results show that the native grass bluebunch wheatgrass grew relatively poorly in soils from a 7-year-old, 15-meter-deep and a 20-year-old, 6-meter-deep topsoil stockpile, regardless of the depth or treatment, compared to a reference field soil from one of the sites. Additionally, despite promising results of native soil additions in other studies (Emam, 2016; Li et al., 2015; Middleton & Bever, 2012; Paluch et al., 2013a; Wubs et al., 2016), there was no evidence that the addition of native field soil at 5% or 10% applications enhanced immediate plant growth in topsoil stockpiles. Discretion should be used when interpreting results from this study because of the inconsistent greenhouse conditions described in the methods section; inaccuracy was a potential concern. If results were more reliable, we may conclude that stockpile depth or native field soil additions has no substantial observed impact on plant growth, and that further manipulations of stockpiled topsoils are required to expedite ecosystem restoration in mine closure procedures. Future studies, in addition to long-term and large-scale research, should investigate effects on other native plant species (early and late-successional) and test the effects of higher ratios of native field soil additions. Furthermore, this study should be repeated due to the greenhouse complications faced in this study.

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APPENDIX B. RAW GEOCHEMICAL DATA FOR NEW AFTON AND QR MILL

Table B-1 Raw geochemical data for QR mill (QR) and New Afton (NA) stockpile and reference soil.

Site	Depth (cm)	C/N		Ca (%)		EC (µS/cm)		K (%)		Mg (%)		Na (mg/kg)		NO_3^- (mg/g)		NH_4 (mg/kg)		OM (%)		P.avail (mg/kg)		pH		S (%)	
		Avg	SE	Avg	SE	Avg	SE	Avg	SE	Avg	SE	Avg	SE	Avg	SE	Avg	SE	Avg	SE	Avg	SE	Avg	SE	Avg	SE
NA	Ref	8.91	1.2	1.01	0.05	67.4	1.9	0.4	0.01	1.37	0.07	767	35	4.63	0.97	5.6	2.1	3.26	0.1	27.7	2.8	7.28	0.09	0.03	0.01
NA	0-61	7.61	0.38	3.18	0.31	1378	55	0.38	0.01	1.68	0.09	2200	82	0.73	0.3	27	7	2.62	0.56	4.83	0.59	8.11	0.04	0.2	0.03
NA	61-152	9	0.62	3.7	0.29	1191	90	0.38	0.02	1.8	0.04	2500	58	0.27	0.15	30.5	4.7	2.82	0.46	4.43	1.63	8.07	0.02	0.29	0.02
NA	152-610	8.81	0.55	3.3	0.23	1245	78	0.41	0.03	1.95	0.1	2000	191	0.63	0.37	21	7.2	2.35	0.45	5.68	2.38	8.18	0.04	0.24	0.02
NA	610-1372	8.45	0.19	3.3	0.09	1446	31	0.4	0.02	1.78	0.03	3025	354	2.58	0.59	13.5	0.9	2.84	0.46	6.18	0.53	8.05	0.02	0.26	0.02
QR	Ref	15.6	1.3	1.3	0.15	119	0.5	0.4	0.02	0.82	0.18	840	44	16.67	2.19	29.07	14.04	10.2	0.29	145	57.95	5.63	0.11	0.04	0.01
QR	0-10	10.7	0.9	1.47	0.07	123	5.7	0.42	0.04	1.03	0.03	683	52	3.6	0.4	7.47	6.27	10	0.63	14	2.08	6.85	0.07	0.04	0
QR	10-20	10.1	0.7	1.5	0.2	61	3.6	0.39	0.06	1.02	0.04	647	34	3.57	0.88	1.3	0.1	6.6	1.14	12	1.15	6.24	0.25	0.03	0
QR	60-120	22.8	12.3	2.38	0.42	131	29.3	0.33	0.01	1.14	0.08	692	37	4.49	3.64	1.48	0.58	4.34	0.45	3.54	1.25	6.6	0.35	0.07	0.02
QR	200-260	12.1	1.1	2.4	0.46	158	9.1	0.35	0.03	1.13	0.12	683	66	18.73	11.2	1.08	0.49	5.9	1.27	4.07	2.58	6.57	0.33	0.1	0.04
QR	350-390	49.7	33.7	3.37	1.38	130	11.1	0.32	0.05	1.19	0.18	670	15	18.77	8.72	1.69	0.76	4.38	1.14	5.07	2.36	6.56	0.32	0.12	0.06
QR	500-575	69.9	28.7	2.7	0.7	172	32.4	0.33	0	1.23	0.12	760	51	18	13.53	2.43	1.91	3.84	0.17	2.73	0.62	6.81	0.19	0.08	0.04

APPENDIX C. SODIUM ADSORPTION RATIO

Sodium adsorption ratio (SAR) is a measure of the concentration of sodium relative to Ca and Mg concentrations. The following calculation was used:

Equation C-1

$$SAR = \frac{[Na^+]\ mmol\ L^{-1}}{(0.5\ x\ [Ca^+ + Mg^+])^{\frac{1}{2}}\ (mmol\ L^{-1})}$$

Depth (cm)	Average SAR	SE
New Afton		
0-61	14.1	1.8
61-152	15.1	1.4
152-610	12.3	4.8
610-1372	19.0	1.7
Reference	7.0	1.5
QR mill		
0-10	6.1	2.3
10-20	5.3	1.0
60-120	5.2	0.7
200-260	5.1	1.2
350-390	4.4	0.2
500-575	5.4	0.8
Reference	8.2	1.1

Table C-1 SAR values at each stockpile depth.

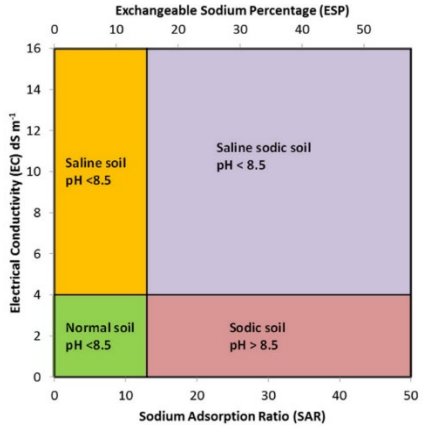

Figure C-1 Chart taken from Kumaragamage et al. (2021).

APPENDIX D. RAREFACTION CURVES

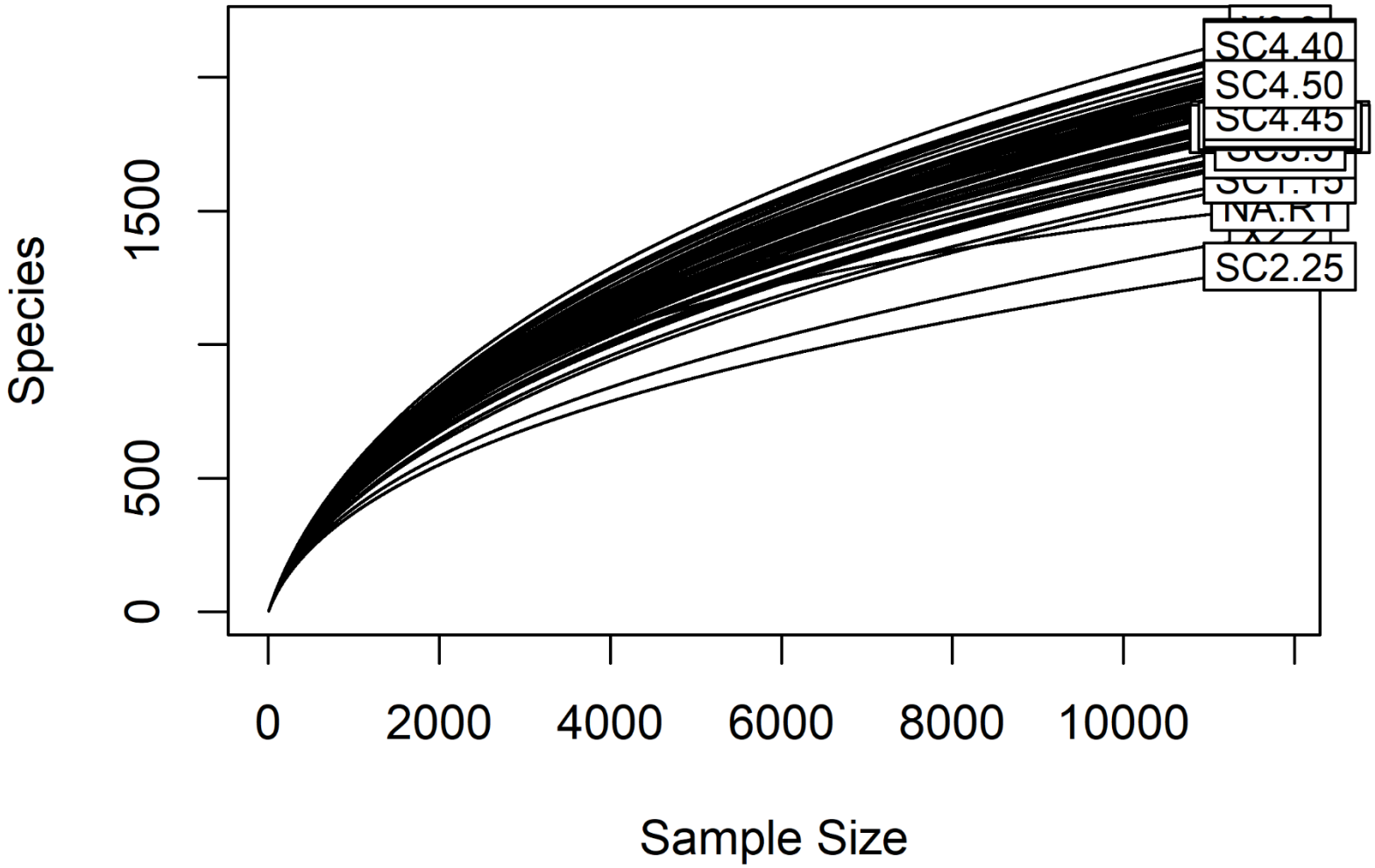

Figure D-1 Rarefaction curve for bacterial OTUs in New Afton

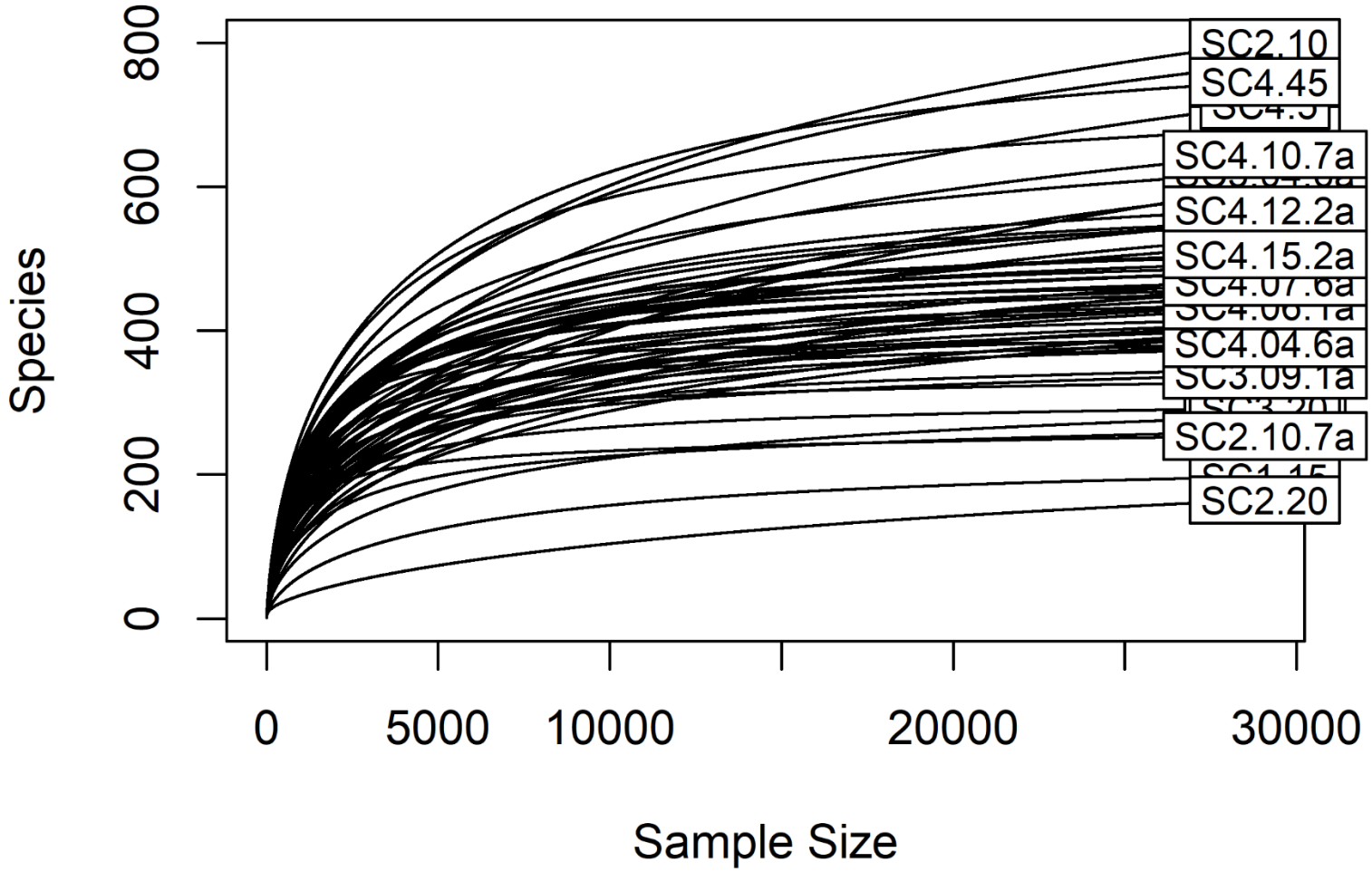

Figure D-2 Rarefaction curve for fungal OTUs in New Afton

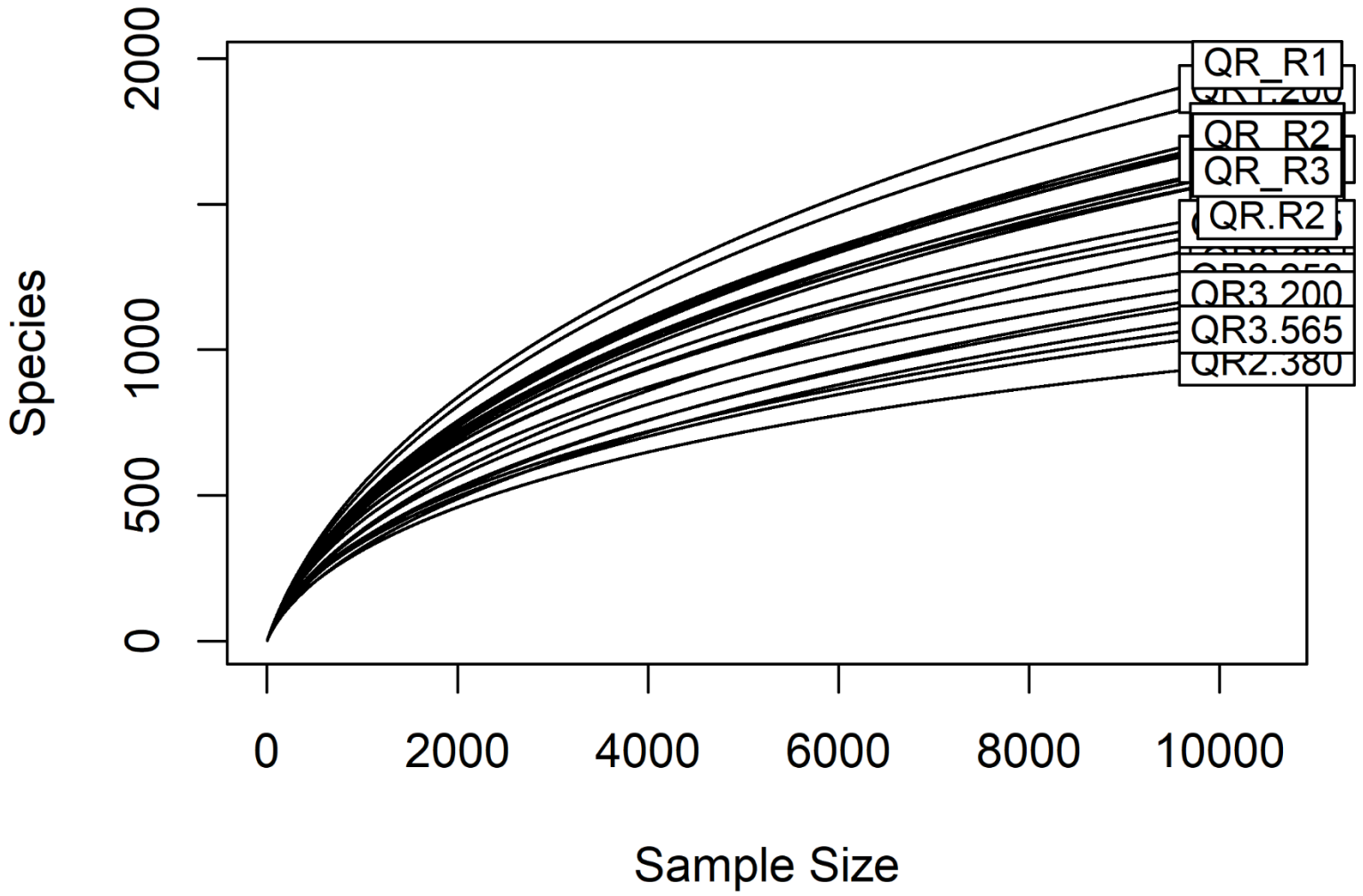

Figure D-3 Rarefaction curve for bacterial OTUs in QR mill

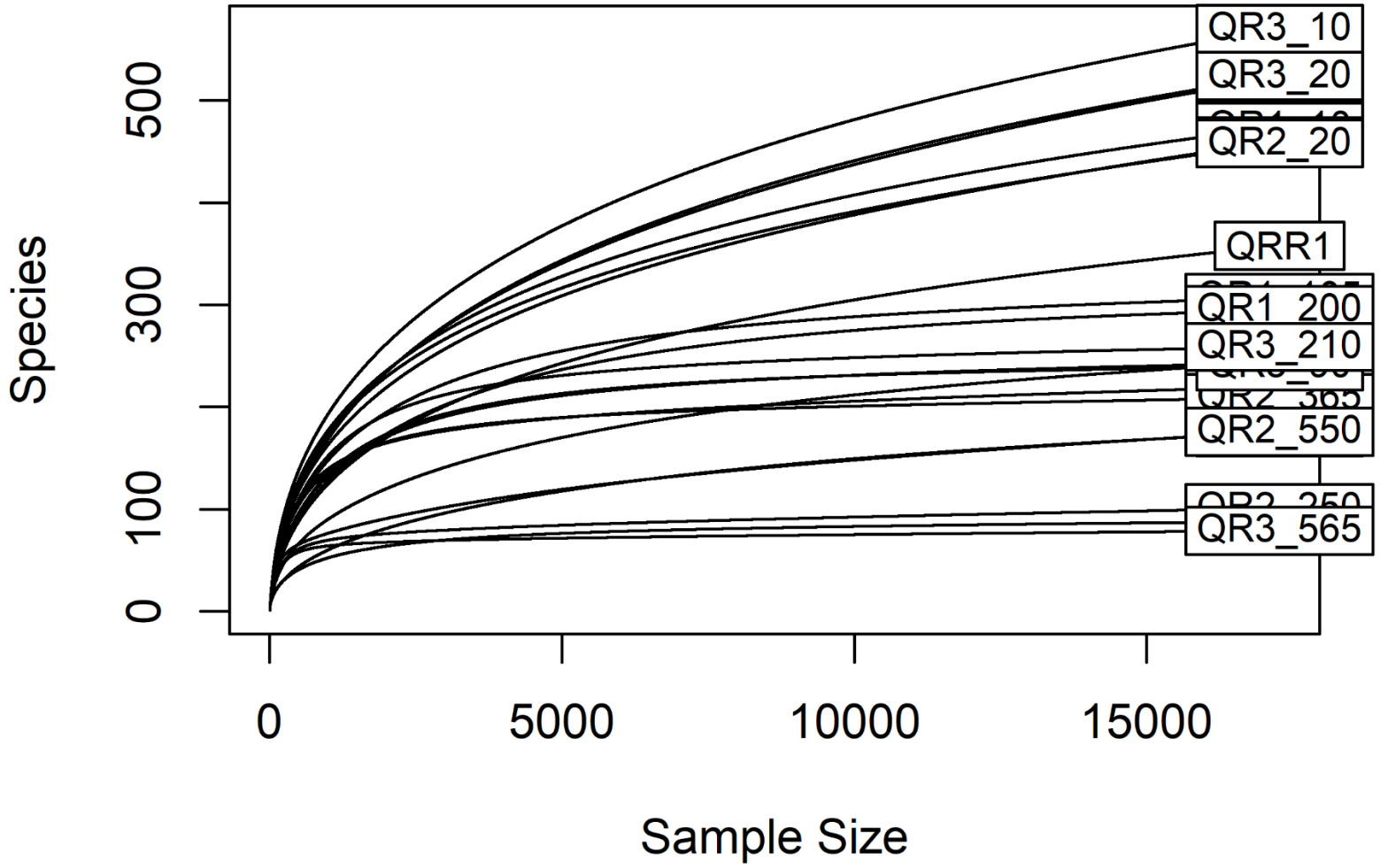

Figure D-4 Rarefaction curve for fungal OTUs in QR mill

APPENDIX E. RELATIVE PROPORTION TABLE AND ANALYSIS FOR MICROBIAL DATA

Table E-1 Relative proportions of bacteria and fungi phylum present above 1% identified in New Afton. P-value was calculated using Type III Analysis of Variance Table with Satterthwaite's method in each bacteria or fungi phylum by stockpile depth in New Afton, excluding the reference soil.

	0-61 cm	61-152 cm	152-610 cm	610-1372 cm	Average	Standard Deviation	P value	Reference
Bacteria								
Acidobacteria	0.123	0.126	0.119	0.128	0.124	0.004	0.916	0.151
Actinobacteria	0.432	0.418	0.422	0.414	0.422	0.008	0.959	0.300
Bacteroidetes	0.032	0.039	0.040	0.031	0.036	0.005	0.338	0.053
Firmicutes	0.017	0.014	0.015	0.044	0.023	0.014	*0.064*	0.003
Proteobacteria	0.177	0.181	0.227	0.186	0.193	0.023	*0.060*	0.314
Unknown	0.190	0.190	0.151	0.173	0.176	0.018	*0.096*	0.133
Fungi								
Ascomycota	0.662	0.724	0.672	0.676	0.684	0.028	0.747	0.344
Basidiomycota	0.128	0.145	0.183	0.214	0.168	0.039	0.416	0.539
Mortierellomycota	0.054	0.047	0.029	0.021	0.038	0.015	**0.012**	0.024
Unknown	0.146	0.078	0.103	0.077	0.101	0.032	0.349	0.079

Table E-2 Relative proportions of bacteria and fungi phyla present above 1% identified in QR mill. P-value was calculated using Type III Analysis of Variance Table with Satterthwaite's method in each bacteria or fungi phylum by stockpile depth in QR mill, excluding the reference soil.

	0-10 cm	10-20 cm	60-120 cm	200-260 cm	350-390 cm	500-575 cm	Average	Standard Deviation	P Value	Reference
Bacteria										
Acidobacteria	0.127	0.148	0.161	0.101	0.110	0.055	0.117	0.038	0.030	0.177
Actinobacteria	0.325	0.296	0.127	0.101	0.084	0.070	0.167	0.113	<0.001	0.217
Bacteroidetes	0.039	0.031	0.056	0.107	0.107	0.173	0.086	0.054	<0.001	0.053
Candidatus_Saccharibacteria	0.012	0.011	0.004	0.005	0.004	0.025	0.010	0.008	0.033	0.014
Chloroflexi	0.002	0.002	0.029	0.037	0.037	0.024	0.022	0.016	0.040	0.001
Firmicutes	0.010	0.010	0.067	0.087	0.223	0.227	0.104	0.099	<0.001	0.004
Proteobacteria	0.326	0.317	0.343	0.211	0.208	0.160	0.261	0.077	0.001	0.352
Spirochaetes	0.000	0.000	0.017	0.061	0.052	0.091	0.037	0.037	0.001	0.000
Unknown	0.119	0.145	0.167	0.260	0.138	0.141	0.162	0.051	0.736	0.145
Verrucomicrobiota	0.023	0.025	0.007	0.004	0.003	0.003	0.011	0.010	0.001	0.017
Fungi										
Ascomycota	0.62	0.605	0.564	0.454	0.516	0.495	0.542	0.065	*0.083*	0.342
Basidiomycota	0.284	0.308	0.389	0.389	0.376	0.363	0.352	0.045	0.486	0.614
Mortierellomycota	0.024	0.026	0.009	0.018	0.016	0.019	0.019	0.006	0.811	0.021
Rozellomycota	0.004	0.002	0.013	0.067	0.040	0.071	0.033	0.031	**0.013**	0.002
Unknown	0.040	0.036	0.011	0.056	0.037	0.040	0.037	0.015	0.543	0.010

APPENDIX F. STACKED BARPLOTS FOR THE TOP 10 OTUS (PIME ANALYSIS)

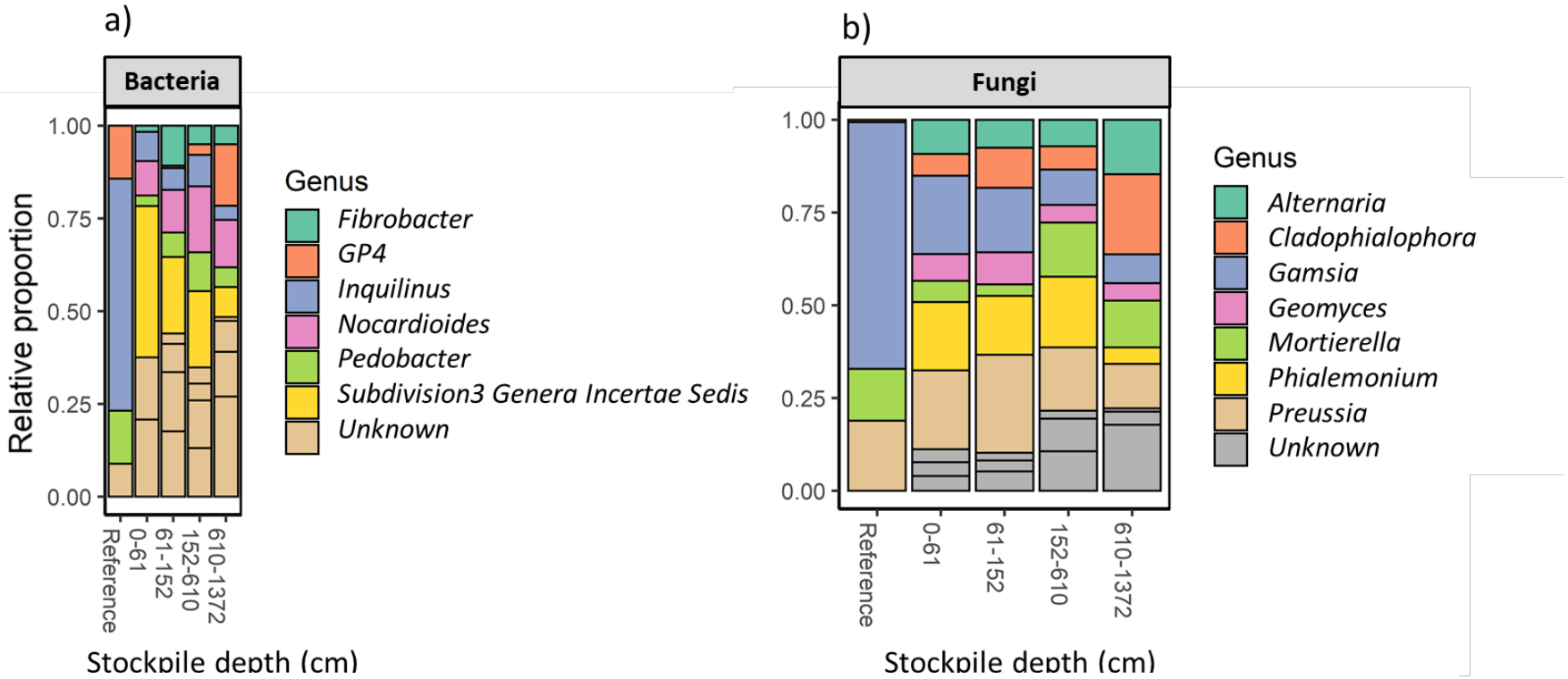

Figure F-1 Coloured barplots showing relative proportion of the top ten a) bacterial and b) fungal genera that are most impacted by stockpile depth in New Afton.

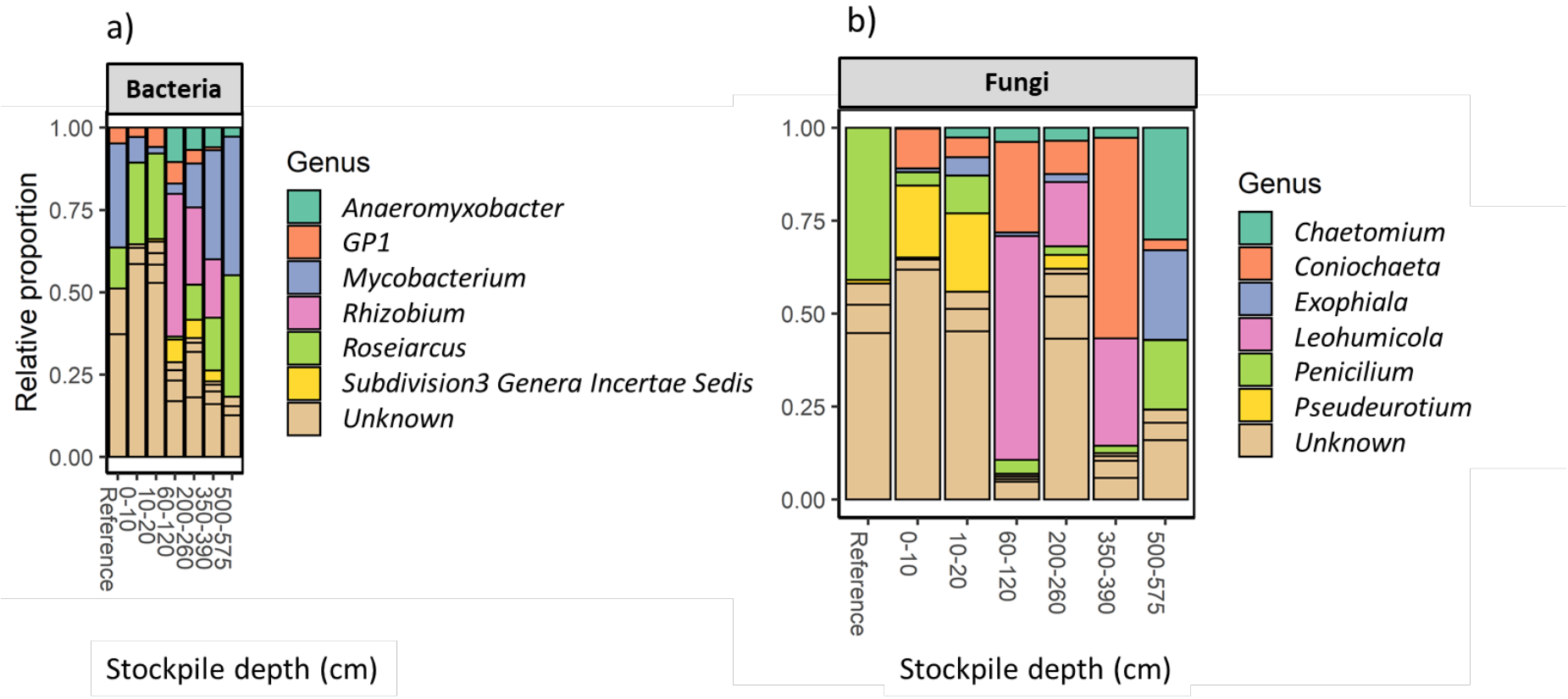

Figure F-2 Coloured barplots showing relative proportion of the top ten a) bacterial and b) fungal genera that are most explained by stockpile depth in QR mill.