Impacts of Soil Stockpiling on Seed Bank Communities and the Availability of Nutrients

by

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Abstract

Soil stockpiles are used around the world to reclaim sites affected by industrial activities. Oil sands surface mining and in situ extraction activities in Alberta, Canada, have directly impacted more than 900 km^2 of land, with more development expected in the future. Soil stockpiles will be essential for the reclamation of large- and small-scale oil and gas sites across Alberta with over half of the disturbed area expected to be reclaimed using stockpiled soils. However, stockpiling soils can lead to the degradation of soil biological, physical, and chemical properties, so it is critical that we understand the potential implications of using this soil in reclamation. Therefore, I studied the impacts of soil stockpiling on seed banks, aboveground plant communities and available nutrients.

To investigate the impacts of soil stockpiling, plant communities and nutrient availability were sampled in eight soil stockpiles of varying ages across Alberta. Four stockpiles and soils of four nearby mature forests were sampled in the Cold Lake region (54.695 °N, 110.730 °W), and four stockpiles and soils of two mature forests were sampled in the Fort McMurray region (57.337 °N, 111.755 °W). Seed bank samples were taken from depths of 0-5 cm, 5-10 cm, 10-20 cm, 20-30 cm, and >50 cm and germinated in a greenhouse using the seedling emergence method. Aboveground vegetation cover was also estimated at these locations. Soil samples were taken from 0-10 cm, 10-20 cm, 20-30 cm, and >50 cm for a lab incubation to estimate the availability of macronutrients (NO_3^-, Ca^{2+}, Mg^{2+}, K^+, $H_2PO_4^-$ /HPO_4^{2-}, SO_4^{2-}) in stockpiled and mature forest soils using plant root simulator probes (PRS; Western Ag Innovations, Saskatoon, SK, Canada).

In Chapter 2 the impacts of soil stockpiling were quantified by seed bank abundance, species richness, functional group composition, and the relationships between the seed bank and aboveground plant communities. In Chapter 3 nutrient availability was compared between stockpiles and mature forests, as well as with depth. Nutrient availability was also compared across stockpiles and mature forests.

The results of the seedling emergence study showed that stockpile seed banks had higher seedling abundance and species richness than that of nearby forests but were dominated by grasses and non-native forbs. Most seeds germinated from the surface layer, with 92% of seeds germinating from the LFH layers in the forests, and 68% from the 0-5 cm layer in the stockpiles. Aboveground and seed bank communities were more similar in mature forests than that expressed in stockpiles.

As indicated by the soil incubation, stockpiles had higher availability of Ca^{2+}, Mg^{2+}, NO_3^- compared to the mature forests. There was also high availability of NO_3^- below 20-90 cm in stockpiles. The variability with depth that was present in the mature forests for Ca^{2+}, Mg^{2+}, clay content, and pH were not found in stockpiles, likely due to mixing. Variability was high across stockpiles for NO_3^-, SO_4^{2-}, and $H_2PO_4^-$/ HPO_4^{2-} compared to mature forests.

Planting or seeding on these stockpiles with desirable reclamation species may be useful in the future to supplement the seed bank. Salvaging fresh topsoil to shallower depths could also help prevent dilution of the seed bank. Variability in nutrient availability across stockpiles could mean that management of stockpiles and reclamation sites should be done on a smaller scale and focus on differences across stockpiles. Overall, all stockpiles were dominated by grasses and non-native forbs, despite higher variability in N, SO_4^{2-}, and $H_2PO_4^-$/HPO_4^{2-}.

Preface

The following thesis is composed of original data generated and analyzed by Jennifer Buss, with no data having been published at the time of submission. Data from Chapter 2 “A comparison between reclamation stockpile and boreal forest plant communities” was presented in an oral presentation at the 2019 American Society of Mining and Reclamation (ASMR) Annual Meeting in Big Sky, Montana, USA. Chapter 2 has been submitted for publication to the journal Restoration Ecology. I was responsible for data collection, analysis, and manuscript composition. B.D. Pinno and S.A. Quideau were involved with the research design, and contributed to analyses, and manuscript edits.

For Chapter 3, I was responsible for data collection, analyses, and manuscript composition. B.D. Pinno and S.A. Quideau were involved with research design, and contributed to analyses, and manuscript edits.

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Chapter 1- Introduction

1.1 Boreal forest region and Central Mixedwood subregion

The Boreal forest region covers approximately 25% of the world's closed canopy forest and 58% of Alberta, Canada (Alberta Parks 2015; Macdonald et al. 2012). The boreal houses a diversity of vegetation, habitats, wildlife, (Alberta Parks 2015) and provides ecosystem services locally, regionally, and internationally (Brandt 2009; Brandt et al. 2013; La Roi 2013). Some of these ecosystem services include nutrient cycling, water storage, wildlife habitat, and carbon storage (Kurz et al. 2013).

Within this region of forest is the Central Mixedwood subregion. The Central Mixedwood subregion covers 25% of Alberta (Natural Regions Committee 2006) with a mean annual temperature of 0.2°C and a mean annual precipitation of 478 mm (Natural Regions Committee 2006). In the upland portions of this subregion aspen, mixedwood and white spruce species dominate the overstory (Natural Regions Committee 2006). Understories of aspen stands in the subregion consist of a diverse array of shrub species including low-bush cranberry, prickly rose, and Canada buffaloberry (Alberta Parks 2015).

Approximately 50% of the Central Mixedwood is made up of well to imperfectly drained uplands with fine glaciolacustrine material, coarse glaciofluvial and eolian sands, and fine-textured till, with an average moisture and nutrient status compared to other stands in the subregion (Natural Regions Committee 2006). Gray Luvisols are typically present on medium to fine-textured upland soils, with Dystric and Eutric Brunisols on coarse-textured sands (Natural Regions Committee 2006). One of the main land uses in the Central Mixedwood subregion is petroleum exploration, ranging from surface oil sands extraction to conventional oil and gas production (Natural Regions Committee 2006).

1.2 In situ and surface mining

It is estimated that mining has disturbed approximately 0.3 (Hooke & Kartin-Duque 2012) to 1 percent (Bridge 2004) of the world's terrestrial land surface. So far, mining activities cover greater than 900 km^2 of land in Alberta, Canada (Alberta Environment and Parks 2017; NRCan 2016), which will only increase with future development. Of the oil sands area, only 3.4% is surface mineable (Government of Alberta 2017). Surface mining occurs when oil sands reserves are less than 75 m from the surface (NRCan 2019). Large shovels and trucks are used to remove the oil sands and transport it to be extracted and upgraded (Natural Resources Canada 2019).

The rest of the oil sands in Alberta lies deep below the surface and is recovered through in situ mining. In situ mining involves drilling wells into the oil sands deposit and using steam to assist in extraction (Government of Alberta 2019). In situ mining disturbs less land per unit of production, however, the disturbance is more dispersed, and results in more fragmentation of the landscape (Jordaan et al. 2009). In situ mining produces a more linear footprint than surface mining due to networks of seismic lines, access roads, pipelines, and well sites (CEMA-SEWG 2008; Jordaan et al. 2009).

Geological deposits or 'overburden' overlies oil sands deposits (Government of Alberta 2019). Some of this material needs to be removed before surface and in situ mining can take place. The volume of soil removed from the surface of these areas is typically proportional to the size of the disturbance, with surface mining resulting in a larger volume of soil moved and stored compared to in situ mining (COSIA 2013). These areas disturbed by mining will eventually need to be reclaimed, returning them to self-sustaining ecosystems (Government of Alberta 2017).

1.3 Stockpiling practices

Prior to mining, surface and subsoil are removed from an area and stored somewhere else until it is needed for reclamation. Upland topsoil is a valuable reclamation material, which can be a source of organic matter, plant propagules, is important for nutrient cycling, and enhances the growth of plants (Alberta Environment and Water 2012; Bowen et al. 2005; Fisher & Binkley 2000). Upland topsoil is also an abundant source of macro and micronutrients (MacKenzie 2006; McMillan 2005). Typically, topsoil is salvaged and stored separately from subsoil (Alberta Environment and Water 2012). Coarse-textured topsoil is salvaged to 15 cm, and fine-textured topsoil to 30 cm (Alberta Environment and Water 2012).

The depth of salvage has a direct impact on the amount of organic matter included in the reclamation material, with a greater salvage depth resulting in dilution of nutrient-rich organic matter from the surface (MacKenzie 2011). Organic matter and nutrient content of a stockpiled soil also depends on slope position and soil texture of the source material (Alberta Environment and Water 2012). Varying salvage depth also impacts the abundance of viable plant propagules present in the salvaged topsoil, with a deeper salvage resulting in dilution of the seed bank (Rokich et al 2000; Tacey & Glossop 1980). Once salvaged, the material is placed into a pile for storage until it is ready to be used for reclamation. In many cases this soil can remain stockpiled for decades, which can negatively impact their viability for reclamation (Ghose 2004).

1.4 Negative impacts of soil stockpiling

When soils are stockpiled it results in changes to their physical, chemical, and biological properties. During the removal and handling of soil with heavy machinery there is often a reduction in aggregate stability, compaction, and increases in bulk density (Abdul-Kareem & McRae 1984). These physical changes can impact soil drainage, aeration, and root growth (Abdul-Kareem & McRae 1984; Potter et al. 1988; Schroeder et al. 2010).

Decreases in aeration can also result in the development of anaerobic conditions of soil stockpiles, especially below 1m (Abdul-Kareem & McRae 1984; Davies et al. 1995). Chemical changes also include fluctuations in available nutrients, pH, and organic matter (Abdul-Kareem & McRae 1984; Ghose 2004; Paterson et al. 2019). For example, anaerobic conditions can result in increases of ammonium (NH_4^+) and decreases in pH (Abdul-Kareem & McRae 1984). Changes to nutrient availability and pH could impact the species that are able to grow in stockpiled soils (Davis et al. 2000; Nordin et al. 2005). It is unclear whether these changes will positively or negatively impact desirable reclamation plant species in Alberta.

Lastly, loss of viable seeds is a biological change that can occur with soil stockpiling (Dickie et al. 1988). The mechanical handling during stockpiling could damage many seeds (Benvenuti & Macchia 1995) and can result in the loss of greater than 65% of the seed bank (Koch et al. 1996; Scoles-Sciulla & DeFalco 2009). Once seeds are damaged, the anaerobic conditions that develop at depth in stockpiles could prevent them from repairing themselves, as oxygen can be required for cellular repair (Ibrahim et al. 1983; Villiers 1973). Therefore, stockpiling could limit the seeds available for revegetation.

1.5 Direct placement

Owing to the potential negative impacts of soil stockpiling, direct placement is preferred for reclamation over using stockpiled soils (Alberta Environment and Water 2012). Direct placement involves removing soil from a nearby unmined forest donor site and placing it directly onto a reclamation site without stockpiling it, thus preserving many of the plant propagules (Naeth et al. 2013). However, directly placing newly salvaged material onto a reclamation site is usually not feasible (Visser et al. 1984). Soil stockpiles will have to be used to reclaim much of the disturbed areas in the oil sands because donor sites and soils are limited. One mine is planning on reclaiming greater than half of their lease with stockpiled soils (I. Sherr 2018, Reclamation Vegetation Specialist, Canadian Natural Resources Limited, personal communication). Therefore, understanding the impact of soil stockpiling on the viability of the material will be important for future reclamation.

1.5 Study objectives

This study investigates the impacts of soil stockpiling and compares seed bank and aboveground plant communities, as well as nutrient availability, between soil stockpiles and soils of mature forests. Having viable seeds present in stockpiled soils will be imperative to ensuring the recovery of the boreal forest after mining. Many of the seeds of boreal forest species are not available commercially and seeding of reclamation sites will not always be feasible (Lanoue & Qualizza 2000). Therefore, further investigation into the seed banks and plant communities present on soil stockpiles is important for future management and reclamation. Chapter 2 aims to determine how soil stockpiling impacts seed bank and aboveground plant communities. Stockpile communities were compared to nearby mature forest communities based on seed bank abundance, species richness, functional group composition, and the relationships between the seed bank and aboveground plant communities.

Nutrient availability is important to plant growth and seed germination of many species (Baskin & Baskin 1998). Therefore, understanding the impacts of soil stockpiling on nutrient availability will be important for future reclamation. Chapter 3 investigates nutrient availability in stockpiled soils compared to that of soils in nearby mature forests. Another aim of this study is to explore variability in nutrient availability with depth to gain further insight into how processes change with stockpiling. Lastly, I investigate spatial variability of nutrient availability across stockpiles compared to the variability across mature forest soils to provide insight into the scale on which stockpiles should be managed.

Chapter 2: A comparison between reclamation stockpile and boreal forest plant communities

2.1 Introduction

Soil stockpiles are used in mining and other industrial operations around the world to store soil for eventual use in land reclamation. Soil stockpiling results in a loss of seed and a change in the plant community composition (Dickie et al. 1988; Golos et al. 2016). Loss of native seeds is problematic for reclamation because native seeds may not always be commercially available and seeding reclamation sites is expensive (Augusto et al. 2001; Lanoue & Qualizza 2000). Oil sands surface mining and in situ extraction activities in Alberta, Canada, have affected greater than 900 km^2 of land, with further expansion planned in the future (Alberta Environment and Parks 2017; NRCan 2016). Much of this disturbed land will need to be reclaimed using stockpiled soil. In fact, one oil sands mine is planning to reclaim 50% of its lease using stockpiled material (I. Sherr 2018, Reclamation Vegetation Specialist, Canadian Natural Resources Limited, personal communication) while another currently has 80 million m^3 of stockpiled soil (Syncrude 2019). Stockpiles will be vital in future reclamation and having quantified the quality and composition of stockpile seed banks will help to make more informed reclamation decisions.

When soils are stockpiled a large proportion of seeds are lost due to mechanical injury from handling and stripping. One study in Australia found that 69% of the original forest soil seed bank was lost immediately after clearing and stockpiling (Koch et al. 1996), while another study in Nevada reported that the majority of the seed bank (79%) was lost immediately after stockpiling (Scoles-Sciulla & DeFalco 2009). Seed dormancy and viability can also decline as a result of anaerobic conditions that may occur in the middle of stockpiles (Abdul-Kareem &

McRae 1984; Benvenuti & Macchia 1995). Due to the loss of viable seeds that occurs during soil stockpiling, it is likely that the diversity of stockpile seed banks will have decreased compared to surrounding forests.

The open conditions and high resource availability associated with stockpile environments (Abdul-Kareem & McRae 1984) create the ideal habitat for weedy forb and grass species to establish and dominate the site (Brothers and Spingarn 1992; Dickie et al. 1988). Grass species such as *Calamagrostis canadensis* (marsh reedgrass) can remain viable in the seed bank for long periods and are adapted for dispersal at long distances (Ahlgren 1960; Conn 1990). A highly invasive species, *Sonchus arvensis* (perennial sow-thistle)*,* is commonly found in disturbed areas (Hansen & Clevenger 2005) and can spread rapidly through vegetative propagation or seed (Lemna & Messersmith 1990). These reproductive and dispersal strategies allow some grass and non-native forb species to take over open areas quickly, such as stockpiles or newly reclaimed sites. Closed canopy forests are less likely to be invaded by non-native species than cleared and open areas because they create a barrier for wind dispersed species and shade out any shade intolerant invasive species (Brothers & Spingarn 1992; Cadenasso & Pickett 2001; Hansen & Clevenger 2005). Therefore, stockpiles will likely have more grass and non-native species than the surrounding forest. Much of the seed bank input comes from existing or surrounding vegetation, so if a stockpile is dominated by non-native forb or grass species it is likely that the seed bank will be as well.

Seed numbers decline with depth in both stockpiles and forests since seeds are mostly added to the soil surface *via* wind or animal dispersal (Qi & Scarratt 1998; Rivera et al. 2012). At the same time, there are losses of seeds belowground due to seed predation, fungal pathogens, and germination of seeds at depth which never reach the surface (MacKay 1972). The result is a gradient of seeds with depth where most of the newly deposited viable seeds are at the surface with the older less viable seeds in the lower soil profile (Harrington 1972). However, when soil is stockpiled, these seeds are mixed up and redistributed throughout the stockpile. Knowing where the seeds are distributed in soil stockpiles can help determine how much seed-dense material is available for reclamation.

The aboveground plant community is typically used to aid in management decisions without sampling the seed bank. However, the aboveground plant community is not always representative of the seed bank. In general, the aboveground vegetation is not a good indicator of the seed bank in forested communities when compared with other ecosystem types (Hopfensperger 2007). This is partially due to the presence of early successional species in the seed bank, which persist belowground, but not aboveground (Bossuyt et al. 2002; Drake 1998). However, after a disturbance the similarity between the seed bank and aboveground vegetation generally increases. Daïnou et al. (2011) found that the relationship between aboveground and seed bank communities was stronger in a logged area than in an undisturbed forest, while Hopfensperger (2007) reported that it decreased with time since disturbance in forests. Considering both perspectives, it is difficult to say how similar the aboveground and seed bank communities will be in formerly forested, stockpiled material.

There are many studies that evaluate stockpile plant communities. For example, there are studies that evaluate the decline in seed viability over time by burying seeds (Mackenzie & Naeth 2019), and other studies compare the seed bank present in stockpiles at different depths (Dickie et al. 1988; Golos & Dixon 2014; Iverson & Wali 1982). However, few studies have looked at the seed bank present in many stockpiles at once and compared those communities to the aboveground plant communities and nearby undisturbed areas. The aim of this study is to evaluate eight soil stockpiles and soils of six mature forests at two study locations in Alberta and determine their reclamation potential as represented by seed bank abundance, species richness, functional group composition, and the relationships between the seed bank and aboveground plant communities.

2.2 Methods

2.2.1 Study area

Sampling took place in two locations in the central mixedwood natural subregion of the boreal forest in Alberta, an oil sands mine north of Fort McMurray, Alberta (57.337 °N, 111.755 °W), and an in situ cyclic steam stimulation (CSS) site near Cold Lake, Alberta (54.695 °N, 110.730 °W). The Fort McMurray site focuses on synthetic crude oil production through surface mining (CNRL 2019a). The stockpiles in this area are on average 10 ha. The Cold Lake site is thermal in situ oil sands, which extracts diluted bitumen from a well by inserting steam (CNRL 2019b). Stockpiles in this area had an average area of 0.3 ha. Both areas have a mean annual temperature between 1 and 2 °C and mean annual precipitation between 419 and 421 mm (Government of Canada 2019a, Government of Canada 2019b). All stockpiles from both locations were classified as upland topsoil stockpiles, which consist of the LFH layer and the underlying mineral soil, of upland soils only, stripped to a depth of approximately 50 cm.

2.2.2 Field and lab methods

In August 2018, we sampled a total of eight stockpiles (four from Fort McMurray and four from Cold Lake), aged 6 months to more than 28 years old, along with six mature forest sites. There were three sampling locations at each stockpile and mature forest ((8 stockpiles + 6 mature forests) x 3 plots = 42 sampling plots). At each sampling plot on the stockpiles, seed bank samples were taken from five different depths: 0-5 cm, 5-10 cm, 10-20 cm, 20-30 cm, and greater than 50 cm.

At the Fort McMurray site, we sampled two mature forests that were approximately an equal distance from all stockpiles on site, while at Cold Lake we sampled four forests directly adjacent to the stockpiles. Forests were dominated by an aspen and white spruce overstory with an average stand height of 11.3 m and a diameter at breast height (dbh) of 11.4 cm. Mature forest seed bank samples were taken from six different depths: LFH, 0-5 cm, 5-10 cm, 10-20 cm, 20-30 cm, and greater than 50 cm.

The surface vegetation was also sampled at all 42 plots. Vegetation plots consisted of four 1 m^2 vegetation quadrats placed in each cardinal direction at 1.78 m from the plot center. In the quadrats, percent cover was determined to species. Soil pits were dug at the plot center after vegetation was sampled.

The seedling emergence method adapted from Buss and Pinno (2019) was used to estimate viable seed numbers and describe the seed bank community for all sites. Samples were mixed and sieved to 4 mm to remove large debris, and then 500 mL was sampled. Samples were then refrigerated at 4 °C for four months. Greenhouse flats were filled with potting mix and 500 mL of sieved soil was spread over the top at a depth of 1 cm. One sample was placed in each flat and flats were placed randomly throughout the greenhouse. Ten control flats were positioned evenly throughout the greenhouse to catch any species that could have come from the potting mix or aerially through the ventilation system. However, no seedlings germinated from the control flats. The greenhouse was set at daytime/nighttime temperatures of 22 °C/18 °C and trays were watered twice a day for the first month and once a day for the remaining three months.

Seed bank samples were left in the greenhouse to germinate for four months. All seedlings were identified to species and recorded. Individuals that could not be identified as seedlings were repotted to identify them at a later growth stage. Five individuals from each unidentified species were repotted and the ratios from these pots were used to account for any potential identification errors. After four months, few seedlings were germinating in the trays, and the trays were emptied.

2.2.3 Statistical analysis

All data analysis was done using R software (version R.3.1.1, R Core Team 2019). To compare species diversity between stockpile and mature forests, seedling abundance and species richness were calculated for each plot (n =3 for each stockpile or mature forest). Seedling abundance was calculated by taking the sum of seedling counts. Species richness was calculated as the sum of species per plot. A hierarchical generalized mixed model (glmm) was used to compare seedling abundance and species richness values between stockpile and mature forests (*lme4* package version 1.1-19, Bates et al. 2015). Random effects were nested and included region (Cold Lake or Fort McMurray), site (stockpile or mature forest), and plot. A Poisson distribution was used owing to the left skewed distribution of the data.

Community compositions of stockpile and mature forest sites were quantified using functional group count and cover for seed bank and aboveground communities. Functional groups included woody, grass, forest forb, native stockpile forb, and non-native forb species. The woody functional group included trees and shrubs, while grasses included all grass, sedge, and rush species. Forbs were split into native and non-native species using the *Flora of Alberta* (Moss and Packer 1994). Native species were further split into forest or stockpile forbs. Forest forbs were defined as native forbs that occurred in any of the forest samples. Stockpile forbs corresponded to native forbs that occurred on stockpile sites only and were not present in the forest samples.

The average count and cover per plot were calculated for each functional group and compared between stockpile and mature forests using hierarchical generalized linear mixed models (glmm), with region, site and plot as random factors. To determine viable seed count variation with depth in both stockpile and mature forests, seedling abundance was compared across sampling depths using hierarchical glmm and pairwise comparisons (*emmeans* package version 1.3.2, Lenth 2019).

Sorensen's index was calculated to quantify the strength of the relationship between the aboveground and surface (to 5 cm) seed bank plant communities. Sorenson's index (S) is a similarity index that can be used to determine how alike two communities are with higher values indicating more similar communities. This was calculated for individual plots, and then averaged by site. Sorenson's index was calculated using the following equation (Legendre and Legendre 2012).

$$S=\frac{2a}{2a+b+c}$$

Where *a* represents the number of species present aboveground and in the seed bank, *b* represents species in the seed bank, but not aboveground, and *c* represents species present aboveground but not in the seed bank. Sorensen's index was chosen because it used presence/absence data, which avoided comparing two different metrics for species abundance (count and cover). Sorenson's index also has a higher weighting for species that are common than for those that are missing, which was desirable for this data because the seed bank has about half as many species as the aboveground community. Therefore, the presence of a species is more informative than the absence of one. Species were omitted from this calculation if the total seed count for all sites was below two, or below a total cover of 5% aboveground to avoid including species that had little effect on the plant community. Hierarchical GLMM was used to compare the Sorensen's index proportions between the stockpile and mature forest and across stockpile sites using a binomial distribution.

2.3 Results

2.3.1 Seed bank diversity

The stockpile sites had a higher total seed bank seedling abundance (p=0.041) and species richness (p=0.003) compared to the soils of the mature forest. A total of 1182 seedlings from 73 species germinated from 60 L of stockpile samples, while 305 seedlings from 42 species germinated from 54 L of mature forest soils. Overall, there were 89 species identified from the seed bank and 149 from the aboveground plant community, with the most abundant species included in Table 1. Only 16 tree seedlings germinated during the experiment, 14 *Betula papyrifera* (paper birch) and two *Picea glauca* (white spruce).

2.3.2 Functional group composition

Stockpile seed banks had more non-native forbs and grasses than soils of mature forests (Table 2.2). Averaged across stockpiles, non-native forbs had the highest seedling count per plot, followed by grasses (Table 2.2). Across all depths, non-native forbs and grasses were the most abundant functional group. Individually, all stockpiles had more grasses and non-native forbs compared to other functional groups (Fig 2.1a). Averaged across mature forests, grasses had the highest number of seedlings per plot (Table 2.2). Across all depths and mature forests, there were more forest forbs or grass species than other functional groups (Fig 2.1a).

Stockpiles had higher non-native forb and grass cover aboveground than mature forest, and mature forest had higher forest forb and woody species cover (Table 2.2). Averaged across stockpiles, grasses had the highest cover per plot (Table 2.2). Individually, all stockpiles had more grasses and non-native forbs compared to the other functional groups except for the 6-month old and 7-year old stockpiles (Fig 2.1b). Averaged across the sampled forests, woody species had the highest cover per plot (Table 2.2), and individually all mature forests had a higher cover of woody species compared to other functional groups (Fig 2.1b).

2.3.3 Seed bank at depth

The greatest number of germinants was from the surface samples in both the stockpile (69% from 0-5 cm) and mature forests (91% from LFH) (Fig 2.2, Table 2.3). Seedling abundance for the mature forests did not differ below the LFH layer, but the stockpile sites had more seedlings germinating from the 5-10 cm depth than the lower depths (Table 2.3). Below 5 cm, stockpiles had a higher total seedling abundance (374) than forests (14, $p<0.001$). The stockpile sites also had a higher species richness below 5 cm (51 species) than the mature forest sites (10 species, $p<0.001$). The three most abundant species below 0-5 cm across all sites were *Agrostis scabra* (tickle grass), *Potentilla norvegica* (rough cinquefoil)*,* and *Polygonum lapathifolium* (pale smartweed). These species each comprised over 9% of the total seedling abundance below 5 cm, adding up to 31% collectively.

2.3.4 Relationship between seed bank and aboveground communities

The mature forests had a stronger relationship between the aboveground and seed bank plant communities than the stockpile sites ($p=0.002$, Fig 2.3) as indicated by their higher Sorenson’s index. The average Sorenson’s index across stockpile sites was 0.4, and 0.6 for mature forests (Fig 2.3). In addition, the Sorensen's index did not vary across stockpile sites of different ages (p=0.361).

2.4 Discussion

Stockpile seed bank diversity was higher than mature forest seed banks, which was unexpected given the loss of seeds that can occur when soils are stockpiled. A study from Australia found that the species richness and seedling emergence were higher on average for stockpiled sites or sites reclaimed using soil stockpiles compared to undisturbed sites (Comino et al. 2004). Alternatively, Tacey and Glossop (1980) found that material stockpiled for two years had a lower species richness and diversity than directly placed soil and undisturbed forest. Lastly, sites in Alberta that were reclaimed using stockpiled material resulted in lower species richness and diversity than a site reclaimed using fresh material (Dhar et al. 2019). If assessing stockpiles solely based on species diversity, the stockpiles we sampled could be a good reclamation material. However, high diversity is not always desirable especially when non-native species make up most of that diversity.

While natural and anthropogenic disturbances can lead to an increase in species diversity, anthropogenic disturbances often lead to a higher abundance of non-native species when compared to other disturbance types (Jauni et al. 2015). Even though the stockpiled sites had more seedlings germinating than that from forest soils, the species I observed may not be desirable for reclamation. The stockpiled sites had more grasses, non-native forbs, and were lacking woody species overall. Bellairs and Bell (1993) also reported a higher percentage of non-native species in stockpiles compared to undisturbed sites, with 15-40% non-native species in stockpiles and only 2.7% in natural sites. The concern is that this trend will continue once stockpiled soil is used for reclamation in the future. Dhar et al. (2019) reported that grasses dominated sites that were reclaimed using stockpiled soils, with an average of 49.5% grass cover 19-23 years after reclamation.

The lack of woody species in the stockpile seed bank is partially because many woody species in the boreal forest are not seed bankers. Most boreal tree species do not have a seed dormancy period longer than nine months, except for white birch (Greene et al. 1999; Zasada et al. 1992). This may explain why *B. papyrifera* was the most abundant tree species germinating in the greenhouse. In the future, desired trees and other woody species may need to be planted because they are not guaranteed to be part of the seed bank.

The seeds that were found in the seed bank were concentrated at shallower depths for both mature forest soils and stockpiles. Dickie et al. (1988) also found that stockpile seedling abundance declined with depth in stockpiles that were less than five years old. A 4-month-old stockpile had 117 seedlings emerge from the surface, and 54 seedlings from a 2 m deep sample, while a 4-year-old stockpile had 130 seedlings from the surface and only 22 from a 2 m sample. In a seed burial experiment, the same trend was discovered, with seed germination declining with depth in soil that had been stockpiled for only six months (Rivera et al. 2012).

In forests across the world most seeds are concentrated near the surface, with most found in the litter layer. In the Acadian forest region of New Brunswick, 90% of seeds germinated from the organic layer, (Moore and Wein 1977) compared to 79% in a coniferous forest in Oregon (Strickler & Edgerton 1976). Having most seeds concentrated at a shallow depth in both older stockpiles and mature forest soils was expected since seeds are deposited on the soil surface from existing vegetation and wind or animal dispersal. Having more seeds in stockpiles below 5 cm in could be positive for reclamation, as there are more seeds available for reclamation.

Mixing of the soil that occurs when soil is stockpiled could redistribute more of the seeds to lower depths in the profile, which is likely why we are seeing more seeds below 5 cm in the stockpiles. However, as with the overall increase in diversity in stockpile sites, this increase in seedling abundance is not always positive. The species that are present below 5 cm are mostly non-native forbs and grasses, with lower numbers of forest forbs and woody species. Of the three species that were the most abundant below 5 cm *A. scabra* is a grass, *P. norvegica* a native forb, and *P. lapathifolium* a non-native forb. All these species produce many seeds each season (Rowe 1983; Staniforth and Cavers 1979; Werner and Soule 1976) and most can remain dormant for long periods in the seed bank (Conn et al. 2006; Staniforth and Cavers 1979). Therefore, they have a greater chance of remaining in the seed bank during storage.

Across most of our stockpiles and mature forests grasses and non-native forbs were consistently the most abundant functional groups despite differences in total seedling abundance. A limitation of seed bank studies in general is that the large spatial variability that exists within seed banks. Across an area, the seed bank composition can change spatially depending on micro topography with wind-dispersed seeds preferentially deposited on rougher surfaces (Vander Wall & Joyner 1998). Seed dispersal can also differ depending on the spatial pattern of aboveground vegetation and their seed outputs (Nathan & Muller-Landau 2000). This makes it difficult to estimate the seed bank community without sampling the whole stockpile, which is not feasible. Despite this spatial variability, most stockpiles were dominated by non-native forbs and grass species.

The mature forests had more similarities between the seed bank and aboveground plant communities than the stockpiles. Typically, older forests have a low Sorenson’s index because of early successional species that remain in the seed bank long after disturbance and canopy closure

(Bossuyt et al. 2002; Drake 1998; Hopfensperger 2007). However, boreal forests do not maintain as much of a seed bank as many other temperate and tropical forests lower in latitude (Johnson 1975). Therefore, the species present aboveground will more closely resemble the seed bank than other forest types resulting in a higher Sorenson's index. Short dispersal distances can also drive high similarity between aboveground and seed bank vegetation (Bossuyt and Hermy 2004), which we see in many of our woody species. An example of a higher Sorenson's index when comparing the aboveground vegetation to the litter layer in a mixed wood boreal forest site in Alberta was found by MacKenzie and Naeth (2007), with a calculated Sorenson's index of 0.65. Since the aboveground and seed bank plant communities are more similar in our mature forests, this raises the question of why the stockpile sites do not see the same pattern.

A low Sorenson's index can occur when there are species present aboveground that are able to produce many small seeds, which can then lead to these species being overrepresented in the seed bank (Eriksson and Eriksson 1997). Although there are more species present aboveground, the seed bank is taken over by a few species. This could be happening on our stockpile sites, which are dominated by non-native species in the seed bank. Several species that are abundant aboveground, produce many seeds (Davy 1980; Staniforth and Cavers 1979; Werner and Soule 1976) and dominate the seed bank on stockpile sites include: *D. cespitosa*, *P. norvegica,* and *P. lapathifolium.*

2.5 Conclusion

Stockpiles may have higher species diversity when compared to soils of mature forests. However, many of the species, such as grasses and non-native forbs present on the stockpiles, are not desirable on reclamation sites. Below 5 cm the stockpiles also have more non-native species. If many non-native species are present, soil should be stripped from deeper depths when salvaging stockpiles for future reclamation sites to dilute the non-native seed bank and increase establishment of native species. On the other hand, if there are many desirable species in a stockpile seed bank, dilution of the seed bank by salvaging stockpiled soil to deeper depths should be avoided.

Overall, it appears that sampling the seed bank of stockpile sites could lead to better reclamation decisions. Since there could be a disconnect between the species observed aboveground and in the seed bank, managers cannot rely on the composition or abundance of aboveground vegetation to predict what species will regenerate from stockpiled soils used to reclaim sites.

2.6 Tables and figures

Table 2.1. The most abundant plant species in the stockpile and soils of mature forests separated by functional groups. Up to three species were chosen per functional group based on the total seedling count or percent cover across all stockpile or forest sites. The total seed bank seedling count or aboveground percent cover is included in parentheses for each category.

Stockpile

Aboveground

Grass	Woody	Forest Forbs	Native Stockpile Forbs	Non-native forbs
Agropyron trachycaulum (246)	*Rubus idaeus* (250)	*Chamerion angustifolium* (69)	*Achillea sibirica* (24)	*Crepis* sp. (41)
Bromus inermis (150)		*Fragaria virginiana* (17)	*Aquilegia brevistyla* (6)	*Polygonum lapathifolium* (30)
Deschampsia cespitosa (192)		*Potentilla norvegica* (15)	*Geranium bicknellii* (25)	*Sonchus* sp. (74)

Seed bank

Grass	Woody	Forest Forbs	Native Stockpile Forbs	Non-Native Forbs
Agropyron trachycaulum (40)	*Rubus idaeus* (26)	*Epilobium ciliatum* (33)	*Achillea sibirica* (3)	*Crepis* sp. (80)
Agrostis scabra (120)		*Potentilla norvegica* (158)	*Collimia linearis* (5)	*Polygonum lapathifolium* (208)
Deschampsia cespitosa (41)		*Urtica dioca* (10)	*Lepidium densiflorum* (20)	*Trifolium hybridum* (97)

Mature Forest

Aboveground

Grass	Woody	Forest Forbs	Non-Native Forbs
Calamagrostis canadensis (68)	*Amelanchier alnifolia* (122)	*Cornus canadensis* (121)	*Taraxacum officinale* (6)
Elymus innovatus (82)	*Picea glauca* (271)	*Galium boreale* (65)	
	Populus tremuloides (988)	*Lathyrus ochroleucus* (73)	

Seed bank

Grass	Woody	Forest Forbs	Non-Native Forbs
Unidentified grass species (83)	*Betula papyrifera* (13)	*Epilobium ciliatum* (15)	*Crepis sp.* (4)
	Rubus ideaus (3)	*Fragaria virginiana* (39)	*Sonchus* sp. *(7)*
		Viola renifolia (22)	

Table 2.2. Average seedling count and species cover (%) across stockpile and mature forest plots by functional group. P values from generalized linear mixed models (glmm) are included for each comparison. For stockpile sites n=24, and for mature forests n=18.

	Seed bank			**Aboveground**		
	Stockpile Count	Mature Forest Count	p	Stockpile Cover	Mature Forest Cover	p
Non-native Forbs	21.3	0.7	0.022	9.5	0.3	0.003
Forest Forbs	9.2	6.7	0.977	12.3	43.5	0.004
Grasses	16.3	8.6	<0.001	48.8	9.7	0.012
Woody	1.2	0.9	0.717	13.5	110.9	<0.001

Table 2.3. P-values from generalized linear mixed model (glmm) pairwise comparisons across depth for stockpile and mature forest seed bank seedling abundance. For stockpiles n=24, and for mature forests n=18.

Stockpile

	0-5cm	5-10cm	10-20cm	20-30cm
5-10cm	<0.001			
10-20cm	<0.001	<0.001		
20-30cm	<0.001	<0.001	0.797	
>50 cm	<0.001	<0.001	0.959	0.375

Mature Forest

	LFH	0-5cm	5-10cm	10-20cm	20-30cm
0-5cm	<0.001				
5-10cm	<0.001	0.748			
10-20cm	<0.001	0.996	0.946		
20-30cm	<0.001	0.748	1.000	0.946	
>50 cm	<0.001	0.374	0.966	0.612	0.966

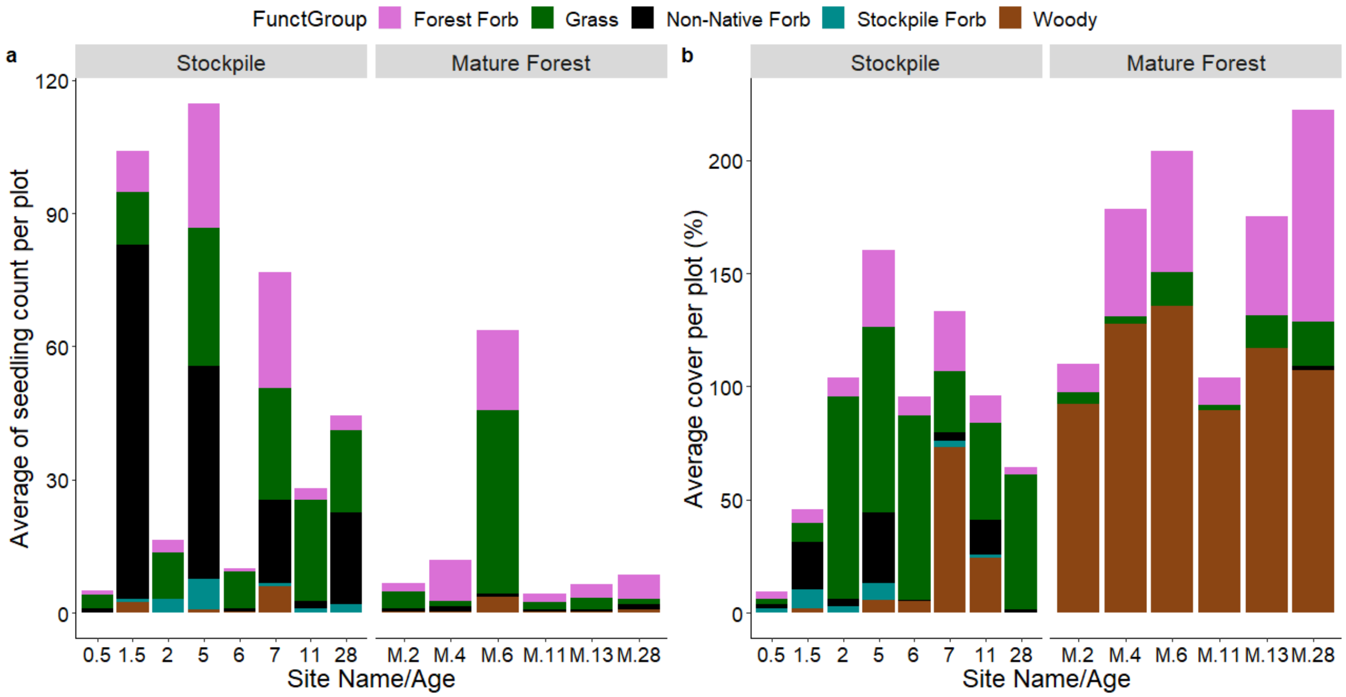

Figure 2.1. Species abundances for aboveground (a), and seed bank (b) communities divided into functional groups (n=3). Site names are included on the x-axis, with all mature forests starting with 'M'.

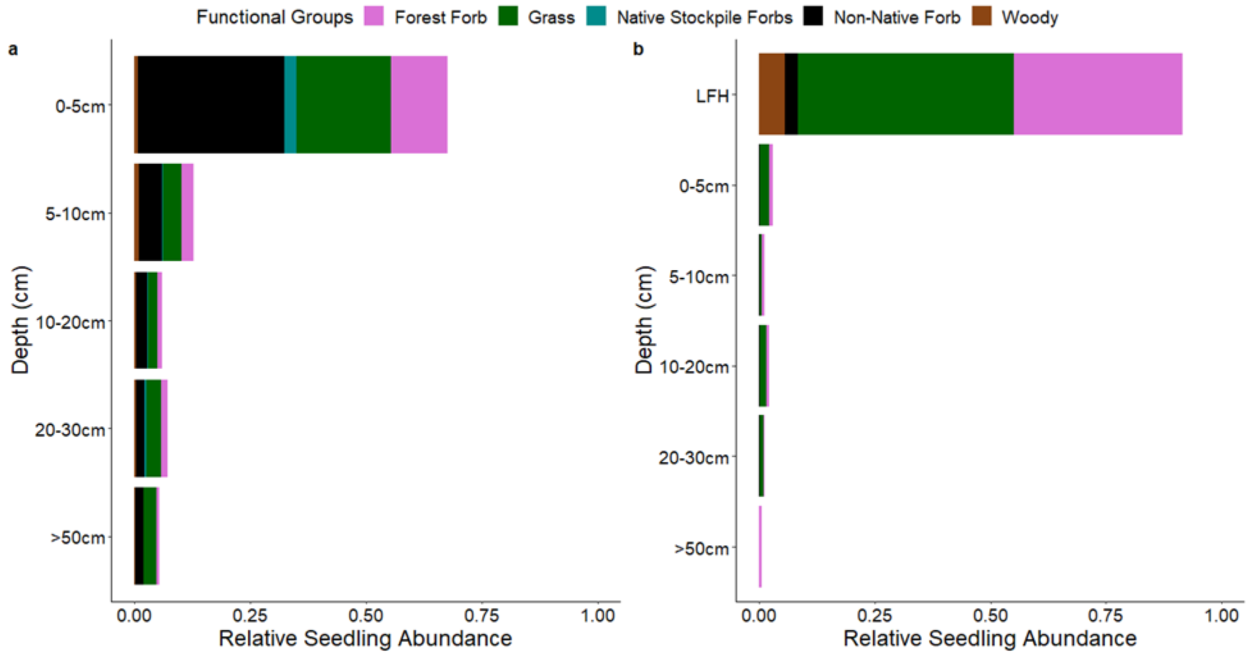

Figure 2.2. Proportion of relative seedling abundance for stockpile (a) and mature forests (b) as a function of soil depth. Relative abundance was calculated by dividing seedling abundance for each functional group at a given depth by the total seedling abundance for that depth.

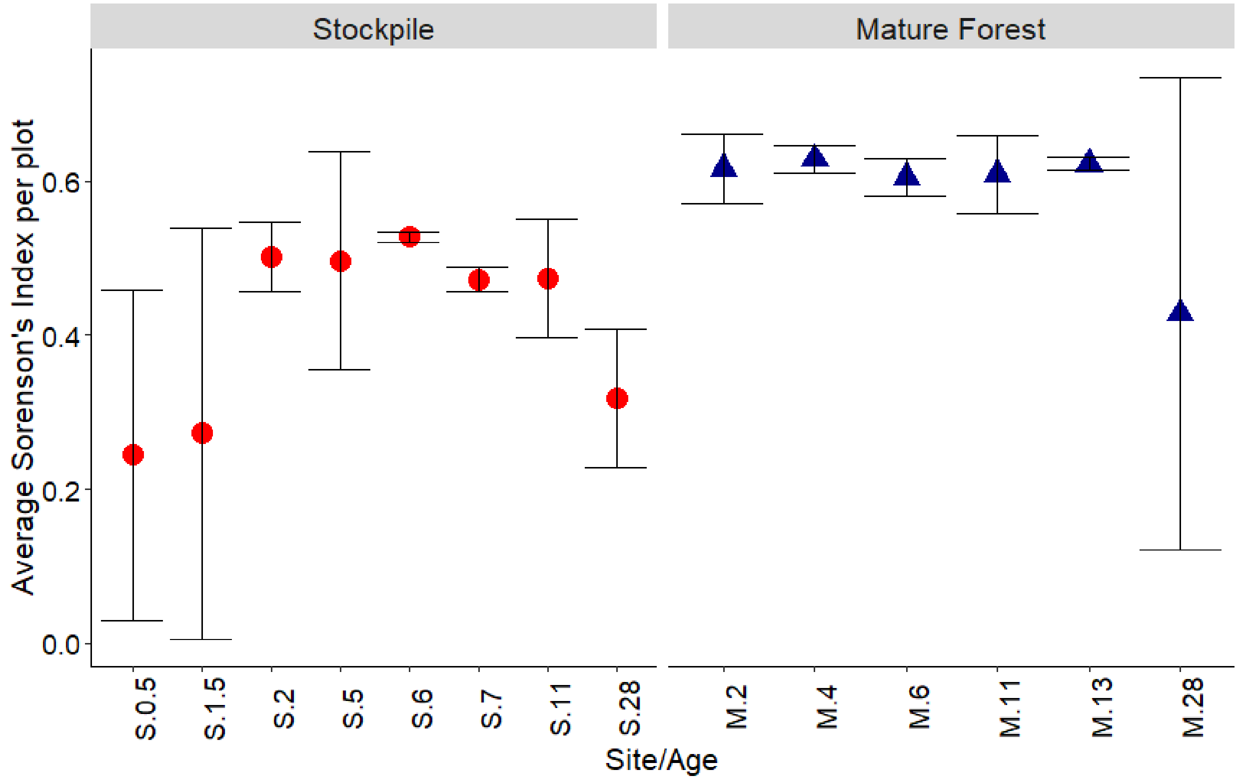

Figure 2.3. Average Sorensen's index per plot. Species with low abundances were excluded. Error bars represent one standard deviation around mean values (n=3). Site names are included on the x-axis, with all mature forests starting with 'M'.

Chapter 3: Availability of macronutrients in stockpiled and mature boreal forest soils

3.1 Introduction

It is estimated that mining has disturbed approximately 0.3 (Hooke & Martin-Duque 2012) to 1 (Bridge 2004) percent of the world's terrestrial land surface. Soil stockpiles will be used around the world to reclaim areas affected by mining. However, soil stockpiling results in many physical and chemical changes to the soil, which can have negative impacts to the environment, for nutrient availability, and plant growth on future reclamation sites.

There has been a lot of research done on soil stockpiling, but there is still uncertainty surrounding how nutrient levels change during stockpiling. When soils are stockpiled, material is handled by heavy machinery and moved, resulting in mixing and compaction. This disturbance can result in a reduction in aggregate stability, and resistance to compaction, changes to pore size distribution, and increased bulk density (Abdul-Kareem & McRae 1984). The breakdown and destabilization of soil aggregates during the stockpiling process could possibly lead to an increase in organic substrates subject to microbial decomposition (Das Gupta et al 2019), and therefore, an increase in nutrient mineralization, and nutrient availability (Larney & Angers 2012; Wick et al. 2008). An increase in decomposition of organic matter can also occur in soil stockpiles as the result of the mixing of topsoil rich in organic matter and subsoil also resulting in an increase in available soil nutrients (Ussiri & Lal 2005; Lorenz & Lal 2007). Conversely, having more available nutrients can cause leaching due to increased mobility, resulting in a net decrease in some nutrients (Ghose 2004; Mushia et al. 2016; Paterson et al. 2019). It is uncertain whether stockpiling will result in an increase or decrease in available nutrients compared to nearby mature forest sites. However, soil stockpiles will be used to reclaim much of the area disturbed by mining, and therefore it is important for managers to understand how nutrient availability could change to ensure the success of future reclamation.

Nutrient availability in the boreal forest and in stockpiled soil impact plant growth and diversity. In particular, macronutrients that may impact plant growth and seed germination include: nitrogen (N), calcium (Ca), magnesium (Mg), potassium (K), phosphorous (P), and sulfur (S), among other nutrients. The boreal forest is considered to be nutrient limited, with N being the most limiting nutrient (Fisher and Binkley 2000; Magnani et al. 2007). Nitrogen is mostly available to plants as NO_3^- and NH_4^+, and is the nutrient most often limiting for plant growth (Nordin et al. 2005; Schulze 1989; Sponseller et al. 2016). Both Ca and Mg are important for photosynthesis, and Ca is needed for cell division and cell wall synthesis (Hepler 2005; Taiz & Zeiger 2010). Potassium (K) is needed for enzyme activation and osmoregulation and if limited, plants become more sensitive to abiotic and biotic stresses (Hawkesford et al. 2012). Another nutrient that can be growth-limiting in the boreal forest is P (Chapin 1980; Maynard et al. 2014; Wieder et al. 2015). Lastly, S can limit tree seedling growth (Ericsson 1995) and, when in excess, can negatively impact plant metabolism, yield, and delay flowering (Rennenberg 1984). Understanding how stockpiling impacts availability of these nutrients will help to inform possible limitations to plant growth in these highly modified soils.

Sampling nutrient availability of natural forests before stockpiling informs managers on the range of availability found in natural forests before soil salvage. If nutrient availabilities stray too far above or below these reference conditions it can have negative impacts on plant growth and diversity. It is important to understand processes and nutrient levels that occur in the natural environment before we can understand trends in stockpiled soils. That is why it is critical to compare stockpiled soils to a reference, or undisturbed mature forest site.

Vertical variability in many soil nutrients, such as Ca, Mg, and N, occurs in boreal forest soils due to pedogenic processes, geological parent material, and inputs of litter. For example, Luvisols, which are a common soil type in the upland Central Mixedwood Subregion, have an increase in clay content at depth due to clay eluviation and illuviation (Howitt & Pawluk 1985). Translocation of clay and mineral weathering of parent material in Luvisols can also result in increases in nutrients such as Ca and Mg in the subsoil (Santos et al. 1986). As microbes decompose litter, nitrogen is released, increasing N at the surface, or at depth due to leaching (Startsev et al. 2008). Therefore, sampling the nearby mature forest sites gives an idea of the processes that occur in nearby undisturbed soil, and trends that occur with depth in these areas. When soils are removed and handled during stockpiling, uneven mechanical mixing of the A, B, and C horizons can occur (Ussiri & Lal 2007). This mixing could decrease variation in nutrient availability with depth.

In addition to vertical variation, nutrients and soil pH may also vary horizontally in forest soils (Bartels & Chen 2010; Bruckner et al. 1999; Laverman et al. 2000). Nutrient variability in forests is influenced by spatial variability in plant community composition. For example, a decrease in pH occurs in coniferous stands due to acidic litter, which can also decrease nitrate production (Ste-Marie & Paré 1999). Spatial patterns in nutrient availability can also impact plant communities, with shifts in N and base cation availability in the boreal forest resulting in shifts in the over and understory plant communities (Giesler et al. 1998). Once stockpiles are created, vegetation is removed and material is mixed and homogenized, which could reduce variabililty in nutrient availability across stockpiles.

Some studies have evaluated changes to pH and nutrients in stockpiles (Abdul-Kareem & McRae (1984), Birnbaum et al. (2017)), however, from the Boreal region of Alberta there are only two studies, to the best of our knowledge, that have researched the effects of stockpiling on nutrients (Das Gupta et al. 2019; Mackenzie & Naeth 2019). Understanding how the availability of macronutrients changes with soil stockpiling will be crucial for estimating nutrient limitations for plant growth on stockpiles in Alberta. In addition, most studies have only sampled two or three stockpiles (Abdul-Kareem & McRae 1984; Das Gupta et al. 2019; Wick et al. 2009), with some studies sampling up to eight stockpiles (Mackenzie & Naeth 2019). Due to the inherent variability of natural soils, combined with the variability introduced during soil stockpiling, we sampled eight stockpiles across Alberta to better understand variability across stockpiles. The aim of this study was to explore the impact of stockpiling soil on the availability of nutrients. More specifically, the objectives included comparing nutrient supply rates of stockpiles to nearby mature forests, and comparing supply rates across stockpiles and at different sampling depths.

3.2 Methods

3.2.1 Soil sampling

A total of eight stockpiles were sampled, along with six natural boreal forest soils; the stockpiles varied in age from six months to greater than 28 years old. Four stockpiles were sampled from an oil sands mine north of Fort McMurray, Alberta and four from an in situ cyclic steam stimulation (CSS) bitumen extraction operation northwest of Cold Lake, Alberta. All stockpiles were vegetated by mostly grasses and non-native forbs (see Chapter 2). Four mature forests were sampled at the Cold Lake site and two at the Fort McMurray site. The overstory of these mature forests were dominated by trembling aspen (*Populus tremuloides*) and white spruce (*Picea glauca*) trees. Both sampling locations were in the Central Mixedwood Subregion of Alberta, Canada, with the soils in the mature forests classified as Gray Luvisols. Three sub-samples were taken from randomly distributed plots at least 10 m apart from each other at each stockpile and mature forest at 0-10 cm. Samples were also taken from 10-20 cm, 20-30 cm and greater than 50 cm at each stockpile and mature forest.

3.2.2 Plant root simulator probes

Plant root simulator probes (PRS; Western Ag Innovations, Saskatoon, SK, Canada) were used to measure availability of soil nutrients (NO_3^-, Ca^{2+}, Mg^{2+}, K^+, $H_2PO_4^-$, HPO_4^{2-}, and SO_4^{2-}). Ammonium, NH_4^+,was also measured, but was not included in the results because it was below detection limits for many of the samples. Soil samples were incubated in the lab rather than in the field to quantify the potential nutrient supply under optimum moisture conditions and a controlled temperature. Given that the stockpiles were from two sampling locations in Alberta, a lab incubation allowed for the soils to be analyzed under the same environmental conditions, and limiting confounding local climate factors (Johnson et al. 2010, 2011).

3.2.3 Soil preparation and incubation

Field capacity was determined for all samples by first sieving samples with a 2 mm sieve. Then samples were placed into rings and onto 1 bar ceramic plates. Samples were fully saturated with deionized water for 24 hours. Samples were then placed into a pressurized extractor at 0.3 bar for 24 hours and oven dried at 105°C for 48 hours. Prior to incubation, samples were stored at 4°C.

Field moist soil samples (100 g each) were thoroughly mixed with 100 g of pure sand to increase aeration, since many samples were above field capacity. Then using the field capacity measurements, each sample was brought to 60% field capacity by adding deionized water. The amount of water added to each sample was calculated on the basis of the 100 g of soil, as the sand was found to be inert, with a minimal water content and water holding capacity. Samples were then pre-incubated for 1 week (Kusbach & Miegroet 2013) at 25°C.

After one week of pre-incubation, PRS probes were added to measure availability of nutrients within the soil. A single pair of probes (1 cation and 1 anion) were added to the samples and removed after 7 days (1-week burial period) incubation. PRS probes were then rinsed with deionized water and returned to Western Ag Innovations for analysis. All nutrients except NO_3^- were quantified using inductively coupled plasma spectroscopy. Nitrate, NO_3^-, supply rates were determined colorimetrically with an automated flow injection analysis system. Ammonium supply rates were also measured, but most of them were below detection limits (Western Ag 2019).

3.2.4 Statistical analysis

All data analysis was done using R software (version R.3.1.1, R Core Team 2019). To account for the effect of sampling location (Fort McMurray and Cold Lake) on nutrient availability, clay content and pH, t-test and Wilcoxon tests were used to compare average supply rates of macronutrients, (NO_3^-, Ca^{2+}, Mg^{2+}, K^+, $H_2PO_4^-$ /HPO_4^{2}, SO_4^{2-}) pH, and clay content between sampling locations (Table A1). A t-test was used if the data was normally distributed, and Wilcoxon test if not normally distributed. If a difference was found, analyses were done separately for a nutrient for the Fort McMurray and Cold Lake locations. The only nutrient that showed a difference between the sampling locations was SO_4^{2-}, as well as clay content.

To compare nutrient supply rates, clay content, and pH between stockpile and soils of mature forests, t-test and Wilcoxon tests were used. T-tests and Wilcoxon test were also used to compare nutrient supply rates, pH, and clay content between the surface (0-10 cm) and subsurface (>50 cm) depths.

The availability of nutrients was also compared at the surface (0-10 cm) and subsurface (>50 cm) depths using ratios of nutrient supply rates. These ratios were calculated by dividing the average supply rate at 0-10 cm by the average supply rate at greater than 50 cm. When ratios are above one, supply rates are greater at 0-10 cm, and when ratios are less than one, rates are greater at greater than 50 cm.

To quantify variability across stockpile and mature forests the coefficient of variation was calculated for all nutrients. The coefficient of variation is calculated by dividing the standard deviation of nutrient availability across the stockpiles by the mean availability for stockpiles. This was also done for mature forests. All depths (0-10 cm, 10-20 cm, 20-30 cm, and 80-90 cm) were included in the standard deviation and mean calculations. Higher coefficients correlate to more variation of nutrients across stockpile or mature forest sites.

3.3 Results

The stockpile sites had higher supply rates of NO_3^-, Ca^{2+}, and Mg^{2+}, but similar rates for K^+, $H_2PO_4^-$ /HPO_4^{2-}, SO_4^{2-}, pH, and clay content compared to the mature forests at 0-10 cm (Table 3.1, Fig 3.1). At greater than 50 cm, clay content was higher in soils of the mature forests compared to the stockpiles, and NO_3^- was higher in the stockpiles (Table 3.1).

The mature forests had more variability in soil properties with depth. Overall, the mature forests had higher pH, clay content, and availability of Mg^{2+} and Ca^{2+} in the subsurface (>50 cm) than at the surface (p values all <0.05). Across all individual mature forests, Ca^{2+} and Mg^{2+} surface to subsurface supply ratios were less than one, meaning that the supply rate was higher at greater than 50 cm compared to 0-10 cm (Fig 3.2).

Overall, the stockpile sites had little variability with depth, having similar nutrient supply rates, clay content, and pH at the surface compared to the subsurface (p values all >0.05). Across individual stockpiles, Ca^{2+} and Mg^{2+} surface to subsurface ratios varied, with some stockpiles having ratios above one, and some below one (Fig 3.2).

Soil pH was also more variable with depth in the mature forests than in stockpiled soils. The stockpile sites had an average soil pH of 6.4 at 0-10 cm, and 6.6 at >50 cm ($p>0.05$), and mature forest soils had an average pH of 5.7 at 0-10 cm and 6.8 at >50 cm (Table 3, $p=0.026$).

Spatial variability among sites, as represented by the coefficient of variation, was higher in the stockpile sites for NO_3^-, SO_4^{2-}, $H_2PO_4^-/HPO_4^{2-}$, and K^+ and higher for mature forest sites for Ca^{2+}, and Mg^{2+} (Table 3.2). Average availability of SO_4^{2-} was higher for the Fort McMurray stockpiles than for the Cold Lake stockpiles ($p=0.023$), with no difference in availability for the mature forests across the two locations ($p=0.533$). The six-month-old stockpile had a neutral pH of 6.4, and the mature forest, M.2, had the highest pH at all depths (Table 3).

3.4 Discussion

Nitrate was higher in the stockpiles than the mature forest soils at both the surface and subsurface depths. Since there is so much variability with N mineralization during stockpiling, I was not sure what to expect for NO_3^- supply rates in the stockpiles compared to soils of mature forests. This higher availability of N in stockpiles could be due to increased mineralization after disturbance (Stark & Redente 1987). A combination of topsoil rich in organic matter and subsoil material, low in organic matter, can result in an increase in N in stockpiles, especially at depth, compared to undisturbed soils. The soil salvaging and stockpiling processes break down and destabilize soil aggregates, which could lead to an increase in decomposition of organic matter (Das Gupta et al 2019), and therefore, an increase in nutrient mineralization, and availability (Larney & Angers 2012; Wick et al. 2008).

Often stockpiles become anaerobic at depth, which results in the accumulation of NH_4^+ (Abdul-Kareem & McRae 1984). However, the lab incubation was done under aerobic conditions. Therefore, it is likely that NH_4^+ that was present in the soil could have been transformed into NO_3^-

during storage or the incubation. Storage of field moist samples for as little as 24 hours to four days may lead to the elevation of NO_3^- (Edmeades et al. 1985; Ross & Bartlett 1990; Van Miegroet 1995). Conversely, these storage conditions were constant across all samples, and simulate processes and transformations that are likely to take place after stockpile placement on a reclamation site. These methods still allow an estimate of N in stockpiled soils.

Both Ca^{2+} and Mg^{2+} availability were higher in the stockpiles at the surface than in soils of the mature forests. It was uncertain whether availability of Ca^{2+} and Mg^{2+} would increase or decrease with stockpiling, but stockpiling would result in a mixing of soil, which could be a factor in the increase of these nutrients at the surface of soil stockpiles (Paterson et al. 2019; Wick et al. 2009).

Variability in nutrients with depth in boreal forest soils is to be expected. The mature forest soils we sampled had more Ca^{2+} and Mg^{2+} and higher clay content in their subsoils compared to the surface. However, when soils are stockpiled, fresh soil is picked up and moved to a new location using heavy machinery. During this process, material that was originally found on the surface can be buried at depth and vice versa. We expected the mixing of soil to influence the variability of nutrients with depth. Once this material was stockpiled and mixed, Ca^{2+} and Mg^{2+} supply rates were not consistently higher at the subsurface, and Ca^{2+}, Mg^{2+}, and clay content were similar between the surface and the subsurface depths. Paterson et al. (2019) also found an increase in clay content at depth in their undisturbed sites, but no increase in clay with depth in the stockpiles. The same trend in variability with depth is found for pH in the mature forest sites. The increase in pH at depth in the mature forest sites is likely correlated with the increase in Ca^{2+} availability. However, once stockpiled, there is also no change in pH with depth.

Spatial variability in nutrients was found across stockpile and mature forest sites. Specifically, NO_3^- was more variable across stockpiles than across the mature forests, likely due to the differences in salvaged material. There is also high spatial variability in nutrients in the boreal forest (Bartels & Chen 2010; Bruckner et al. 1999; Laverman et al. 2000), and when material is salvaged there is even more variability introduced by the uneven mixing of soil horizons during the salvaging process. When material is stockpiled, the organic matter is mixed unevenly throughout the pile, resulting in variation in N mineralization, and therefore N availability. The variability in N, which is one of the most limiting nutrients in forest soils (Nordin et al. 2005; Schulze 1989; Sponseller et al. 2016), will impact management of stockpiled soils. Stockpiles will have to be managed and monitored individually to account for spatial variation present in boreal forests as well as variation introduced during the salvaging process.

Due to the proximity of industrial operations, variability in SO_4^{2-} increased in the stockpiles when compared to the mature forest sites. The availability of SO_4^{2-} was higher on stockpiles at the Fort McMurray site than the Cold Lake sites, with no differences between locations for the mature forest sites. Percy et al. (2012) also found higher availability of SO_4^{2-}, using PRS probes, closer to oil sands mining and extraction facilities. Sulphate could be deposited as a result of oil sands mining operations (Sorenson et al. 2017). The availability of SO_4^{2-} may not be as high in soils of the Fort McMurray mature forests because concentrations decrease further from the forest edge (Percy et al. 2012).

Trends with $H_2PO_4^-/HPO_4^{2-}$, and Ca^{2+} are likely linked to changes in pH. Phosphate availability was similar across stockpiles and mature forests except for the six-month-old stockpile. The increase in phosphate availability in the six-month-old stockpile corresponds to the neutral pH value (6.4), as phosphate is the most available at an intermediate pH around 6-7 (Dickinson

2002; Price 2006). Variability in Ca^{2+} across soils could also be related to pH. Ca^{2+} was more variable in the mature forests than the stockpile sites, however, this could be partially attributed to the high availability of Ca^{2+} in one mature forest (M.2). This site (M.2) also has the highest pH of all the mature forests (7.4) and Ca^{2+} is more available at a pH above 7 (Dickinson 2002; NRCS 1998).

Soil pH moderates the availability of nutrients to plants, and N, P, Mg, K, and S are the most available at an intermediate pH (Dickinson 2002). A soil pH between 6.5 and 7.5 is optimum for plant nutrient availability (Ghose 2004), and the average pH across all stockpiles was within this range for all depths. As well, the soil pH of stockpiles was comparable to the reference mature forest sites, which is likely good news for nutrient availability.

3.5 Conclusion

Having more available N in stockpiled soil can be an advantage since N is often a limiting nutrient, although, N could also leach out of the soil. The mixing of soil during soil stockpiling impacts Ca^{2+}, Mg^{2+}, clay content and pH variability with depth. However, over time soil processes will occur, and these nutrients could move down the profile along with clay. Also, increased spatial variation in NO_3^-, SO_4^{2-}, and $H_2PO_4^-/HPO_4^{2-}$ across stockpiles could indicate that management of stockpiles and reclamation sites should be executed on a smaller scale and focus on differences across individual stockpiles. Moreover, if soils are salvaged from the same area they are likely to be more variable once stockpiled. Thus, sampling should examine individual stockpiles in order to optimize utilization.

3.6 Tables and figures

Table 3.1. Average nutrient supply rate (µg/10 cm2/7 days), pH, and clay content (%) for stockpile and soils of mature forests at 0-10 cm (surface) and >50 cm (subsurface). Standard deviation (n=8 for stockpiles, n=6 for mature forests) is included in parentheses. Results of t-test and Wilcoxon test are included as p-values.

Surface

	Stockpile	Mature Forest	p
NO_3^-	15.3 (7.3)	2.6 (2.4)	0.001
Ca^{2+}	576.0 (453.1)	231 (312.5)	0.02
Mg^{2+}	109.7 (49.2)	53.8 (60.9)	0.059
SO_4^{2-} (Fort McMurray)	42.2 (32.8)	6.7 (0.4)	0.133
SO_4^{2-} (Wolf Lake)	8.1 (0.6)	8.3 (1.8)	0.886
$H_2PO_4^-$ /HPO_4^{2-}	5.6 (9.4)	2.4 (2.0)	0.852
K^+	49.5 (42.0)	37.2 (12.5)	0.454
pH	6.4 (1.0)	5.7 (0.8)	0.228
Clay (Fort McMurray)	26.9 (7.1)	29.6 (15.0)	0.800
Clay (Wolf Lake)	11.9 (2.6)	11.5 (2.8)	0.686

Subsurface

	Stockpile	Mature Forest	p
NO_3^-	44.6 (38.4)	2.0 (1.7)	0.016
Ca^{2+}	816.6 (491.0)	605.2 (508.3)	0.451
Mg^{2+}	188.8 (125.0)	156.5 (95.6)	0.594
SO_4^{2-} (Fort McMurray)	83.9 (94.5)	6.5 (1.0)	0.133
SO_4^{2-} (Wolf Lake)	12.2 (7.4)	9.2 (5.8)	0.486
$H_2PO_4^-$ /HPO_4^{2-}	2.6 (2.2)	1.6 (0.7)	0.604
K^+	93.3 (58.5)	32.8 (47.3)	0.054
pH	6.3 (1.0)	5.7 (0.8)	0.366
Clay (Fort McMurray)	26.8 (8.9)	54.8 (4.7)	0.008
Clay (Wolf Lake)	11.5 (3.1)	24.2 (3.7)	0.002

Table 3.2. Coefficient of variation (CV) for stockpile and mature forests (0-10 cm, 10-20 cm, 20-30 cm, and >50 cm depths included in this calculation).

	Stockpile	**Mature Forest**
NO_3^-	1.36	1.13
Ca^{2+}	0.78	1.22
Mg^{2+}	0.69	1.02
SO_4^{2-}	1.38	0.39
$H_2PO_4^-$ /HPO_4^{2-}	2.85	1.56
K^+	0.93	0.77

Table 3.3. pH and clay content across stockpile and mature forests with depth. Standard deviation is included in parentheses. Data was collected by Kyle Stratechuk.

Stockpile

Site	pH (0-10cm)	pH (10-30cm)	pH (>50cm)	clay (0-10cm)	clay (10-30cm)	clay (>50cm)
0.5	6.3	6.4	6.6	18.2	20.1	20.7
1.5	5.3	5.4	6.2	30.5	29.9	38.6
2	7.0	6.8	6.7	9.4	10.2	11.6
5	6.9	6.5	6.6	34.4	32.5	38.8
6	6.9	7.4	6.4	13.6	9.7	10.3
7	5.2	5.0	5.3	24.3	23.7	19.0
11	5.9	5.6	6.9	9.9	9.2	16.4
28	8.1	8.0	7.9	14.7	12.1	16.9
Average	6.4 (1.0)	6.4 (1.1)	6.6 (1.0)	19.4 (10.1)	18.4 (9.8)	21.5 (1.0)

Mature Forest

Site	pH (0-10cm)	pH (10-30cm)	pH (>50cm)	clay (0-10cm)	clay (10-30cm)	clay (>50cm)
M.2	6.6	7.3	8.2	12.9	22.7	21.7
M.4	5.5	5.3	6.0	19.0	44.4	53.6
M.6	5.6	5.8	6.0	12.7	18.4	26.9
M.11	5.5	6.6	6.8	13.1	18.1	24.9
M.13	4.4	4.6	7.1	40.2	52.6	59.5
M.28	6.6	6.8	6.8	7.3	19.3	29.2
Average	5.7 (0.9)	6.0 (1.1)	6.8 (1.0)	17.5 (13.4)	29.2 (16.7)	36.0 (16.3)

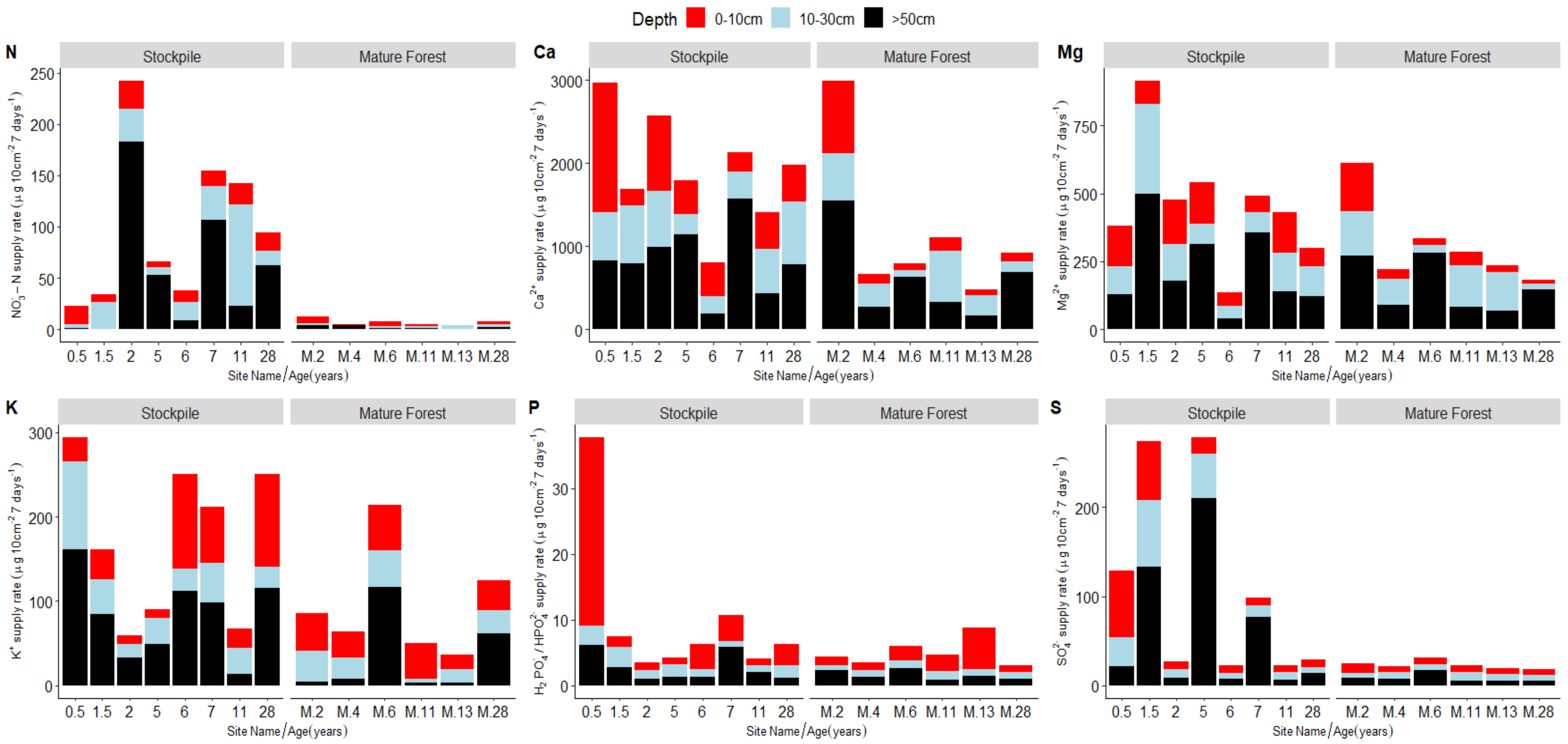

Figure 3.1. Nutrient supply rates (NO3-, Ca2+, Mg2+, K+, H2PO4- /HPO42, SO42-) across stockpile and soils of mature forests.

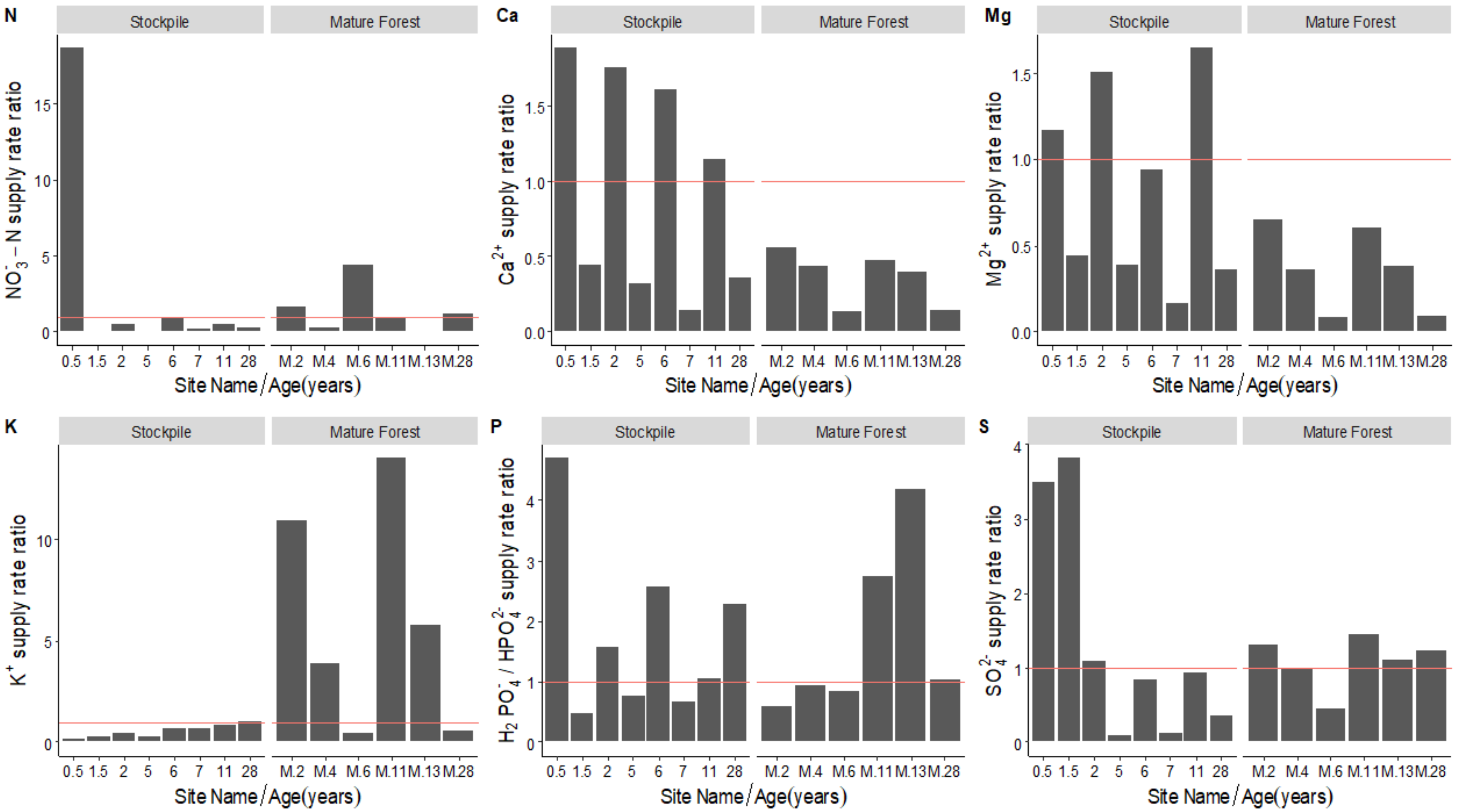

Figure 3.2. Ratio of nutrient supply rates (NO3-, Ca2+, Mg2+, K+, H2PO4- /HPO42, SO42-) at 0-10 cm >50 cm. The red line represents a ratio of 1, where the supply rate at 0-10 cm is equal to the rate at >50 cm.

Chapter 4: Conclusion

4.1 Study implications and management recommendations

In Chapter 2 I found that stockpiles had a higher abundance of viable seeds than soils of mature forests, but most of them were present in the surface samples (0-5 cm), and the LFH layers for the mature forests. Salvaging down to 15-30 cm from forests will likely dilute the seed bank. However, when using stockpiles with high concentrations of weedy or undesirable species for reclamation, it might be beneficial to dilute the seed bank to give native species a better chance to establish on new reclamation sites.

The stockpiles had higher diversity than the mature forests, but most of the species present above ground and in the seed bank were grasses and non-native forbs. To prevent the takeover of grasses and non-native forbs on future reclamation sites, vegetation management may be required. As the aboveground vegetation did not match the seed bank as closely on the stockpiles compared to the mature forests, sampling of the stockpile seed bank may be beneficial for planning seeding and vegetation management on future reclamation sites. For example, some desirable species in the seed bank may not be represented aboveground. In this case, sampling only the aboveground vegetation could lead to seeding or planting of species that are already present in the seed bank.

Results from Chapter 3 demonstrated that N in the stockpiled soils was higher than in the mature forest soils. Increased N in the stockpiles could be beneficial for plant growth but can lead to an increase in seed germination in buried seeds, which will result in seed death (Baskin & Baskin 1998). Invasion of unwanted weedy species is also more likely to occur with higher N availability (Davis et al. 2000; Nordin et al. 2005).

The increased Ca^{2+} and Mg^{2+} availabilities in the surface layers of the stockpiles, compared to soils of the mature forests, could be beneficial for plant growth, as they aid in photosynthesis (Hepler 2005; Taiz & Zeiger 2010). However, N is considered to be the most limiting nutrient in the boreal forest (Fisher & Binkley 2000; Magnani et al. 2007), and therefore an increase in Ca^{2+} and Mg^{2+} may not positively impact growth. Once this stockpiled material is placed on a reclamation site it will likely be mixed again, leading to dilution in both Ca^{2+} and Mg^{2+}.

Even when stockpiles are salvaged from the same area, the salvaging and stockpiling process can result in nutrient variability across stockpiles. Stockpiles should be managed individually to account for the variability that can occur in NO_3^-, SO_4^{2-}, and $H_2PO_4^-/HPO_4^{2-}$ across stockpiles. Particularily, differences in N availability could impact plant communities if not managed for accordingly. Despite large variability in the availability of nutrients across stockpiles, grasses and non-native forb species dominated all stockpiles. Increases in N could be a factor in the outcompeting of native forest forbs and woody species. In any case, vegetation management will be required in order to reach desired plant communities in the future.

4.2 Study limitations

It is possible that our methods could have allowed for an underestimation of the number of viable seeds in the seed bank. The length of time for the germination study, as well as the lack of mixing/scarring, or treatment of the seeds could have limited the species that germinated in the greenhouse, as well as the seedling numbers. However, if seedlings are not able to germinate within four months in the field, they could be outcompeted by other species that are able to take advantage of the open conditions. Our goal was to have a realistic idea of the species that could colonize stockpiles, or sites reclaimed using stockpiles.

There were also limitations involved with the lab incubation. PRS probes are a useful tool to determine nutrient availability. However, when using field moist soils during the lab incubation, there were some concerns with samples becoming anaerobic, which could have affected the results. Another limitation with the lab incubation is that the samples were stored for seven months before being incubated, which likely influenced the nutrient availabilities that were found, especially for N (Van Miegroet 1995).

4.3 Future research

Future research should focus on determining changes in nutrient availability and seed viability in individual stockpiles over time. For example, sampling the same stockpiles multiple times throughout storage would be useful to examine seed loss over time. However, the challenge with sampling the same stockpiles over time is the spatial variability in seed banks, as well as the mixing and disturbance that occurs during sampling.

Knowing more about the source material would be useful to determine what was in the seed bank prior to stockpiling. A study that samples soil before stockpiling, after stockpiling, and after stockpile material placement on a reclamation site would be useful in determining changes to seed bank and chemical properties to soils after each lift. Tracking leachates from stockpiles would also be useful to investigate nutrient leaching over time during stockpiling.

Lastly, mixing during the soil stockpiling process appears to be impacting variability in seed numbers and nutrient availability with depth. However, there is still uncertainty in how long it would take trends with depth observed in mature forest sites to reappear in sites reclaimed with stockpiles, or if they would reappear at all. Tracking trends in nutrient availability with depth over time in stockpiles or sites reclaimed with stockpiles would give more information on how soil forming processes are impacted by the soil stockpiling process.

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Appendix

Table A1. Average probe supply rate (µg/10 cm2/7 days) of nutrients, pH and clay content (%) for the stockpiles and soils of mature forests across sampling locations. Surface samples were taken from 0-10 cm, and subsurface from >50 cm. Standard deviation is included in parentheses. Results of t-test and Wilcox test are included as p-values.

Stockpiles

	Fort McMurray	Cold Lake	p
NO_3^-	19.5 (11.7)	30.2 (15.7)	0.323
Ca^{2+}	625.8 (326.7)	556.4 (205.5)	0.886
Mg^{2+}	149.3 (32.7)	108.3 (43.7)	0.189
SO_4^{2-}	49.1 (18.9)	8.8 (1.2)	0.023
$H_2PO_4^-$ /HPO_4^{2-}	5.8 (7.1)	1.8 (0.8)	0.886
K^+	54.1 (23.7)	53.2 (38.9)	0.969
pH	5.9 (0.9)	6.9 (0.9)	0.132
Clay	27.3 (6.6)	11.8 (1.6)	0.029

Mature Forest

	Fort McMurray	Cold Lake	p
NO_3^-	1.3 (0.2)	2.9 (1.3)	0.159
Ca^{2+}	169.8 (38.7)	400.7 (328.7)	0.267
Mg^{2+}	67.5 (5.4)	96.4 (64.8)	0.800
SO_4^{2-}	7.0 (0.4)	8.0 (1.2)	0.533
$H_2PO_4^-$ /HPO_4^{2-}	2.4 (1.8)	1.5 (0.4)	0.800
K^+	19.8 (7.4)	38.9 (15.7)	0.267
pH	5.3 (0.4)	6.6 (0.8)	0.267
Clay	37.5 (3.7)	16.1 (1.3)	0.133