The role of soil reconstruction and soil amendments in forest reclamation

by

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Abstract

The physical and chemical properties associated with different soil materials are arguably the most important factor affecting early seedling growth and survival as they determine the amount of rooting space, nutrients and water available to planted seedlings. The likelihood of successful reforestation after severe anthropogenic disturbances, such as those caused by surface mining, depend heavily on the quality and quantity of available soil materials, which are likely to differ significantly from site to site. In some cases, there may be an opportunity to salvage high quality soil materials as part of the ongoing mining operations and later use these materials to construct a suitable growing medium for reforestation. In other cases, such materials may not be readily available in which case externally sourced soil amendments may offer a way to improve soil conditions to the point where planted seedlings can be supplied with sufficient water and nutrients to ensure successful establishment. However, given the variety of available soil materials and amendments, as well the varying requirements associated with different tree species, it is important to understand how and when to use these materials in forest land reclamation. For my thesis, I conducted two field experiments to test the effects of (1) different soil materials and placement techniques and (2) different soil amendments on soil properties as well as growth and establishment of three important boreal forest species, trembling aspen (*Populus tremuloides* Michx.), jack pine (*Pinus banksiana* Lamb.) and white spruce (*Picea glauca* (Moench) Voss.). The results of my first study revealed that the topsoil material type (e.g. peat from a lowland and forest floor material (FFM) from an upland forest site) and application thickness (e.g. 30 cm peat vs. 10 cm peat) were the main drivers for the observed differences in seedling growth, while subsoil layer and total capping thickness had only limited effects on

seedling growth at this early stage (three years) of reforestation. The persistently low soil temperature associated with the 30 cm peat thickness was likely the main cause for poor seedling growth performance as low soil temperatures can interfere with physiological function such as root growth and water and nutrient uptake. Due to different nutrient requirements and differences in nutrient availabilities among soil materials all planted species revealed nutrient deficiencies on both topsoil material types, although the form of nutrient deficiency varied by species. In my second study I found that fertilization with a slow release fertilizer increased seedling height after three growing seasons. However, neither the addition of biochar to the soil nor the combination of biochar and fertilizer resulted in the expected beneficial effects on seedling growth. The addition of biochar reduced the amount of plant available nitrate, while it marginally increased soil moisture and thus might have contributed to the lower observed seedling mortality. In combination, my experiments showed that adding salvaged soil materials with a high organic matter content or organic matter rich soil amendments can be a tool to improve soil conditions and thus promote the successful establishment of planted tree seedlings. However, my findings also highlight the need to understand the different requirements associated with different tree species and the respective physical and chemical soil properties associated with each material so that they can be applied in adequate amounts to ensure fast seedling growth and low mortality.

Preface

The following thesis is an original work, with data being collected and analyzed by Jana Bockstette. No part of this thesis has been previously published.

The first experiment "AURORA soil capping study" was part of a research collaborative between Syncrude Canada Ltd. and researches of the University of Alberta. The measurements regarding seedling height, survival, biomass and foliar nutrient concentrations were collected and statistically analyzed by myself. The measurements regarding soil properties (soil temperature, soil water content, soil nutrients) were collected by NorthWind Land Resources Inc. and statistically analyzed by myself. Parts of Chapter 2 "Rebuilding soils and forests: Soil capping materials, thickness and arrangement affect seedling development on an upland boreal forest reclamation site" have been presented in an oral presentation at the Mine Closure 2015 conference in Vancouver, BC, Canada.

The second experiment "Whitewood coal mine reclamation project" was part of a research collaborative between TransAlta Corp. and researchers of the University of Alberta. The measurements regarding seedling height, survival, biomass and foliar nutrient concentrations, soil water content and soil nutrient availability in 2013 were collected and statistically analyzed by myself. The measurements regarding soil nutrient availability in 2012 were collected by Jinhu Liu and were statistically analyzed by myself. Data from Chapter 3 "Aspen seedling establishment on reclamation sites lacking topsoil cover – impacts of biochar and fertilizer amendments" have been presented at the following conferences: poster at the 2013 3rd HAI Science Forum, Edmonton, AB, Canada; oral presentation at the 4th HAI-Energy and Environment Science Forum, Edmonton, AB, Canada

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Chapter 1 - General Introduction

1.1 Surface mining and reclamation

Globally, forests cover about 33% of the terrestrial areas and play a crucial role in providing habitat, food and other resources for plants, animals and humans. With about 1.9 billion hectares, the boreal biome is, after the tropical forest biome, the second largest forested region globally (NRCAN 2017). The boreal forest zone encompasses large areas of the circumpolar northern hemisphere, covering about 29% of the North American continent and vast areas of Europe and Asia (Brandt 2009; Runesson 2014). The climate of this zone is characterized by short, relatively warm growing seasons and cold, long winters (Bonan 1989) and the main limiting factors for plant productivity are low temperatures and the short growing seasons (Vitousek and Howarth 1991). The boreal forest provides a large number of ecosystem services and resources, such as climate regulations, water and nutrient cycling, carbon storage, timber, minerals, oil and gas (Brandt et al. 2013; Venier et al. 2014). Forest ecosystems worldwide are exposed to a range of natural and anthropogenic disturbances. While most forest ecosystems are adapted to natural disturbances within the natural range of intensity and severity, some anthropogenic disturbances produce relatively novel disturbance regimes with unprecedented intensity and severity, to which these forests might not be well adapted.

One such anthropogenic disturbance is surface mining for underground resources such as minerals, coal, oil and gas. Large scale surface mining requires the complete removal of all existing vegetation, surface soils and overburden material, before the resource can be extracted. Once the mining process has come to an end, the reclamation process usually begins with re-contouring the excavated area with topographical features using the overburden and surface soil materials removed before. The mining and reclamation process severely changes physical, chemical and biological soil properties, which often include the transformation and loss of nutrients, changes of pH values and soil temperature, increases in bulk density, decrease of soil moisture, soil organisms, soil organic material and propagule bank (Schafer et al. 1979; Haigh 1992; Davies et al. 1995; Wick et al. 2009; Sheoran et al. 2010; Macdonald et al. 2015). One of the goals of land reclamation in Alberta is to “return the specified land to an equivalent land capability” (Government of Alberta 1993); however, poor topsoil and subsoil material qualities can make the natural recovery of a native ecosystem challenging. Thus the careful storage and handling of soil materials, such as short-term storage or direct placement of soil materials on reclamation sites, limiting traffic of heavy machinery and planting of native tree species may be of utmost importance for the rapid and successful forest reclamation.

1.2 Revegetation

Planting tree seedlings on reclamation sites is an effective measure to initiate the restoration of forests and their associated functions. A developing tree canopy can suppress the establishment of weedy species and promote organic matter (OM) accumulation in the soil by producing litter (Macdonald et al. 2012). The boreal forest naturally contains a rather low number of native tree species, which derive from an even narrower range of genera. Trembling aspen (*Populus tremuloides* Michx.) and jack pine (*Pinus banksiana* Lamb.) are some of the commonly planted tree species in forest reclamation, because they are fast growing, light and drought tolerant and they are the dominant tree species in many upland forest types in central and northern Alberta (Natural Regions Committee 2006). White spruce (*Picea glauca* (Moench) Voss.) is also planted in reforestation and reclamation projects, because it is a commercially important species, which naturally occurs in many forest types across Canada. As a shade tolerant, climax species, it plays an important role in the late successional forests in northern and central Alberta (Natural Regions Committee 2006).

1.3 Soil reconstruction and soil amendments

The soil is one of the most important components that drive a myriad of above and below-ground ecosystem processes in terrestrial ecosystems (Nyle and Weil 2004). These processes have significant impact on the vegetation that depends on soils through the provision of essential resources such as water, nutrients and rooting space. The formation of natural soils and their components over time is driven by the action and interaction of biotic (soil organisms, plants, animals and humans) and abiotic (climate, topography, parent material) variables (Jenny 1994).

The soils shaped by these factors will in-turn mediate plant community development and composition and their performance via resource availability (Coyne and Thompson 2006; Binkley and Fisher 2013).

The most important, but also challenging step in forest reclamation is the reconstruction of a suitable rooting medium, which can support the development of a forest ecosystem for decades to come (Burger et al. 2005; Burger and Zipper 2011; Zipper et al. 2013; Macdonald et al. 2015). This rooting medium should therefore consist of materials which can supply and store sufficient nutrients and moisture to promote mature forests. Those materials are high quality topsoil and subsoil materials, which were extracted from newly mined areas or were stockpiled for later usage in reclamation. Recent practices attempt to create reclamation soils with distinct soil horizons by salvaging the soil materials horizon by horizon and putting them onto the reclamation site in the reverse order they were dug up. However, those reconstructed soils may differ considerably from undisturbed soils in terms of number of horizons, horizon sequence, thickness and materials. Due to the greater expansion of lowland forests (i.e. treed bogs and fens) in the boreal eco-zone, with several meters of organic soil (peat) over mineral soil horizon, peat is commonly used in forest reclamation as a topsoil cover at various thicknesses or as a soil amendment, because it contains a high amount of OM (Fung and Macyk 2000; Macdonald et al. 2015). Other mineral soil materials originating from upland forests are typically less available, because soil horizons over the parent material might not be as thick and salvaged areas might not be as large as the more common lowland areas (Soil Classification Working Group 1998; Rooney et al. 2012). Recent studies have shown that directly transplanted forest floor material (FFM), salvaged from upland forest sites and consisting of the organic layers (LFH), mineral A horizons and some of the B horizon, can enhance the recovery of desired forest tree and understory species (Hahn and Quideau 2013; Naeth et al. 2013), soil microbial communities (McMillan et al. 2007; MacKenzie and Quideau 2010; Beasse 2011), as well as mycorrhizal associations (Gaster et al. 2015; Hankin 2015). While the FFM can only be salvaged up to a depth of several centimeters, the underlying subsoil horizons, which comprise varying thicknesses of B and C materials, can also be used to reconstruct the distinct soil horizons mentioned above on the reclamation site.

However, in some instances, suitable soil materials may not be available for reclamation purposes. This may be the case when surface mining has come to an end due to the total exploitation of the desired resource in a certain area. Moreover, before 1983 topsoil was often transported off site, as regulations were not as strict as today, leaving little or no topsoil material for today's reclamation (Larney et al. 2005). Under those circumstances it may be necessary to proceed with the reclamation operation without the use of a topsoil capping material and plant tree seedlings directly into the unconsolidated mineral soil material (overburden). This, however, may be problematic, as those materials may be lacking nutrients and may have poor water holding capacity (Frouz et al. 2008, 2009; Larney and Angers 2012; Macdonald et al. 2015).

To overcome those limitations, soil amendments, which are added to the soil in order to improve physical, chemical and biological properties of the growing medium, may be an option. Soil amendments can be separated into inorganic (sand, vermiculite, perlite, crumb rubber, lime, inorganic fertilizer, etc.) and organic (straw, compost, manure, peat, biochar, organic fertilizer, etc.) materials, which each have their specific properties and effects on the soil, soil biota and plants. Inorganic soil amendments are usually used to improve physical soil characteristics, such as gas exchange, water dynamics and soil temperature or chemical soil properties, such as soil acidity and heavy metal contamination or add nutrients to the soil (Gruenthal 1999; Hazelton and Murphy 2016a, b; Lwin et al. 2018). Organic soil amendments, on the other hand, can also be used to improve chemical and biological soil properties, such as nutrient supply, ion exchange capacity, pH and soil microbial activity (Hazelton and Murphy 2016b). This is usually achieved by adding OM to the growing medium, which can increase soil microbial activity by supplying the soil fauna with food, adding nutrients to the soil by decomposition of the material, and improving water holding capacity by increasing soil porosity (Cooperband 2002).

1.4 Biochar

One such material recently attracting a lot of attention in forest reclamation is pyrolysed biomass (charcoal), which is called biochar when it is used as a soil amendment to alter soil properties. Biochar is defined as fine grained charcoal, which is high in organic carbon and highly resistant to decomposition (Lehmann and Joseph 2009). It is produced by pyrolysis of organic material (feedstock), which is a slow combustion method under oxygen-poor conditions and at temperatures between 100 and 1000 ºC. The feedstock for biochar production can be anything from animal manure (chicken, cow, pig), energy crops (corn, cereals, palm oil) and agricultural waste (straw, nut shells), to any kind of wood (twigs, branches, stems, roots) (Sohi et al. 2010). Scott et al. (2014) suggest to separate biochar into two groups depending on the feedstock: plant derived biochar (PDB) and animal derived biochar (ADB), because PDBs generally has higher total C, lower ash content, lower nutrient contents and lower cation exchange capacity (CEC) than ADBs. Depending on the feedstock material and pyrolysis process (pyrolysis temperature, duration and oxygen level), biochar can have very heterogeneous physical, chemical and biological characteristics (Singh et al. 2010; Sohi et al. 2010; Zhao et al. 2013; Suliman et al. 2016) and thus, can have varying impacts on soil properties and plant growth (Wardle et al. 1998; Robertson et al. 2012; Omil et al. 2013; Pluchon et al. 2014).

For instance, biochar can have a highly porous structure and large inner surface area with many micropores (internal diameter < 2 nm), mesopores (internal diameter < 50 nm) and macropores (internal diameter > 50 nm) (Verheijen et al. 2009; Mukherjee et al. 2011; Soucémarianadin et al. 2013). Several studies found that increasing pyrolysis temperatures increased the surface area of a biochar (Keiluweit et al. 2010; Břendová et al. 2012; Zhao et al. 2013; Scott et al. 2014), while pore size distribution will largely depend on the feedstock material (Quilliam et al. 2013). Břendová et al. (2012) found that the specific surface area was higher for biochar derived from wood than for biochar derived from maize or meadow grass, while Zhao et al. (2013) found that biochar derived from sawdust had the highest surface area and pore volume out of 14 different feedstock materials tested.

Biochar has a very high carbon content, which is caused by the carbonaceous skeleton remaining from the cell walls and capillary system of the feedstock material (Laine et al. 1991). Mukherjee and Zimmerman (2013) found that biochar derived from oak wood had relatively higher total carbon content (62.6 – 81.3 %) than biochar derived from grass (49.4 – 70.4%), while relative carbon content increased with higher pyrolysis temperature (250°C < 650°C), for both oak and grass. The carbon found in biochar can be separated into recalcitrant, labile, leachable and aromatic carbon, which influences decomposition rate and availability of carbon as an energy resource for microorganisms (Lehmann et al. 2011). The generally highly recalcitrant nature of biochar results in a mean residence time between 90 and 1600 years (Singh et al. 2012).

Biochar contains a certain amount of nutrients that generally depends on the relative ash content of the final product. The ash content on the other hand is again highly dependent on the feedstock material and the pyrolysis process (Lehmann and Joseph 2009; Mukherjee and Zimmerman 2013). Raveendran et al. (1995) and Bourke et al. (2007) found high variability of ash contents and nutrients contained within biochars derived from different agriculatural waste materials. Furthermore, experiments using different temperatures during pyrolysis found that potassium and chlorine vaporize easily at relatively low temperatures, while calcium and magnesium both need much higher temperatures to vaporize. Phosphorus and sulfur are organically bound to the cell structures and are therefore relatively stable during pyrolysis (Lehmann and Joseph 2009).

1.4.1 Biochar effect on soil properties

Due to the high porosity and large surface area, biochar has been found to retain water and nutrients in soil (Lewis et al. 2006; Gaskin et al. 2007; Ouyang et al. 2013; Raave et al. 2014; Prober et al. 2014; Dan et al. 2015) through water infiltration into the porouse structure and adsorption of nutrients to the surface area. Biochar can increase the cation exchange capacity (CEC) of a soil due to its mostly negative charge and large surface area (Mukherjee et al. 2011; Sizmur et al. 2015). Thus, it can decrease the leaching of cations such as potassium and ammonium (Omil et al. 2013; Sika and Hardie 2014), but also of anions such as nitrate (Ding et al. 2010, 2017). This can be a short-term improvement, but due to the high stability of biochar in soil (high amount of recalcitrant carbon) it can also provide a long-term improvement of water holding capacity, especially of sandy soils (Sohi et al. 2010).

Some studies have found that biochar has the ability to change the pH of a soil, due to biochar's own pH (Verheijen et al. 2009; Mukherjee et al. 2011; Yuan et al. 2011). Yuan et al. (2011) found that the pH of an acidic Utisol can be increased or decreased by the addition of different biochars derived from crop straws, depending on the alkalinity of the feedstock material and the pyrolysis process. Mukherjee et al. (2011) and Yuan et al. (2011) found that the pH of a biochar increases with increasing pyrolysis temperature and duration, both suggested that biochars produced at high temperatures will be more suitable to increase soil pH.

Omil et al. (2013) found that soil OM increased with the addition of charcoal to a sandy and fine soil, not only from addition of biochar, but also from higher OM turnover due to increased microbial activity. Microbial biomass and activity has been found to increase with the addition of biochar to soil (Pietikäinen et al. 2000; Lehmann et al. 2011), which can be attributed to the large surface area and carbon content of the biochar. Jones et al. (2012) found that biochar (derived from woodchips of temperate deciduous tree species) incorporated into a sandy clay loam increased bacterial and fungal growth after 2 growing seasons.

1.4.2 Biochar effect on plant growth

Biochar has been extensively used in agriculture to improve crop production since the late 1800's. Even earlier evidence of biochar-type substances used by ancient Amerindian populations are reported from the Amazon region, where the very fertile Terra Preta was discovered (Lehmann and Joseph 2009). In agricultural trials biochar is commonly used in large amounts (up to 200,000 kg ha^{-1}; Kammann et al. 2011) and in combination with fertilizer, where it improved crop yield, nutrient and water uptake efficiency, thus making the crop more resilient to drought (Kammann et al. 2011; Jones et al. 2012; Quilliam et al. 2013; Mulcahy et al. 2013). However, without the addition of fertilizer biochar can have detrimental effects on plants due to the adsorption of nutrients by the biochar and the resulting reduction of nitrogen uptake by plants, which can cause decreased biomass production (Asai et al. 2009).

The first research regarding biochar application to soil and tree seedling growth was conducted by Retan (1915), who found that application of biochar to a heavy clay in the Mont Alto nursery at the Pennsylvania State Forest Academy increased growth and weight of seeded white pine (*Pinus strobus* L.) two fold after two growing seasons compared to the untreated control. Since then, several studies confirmed the benefits of biochar and fertilizer applied to the growing medium on tree seedling growth (Robertson et al. 2012; Omil et al. 2013; Pluchon et al. 2014), as well as for mature trees (Thomas and Gale 2015). However, the positive effect of biochar always highly depends on the biochar properties itself and the substrate it was added to, with the most improvements of tree growth seen, when biochar and fertilizer was added to coarse textured and/or acidic soil substrates (Spokas et al. 2012; Thomas and Gale 2015).

In recent years, biochar has been increasingly used in reclamation settings, where it is mainly applied for the restoration of contaminated soils and water with heavy metals and organic and inorganic pollutants (Beesley et al. 2011; Fellet et al. 2011; Sizmur et al. 2015; Lwin et al. 2018).

1.5 Thesis outline and Objectives

This thesis consists of two individual research chapters. The overall objective of this work was to study the effects of different reclamation materials (peat and sandy soil materials), their placement depth and sequence, as well as the addition of soil amendments (biochar and fertilizer) to an unconsolidated mineral material on planted tree seedling establishment, growth and nutritional status, as well as the impact on soil physical and chemical properties in upland boreal forest reclamation following surface mining. The results of both studies are intended to be used to improve best management practices.

In my second chapter I examined the effect of (1) various soil materials, in particular the peat from a lowland area and A horizon (FFM) of a salvaged Brunisol, (2) cover soil placement depth, (3) the underlying subsoil material type (different subsoil horizons of a salvaged Brunisol (Bm, B, C)) and (4) total capping material depth on early seedling establishment and growth of three common boreal upland tree species (trembling aspen (*Populus tremuloides* Michx.), jack pine (*Pinus banksiana* Lamb.) and white spruce (*Picea glauca* (Moench) Voss.)), as well as the impact of these different soil capping treatments on soil physical (soil temperature and water content) and chemical (i.e. nutrient availability) properties in an oil sands mine reclamation project.

In my third chapter I studied the impact of (1) biochar, (2) fertilizer and (3) the combination of both added to an unconsolidated mineral soil material on aspen seedling establishment, growth and soil water and nutrient availability in a coal mine reclamation project, which was lacking suitable topsoil cover.

Chapter 2 - Rebuilding soils and forests: Soil capping materials, thickness and arrangement affect seedling development on an upland boreal forest reclamation site

2.1 Introduction

After severe anthropogenic disturbances, such as surface or open pit mining, intimate relationships and interactions between landscape, plants, and soils are disturbed or destroyed (Pickell et al. 2013; Macdonald et al. 2015). Open pit mining requires the complete removal of all existing vegetation and soil materials (topsoil, subsoil, and geological overburden materials) before the resource can be accessed and extracted. After mining and as part of the reclamation and restoration process, the topography, including soils and vegetation, of entire landscapes need to be reconstructed (Rooney et al. 2012; Macdonald et al. 2015). At these large scales, surface mines have no natural disturbance analogues, and therefore the reclamation and re-establishment of forests and their functional processes require novel approaches for their recovery (Jacobs et al. 2015).

A challenge in the reclamation process is the reconstruction of soils that have the ability to provide a suitable growing medium and resources necessary to establish and sustain both the planted and volunteer vegetation. Soils play a particularly important role in forest restoration, as they have to sustain deep-rooted long-lived plants (i.e. trees) over decades or centuries, over which these ecosystems can be exposed to a wide range of climatic conditions, disturbances and other biotic or abiotic stresses. While it is impossible to recreate the exact soils with all its distinct horizons and differences in properties, reclamation practices can attempt to emulate some of the characteristics that provide short- and long-term benefits similar to undisturbed soils (Burton and Macdonald 2011; Zipper et al. 2011, 2013; Macdonald et al. 2015).

The first step in operational land reclamation is the reconstruction of geo-technically stable landforms using overburden material. Once the landforms are built, salvaged subsoil materials are placed on top of the overburden material and subsequently covered with salvaged topsoil materials (Oil Sands Vegetation Reclamation Committee 1998). Creating a suitable soil cover design with appropriate soil material horizons and thicknesses can help minimize the economic and ecological risk of failure of reclamation areas (Macdonald et al. 2015). The topsoil layer and the soil organic material (SOM) contained within, play an important role in short-term nutrient cycling, as well as water infiltration and storage. The shallower subsoil horizons (e.g. B-horizon), in contrast, are typically characterized by lower SOM, microbe activity and nutrient content than the topsoil, but may have higher availability of specific nutrients, such as phosphorous, than the deeper parent material (C-horizon). However, the C-horizon is essential for the long-term availability of mineral nutrients through weathering. Moreover, these deeper subsoils provide structural support for plants, as well as long-term water storage (Jenny 1994; Soil Classification Working Group 1998; Nyle and Weil 2004).

The handling, moving and storing of salvaged soil materials during the mining process can drastically change physical, chemical and biological soil properties and thus impact reclamation success (Sheoran et al. 2010; Audet et al. 2015). The different salvaged soil materials can either be stockpiled and stored for later use, which can reduce their quality, especially the topsoil material (Johnson et al. 1991; Harris et al. 1993; Sheoran et al. 2010), or can be placed directly on a reclamation area after salvaging without stockpiling. Direct-placement has been found to be beneficial for forest restoration as these materials contain more viable soil organisms and propagules (Harris et al. 1989; Koch et al. 1996; Mackenzie and Naeth 2010; Beasse 2011; Naeth et al. 2013) and may be more cost efficient due to reduced transportation. In the boreal forest region of northern Alberta, many areas that are currently being disturbed by open-pit mining are dominated by organic soils (peat) and so peat material salvaged from these lowland forests (i.e. treed bogs and fens) are commonly used at various thicknesses as a topsoil soil cover in forest reclamation (Fung and Macyk 2000; Macdonald et al. 2015).

In 2011, the Aurora soil capping study (ASCS) was established to test a range of questions related to the impact of soil capping prescriptions on early upland forest establishment at an operational scale. Different soil materials, in particular the different horizons of a salvaged Brunisol (LFH, A, Bm, B, C) and the peat from a lowland area, were used to construct thirteen different soil capping treatments, which differed in their soil material type, horizon configuration and application thickness. The effect of those soil capping treatments on early seedling establishment and growth of three common boreal upland tree species, trembling aspen (*Populus* *tremuloides* Michx.), jack pine (*Pinus banksiana* Lamb.) and white spruce (*Picea glauca* (Moench) Voss.) and the impact of these different soil capping treatments on soil physical (soil temperature and water content) and chemical (i.e. nutrient availability) properties was assessed. The results of this study will be used to improve best management practices.

My study hypotheses were:

(1) Due to the high organic matter content and higher nitrogen and water availability of peat, seedlings of all three species would have better growth, with less allocation to root mass, and higher foliar nitrogen concentrations, compared to seedlings growing in the coarse-textured Forest Floor Material (FFM, defined later in chapter) coversoil topsoil.

(2) Because topsoil materials typically have higher water and nutrient availability than subsoil materials, thicker application of topsoil (with topsoil thicknesses ranging from 10 to 30 cm) will result in better growth and higher foliar nutrient concentrations.

(3) Integrating a selectively salvaged subsoil material that has higher phosphorous concentrations (Bm) rather than a blended subsoil salvaged from greater depths (B/C and C horizon) will improve seedling growth, irrespective of topsoil material.

(4) Thicker total soil caps (with capping thicknesses ranging from 30 to 150 cm) over the overburden material (lean oil sand) will improve seedling growth due to greater rooting space within the soil cover.

2.2 Methodology

2.2.1 Site description

The Aurora Soil Capping Study (ASCS) site, a large-scale (36 ha) reclamation experiment, is located in the Syncrude Aurora North-Mine lease, about 80 km north of Fort McMurray, Alberta, Canada (57°20' N, 111°31' W). The reclamation site is on a relatively flat plateau with a gentle slope (< 5 %) and an east – west aspect at an elevation of 350 m on an overburden mine dump. The mine lease is located within the Central Mixedwood Natural Subregion, which is composed of a mixture of forests and wetlands on upland and lowland sites. Trembling aspen and white spruce mixedwood stands characterize upland sites on medium to fine textured Gray Luvisolic soils, while jack pine dominated stands occur on coarser textured Brunisolic soils. The poorly drained lowland sites are typically dominated by black spruce and tamarack dominated fens and bogs on organic soils (Soil Classification Working Group 1998; Natural Regions Committee 2006).The climate in the Central Mixedwood Natural Subregion is characterized by short growing seasons (May through September) of about 97 frost free days (Natural Regions Committee 2006). The climate normal during the growing season (May – September) for this region (1981-2010) is 13.4°C mean air temperature and 284.3 mm total precipitation (Government of Canada 2016). Mean air temperature for the 2012 through 2014 growing seasons was 16.4, 16.0 and 14.5°C and total accumulated precipitation was 242, 319 and 343 mm, respectively.

2.2.2 Site construction and material description

The ASCS was established on a large lean oil sand (LOS) overburden dump. Regulatory approvals for mine operators characterize lean oil sand overburden material as being unsuitable for plant growth which requires a soil reclamation cover. It has a mean texture of sandy loam, a neutral pH, and an oil concentration of 2.7% (Table 2-1), which is below the economical concentration for extraction (approximately 7-8%). The ASCS surface landform grading of the overburden was completed in 2011, with soil reclamation placement completed prior to the 2012 growing season.

Five different types of capping materials were salvaged within the mine development footprint for the study. Capping materials included two topsoil materials: Peat salvaged from organic horizons up to approximately 4 m in depth that were lowland bogs and fens and Forest Floor Material (FFM) from coarse-textured, Brunisolic Order soils dominated by jack pine forests. FFM consisted of the surface LFH and A horizons, and potentially a portion of the B horizon salvaged to a depth of approximately 15 cm. Three subsoil materials were investigated, derived from the native Brunisolic Order soils which differed in their depth of salvage: a selectively salvaged Bm horizon from approximately 15 to 50 cm, a blended BC and C horizons (B/C) from approximately 50 to 100 cm, and a deep salvage of approximately 15 to 250 cm that is dominantly C horizon material. The FFM, Bm, B/C and C materials were all salvaged from the same area of the mine lease, as such they all have the same glaciofluvial geologic parent material. Peat and FFM were topsoils placed over subsoil and they were directly placed (not in stockpile) from their source of salvage. The FFM has a sandy texture with a slightly acidic pH of 5.6 compared to Peat (pH 7.4). It has lower nitrogen and sulfur concentrations, but higher phosphorus and potassium concentrations compared to the Peat (Table 2-1).

The Peat contains 34% organic matter with only a small proportion of mineral material (Table 2-1), relative to FFM which contained 2.6 % organic matter. The Peat has higher total nitrogen, nitrate and sulfur than FFM, while the phosphorus concentration was significantly lower (Table 2-1).

Prior to placement in 2011, the Bm and B/C materials had been in stockpile since the winter of 2007/08. This material has a sandy texture and a lower pH (6) than the other subsoil materials (B/C and C; Table 2-1). While this material has similar concentrations of ammonium, nitrate, potassium and sulfur to the other subsoil materials, the phosphorous concentration was much higher than those found in the other subsoil materials, but very similar to the FFM topsoil (Table 2-1).

Once the Bm material was removed, the remaining B and underlying C horizons were salvaged between a depth of 50 and 100 cm (B/C material). This material had also been stockpiled until its placement in 2011. It has a higher pH (7.3) than the other subsoil materials.

Only the phosphorus concentration in the B/C material was lower than in the Bm, while all other soil nutrients measured were similar to the other subsoil materials (Table 2-1).

The C material was salvaged adjacent to the Bm and B/C salvage area and was salvaged to approximately 250 cm following the salvage of the FFM. It was directly placed at the ASCS. The concentrations of ammonium, nitrate, potassium and sulfur did not differ from the other subsoil materials, but the phosphorous concentration was lower than in the Bm material (Table 2-1).

2.2.3 Experimental design

The ASCS was set up as a split-plot design, with soil capping treatment as the plot effect and tree planting treatment as the split effect. Thirteen different soil capping treatments were tested in the ASCS. Each treatment was replicated three times and randomly located on the reclamation site (approx. 36 ha; Fig. 2-1). Each treatment cell was 1 ha in size. Four tree plots (25 × 25 m) were established within each of the treatment cells and separated by a minimum 10 m buffer. Three of the tree plots were planted with one of three tree species each: trembling aspen, jack pine and white spruce, while the fourth tree plot was planted with an even mixture of all three species. All seedling stock was grown from local seed sources and produced at a commercial nursery (Smoky Lake Forest Nursery Ltd.) under operational protocols described in Landhäusser et al. (2012). Aspen and spruce seedlings were sown into 615A Styroblock™ (Beaver Plastics Ltd, Acheson, Alberta), while pine were sown into 412A Styroblock™ (Beaver Plastics Ltd, Acheson, Alberta). All seedlings were planted as one-year-old seedlings. Aspen were 30 cm, spruce were 29 cm and pine were 18.2 cm in height at time of planting (Table 2-2). Seedlings were hand-planted in June 2012 at a regular 1 × 1 m spacing (equivalent of 10,000 stems per hectare (sph)). The area for each treatment cell outside the tree plots was planted with a mixture of the same tree species at a density of approximately2,000 sph, as well as, three native shrub species: pincherry (*Prunus pensylvanica* L.f.), green alder (*Alnus crispa* Chaix.) and saskatoon (*Amelanchier alnifolia* (Nutt.) Nutt. ex M. Roem.) at a density of about 800 sph.

2.2.4 Soil temperature and soil water content

Data Acquisition Systems with CR1000 dataloggers (Campbell Scientific, Edmonton, Canada) were installed in all treatment cells, outside of the tree plots in spring and summer 2012. Volumetric water content (VWC) was measured with time domain refectory sensors (model 616 Campbell Scientific) and soil temperature was measured with thermal conductivity sensors (model 229 Campbell Scientific) six times a day. They were installed in each soil material at various depths and in LOS. For this study I used data from sensors at 15 cm (topsoil) and 35 cm (subsoil) depths to determine VWC and soil temperature. The four hourly readings were averaged per day for the years 2013 and 2014. Daily means from May through September were averaged to determine the average growing season soil temperature and VWC. Further, the numbers of days with daily mean soil temperatures above 5°C were calculated for 2013 and 2014, because root growth of most boreal tree species is considered to be severely restricted below 5°C soil temperature (Chapin III 1977).

2.2.5 Mortality and seedling growth

Seedling heights were measured over three growing seasons (2012-2014) in the single tree plots at the end of each growing season (August) to the nearest 0.5 cm. About twenty-four seedlings per tree plot (subsamples) were measured within two circular permanent sampling subplots (2 m radius), with the first one located in the north-west corner and the second one located in the south-east corner of each tree plot. All seedlings that had their stem inside the circular sampling subplots were measured. In addition, 16 individually tagged seedlings in the center of each tree plot were measure for height and root collar diameter (RCD). RCD was measured to the nearest 0.1 mm and used in 2014 to identify representative seedlings per treatment for biomass harvest (data not presented) (see below: Biomass and biomass allocation). Average annual seedling growth was calculated for each of the three growing seasons (2012-2014) by subtracting the average height measured in each subplot of the previous year from the average height measured in the current year. Plot averages were calculated from the three subplots in each treatment cell. Seedling mortality was assessed by monitoring and counting all dead or missing trees within the sampling subplots and averaging them across the plot.

2.2.6 Biomass and biomass allocation

To determine above- and belowground seedling biomass and biomass allocation within species in response to topsoil materials with contrasting soil nutrient concentrations (FFM and Peat), nine seedlings of each species (representative of the average seedling in the respective treatment) were excavated in fall 2014.

To identify representative seedlings in each treatment, RCD was measured on the 16 permanently tagged seedlings and averaged. Three seedlings (subsamples) with a RCD equal to the plot average RCD were then selected in each tree plot, but outside of the three tree subsampling plots, and excavated carefully to capture the majority of the root system. In total, 54 seedlings were excavated. The excavated seedlings were cold stored in the field and frozen in the laboratory. During processing, roots, stems and leaves were separated, and roots were carefully washed to remove the attached soil. The different organs (leaves, stems and roots) were then dried at 70°C to constant weight and their dry mass was measured. The average leaf area was determined by taking a leaf or needle subsample from the harvested trees. Aspen leaves were scanned with a LI-3100 Area Meter (LI-Cor Inc., Lincoln, USA), while pine and spruce needles were scanned using a STD4800 scanner and analysed using the WinSEEDLE™ software (Regent Instruments Inc., Quebec, Canada). The subsamples were then dried in a *ventilated drying oven at 70°C and weighed. Root to shoot ratio (RSR) was calculated by dividing the root dry weight by the shoot (leaves and stems combined) dry weight.* The allocation to the different organs was determined as mass ratios, leaf mass ratio (LMR), stem mass ratio (SMR) and root mass ratio (RMR) and was calculated by dividing leaf, stem, or root dry mass by the total dry mass of a seedling within each treatment. Mass ratios allow to explore biomass allocation in plants (Thornley 1972), which can change depending on nutrient supply and growing conditions. For example, plants can compensate for low nitrogen or water availability by increasing root growth compared to shoot growth (Wilson 1988).

2.2.7 Foliar nutrient concentrations

Foliar nutrient sampling took place in August 2014. The foliage of nine to twelve seedlings per tree plot were combined into one sample. Leaves and needles were collected using scissors (to minimize damage to the seedling) from seedlings outside the three tree sampling subplots in order not to interfere with their growth. Aspen leaves were randomly collected from within the entire crown, while pine and spruce needles were collected from the current year growth of the terminal leader and branches. Leaves and needles were then cold stored in the field and frozen in the laboratory before processing. Leaves were dried at 70°C to constant weight and subsequently ground with a Wiley mill to 40 mesh (0.4 mm) and sent for nutrient analysis to a commercial laboratory (Natural Resources Analytical Laboratory (NRAL), University of Alberta). The samples were analyzed for total nitrogen (N) concentration, measured by combustion. The phosphorus (P) concentration was measured by microwave digestion (using HNO_3) followed by inductively coupled plasma optical emission spectrometry (ICP-OES) and subsequently converted into per cent concentration (%). Concentrations of N and P were used to calculate the N:P ratios per species and capping treatment.

2.2.8 Data analysis

Of the 13 capping prescription treatments, 11 treatments were used to compare seedling growth in the single species tree plots. Seedling growth responses were compared in response to:

(1) topsoil material type (FFM and Peat), two treatments were used ((**FFM** (20 cm FFM over 30 cm Bm over 100 cm C) and **Peat** (30 cm Peat over 30 cm Bm over 90 cm C)) (Fig. 2-2(a)).

(2) topsoil application thickness, four treatments were used (**FFM10** (10 cm FFM over 140 cm C); **FFM20** (20 cm FFM over 130 cm C); **Peat10** (10 cm Peat over 140 cm C); and **Peat30** (30 cm Peat over 120 cm C)) (Fig. 2-2(b)).

(3) topsoil type with different subsoil configurations, six treatments were used (**FFM/Bm** (20 cm FFM over 30 cm Bm over 100 cm C); **FFM/B/C** (20 cm FFM over 130 cm B/C); **FFM/C** (20 cm FFM over 130 cm C); **Peat/Bm** (30 cm Peat over 30 cm Bm over 90 cm C); **Peat/B/C** **(**30 cm Peat over 120 cm B/C); and **Peat/C** (30 cm Peat over 120 cm C)) (Fig. 2-2(c)).

(4) total capping thickness, four treatments were used ((**Cap30** (30 cm Peat over LOS); **Cap60** (30 cm Peat over 30 cm B/C); **Cap100** (30 cm Peat over 70 cm B/C); and **Cap150** (30 cm Peat over 120 cm B/C)) (Fig. 2-2(d)).

All physical and chemical properties of the ASCS soil materials were analysed using a one-way analysis of variance (ANOVA) based on a full factorial design (Table 2-1). The factor topsoil with two levels (FFM and Peat), factor topsoil application thickness with four levels (FFM10, FFM20, Peat10 and Peat30), and the factor total capping thickness with four levels (Cap30, Cap60, Cap100 and Cap150) were analysed using a one-way ANOVA based on a full factorial design. The factors topsoil by subsoil with two levels for topsoil (FFM and Peat) and three levels for subsoil (Bm, B/C and C) were analysed using a two-way ANOVA based on a full factorial design. All response variables describing seedling growth performance (annual seedling growth, final seedling height, seedling biomass and mass fractions, foliar nutrient concentrations and N:P ratios) were analysed for each tree species separately, due to varying species-specific ecology as well as differences in nursery stock (Table 2-2). The average annual and seasonal soil temperature, the seasonal VWC, and the number of days above 5°C soil temperature for 2013 and 2014 were analysed using a repeated measure ANOVA with years as the repeated factor with two levels (2013 and 2014). Annual seedling growth was also analysed as a repeated measure ANOVA with years as the repeated factor with three levels (2012, 2013 and 2014). Each repeated measure ANOVA was executed using SPSS 20.0 (IBM Corp., 2011). All other response variables were analysed using a general linear model (GLM) in RStudio (version 3.0.2, The R Foundation for Statistical Computing, R Core Team 2014). Prior to each statistical analysis, normality of data and homogeneity of variances was tested on residuals using Shapiro-Wilk and Levene's test using the package "car". If main effects were significant in the ANOVA ($p = 0.1$), Fisher's LSD tests were performed using the package "agricolea". A significance level of $\alpha = 0.1$ was chosen due to the low replication of this operational scale study (n = 3), this has been done in several other studies conducted on this reclamation site (Jones 2016; Bockstette 2017).

2.3 Results

2.3.1 Mortality

Overall, seedling mortality during the three years of the study was low across the study area and did not differ among capping treatments. Aspen had the highest mortality with about 6%, while pine and spruce had an average mortality of 1.3%.

2.3.2 Topsoil material type

2.3.2.1 Soil temperature and soil water content

Treatments with a Peat cover soil had consistently lower annual and seasonal soil temperatures at 15 cm soil depth than FFM in both years (2013, 2014) (topsoil effect: $p < 0.02$). The Peat was also cooler, annually and seasonally, in 2014 than in 2013 (year effect: $p < 0.06$; Table 2-3). Peat was on average 2.6°C colder during the growing season than FFM and as a result, the Peat had 11 fewer days where daily average soil temperatures were above 5°C than the FFM (topsoil effect: $p = 0.002$; Table 2-4). Volumetric soil water content (VWC) at 15 cm soil depth was higher in peat than in FFM in both growing seasons, but there were no differences between the two years (topsoil effect: $p < 0.001$; Table 2-4).

2.3.2.2 Seedling growth

Aspen seedling height growth was similar on both topsoil materials (FFM and peat) (27 cm) in 2012. In the following years, however, aspen steadily increased height growth on FFM with 37 cm in 2013 and 47 cm in 2014, while aspen on Peat had decreased height growth in 2013 (21 cm) which remained similar in 2014 (27 cm) (topsoil by year interaction: $p = 0.074$; Fig. 2-3(a)). The final height of aspen seedlings after three growing seasons was 140 cm on FFM and 100 cm on Peat ($p = 0.108$; Fig. 2-3(b)). The RCD in 2014 of aspen seedlings was higher on FFM (19.6 mm) than on Peat (12.9 mm) ($p = 0.07$) (data not shown).

Similar to aspen, pine seedling height growth was not different on both topsoil materials (15 cm) in the first growing season (2012), while it was higher on FFM than on peat in 2013 and 2014 (topsoil by year interaction: $p = 0.04$; Fig. 2-3(c)). In 2013, pine height growth remained stable on FFM (19 cm), while it decreased on peat (8 cm). In 2014, height growth of pine on FFM and peat both increased but was still higher on FFM (39 cm) than on Peat (19 cm; Fig. 2-3(c)). After three growing seasons, pine seedlings where 82 cm tall on FFM and 52 cm on Peat ($p = 0.01$; Fig. 2-3(d)). The RCD in 2014 of pine seedlings was higher on FFM (18.7 mm) than on Peat (13.1 mm) ($p = 0.04$) (data not shown).

Spruce seedling height growth was similar on both topsoil materials for all three growing seasons (2012 = 15 cm, 2013 = 3 cm, 2014 = 14 cm; Fig. 2-3(e)) and as a result, spruce seedlings were similar in height (52 cm) on both topsoil materials ($p = 0.50$; Fig. 2-3(f)). The RCD in 2014 of spruce seedlings was similar on both topsoil materials ($p = 0.98$).

2.3.2.3 Biomass and biomass allocation

After three growing seasons (2012-2014), total biomass of aspen seedlings was nearly double for seedlings growing on FFM than on Peat (Table 2-5); however, these averages were not statistically different due to the large variation in the size of seedlings growing on Peat ($p = 0.11$). This was likely driven by one of the three replication cells, which had 38 – 49 % smaller aspen seedlings than the other two replication cells. Aspen leaf mass (Table 2-5) and leaf area (Table 2-6) were both similar on the topsoil materials (both $p > 0.14$), while aspen stem and root mass were both higher on FFM than on Peat (both $p < 0.098$; Table 2-5). Biomass allocation to leaves (LMR) was higher on Peat than on FFM ($p = 0.006$; Table 2-6). However, stem mass ratio (SMR), root mass ratio (RMR) and root to shoot ratio (RSR) in aspen seedlings were similar regardless of topsoil material (all $p > 0.35$; Table 2-6)).

Pine seedlings had greater total biomass, leaf, stem and root mass, on FFM than on Peat (all $p < 0.06$; Table 2-5). Pine needle area was also greater when seedlings grew on FFM ($p = 0.04$; Table 2-6). However, the allocation to needles (LMR), stem (SMR) and roots (RSR and RMR) was not different in pine on both topsoil materials (all $p > 0.15$; Table 2-6).

Although spruce seedlings had similar total biomass, needle mass, stem mass and root mass on both topsoil materials (all $p > 0.24$; Table 2-5), LMR was greater in seedlings on FFM ($p = 0.003$), while SMR and RMR were both greater on Peat (both $p < 0.03$; Table 2-6). Only RSR was similar for both topsoil materials ($p = 0.37$; Table 2-6).

2.3.2.4 Foliar nutrient concentrations and N:P ratios

Compared to seedlings growing on FFM, foliar N concentration of aspen in 2014 was about 54% higher on Peat ($p < 0.001$), while foliar P was 33% lower ($p = 0.09$). Accordingly, the N:P ratio in aspen leaves was significantly higher on Peat than on FFM material ($p = 0.001$; Table 2-7). The concentration of foliar nutrients and N:P ratio did not differ in pine seedlings on both topsoil materials (all $p > 0.1$; Table 2-7). Foliar N concentration of spruce was 47% higher on Peat compared to FFM ($p = 0.03$), while foliar P concentration was not different in spruce between the two topsoil materials ($p = 0.45$). These differences resulted in a higher foliar N:P ratio in spruce seedlings growing on Peat ($p = 0.003$; Table 2-7).

2.3.3 Topsoil thickness

2.3.3.1 Soil temperature

The application thickness of topsoil material affected soil temperature at 35 cm depth only in Peat during both growing seasons (2013 and 2014). The soil temperature in Peat30 was about 2°C cooler in 2013 and 3°C cooler in 2014 compared to the soil temperature in Peat10. Soil temperature in Peat10 in both years was not statistically different from the soil temperature in FFM10 (treatment by year interaction: $p = 0.003$; Table 2-8).
For both Peat thicknesses the number of days with soil temperatures above 5°C was not statistically different in 2013; however, in 2014 this period was significantly shorter (14 days) for Peat30 than Peat10. In Peat10, the number of days above 5°C soil temperature was similar to those for both FFM application thicknesses (163 days on average) (treatment by year interaction: $p = 0.04$; Table 2-8).

2.3.3.2 Seedling growth

Application thickness of FFM as topsoil material did not have an effect on seedling growth in all three species. Conversely, the application thickness of Peat affected seedling growth during the first three growing seasons after planting.

In the 2012 growing season, aspen seedlings grew more on FFM10 (24 cm) than on Peat10 (16 cm), which was also less than on Peat30 (24 cm). However, during the following two growing seasons (2013 and 2014) aspen seedlings grew significantly more on FFM10, FFM20 and Peat10 when compared to Peat30 (treatment by year interaction: $p = 0.003$; Fig 2-4(a)). After three growing seasons this resulted in aspen seedlings that were on average 117 cm tall on FFM10, FFM20 and Peat10, while on Peat30 seedlings were only 70 cm tall ($p = 0.04$; Fig 2-4(b)).

In 2012, pine seedlings grew less on FFM20 (13 cm) than on Peat30 (15 cm), while in 2013, seedlings on both FFM treatments (FFM10 and FFM20) (18 cm) grew more than on both Peat treatments (Peat10 and Peat30) (8 cm). In 2014 this trend continued and pine grew 38 cm in both FFM treatments compared to 26 cm in the Peat10 and 14 cm in the Peat30 treatment (treatment by year interaction: $p < 0.001$; Fig 2-4(c)). At the final measurement in 2014 pine seedlings were tallest on FFM20 and FFM10 (79 cm) when compared to Peat10 (60 cm) and Peat 30 (47 cm) ($p = 0.002$; Fig 2-4(d)).

Spruce seedling growth did not differ among treatments in 2012 with an overall mean of 9 cm. In 2013, spruce seedlings showed the highest growth on FFM20 (7 cm). Seedlings on FFM10 grew 5 cm and seedlings on Peat30 grew 2 cm; however, this difference was not statically significant. Spruce showed the least growth on Peat10 with only 0.1 cm. In 2014, however, spruce seedling growth on FFM10, FFM20 and Peat10 was similar (13 cm), while seedlings on those three treatments grew significantly more than on Peat30 (5 cm) (treatment by year interaction: $p = 0.003$; Fig 2-4(e)). In 2014 spruce seedlings on both FFM treatments were 53 cm tall, compared to 47 cm on Peat10 and 42 cm on Peat30 ($p = 0.006$; Fig 2-4(f)).

2.3.3.3 Foliar nutrient concentrations

Aspen seedlings growing on both Peat treatments (Peat10, Peat30) had higher foliar N concentrations than those on the FFM treatments (FFM10, FFM20) ($p < 0.001$). Foliar N concentrations did not differ within the FFM and Peat treatments. Foliar P concentrations, as well as N:P ratios were similar across all four treatments (both $p > 0.11$; Table 2-9).

For pine, only foliar P concentrations differed significantly across treatments, with pine seedlings in Peat30 showing similar concentrations to those in Peat10, but lower than those in the two FFM treatments ($p = 0.07$; Table 2-9).

Foliar N concentrations in spruce seedlings were higher on Peat30 than on both FFM treatments, but similar to those on Peat10 ($p = 0.01$). Foliar P concentrations in spruce seedlings were lower on Peat30 than on both FFM treatments, but, again, similar to those on Peat10 ($p = 0.07$). The N:P ratios were highest on Peat30, followed by Peat10, followed by both FFM treatments ($p < 0.001$; Table 2-9).

2.3.4 Subsoil material type

2.3.4.1 Seedling height in 2014

After three growing seasons (2014), only aspen final height was affected by the subsoil material type underlying the topsoil (subsoil effect: $p = 0.04$). The tallest seedlings (120 cm) were in the soil caps with selectively salvaged Bm as a subsoil material, while seedlings were much shorter on B/C (101 cm) and seedlings on C (94 cm), which were not statistically different from each other (Fig. 2-5(b)). However, final seedling height of all species was affected by the topsoil material (topsoil effect: all $p < 0.001$). Final height of aspen was 127 cm on FFM and 83 cm on peat (Fig. 2-5(a)), while pine was 81 cm on FFM and 47 cm on Peat (Fig. 2-5(c) and spruce was 54 cm on FFM and 45 cm on Peat (Fig. 2-5(d)).

2.3.4.2 Foliar nutrient concentrations

Foliar nutrition and N:P ratio of aspen seedlings (2014) were not affected by subsoil material type. However, foliar N was higher when seedlings grew on Peat (2.5 %) compared to FFM (1.6 %). Foliar P concentrations did not differ, while the N:P ratios of aspen leaves were higher for seedlings growing on Peat (23 %) than on FFM (11 %).

Foliar P concentrations of pine seedlings were affected by subsoil materials, with the highest foliar P concentrations found when Bm (0.117 %) and C (0.108 %) were used as subsoil materials compared to B/C (0.099 %). Foliar P concentration in pine needles was also lower on Peat (0.102 %) than on FFM (0.114 %). Foliar N concentrations of pine seedlings were only affected by topsoil material (Peat (1.5 %) and FFM (1.3 %)). Consequently, the N:P ratios in pine needles were higher when seedlings grew on Peat (15 %) than on FFM (11 %).

Foliar N concentrations were not affected by subsoil material in spruce seedlings but were higher when growing on Peat (1.8 %) than on FFM (1.3 %). Foliar P concentrations and the calculated N:P ratios of spruce seedlings, however, were affected by both topsoil and subsoil material types (topsoil by subsoil interaction). Foliar P concentrations of spruce were only higher on FFM than on Peat within subsoil C. Within the FFM topsoil, all foliar P concentrations were statistically not different across all subsoil material types (Bm (0.135 %), B/C (0.130 %), C (0.107 %)), while within the Peat topsoil, foliar P concentration found on Bm (0.148 %) were higher than on B/C (0.110 %) and C (0.107 %). The calculated N:P ratios of spruce needles were always higher on Peat than on FFM (across all subsoil material types). Within the FFM topsoil, N:P ratios in spruce were similar across all subsoil material types (Bm (9 %), B/C (10 %), C (9 %)), but for Peat, they were higher on B/C (15 %) and C (16 %) than on Bm (12 %).

2.3.5 Total capping thickness

2.3.5.1 Seedling growth

Aspen seedling height growth was not affected by the total capping thickness during the first three growing seasons (2012-2014) ($p = 0.38$). In 2012, aspen seedling height growth was not statistically different across all capping depth treatments (27 cm on average), but they decreased their height growth to 10 cm on average in 2013 across all treatments (year effect: $p < 0.001$). In 2014, height growth across all treatments was similar to 2013. However, there was a trend of increased height growth on Cap60 (14 cm), Cap100 (17 cm) and Cap150 (16 cm), while height growth on Cap30 (3 cm) trended downwards (Fig. 2-6(a)). This growth performance of aspen seedlings resulted in slightly taller seedlings in 2014 on Cap100 (which was 14 cm taller compared to Cap30 ($p = 0.10$; Fig. 2-6(b)).

Similar to aspen, pine seedling height growth was not affected by the thickness of the cap during the first three growing seasons (treatment effect: $p = 0.60$). In 2012, pine growth was not different across all capping depth treatments with 15 cm on average, and similarly decreased (7 cm) in 2013 (year effect: $p < 0.001$). In 2014, pine seedlings growing on Cap100 (13 cm) and Cap150 (10 cm) increased their growth, while seedling growth on Cap30 (8 cm) and Cap60 (6 cm) was not different to 2013 (Fig. 2-6(c)). This, however, did not affect final seedling height in 2014 (43 cm across all treatments) after three growing seasons ($p = 0.61$; Fig. 2-6(d)).

Spruce seedling growth did not respond to cap thickness in 2012, with 11 cm on average. Height growth decreased significantly in 2013 to only 3 cm while it improved in 2014, spruce seedlings growing on Cap60 (6 cm), Cap100 (8 cm) and Cap150 (7 cm), while it remained similar to 2013 on Cap30 treatment (3 cm) compared to 2013 (year by capping depth interaction: $p = 0.09$; Fig. 2-6(e)). Nevertheless, this response did not affect the final height of spruce (44 cm across all treatments) after three growing seasons ($p = 0.31$) (Fig. 2-6(f)).

2.4 Discussion

The selection of topsoil material had the greatest effect on early seedling establishment and height growth in this study. In my first hypothesis I suggested that due to the high organic matter content of peat, seedlings of all three species would grow better in peat than in the sandy FFM topsoil, but my findings did not support this hypothesis. This outcome was somewhat unexpected as several studies comparing growth performance of tree seedlings in different soil materials found that seedlings performed better when growing on a peat-mineral mix (PMM) than on a forest floor material (FFM) (Pinno et al. 2012; Quideau et al. 2013; Pinno and Errington 2015). However, the PMM used in these studies contained only 16-26% SOM, which was considerably lower than the 34% SOM of the peat material used in this study. Furthermore, the peat material used can have very different physical and chemical properties, depending on its origin (e.g. peat building species) and the degree of decomposition (e.g. salvage depth) (Boelter 1966; Hayward and Clymo 1982; Rezanezhad et al. 2010; Rahgozar and Saberian 2016) and inclusions of other materials.

Soil organic matter is often considered a key indicator of reclamation soil quality (Rajan et al. 2010; Bodlák et al. 2012) because it plays an important role in nutrient cycling and water holding capacity, which has been linked to improved growth performance of plants (Berg and Laskowski 2005; Wolken et al. 2010). However, in this study the material with the highest OM content (peat) did not result in the best seedling growth performance. This was likely the result of the observed excessive soil moisture and low growing season soil temperatures. The Peat took much longer than the FFM to warm up in the spring, likely due to the peat's insulating properties. Rapid soil warming in the spring is particularly important in cold climates with short growing seasons as low soil temperatures can inhibit plant growth (Bonan and Shugart 1989; Bonan 1989; Landhäusser et al. 1996, 2001, 2003; Wan et al. 1999; Peng and Dang 2003; Wolken et al. 2011). Excessively high soil moisture, low soil aeration and low soil temperatures have all been linked to reduced biological activity including restricted tree root growth (Islam and Macdonald 2005). Differences in soil temperature between Peat and FFM were particularly prominent in the capping treatments with 30 cm peat as topsoil, in which the soil temperature at 35 cm depth was much lower (4ºC lower on average) in the 2013 and 2014 growing season than in the treatments with FFM. When reducing the thickness of the peat to 10 cm, soil temperatures were similar to those in the FFM. This suggests that pure peat material can have negative effects on early seedling growth, which contradicts my second hypothesis that thicker topsoil application would result in better seedling growth due to greater water and nutrient availability than subsoil. My findings do not support this hypothesis, because none of the species had better growth when the topsoil layer increased in thickness. While there was no difference in FFM, growth was reduced with the thicker peat material (Peat30), which likely was related to the soil temperature limitations outlined above. Interestingly aspen seedlings on both FFM thicknesses and the Peat10 treatments were similar in height after three growing seasons, which also indicates that the presence of higher soil organic matter in the surface layer alone will not result in better seedling growth. Several studies conducted in natural stands in the Canadian boreal forest suggest that seedling establishment and growth can be greatly affected by peat thickness (Greene et al. 2007; Gewehr et al. 2014; Lafleur et al. 2015). Greene et al. (2007) found that seedling survival of trembling aspen, jack pine and black spruce was negatively correlated with peat thickness after fire disturbance, while Gewehr et al. (2014) and Lafleur et al. (2015) went even further suggesting that a threshold of peat thickness of 20 – 30 cm limits trembling aspen distribution. They concluded that peat accumulation greater than 25 cm leads to waterlogged conditions, low nitrogen availability and low soil temperatures.

Root growth of most boreal tree species is considered to be severely restricted below 5°C soil temperature (Chapin III 1977), although some species, including white spruce, are more tolerant of low soil temperatures. Landhäusser et al. (2001) found that one-year-old trembling aspen seedlings had no measurable root growth at 5°C soil temperature, while spruce seedlings increased root mass. Height growth was similarly affected by low soil temperature, with aspen seedlings growing at 5°C soil temperature being much smaller than those growing at 25°C, while spruce height growth showed no change in response to the different soil temperatures. In addition, pine seedlings showed little to no root and shoot growth at soil temperatures below 5°C (Black and Bliss 1980; Lopushinsky and Max 1990; Peng and Dang 2003; Karst and Landhäusser 2014). Moreover, nutrient and water uptake is severely restricted at low soil temperatures (Cooper 1973; Bowen 1996; Wan et al. 1999). For example, Dong et al. (2001)

found that one-year-old apple trees (*Malus domestica* Borkh) increased ^{15}N uptake with increasing soil temperatures above 12ºC.

Peat used in this study had a pH of 7.4, while the pH of FFM was only 5.6 (Table 2-1). Soil pH is known to have a strong effect on nutrient availability (Van Den Driessche 1978; Jenkins 2013), water uptake (Tang et al. 1993) and plant growth (McCormick and Amendola 1983; Böhlenius et al. 2016). In hydroponic systems, trembling aspen and jack pine were found to have reduced growth above a neutral pH, while spruce growth appears to be less sensitive to solution pH (Zhang et al. 2013). The results from my study appear to support these findings as aspen and pine seedlings growing on FFM had about two times greater seedling dry mass than seedlings growing on peat, while spruce dry mass was similar on both topsoil materials (Table 2-5).

In my first hypothesis I suggested that seedling growth on FFM would be limited by nitrogen, which would be expressed by lower foliar N concentrations compared to seedlings growing on peat topsoil. That hypothesis was only partially supported by the data, as foliar N was only lower in aspen leaves (1.66 %) and spruce needles (1.25 %) when growing on FFM, while foliar N in pine was similar when growing on FFM and Peat (1.41 %). Further, foliar nutrient concentrations were not affected by topsoil application thickness (see above), but only by topsoil material. According to recommendations of Van den Burg (1990) and Swan (1971), the N concentrations of aspen leaves and spruce needles in my study indicate a slight nitrogen deficiency when growing on FFM compared to a sufficient supply of nitrogen on peat. Foliar P concentration in aspen leaves (0.12 %) indicated a deficiency when seedlings were growing on peat (Van den Burg 1990). This was expected, because phosphorus concentrations in the Peat treatments were five times lower than the concentrations in the FFM material (Table 2-1). Interestingly, spruce had lower foliar phosphorus concentrations when growing on FFM (0.13%) compared to peat (0.15 %), which is considered adequate (Swan 1971). Foliar N and P concentrations in pine needles were not statistically different between the two topsoil materials, and for both materials phosphorus could be considered deficient (0.12 %) according to Swan (1972) and Weetman et al. (1985). This was somewhat unexpected and seems to suggest that each tree species has very species-specific nutrient requirements that also depend on growth rate, and suggest that foliar nutrient concentrations cannot be predicted readily by soil nutrient concentrations (Hogberg 2017). It also indicates that the different tree species would benefit from species-specific fertilization regimes: pine could potentially benefit from phosphorus addition on both topsoil materials; aspen might benefit from nitrogen fertilization on FFM and phosphorus additions on Peat; and spruce could benefit from a nitrogen and phosphorus combination on FFM.

More recent literature suggests that N:P ratios can be used as an indicator for nitrogen- or phosphorus-limitation (Tessier and Raynal 2003). High N:P ratios (>16) are generally associated with phosphorus limitation, while low N:P ratios (<14) are associated with nitrogen limitation (Koerselman and Meuleman 1996). Taking those thresholds as indication for nutrient limitations, and also considering the thresholds for foliar nutrient concentration, only aspen N:P ratios were consistent with the findings. Aspen had an N:P ratio of 10 on the FFM material, which indicates nitrogen limitation, while it had an N:P ratio of 21 on Peat indicating a phosphorus limitation. Pine and spruce N:P ratios (9 and 12), however, always indicated nitrogen limitation, which was not consistent with the findings from the foliar nutrient measurements. This supports the suggestion that N:P ratios are influenced by the species, its age and what plant organ is measured (Güsewell 2004), thus there might be a need to determine species specific N:P ratios, which can then be used as thresholds, in order to define possible nutrient limitations and thus develop targeted fertilization prescriptions.

Tree seedlings may respond to nutrient limitations, particularly nitrogen and phosphorus limitations, with increased allocation to root growth and higher root mass ratio (RMR) (Wilson 1988; Ingestad and Agren 1991; Ericsson 1995; McDonald et al. 1996). In my study, however, allocation to root and stem appeared to be unaffected by soil material and soil nutrient concentration for aspen and pine seedlings, despite greater total biomass on the FFM material. Spruce seedlings, however, located more biomass to stems and roots when growing on the Peat treatments, which would indicate an overall nutrient limitation when growing on peat. This is, however, inconsistent with the findings of the foliar nutrient concentrations, where spruce had N and P deficiencies when growing on FFM. This indicates that in our setting, biomass allocation is not a good predictor for nutrient limitations. Other sources identified light (Monnier et al. 2013; Chmura et al. 2017), water (Brouder and Volenec 2008), competition with other plants

(Bockstette et al. 2017) and the plant species itself (Shukla and Ramakrishnan 1984; Islam and Macdonald 2005) as possible drivers for different biomass allocation. There is also an ongoing discussion as to whether biomass allocation is driven by environmental conditions (balanced growth hypothesis (Thornley 1972; Hunt 1975)) or is genetically predetermined (allometric growth hypothesis (Pearsall 1923; Troughton 1956)) (Müller et al. 2000; Shipley and Meziane 2002). Only aspen and spruce allocated more biomass to leaves when growing on the Peat treatments, which may be a response to the greater water availability during the growing season. The volumetric water content (VWC) of peat never went below 0.25 cm^3 cm^{-3} during the three growing seasons (Stack unpublished data), which was close to the approximate permanent wilting point (PWP) of peat (Ojekanmi and Chang 2014). However, seedlings growing on the FFM material experienced periods of drought, where the VWC went below the PWP (0.05 cm^3 cm^{-3}) several times and for longer periods during the growing season.

At this early regeneration stage, the subsoil materials appear to have very limited effect on seedling growth (third hypothesis: Integrating a selectively salvaged subsoil material that has higher phosphorous concentrations (Bm) rather than a blended subsoil salvaged from greater depths (B/C and C horizon) will improve seedling growth, irrespective of topsoil material). Only aspen growth was greater when topsoil was underlain by the subsoil with higher phosphorus availability (Bm) (Fig. 2-5 (b)). This response became apparent in the third growing season after planting, when the seedlings had the majority of their roots in the Bm subsoil layer (Bockstette 2017). Aspen reacts positively to phosphorus fertilization with increased height growth, seedling survival and foliar P concentrations (van den Driessche et al. 2003, 2005; Liang and Chang 2004; Pinno et al. 2012).

In my fourth hypothesis I suggested that a thicker cap over the lean oil sand (LOS) would improve seedling growth. This was partially confirmed in this study, as seedlings of all species showed better growth on treatments with capping depths greater than 30cm in the third growing season (2014); however, there were no differences among the caps of 60 to 150 cm (Fig. 2-6(a;c;e)). In addition, this difference only became apparent in the last year of observation, indicating the Peat treatments played a greater role this early in the seedling growth than the total thickness of the cap. It is suspected that the response could have been more pronounced if the topsoil material had been FFM and the seedlings would have had greater growth and development of a root system. The response is not clear at this early stage of development, but it could be expected that growth and productivity will separate more in time, while these trees get bigger and the root systems occupy larger soil volumes. McConnaughay and Bazzaz (1991) demonstrated that a greater accessible soil volume alone significantly increased biomass of four herbaceous species, although they also found sensitivity to soil volume varied among species. In this study, the soil volume in the Cap30 treatment was considerably smaller than the soil volume in the other three thicker caps (Cap60-150). However, this treatment had 30 cm of peat directly underlain by lean oil sands overburden (LOS), which might have also been a barrier for root expansion, as observational data indicate that roots did not penetrate into the LOS material at this early stage in regeneration. The underlying reasons for this are not clear; physical (e.g. bulk density) or chemical (e.g. hydrocarbon content) properties of the LOS might play a role (Paragon Soil and Environment Consulting Inc. 2006; Kessler et al. 2010; Fleming et al. 2012; Korbas 2013). Regardless, the smaller available rooting volume in combination with the poor growing conditions in the Peat treatments (see above) likely contributed to the poor performance of the seedlings in this treatment. The observed responses of seedlings to total capping thickness should, however, be viewed with caution, as seedlings are still small and root systems have not yet fully developed. It is expected that potential differences should become more evident when these stands develop maximum leaf area.

In summary, the topsoil material type appears to be the main driver for seedling growth during early regeneration, with seedlings performing much better when growing on FFM than Peat. Low soil temperature in the Peat was apparently the main factor restricting seedling growth; however, nutrient limitations (i.e. phosphorous) might have also played a role. This response became most apparent when comparing the thickness of the Peat layer, where seedlings growing with 10 cm Peat performed as well as seedlings with FFM topsoil. This suggests that salvaged peat material should only be used sparingly, or potentially mixed with underlying mineral soil material to reduce the insulating qualities of this topsoil material. It is also uncertain if the peat material of this study is unique and if similar results would occur across the range of peats used in oil sands reclamation. In contrast, the thickness of the FFM material did not affect seedling growth performance. Subsoil had only little effect on seedling growth during this early regeneration stage, with only larger aspen seedlings responding to subsoil conditions. Overall, aspen appears to be the species most sensitive to the treatments I tested, likely due to its faster growth rates and its need to quickly develop a large root system.

2.5 Tables

Table 2-1: Physical and chemical properties (mean (SD)) of ASCS soil materials measured in 2012. Different letters indicate statistically significant differences among material types ($\alpha = 0.1$) (n = 3).

Soil materials	Topsoil		Subsoil			Overburden
	FFM	Peat	Bm	B/C	C	LOS
Salvage depth (cm)	0-15	0-mineral contact*	15-50	50-100	15-250	n/a
BD (g cm^{-3})	1.1 (0.06) **d**	0.6 (0.04) **e**	1.6 (0.05) **bc**	1.7 (0.04) **a**	1.5 (0.03) **c**	1.6 (0.03) **b**
Sand (%)	91.6 (1.2) **b**	n/a	93.2 (0.4) **ab**	94.7 (0.3) **a**	94.4 (1.7) **a**	59 (1.7) **c**
Silt (%)	4 (0.9) **b**	n/a	2.6 (0.5) **c**	1.2 (0.2) **d**	2.1 (1.1) **cd**	28.7 (1.3) **a**
Clay (%)	4.4 (0.3) **b**	n/a	4.3 (0.2) **bc**	4.2 (0.2) **bc**	3.7 (0.7) **c**	12.3 (0.4) **a**
Bitumen (%)	n/a	n/a	n/a	n/a	n/a	2.7 (0.9)
SOM (%)	2.6 (0.5) **b**	34.1 (2.6) **a**	n/a	n/a	n/a	n/a
TOC (%)	1.3 (0.3) **b**	17 (1.3) **a**	n/a	n/a	n/a	n/a
TON (%)	0.04 (0.01) **b**	0.74 (0.04) **a**	n/a	n/a	n/a	n/a
pH	5.6 (0.2) **e**	7.4 (0.1) **b**	6 (0.3) **d**	7.3 (0.1) **b**	6.7 (0.2) **c**	7.7 (0.01) **a**
EC (ds m^{-1})	0.2 (0.004) **c**	1.2 (0.1) **b**	0.2 (0.02) **c**	0.3 (0.03) **c**	0.2 (0.04) **c**	2.4 (0.1) **a**
SAR	0.2 (0.01) **b**	0.6 (0.07) **b**	0.2 (0.01) **b**	0.3 (0.05) **b**	0.3 (0.06) **b**	5.3 (0.6) **a**
Sat (%)	40 (3.8) **b**	207 (18) **a**	31 (0.7) **b**	30 (0.4) **b**	33 (0.5) **b**	40 (0.6) **b**
N (mg kg^{-1})	4.2 (1.1) **b**	13.8 (2.5) **a**	2.6 (0.3) **bc**	2.3 (0) **c**	2.4 (0.1) **c**	n/a
NO_3^- (mg kg^{-1})	2 (0) **b**	12.4 (2.5) **a**	2.3 (0.4) **b**	2 (0) **b**	2 (0) **b**	n/a
NH_4^+ (mg kg^{-1})	2.2 (1.1) **a**	1.3 (0.1) **b**	0.3 (0.05) **c**	0.3 (0) **c**	0.4 (0.1) **c**	n/a
P (mg kg^{-1})	24.6 (0.4) **a**	5 (0) **c**	27.6 (4.6) **a**	10.8 (0.9) **b**	9.7 (2.4) **b**	n/a
K^+ (mg kg^{-1})	41.2 (7.6) **a**	35.6 (0.8) **b**	26.5 (2.7) **c**	24.8 (1) **c**	24.9 (0.3) **c**	11.2 (4.5) **d**
SO_4^- (mg kg^{-1})	5 (0.6) **c**	482 (149) **a**	2.7 (0.8) **c**	4 (0.9) **c**	4.7 (4.4) **c**	207 (118) **b**

BD = dry bulk density; SOM = soil organic material; TOC = total organic carbon; TON = total organic nitrogen; EC = electrical conductivity; SAR = sodium adsorption ratio; Sat = saturation; N = nitrogen; NO_3^- = nitrate; NH_4^+ = ammonium; P = phosphorus; K^+ = potassium; SO_4^- = sulfur.

*Maximum salvage depths were approximately 300 to 400 cm.

Table 2-2: Initial seedling characteristics (mean (SD)) at out planting in 2012.

Species	Height (cm)	Shoot mass (g)	Root mass (g)	RSR	Plug height (cm)	Plug diameter (cm)	Plug volume (ml)
Aspen	30 (8.6)	0.7 (0.4)	2.5 (1.2)	3.4	15	6	340
Pine	18.2 (2.8)	2.2 (0.7)	1.3 (0.5)	0.6	12	4	125
Spruce	29 (5.5)	4.5 (1.6)	2.9 (1.4)	0.7	15	6	340

RSR = Root shoot ratio

Table 2-3: Annual (2013 – 2014) and growing season (May – September) soil temperature (ºC) (mean (SD)) at 15 cm depth for the FFM and Peat treatments. Letters indicate statistically significant effects ($\alpha = 0.1$) ($n = 3$).

Year	Topsoil material	Annual soil temperature (°C)	Seasonal soil temperature (°C)
2013	FFM	6.3 (0.06) **a**	19.2 (0.3) **a**
	Peat	4.9 (0.78) **b**	17 (0.03) **b**
2014	FFM	5.7 (0.11) **a**	18.7 (0.9) **a**
	Peat	3.6 (1.04) **c**	15.8 (1) **c**

Table 2-4: Volumetric soil water content (VWC) (cm3 cm-3) during the growing season (May – September) and number of days with daily soil temperature above 5°C (mean (SD)) at 15 cm depth for the FFM and Peat treatments. Letters indicate statistically significant topsoil effect ($\alpha = 0.1$) ($n = 3$).

Topsoil material	Soil water content (cm^3 cm^{-3})	No of days > 5°C soil temperature
FFM	0.09 (0.03) **a**	168 (1) **a**
Peat	0.56 (0.014) **b**	157 (1) **b**

Table 2-5: Total biomass, leaf, stem and root mass (g) (mean (SD)) in 2014 of aspen, pine and spruce on FFM and Peat treatments. Different letters indicate statistically significant topsoil effects within each species (α = 0.1) (n = 3).

		(g)			
Species	Topsoil material	Total biomass	Leaf mass	Stem mass	Root mass
Aspen	FFM	338 (13) **a**	54.5 (2.3) **a**	122.6 (10.2) **a**	161.3 (7) **a**
	Peat	180 (130) **a**	32.9 (24.4) **a**	61.4 (48.2) **b**	85.5 (59.5) **b**
Pine	FFM	246 (37) **s**	123.4 (21.5) **s**	91.7 (11) **s**	31.3 (6.9) **s**
	Peat	108 (68) **t**	55.3 (34.4) **t**	38 (26.2) **t**	15 (8.1) **t**
Spruce	FFM	121 (30) **x**	56.1 (12.1) **x**	44.8 (11.8) **x**	20.4 (6.6) **x**
	Peat	100 (58) **x**	36.3 (21.9) **x**	43.2 (25.2) **x**	20.2 (11) **x**

Table 2-6: Leaf area (cm2), root shoot ratio (RSR), leaf mass ratio (LMR), stem mass ratio (SMR) and root mass ratio (RMR) (g g-1) (mean (SD)) in 2014 of aspen, pine and spruce on FFM and Peat treatments. Different letters indicate statistically significant topsoil effects within each species (α = 0.1) (n = 3).

		(cm^2)		($g\ g^{-1}$)		
Species	Topsoil material	Leaf area	RSR	LMR	SMR	RMR
Aspen	FFM	5825 (503) **a**	1.3 (0.15) **a**	0.16 (0.003) **b**	0.37 (0.02) **a**	0.48 (0.02) **a**
	Peat	3099 (2506) **a**	1.4 (0.25) **a**	0.18 (0.005) **a**	0.34 (0.03) **a**	0.48 (0.04) **a**
Pine	FFM	6125 (1091) **s**	0.3 (0.06) **s**	0.5 (0.02) **s**	0.37 (0.03) **s**	0.13 (0.01) **s**
	Peat	2592 (1636) **t**	0.5 (0.12) **s**	0.5 (0.05) **s**	0.34 (0.03) **s**	0.15 (0.04) **s**
Spruce	FFM	2852 (692) **x**	0.4 (0.06) **x**	0.47 (0.02) **x**	0.37 (0.009) **y**	0.17 (0.01) **y**
	Peat	1924 (1237) **x**	0.5 (0.1) **x**	0.36 (0.02) **y**	0.43 (0.01) **x**	0.21 (0.02) **x**

Table 2-7: Foliar nutrient concentrations (%) of N and P measured in 2014 and calculated N:P ratio (mean (SD)) of aspen, pine and spruce growing on FFM and Peat treatments. Different letters indicate statistically significant topsoil effects within each species (α = 0.1) (n = 3).

		Foliar nutrient concentrations (%)		
Species	Topsoil	N	P	N:P
Aspen	FFM	1.66 (0.09) **b**	0.16 (0.02) **a**	10 (0.6) **b**
	Peat	2.55 (0.1) **a**	0.12 (0.02) **b**	21 (2) **a**
Pine	FFM	1.34 (0.06) **s**	0.12 (0.006) **s**	11 (0.6) **s**
	Peat	1.48 (0.12) **s**	0.12 (0.01) **s**	12 (0.6) **s**
Spruce	FFM	1.25 (0.06) **y**	0.13 (0.01) **x**	9 (0.6) **y**
	Peat	1.84 (0.3) **x**	0.15 (0.03) **x**	12 (0.6) **x**

Table 2-8: Growing season (May – September) soil temperature (°C) and number of days with average daily soil temperature above 5°C (mean (SD)) at 35 cm depth for the treatments FFM10, FFM20, Peat10 and Peat30. Different letters indicate statistically significant effects (α = 0.1) (n = 3).

		Soil temperature (°C)	
Year	Treatments	Growing season	No of days > 5°C soil temperature
2013	FFM10	16.3 (0.9) **ab**	169 (3) **a**
	FFM20	16.8 (0.8) **ad**	167 (1) **a**
	Peat10	15.4 (0.8) **bc**	156 (7) **d**
	Peat30	13.5 (0.6) **f**	158 (4) **cd**
2014	FFM10	15.8 (1.1) **cde**	166 (2) **ab**
	FFM20	16.1 (0.6) **bc**	163 (1) **abc**
	Peat10	14.6 (1.2) **ef**	160 (2) **bcd**
	Peat30	11.5 (1) **g**	146 (10) **e**

Table 2-9: Foliar N and P concentrations (%) measured in 2014 and calculated N:P ratio (mean (SD)) of aspen, pine and spruce growing on FFM10, FFM20, Peat10 and Peat30 treatments. Different letters indicate statistically significant treatment effects within each species ($\alpha = 0.1$) (n = 3).

		Foliar nutrient concentrations (%)		
Species	Treatments	N	P	N:P
Aspen	FFM10	1.59 (0.04) **b**	0.15 (0.007) **a**	10.7 (0.8) **a**
	FFM20	1.69 (0.03) **b**	0.16 (0.01) **a**	10.9 (0.6) **a**
	Peat10	2.18 (0.26) **a**	0.12 (0.003) **a**	17.9 (1.8) **a**
	Peat30	2.42 (0.13) **a**	0.16 (0.1) **a**	19.3 (9.1) **a**
Pine	FFM10	1.36 (0.12) **s**	0.11 (0.002) **s**	11.9 (1) **s**
	FFM20	1.34 (0.06) **s**	0.12 (0.005) **s**	11.6 (0.3) **s**
	Peat10	1.25 (0.04) **s**	0.11 (0.009) **st**	11.5 (0.6) **s**
	Peat30	1.43 (0.18) **s**	0.1 (0.007) **t**	14.4 (2.7) **s**
Spruce	FFM10	1.22 (0.05) **y**	0.14 (0.01) **x**	8.7 (0.6) **z**
	FFM20	1.25 (0.17) **y**	0.14 (0.02) **x**	9.1 (0.4) **z**
	Peat10	1.52 (0.1) **xy**	0.13 (0.005) **xy**	12 (0.7) **y**
	Peat30	1.73 (0.23) **x**	0.11 (0.02) **y**	16.2 (1.1) **x**

2.6 Figures

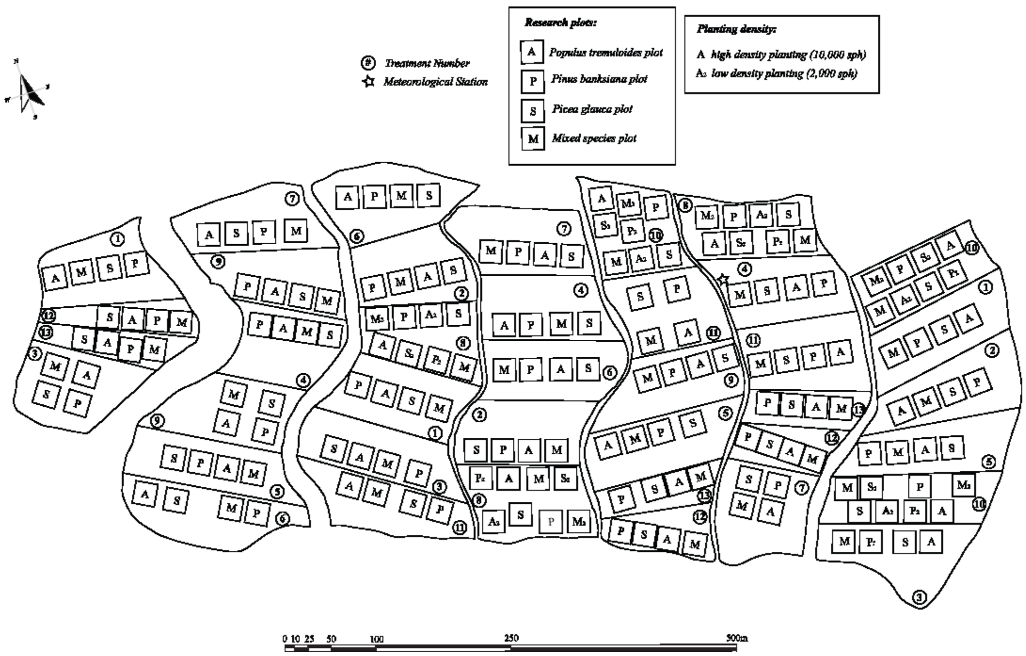

Figure 2-1: Map of Aurora Soil Capping Study (ASCS) including all treatments, plantings and planting densities.

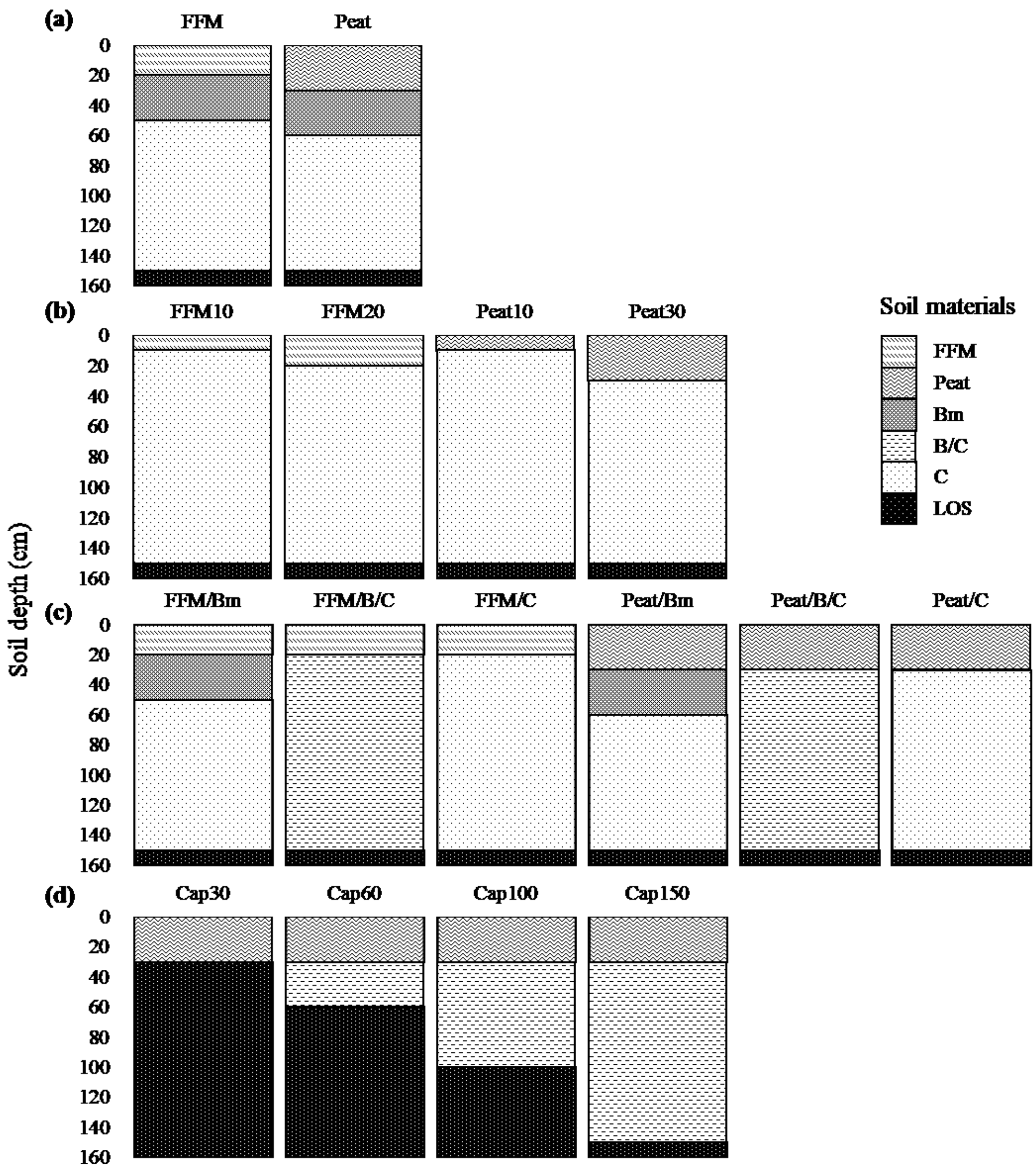

Figure 2-2: Capping treatments from the Aurora Soil Capping Study (ASCS) used for comparisons of (a) topsoils (FFM vs Peat), (b) topsoil application thickness (FFM10 vs FFM20 vs Peat10 vs Peat30), (c) topsoil (FFM, Peat) by subsoil (Bm, B/C, C) and (d) total capping thickness (Cap30 vs Cap60 vs Cap100 vs Cap150).

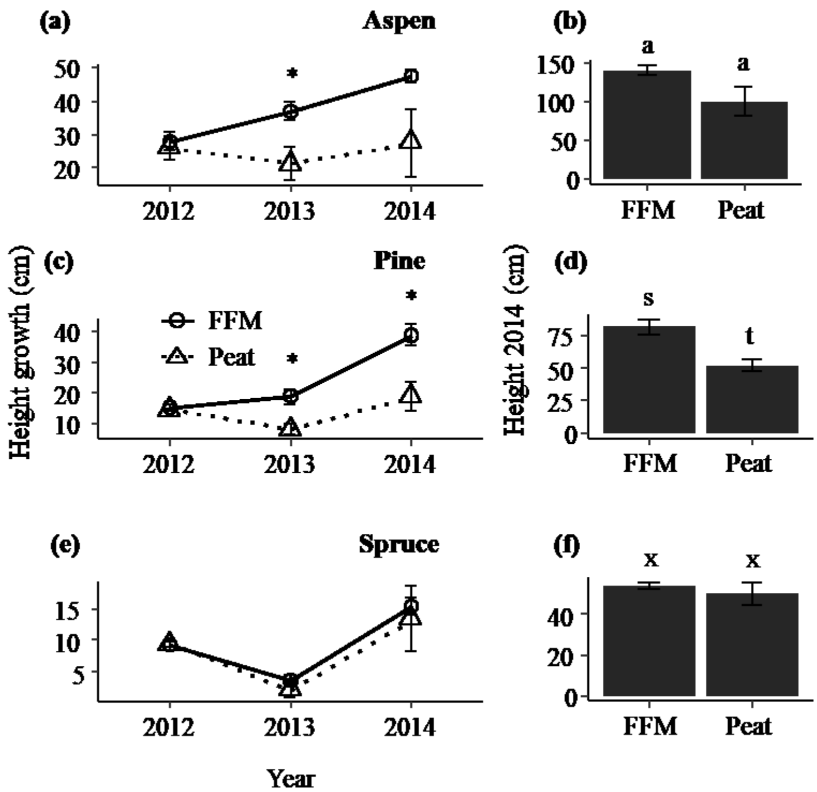

Figure 2-3: Annual height growth (2012-2014) of (a) aspen, (c) pine and (e) spruce and final heights in 2014 of (b) aspen, (d) pine and (f) spruce in response to topsoil materials (forest floor material (FFM) and Peat (Peat)). Error bars are one standard error of the mean (± SEM) (n = 3). Asterisks represent significant topsoil effects within year and species. Different letters represent statistically significant differences among treatment means within each species (α = 0.1).

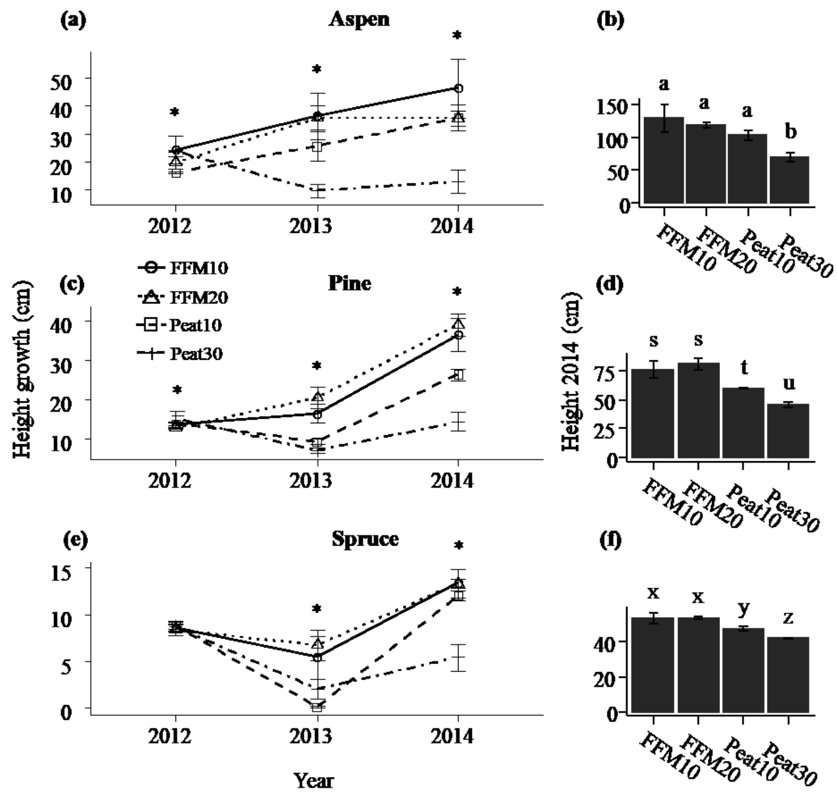

Figure 2-4: Annual height growth (2012 – 2014) of (a) aspen, (c) pine and (e) spruce and final heights in 2014 of (b) aspen, (d) pine and (f) spruce in response to the treatments FFM10, FFM20, Peat10 and Peat30. Error bars are one standard error of the mean (± SEM) (n = 3). Asterisks represent significant treatment effects within year and species. Different letters represent statistically significant differences among treatment means within each species (α = 0.1).

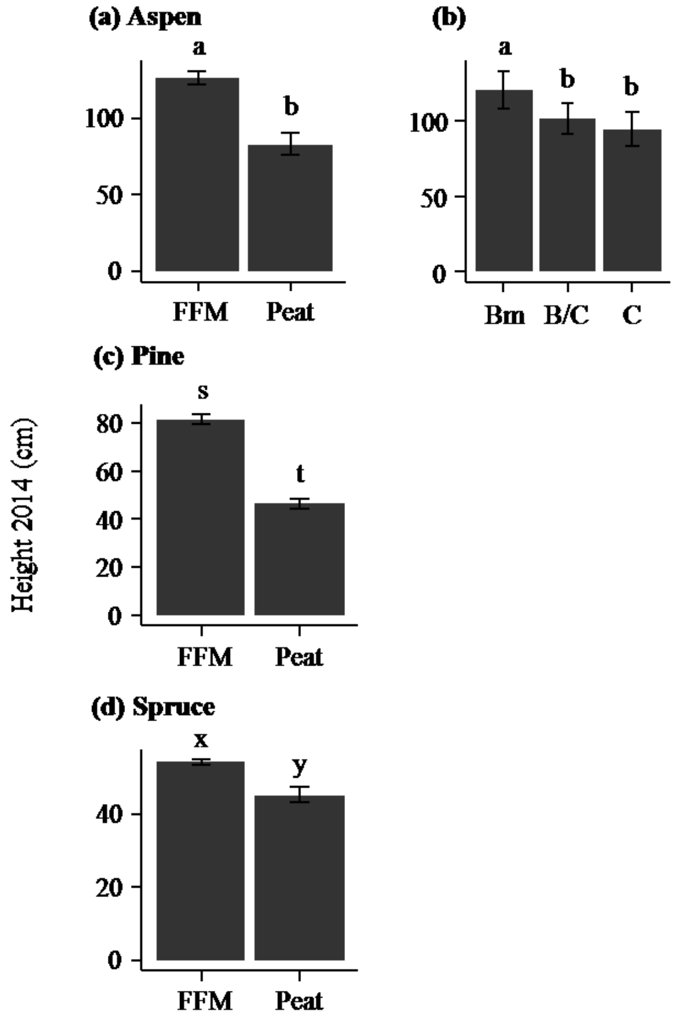

Figure 2-5: Final heights of (a) aspen, (c) pine and (d) spruce in response to topsoil materials (FFM and peat) (n = 9) and (b) aspen in response to subsoil materials (Bm, B/C and C) (n = 6) in 2014. Error bars are one standard error of the mean (± SEM). Different letters represent statistically significant differences among treatment means within each species ($\alpha = 0.1$).

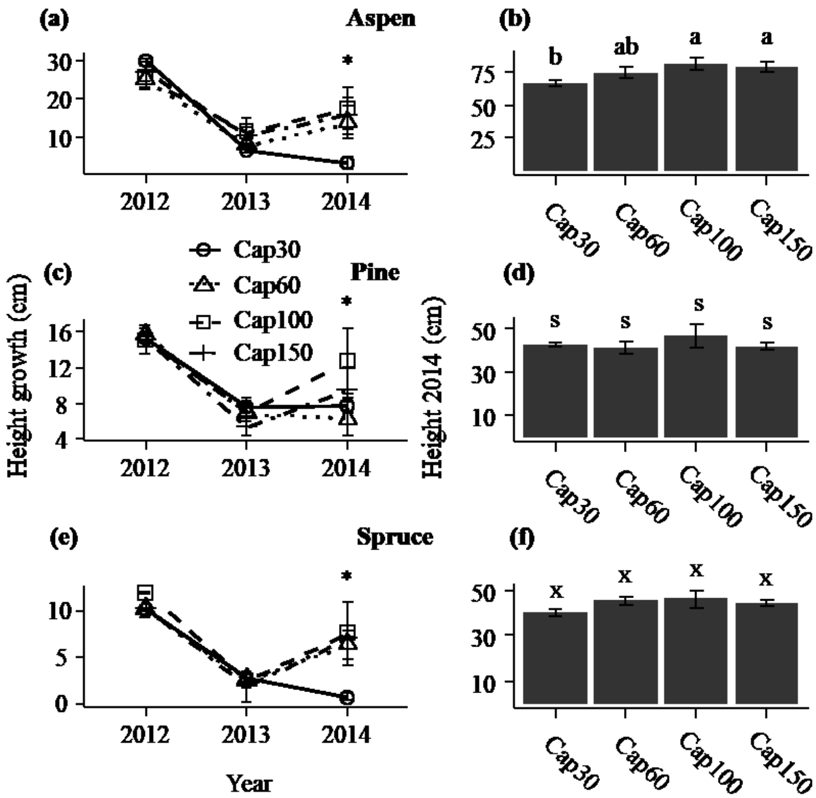

Figure 2-6: Annual height growth (2012 – 2014) of (a) aspen, (c) pine and (e) spruce and final heights in 2014 of (b) aspen, (d) pine and (f) spruce in response to total capping depth Cap30, Cap60, Cap100 and Cap150. Error bars are one standard error of the mean (± SEM) (n = 3). Asterisks represent significant treatment effects within year and species. Letters represent statistically significant differences among treatment means within each species (α = 0.1).

Chapter 3 - Aspen seedling establishment on reclamation sites lacking topsoil cover – impacts of biochar and fertilizer amendments

3.1 Introduction

Surface mining for resources such as minerals, bitumen and coal creates large areas where all vegetation, soil, and geological materials (overburden) are removed to access the resource. After surface mining is completed, reclamation of these areas takes place to create a landscape which can be used by humans (agricultural or recreational land) or wildlife (wildland areas). After constructing the landforms with overburden material, reclamation practices typically involve the placement of salvaged surface or topsoil materials that supply essential resources such as water and nutrients and support plant growth. This soil material is often salvaged from areas that are prospected for further resource extraction, and it is either directly placed on areas to be reclaimed, or stored for future use. However, once the resources are exhausted and mining activities are to be terminated, there may be a shortage of these topsoil materials, and thus final reclamation operations must rely on less suitable, unconsolidated mineral soil materials. Unconsolidated mineral soil material, unlike topsoil, contains little to no soil organic matter (SOM), which plays an important role in nutrient cycling and water storage (Berg and Laskowski 2005). As a result, regenerating plants may experience severe nutrient and water limitations (Larchevêque et al. 2014), which can seriously restrict their establishment and early growth performance and therefore hinder the development of a vegetation cover. Mining areas that are located in an agricultural setting often use the salvaged topsoils to reclaim agricultural fields and pasture. Areas that lack these topsoils are considered marginal and are often slated for forest reclamation.

One measure to allow for forest establishment on these marginal sites is to select appropriate tree species and nursery stock types that may withstand site limiting conditions. In Alberta, prime agricultural areas are located in the prairie-boreal forest ecotone (Parkland) where trembling aspen (*Populus tremuloides* Michx.) dominates the forested landscapes (Natural Regions Committee 2006). Aspen has become a widely used tree species in afforestation and reclamation of disturbed sites in Alberta because it is a relatively drought tolerant native tree species, and can provide early crown closure due to its rapid height and lateral shoot growth

(McDonough 1979; Swanson et al. 2010; Schott 2013). Aspen seedling stock characteristics have been identified that result in improved establishment under stressful conditions (Landhäusser et al. 2012; Kulbaba 2014). Further, aspen's ability to produce suckers from a large root system (Peterson and Peterson 1992; Frey et al. 2003; Fitzgerald 2010) provides increased resiliency to these newly developing forests (Macdonald et al. 2012, 2015).

Another measures to overcome these soil limitations is the use of soil amendments that can be applied or incorporated into the surface soil using a range of organic (e.g. straw, sawdust, compost, sludge, manure) or inorganic (e.g. sand, vermiculite, perlite, crumb rubber, fertilizer) materials. Depending on the soil amendment and its properties, the soils physical (e.g., water holding capacity and soil structure) (Gruenthal 1999) and chemical properties can be affected (Land Resource Network and Land Resource Network Ltd. 1993; Larney and Angers 2012).

An organic material currently attracting a lot of attention is pyrolysed biomass, which, when used to alter soil quality, is referred to as biochar. Biochar has been shown to increase agricultural yields on marginal soils by increasing soil moisture and nutrient retention while reducing leaching (Asai et al. 2009; Ding et al. 2010; Kammann et al. 2011; Jones et al. 2012; Mulcahy et al. 2013). In recent years, biochar has been increasingly used in reclamation settings, where it is mainly applied for the restoration of contaminated soils with heavy metals and organic and inorganic pollutants (Beesley et al. 2011; Fellet et al. 2011; Sizmur et al. 2015; Lwin et al. 2018). Depending on the feedstock material (e.g. wood, straw, chicken manure) and pyrolysis process (temperature and duration of pyrolysis) (Mukherjee et al. 2011), biochar has a very large surface area due to its porous structure (Beesley et al. 2011; Sizmur et al. 2015). These pores can be infiltrated by water, which can then be slowly released back into the surrounding soil material decreasing evapotranspiration (Tryon 1948; Scott et al. 2014) and, in turn, increasing water holding capacity (Chan et al. 2007; Karhu et al. 2011) and water availability for plants (Tryon 1948). Its large surface area and negative charge (Mukherjee et al. 2011; Sizmur et al. 2015) can further function as an interface for cation exchange and thus improve the cation exchange capacity (CEC), thereby reducing the leaching of plant available soil nutrients such as K^+ and NH_4^+ (Sika and Hardie 2014). Particularly in combination with fertilization, the use of biochar is thought to prolong nutrient availability (Atkinson et al. 2010), suggesting that this combination might be a suitable amendment that could improve nutrient availability in these unconsolidated mineral subsoil materials.

The objectives of this field experiment were to test the effects of (i) biochar on survival, growth, and allocation of planted aspen *(Populus tremuloides* Michx.) seedlings with and without a mineral fertilizer addition, and (ii) to evaluate how these applications affect water and nutrient availability for the planted aspen. The results of this study will be used to improve best management practices.

Based on the evidence in the literature, my underlying hypotheses were:

(1) A soil amendment of biochar will improve soil water availability and thus aspen seedling survival and growth, and

(2) In combination with the addition of a fertilizer, biochar will improve soil nutrient availability and aspen seedling growth further.

3.2 Methodology

3.2.1 Site description

The study site is located at the Whitewood coal mine (53° 33’ N, 114° 29’ W), near the town of Wabamun, Alberta, approximately 70 km west of Edmonton. Strip mining for coal occurred from 1962 to 2010, and reclamation began in the late 1980s with the East Pit Lake (Brocke and Chymco 1991) and has occurred progressively across the 1900 ha of mined land (Hudson 2013). Reclamation included the return to both agriculture and wildland areas, with the research site located within the wildlands area of the mine, which has a lack of suitable topsoil as well as abundant erosion. The study area is located in the Dry Mixedwood natural subregion of the boreal forest, with trembling aspen (*Populus tremuloides* Michx.), jack pine (*Pinus banksiana* Lamb.) and white spruce (*Picea glauca* (Moench) Voss.) in pure or mixed stand forests (Natural Regions Committee 2006).

The upland soils in these aspen forests of this subregion are typically Orthic Gray Luvisols, which have eluvial Ae and Bt horizons that develop when the mean annual soil temperature is < 8°C. Dark Gray Luvisols are found in the cultivated areas of this subregion, and have eluvial features combined with an Ah and Ahe horizon of ≥ 5 cm in thickness (Soil Classification Working Group 1998; Natural Regions Committee 2006). No topsoil was available for reclamation of this study area and as a result the surface soil material within the study area was composed of unconsolidated mineral subsoil material with pH of 8.1, total nitrogen (N) of 12.14 mg kg^{-1}, nitrate (NO_3^-) of 10.7 mg kg^{-1} and phosphorus (P) of 0.58 mg kg^{-1} (Table 3-1; Liu 2014). According to the “Soil Quality Criteria relative to Disturbance and Reclamation” (Soil Quality Working Group 2004) the texture (sandy loam) and pH are considered “fair”; while the nutrient contents of total nitrogen, nitrate and phosphorus, and the overall nutrient supply was considered “very low” (Hazelton and Murphy 2016b). Competition by ground vegetation was low (< 10% ground cover) across all blocks and treatments and consisted mainly of early successional, introduced species, which are common for early reclamation sites (Wilson 2016).

The climate in the Dry Mixedwood natural subregion is characterized by cold winters and short growing seasons (May – September) of about 98 frost free days (Natural Regions Committee 2006). The climate normal during the growing season (May – September) for this region (1981-2010) is 13.9°C mean air temperature and 343.4 mm total precipitation (Government of Canada 2018). Air temperature for the 2012 through 2014 growing season was 14.8, 14.8 and 14.1°C and total accumulated precipitation was 368, 242 and 252 mm, respectively (weather station: Edmonton Stony Plain CS; 53°32'50.080" N, 114°06'30.070" W).

3.2.2 Experimental design

A complete block design was used to setup the study on approximately 2.5 ha of land at the Whitewood mine, creating five blocks (each 0.5 ha); each block included all treatments (Fig. 3-1). One-year old trembling aspen stock with container plug dimensions of 6 cm diameter and 15 cm depth (615A Styroblock™; 340 ml) (Beaver Plastics Ltd, Acheson, Alberta) (Table 3-2) were commercially grown at the Smoky Lake Forest Nursery Ltd. (Smoky Lake, Alberta, Canada) using operational protocols described in Landhäusser et al. (2012). The seedlings had an average initial height of 28 (± 1.5 SD) cm, and were hand-planted at regular 1.3 x 1.3 m spacing throughout the research blocks at a density equivalent to 7700 stems ha^{-1} in May 2012. Two different soil amendments were used in this study: biochar and a slow release fertilizer. The biochar was derived from lodgepole pine (*Pinus contorta* Dougl.) biomass and was produced by a slow pyrolysis process (two hours at 400-500ºC), had a pH of 7.3, total organic carbon of 56%, total organic nitrogen of 1.3 %, and a bulk density of 232 kg m^{-3} (Table 3-1). The biochar was applied at two levels: no biochar and 1670 kg ha^{-1} (0.54 % by volume). Biochar was spread manually in October 2011 and was disked into the soil to a depth of 20 cm, using a tractor with a disk harrow (Liu 2014). Polyon® nursery blend 19-6-13 (N-P-K) plus minors controlled-release fertilizer (Agrium Advanced Technologies Direct Solutions, Calgary, Canada) with an 8-9 month nutrient release period was used for the fertilizer amendment. The fertilizer contained 19 % total nitrogen from ammonium, nitrate and urea nitrogen, 6 % phosphoric acid, 13 % soluble potash, 5 % sulphur and ≤ 1 % iron, manganese, molybdenum, zinc and copper. Application of fertilizer occurred in June 2012 using a manual seed spreader. The fertilizer was applied at three levels: no fertilizer, fertilizer at an equivalent of 50 kg N ha^{-1} (low), and fertilizer at an equivalent of 100 kg N ha^{-1} (high). To limit the potential of fertilizer contamination of the unfertilized plots from soil leaching and run-off, we applied fertilizer to the treatment plots located downslope. Together these resulted in six treatment combinations: an unamended control (C), biochar only (B), fertilizer only (low rate equiv. 50 kg N ha^{-1}; FL), fertilizer only (high rate equiv. 100 kg N ha^{-1}; FH), combined biochar and low rate fertilizer (BFL) and combined biochar and high rate fertilizer (BFH) (Fig. 3-1).

3.2.3 Soil water content and plant available soil nutrients

To determine if biochar affected soil moisture conditions, volumetric soil water content (VWC) was measured at a soil depth of 10 and 30 cm using 5TM sensors connected to EM50 data loggers (Decagon Devices Inc., Pullman, Washington, USA). One 5TM sensor per each depth (10 and 30 cm) was installed in the biochar and the no biochar treatments of each block (Fig 3-1). VWC was recorded in two-hour intervals and then averaged for each day for the years 2013 and 2014. The daily means from May through September were then averaged to determine the average growing season VWC at 10 and 30 cm soil depth. Due to some sensor malfunctions at the 10 cm soil depth, only three replications (n = 3) could be used for VWC analysis at this depth, while a replication of five (n = 5) was used for the VWC at 30 cm soil depth.

To estimate the effect of biochar and fertilizer on plant available soil nutrients, Plant-Root-Simulator (PRS™) - probes were installed in each tree measurement plot. One pair of PRS™ - probes (one anions probe and one cations probe) were buried on July 12th, 2013 to a depth of 15 cm in every corner of the tree measurement plots (described below: "Mortality and seedling growth"). They were recovered on September 4th, 2013 after an eight-week burial period. The PRS™ - probes were cleaned with de-ionized water, placed in plastic bags and sent to Western Ag Innovations (Saskatoon, Canada) for analyses. PRS™ - probes capture a range of ions, but we were mostly interested in nitrogen (nitrate (NO_3^-) and ammonium (NH_4^+)), phosphate ($H_2PO_4^-$), potassium (K^+) and sulphate (SO_4^{2-}) (2010 Western Ag, Saskatoon, Canada). Nitrate and ammonium were measured colorimetrically by an automated flow injection analysis system using the Lachat QuickChem AE Automated Flow Injection Ion Analyzer (United States Environmental Protection Agency, 1991). Nitrate and ammonium were combined to calculate total nitrogen (N). All other nutrients were measured by inductively coupled plasma spectrometry (ICP-OES, Thermo Fisher, USA).

3.2.4 Mortality and seedling growth

In the center of each treatment plot a tree measurement plot (10 × 10 m) was established, which contained 64 aspen seedlings each (8 x 8 seedlings) at the start of the study (64 subsamples). Between 2012 and 2014, seedling height and root collar diameter (RCD) were measured at the end of each growing season (August) and averaged per treatment plot. RCD was measured to the nearest 0.1 mm and used in 2013 to identify representative seedlings per treatment for biomass harvest (data not presented) (see below: "Biomass, leaf area and biomass allocation"). Seedling height was measured in a pre-determined pattern identical for each tree measurement plot, in order to calculate individual seedling height growth. Height growth was calculated by subtracting the last year's height from the current year's height. To determine seedling mortality, all dead or missing seedlings were counted within the tree measurement plots.

3.2.5 Biomass, leaf area and biomass allocation

To determine above and below ground biomass and biomass allocation of the aspen seedlings after two growing seasons, seedlings were harvested in August/September 2013 and May 2014. To identify representative seedlings in each treatment, RCD was measured in the tree measurement plots in 2013 and averaged. Three seedlings (subsamples) with that average RCD were then selected in each treatment plot, but outside of the tree measurement plots, and excavated carefully to capture the majority of the root system. In total, 90 seedlings were excavated (three per treatment plot). The aboveground part (stem and leaves) of the seedlings was harvested in August/September 2013 and root systems were excavated in May 2014. The excavated seedlings were cold stored in the field and frozen until processing. During processing, roots, stems and leaves were separated, and roots were carefully washed to remove the attached soil. The stems and roots were then dried at 70°C to constant weight. To determine seedling leaf area, all leaves from the harvested trees were separated from the stem and scanned with a LI-3100 Area Meter (LI-Cor Inc., Nebraska, USA). All leaves were then dried at 70°C and weighed. To explore changes in biomass allocation between organs in response to treatments, leaf mass ratio (LMR), stem mass ratio (SMR) and root mass ratio (RMR) were calculated by dividing leaf, stem, or root dry mass by the total dry mass for each individual seedling.

3.2.6 Foliar nutrient analysis

Leaf samples for foliar nutrient analysis were collected in September 2013 within the tree measurement plots. To avoid spatial differences in the tree measurement plots, leaf samples were collected from every second seedling in every second row across the tree measurement plots (16 seedlings in total). Leaves (3-4 per seedling) were carefully removed to minimize damage to the seedling, placed in a cooler for transport and subsequently stored at -15°C until they were processed. Samples were dried at 70°C to constant weight, ground with a Wiley mill (Thomas Scientific, New Jersey, USA) to 40 mesh (0.4 mm), and sent for nitrogen (N) and phosphorus (P) analysis to a commercial laboratory (Natural Resources Analytical Laboratory (NRAL), University of Alberta). N concentration (%) was measured by combustion and P concentration ($\mu g\ g^{-1}$) was measured by microwave digestion (using HNO_3) followed by inductively coupled plasma optical emission spectrometry (ICP-OES). P was subsequently converted into per cent concentration (%). Concentrations of N and P were used to calculate the N:P ratios per treatment plot.

3.2.7 Data analysis

All analyses used linear mixed effect models with block as a random factor with the "lme" function from the "nlme" package in RStudio (version 3.0.2, The R Foundation for Statistical Computing, 2013). To test for possible year by fertilizer, year by biochar, or year by fertilizer by biochar interactions, annual seedling height growth was analyzed as repeated measure three-way ANOVA. The treatment variable fertilizer had three levels (0, 50, 100 kg N ha^{-1}), the treatment variable biochar had two levels (biochar and no biochar) and measurement year had three levels (2012, 2013 and 2014). The model was y (seedling height growth) = fertilizer * biochar * year, with block as random factor (error) and year as the repeated variable. Volumetric soil water content (VWC) was analyzed as a two-way ANOVA between year (2013, 2014) and biochar with two levels (biochar and no biochar) to test for a possible year by biochar interaction. All other response variables (final height, RCD, biomass, biomass fractions, foliar nutrient concentrations and ratios, mortality and plant available soil nutrients) were analyzed using two-way ANOVAS with the factor biochar with two levels (biochar and no biochar) and the factor fertilizer with three levels (0, 50, 100 kg N ha^{-1}), to test for a possible biochar by fertilizer interaction. Prior to each statistical analysis, normality of data and homogeneity of variances were tested on residuals using Shapiro-Wilk and Levene's test using the package "car". If main effects were significant in the ANOVA ($p = 0.05$), Fisher's LSD tests were performed using the package "agricolea". A significance level of $\alpha = 0.05$ was chosen with five replications ($n = 5$) (VWC at 30 cm soil depth, final height, RCD, biomass, biomass fractions, foliar nutrient concentrations and ratios, mortality and plant available soil nutrients) and with three replications ($n = 3$) (VWC at 10 cm soil depth).

3.3 Results

3.3.1 Volumetric soil water content and available soil nutrients

Volumetric water content (VWC) at 10 cm (21%) and 30 cm (23%) soil depth was similar across all treatments and years (all $p > 0.07$), no biochar effect was detected (all $p > 0.07$).

Only plant available nitrate was higher in treatments without biochar application (biochar effect: $p = 0.03$; Fig. 3-2), but it was not affected by fertilization (fertilizer effect: $p = 0.51$) or the combination of both soil amendments (fertilizer by biochar interaction: $p = 0.94$). All other tested soil nutrients (total nitrogen, ammonium, phosphate, potassium and sulphate) were similar across all treatments (all $p > 0.06$).

3.3.2 Seedling responses

After three growing seasons (2012-2014), mortality was 16% across all treatments. Biochar, fertilization or the combination of both had no effect on seedling mortality (all $p > 0.08$). Root collar diameter (RCD) was similar across all treatments with 9.8 mm (all $p > 0.12$).

Annual height growth of the seedlings was similar in 2012 and 2014 (14 and 15 cm, respectively) across all treatments; however, in 2013, aspen seedling height growth was less than 2012 and 2014 with an average of 11 cm (year effect: $p = 0.005$; Fig. 3-3). Seedling height growth was greater in the treatments with a high fertilizer application rate (15 cm) than in the no fertilizer treatment (11 cm), while height growth in the low fertilizer treatment (13 cm) was similar to the high and no fertilizer treatment (fertilizer effect: $p = 0.009$; Fig. 3-4). Biochar had no effect on seedling height growth ($p = 0.53$) across all years. After three growing seasons (2012-2014), aspen seedlings were taller in treatments with the high fertilizer application (77 cm) than in the control (63 cm), while seedling height in the low fertilizer treatment was similar to the high and no fertilizer treatments (fertilizer effect: $p = 0.01$; Fig. 3-5). Biochar had no effect on seedling height after three growing seasons (biochar effect: $p = 0.65$).

Total biomass, stem, root and leaf mass, as well as leaf area, RMR and LMR of aspen seedlings after two growing seasons (2013) were similar across all treatments. Biochar, fertilization or the combination of both had no effect on those seedling traits (all $p > 0.06$). Only SMR was higher in seedlings that grew in treatments that had biochar added (biochar effect: $p =$

0.03; Table 3-2) and RSR was higher in treatments without biochar (biochar effect: $p = 0.04$; Table 3-2). Fertilization had no effect on SMR or RSR (all $p > 0.57$).

Foliar N and P concentrations and the N:P ratio (18) were not different among all treatments (all $p > 0.53$). Foliar nitrogen (N) concentration was 1.9 % (± 0.2) and phosphorus (P) concentration was 0.10 % (± 0.01) in 2013 averaged across all treatments.

3.4 Discussion

I had hypothesized that amending a surface soil material composed of unconsolidated mineral subsoil material with biochar during mine reclamation would increase water and nutrient availability and thereby improve seedling survival and growth (first hypothesis). This hypothesis was not supported by my findings. The addition of biochar did not increase soil water content in 10 or 30 cm soil depth. This was somewhat surprising, as other studies have found strong positive evidence of biochar on soil water holding capacity (Gaskin et al. 2007; Ouyang et al. 2013; Raave et al. 2014; Prober et al. 2014). For example Prober et al. (2014) found a 6 – 25 % increase in VWC when a green-waste biochar was applied to a clay loam at 20,000 kg ha^{-1}. Ouyang et al. (2013) added 2 % (by weight) of a dairy manure biochar to different soil types and found a 5.2 % increase in water content for a silty clay and a 10.6 % increase for a sandy loam. The reclamation substrate in this study had a clay loam texture. These findings suggest that the effect of added biochar on soil hydrological properties depend on soil texture, as well as the type and amount of biochar used (Lewis et al. 2006; Mulcahy et al. 2013; Dan et al. 2015; Omondi et al. 2016; Teβin 2016; Li et al. 2018). However, the difference of VWC at 30 cm soil depth between the biochar and no biochar treatments was close to be statistically significant ($p = 0.07$) and this difference resulted in soils being on average below the wilting point in the no biochar treatments (21%), but above the wilting point in the biochar amended treatments (24%) during the 2013 and 2014 growing season. According to Saxton and Rawls (2006) the wilting point for the clay-loam soil texture is around 22 % VWC. Further, the addition of biochar had a marginal positive effect on seedling survival ($p = 0.08$). Seedling mortality was 18 % in plots without biochar, but only 14 % in plots with biochar. It is possible that the somewhat higher water content in the biochar treatments was responsible for this marginal decrease in seedling mortality. This leads me to the assumption that the quantity of biochar added during this experiment (1670 kg ha^{-1}) was not large enough to see conclusive improvements in VWC and seedling survival.

Further, the biochar subsoil amendment in this study did not lead to an increase in soil nutrient availability. In fact, nitrate availability was two times lower in treatments with biochar (Fig. 3-2). The addition of biochar has been shown to increase the cation exchange capacity (CEC) of the soil and thus increase the retention of nutrients (Sohi et al. 2010; Fellet et al. 2011; Scott et al. 2014). This is generally attributed to biochar's large surface area, high porosity and negative charge (Atkinson et al. 2010; Mukherjee et al. 2011; Sizmur et al. 2015). Raave et al. (2014) tested the effect of biochar on nitrogen leaching from a sandy loam soil and found that leaching of nitrate, but not ammonium, was reduced. In a similar experiment with a sandy soil, Sika and Hardie (2014) also found that biochar-amended soils contained less exchangeable nitrate than the control soils without biochar. They argued that the inorganic nitrogen may have been physically absorbed by the biochar, assimilated by microbes or lost in gaseous form (Sika and Hardie 2014).

The addition of biochar did not increase seedling growth, height or RCD after three growing seasons. Accordingly, leaf, shoot and root biomass, as well as leaf area, RMR and LMR were not affected by the addition of biochar. However, SMR and RSR were both affected by biochar with seedlings allocating more to shoot mass than to root mass in the biochar treatments. Pluchon et al. (2014) found similar effects of biochar on white birch (*Betula pubescens* Ehrh.) and scots pine (*Pinus sylvestris* L.), which had lower root-to-shoot ratios with the addition of biochar at 3000 kg ha^{-1}. Not all of the nine biochars examined in this study (derived from different boreal woody species), however, generated such effects. In addition, Wardle et al. (1998) found that root-to-shoot ratios of silver birch (*Betula pubescens* Ehrh.) and scots pine decreased with addition of biochar at 2000 kg ha^{-1}, but only in one of the three humus substrates used, which all differed in origin and soil fertility. This suggests that the effect of biochar on biomass partitioning depends highly on biochar's origin and soil material used. For instance, Jeffery et al. (2011) found a significant increase in crop productivity when biochar was added to coarse and medium textured soil, but not to fine textured soil.

I had hypothesized further that biochar in combination with the addition of a slow-release fertilizer, will improve soil nutrient availability and positively affect aspen seedling growth (second hypothesis). This hypothesis was not confirmed, as the addition of biochar alone and biochar in combination with fertilizer did not increase aspen seedling growth or height, nor did it change foliar nutrient concentrations or the N:P ratio in foliage. According to van den Burg (1990) the foliar N concentration (1.9 %) of aspen seedlings in 2013 indicate sufficient supply with nitrogen, while the foliar P concentration (0.10 %) indicates a slight phosphorus deficiency across all treatments. Also the N:P ratio (18) of aspen foliage indicates a phosphorus shortage according to Koerselman and Meuleman (1996).

Only the greater application of fertilizer led to a significant increase in seedling height growth after three growing seasons, even though measured available soil nutrient in 2013 were similar across all treatments. However, Liu (2014), who tested the efficacy of biochar on soil microbial biomass, soil respiration and heavy metal adsorption in this field study, found that total nitrogen was three to four times higher, while nitrate was eleven to fourteen times higher in FL and FH treatments in 2012, just after the fertilizer application. This is somewhat surprising, as other studies found a carry-over effect of a controlled release fertilizer up to three years (Fan et al. 2002; Moore et al. 2002; Sloan and Jacobs 2013). This lack of a second year fertilizer effect was likely due to the low vegetation cover (< 10%), strong wind and water erosion (personal observation), as well as the fact that the fertilizer had not been incorporated into the soil after broadcasting. Many studies evaluating the growth of planted tree seedlings in response to different fertilizers and application rates found that seedlings grew best with the highest fertilizer applications (Walker and del Moral 2002; DesRochers et al. 2003; van den Driessche et al. 2003, 2005; Walker 2012; Sloan and Jacobs 2013). Especially on poor substrates, like the unconsolidated mineral subsoil material used in this study that had very low total nitrogen, nitrate and phosphorus levels (Hazelton and Murphy 2016b), planted tree seedlings normally respond positively to fertilizer amendments (Walker 2002, 2008, 2012).

Fertilizer appears to be the main driver for the increased seedling growth observed this early on in our study, particularly on this subsoil material. Intra-specific competition effects often observed with fertilization of forest reclamation sites (Schott et al. 2016; Sloan and Jacobs 2013), did not play a role here, likely due to the overall very poor soil nutrient conditions (Wilson 2016). Biochar did not have the desired effect on seedling height growth; however, biochar likely affected soil moisture positively and thus might have reduced seedling mortality. In this study, a relatively low amount of biochar (1670 kg ha^{-1}) was applied to the soil. This amount is comparable to naturally occurring quantities of charcoal in boreal forest soils from past fire events that can range anywhere from 300 to 6000 kg ha^{-1} (Zackrisson et al. 1996; Bélanger et al. 2004; MacKenzie et al. 2008; Soucémarianadin et al. 2015). Agricultural and remediation studies have applied biochar at rates between 8000 (Asai et al. 2009) and 200,000 kg ha^{-1} (Kammann et al. 2011). I suspect, that if we had used more biochar for this study, the marginal effects of biochar alone and biochar in combination with fertilizer would have been more pronounced. However, Kammann et al. (2011) found that a high biochar application rate of 200,000 kg ha^{-1} did not improve plant growth of *Chenopodium quinoa* Willd. (pseudo-cereal) in a greenhouse study compared to a lower application of 100,000 kg ha^{-1}. This indicates that there may be an upper limit at which biochar is beneficial for improving seedling establishment and growth.

3.5 Tables

Table 3-1: Soil characteristics (mean (SD)) of subsoil material found at Whitewood mine, adapted from Liu 2015.

	(%)				(mg kg^{-1})		(kg m^{-3})	
Substrate	TON	TOC	pH	NO_3^-	NH_4^+	P	BD	Texture
unconsolidated geological subsoil material	0.05 (0.02)*	2.4 (1.4)	8.1	10.7	1.34	0.58	n/a	clay loam
Biochar	1.3	56	7.3	n/a	n/a	n/a	232	n/a

TON = total organic nitrogen; TOC = total organic carbon; NO_3^- = nitrate; NH_4^+ = ammonium; P = phosphorus; BD = dry bulk density; n/a = data not available.

*Standard deviation only available for TON and TOC

Table 3-2: Shoot mass ratio (SMR) and root to shoot ratio (RSR) (mean (SD)) of aspen seedlings in the biochar and no biochar treatments. Letters indicate statically significant differences (α = 0.05) (n = 5).

Treatment	SMR	RSR
Biochar	0.34 (0.03) **a**	1.51 (0.20) **b**
No biochar	0.32 (0.03) **b**	1.69 (0.24) **a**

3.6 Figures

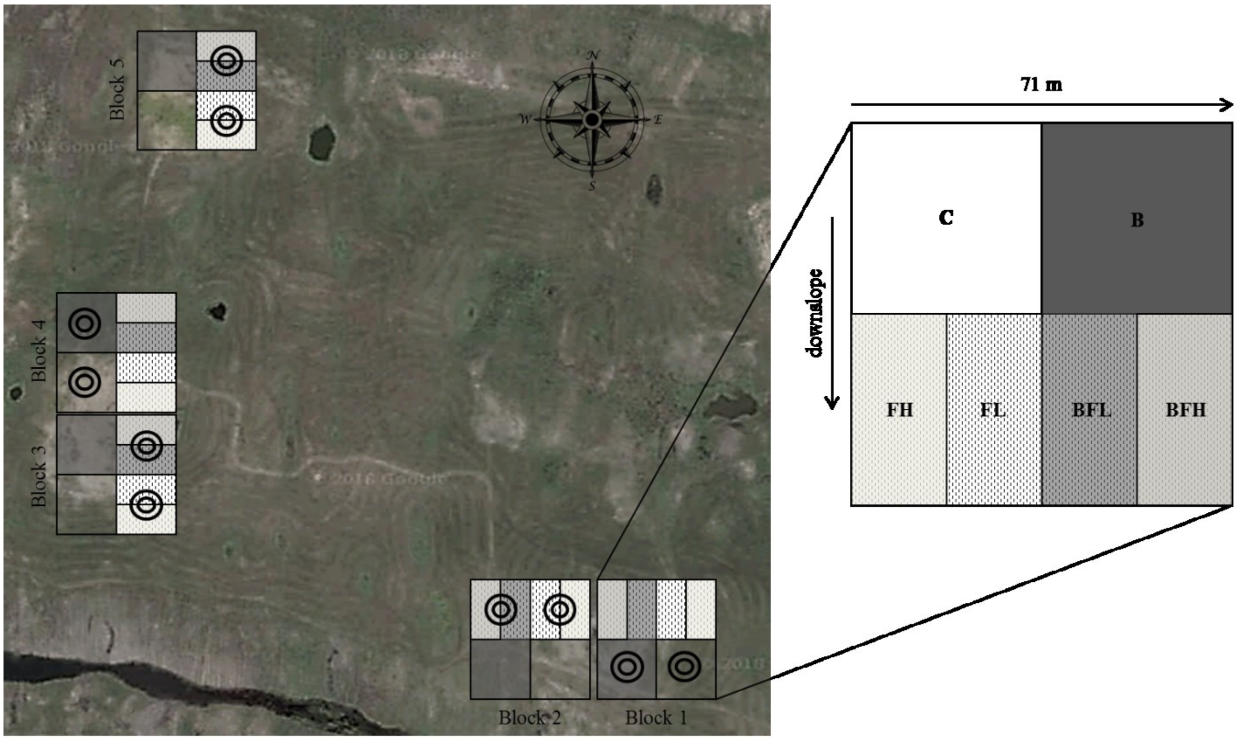

Figure 3-1: Location and orientation of research blocks (1-5) within the Whitewood coal mine. Circles represent the location of the EM50 data loggers (Decagon Devices Inc., Pullman, Washington, USA) with 5TM sensors at 10 and 30 cm soil depth. The background picture was adjusted from Google Earth (July 2018). The figure to the right represents the experimental layout for one of the five blocks (0.5 ha). The treatments are unamended control (C), biochar only (B), fertilizer only (low rate equiv. 50 kg N ha^{-1}; FL), fertilizer only (high rate equiv. 100 kg N ha^{-1}; FH), combined biochar and low rate fertilizer (BFL) and combined biochar and high rate fertilizer (BFH).

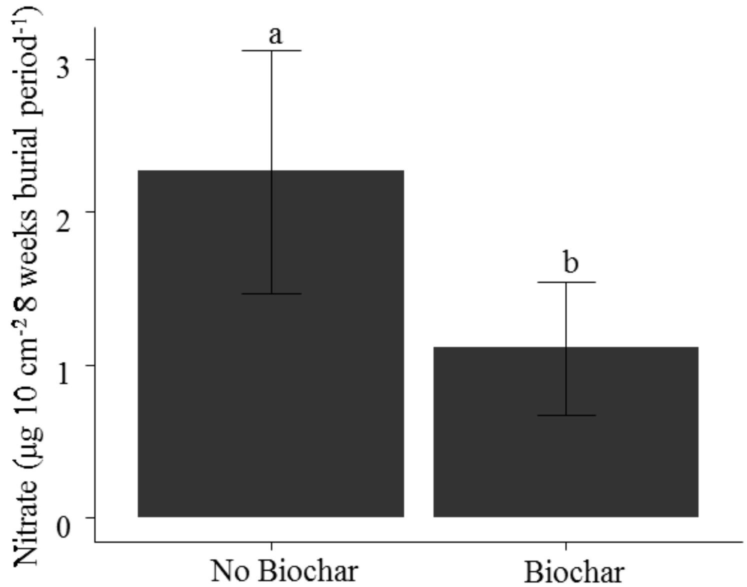

Figure 3-2: Plant available nitrate (µg 10 cm^{-2} 8 weeks burial $period^{-1}$) for the treatments with and without biochar. Error bars are one standard error of the mean (± 1 SEM) (n = 5). Letters indicate significant treatment effects (α = 0.05).

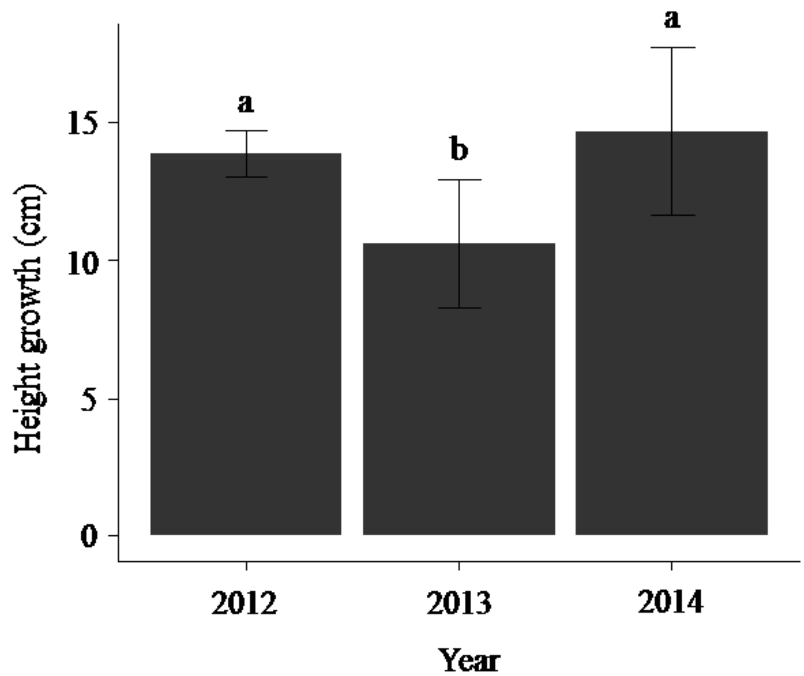

Figure 3-3: Aspen height growth (cm) between 2012 and 2014 across all treatments. Error bars are one standard error of the mean (± 1 SEM) (n = 5). Letters indicate significant treatment effects (α = 0.05).

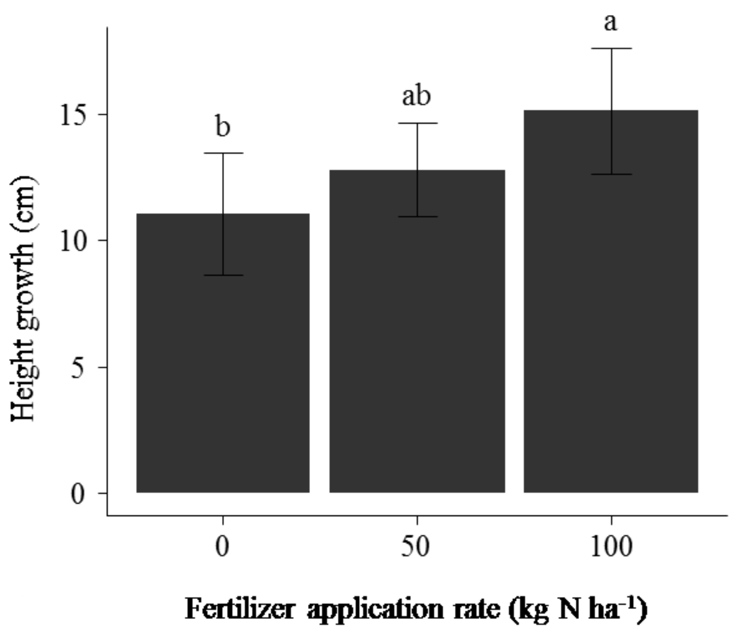

Figure 3-4: Aspen height growth (cm) between 2012 and 2014 for the treatments without fertilizer, fertilizer only (low rate equiv. 50 kg N ha^{-1}), fertilizer only (high rate equiv. 100 kg N ha^{-1}). Error bars are one standard error of the mean (± 1 SEM) (n = 5). Letters indicate significant treatment effects ($\alpha = 0.05$).

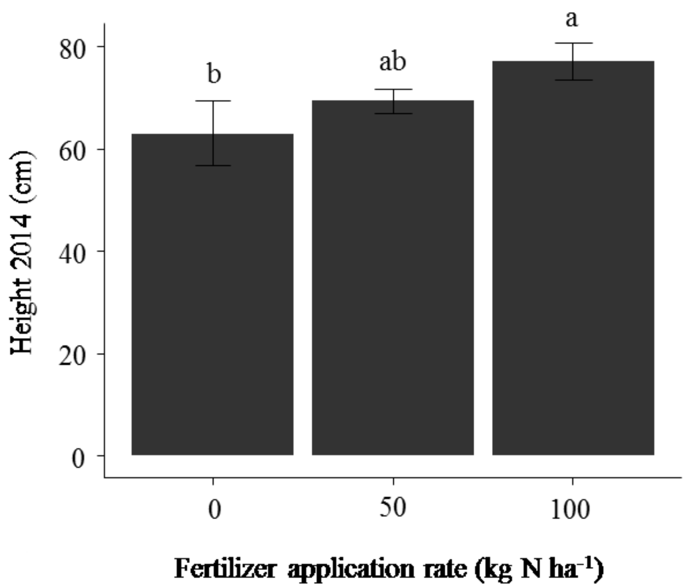

Figure 3-5: Aspen height in 2014 for the treatments without fertilizer, fertilizer only (low rate equiv. 50 kg N ha^{-1}), fertilizer only (high rate equiv. 100 kg N ha^{-1}). Error bars are one standard error of the mean (± 1 SEM) (n = 5). Letters indicate significant treatment effects (α = 0.05).

Chapter 4 - General Discussion and Conclusions

4.1 Research summary

The main objective of my thesis was to explore the effects of different reclamation substrates, placement techniques and soil amendments on seedling development and edaphic properties in forest reclamation following surface mining. In my first study I looked at the impact of (1) various cover soil materials, (2) cover soil thickness, (3) the subsoil material type, and (4) total capping depth on early (first three years after planting) seedling survival, height growth and foliar nutrients of three common boreal upland tree species, as well as the impact of these different soil capping treatments on physical and chemical soil properties in an oil sands mine reclamation project. The results of this study revealed that the topsoil material type was the main driver for the observed differences in seedling growth during the early establishment phase. Aspen and pine seedlings, as the faster growing, early successional species performed much better when growing on FFM than the thick peat layer (30 cm). Spruce, however, as a slower growing and considered later successional species did not show any preferences. The main factors restricting seedling growth in the 30cm thick peat were likely the high moisture content and low soil temperature, as well as low phosphorus availability and high pH. The low soil temperatures associated with the 30 cm peat thickness were likely due to the insulating properties of peat which is highly porous and has a low thermal conductivity once it dries out near the surface in combination with high moisture content at greater depth. Accordingly, seedlings from all three species grew less on the 30 cm peat cover, when compared to the 10 cm thickness. An analysis of leaf and needle tissues revealed that the two contrasting topsoil materials affected foliar nutrient concentrations in aspen and spruce, but not in pine. This was probably a consequence of the varying nutrient availabilities in the different topsoil materials and the different nutrient requirements of the species. I was also able to demonstrate that all three species showed nutrient deficiencies on both materials. Moreover, the form of nutrient deficiency varied by species. Subsoil material and total capping depth had only little effect on seedling growth at this early stage of regeneration, with only larger aspen seedlings responding to subsoil conditions and deeper total soil cappings. Overall, aspen appears to be the species most sensitive to the treatments I tested, while spruce was least responsive to the treatments.

It should be noted that I only observed the first three years of forest development. It remains unclear how the different soil materials will affect seedling growth performance in the long-term as soil development progresses.

In my second study I investigated the impact of (1) biochar, (2) fertilizer and (3) the combination of both as amendments to an unconsolidated mineral soil material on seedling establishment and development, as well as soil water and nutrient availability in a reclamation project, which was lacking suitable topsoil material. The results of this study suggest that fertilization increased seedling height after three growing seasons, while biochar or the combination of biochar and fertilizer did not show the expected cumulative effects on seedling development. However, biochar reduced the amount of plant available nitrate, which was likely absorbed and immobilized by the biochar material due to its high C:N ratio. Biochar also likely increased soil moisture and thus might have reduced seedling mortality.

Compared to other studies, I added a relatively low amount of biochar to the soil (1670 kg ha^{-1}), which may have been the main limitation of this experiment. For comparison, other studies used rates between 8000 (Asai et al. 2009) and 200,000 kg ha^{-1} (Kammann et al. 2011) of biochar in order to improve plant growth. Moreover, the low degree of ground vegetation cover, combined with high wind and water erosion, as well as surface application of the fertilizer likely caused a significant loss of biochar and fertilizer early in the study. This probably explains the limited effects I detected. I suspect, that had we used more biochar, the positive effect of the biochar would have been more pronounced.

In combination, the two studies I conducted showed that, both available soil materials containing OM (e.g. FFM and peat) as well as soil amendments from external sources (e.g. biochar and fertilizer) can be used to promote seedling growth and establishment by increasing nutrient and water availability. However, it is important to understand the specific physical and chemical soil properties associated with each material so they can be applied in adequate amounts to ensure fast seedling growth and low mortality. Low mortality and steady seedling growth are, of course, highly desirable as they are an indicator of successful reforestation (Macdonald et al. 2015).

4.2 Management implications and further research

The results of my research illustrate that the addition of soil materials and amendments containing OM can promote seedling growth and development by improving soil physical and chemical properties of unconsolidated mineral soil materials commonly found on mine reclamation sites. Based on my findings, as well as findings from studies conducted by Greene et al. (2007), Gewehr et al. (2014) and Lafleur et al. (2015), I suggest not using a thick layer of pure peat as a cover soil material. Greene et al. (2007), Grwehr et al. (2014) and Lafleur et al. (2015) suggest that peat accumulation greater than 25 cm could lead to waterlogged conditions, low nitrogen availability and soil temperatures and thus affect seedling survival and development negatively. Instead, I suggest to use a mixture of peat and mineral soil material (peat mineral mix) as the higher mineral content may counter some of the adverse effects (low soil temperature and excessive soil moisture) observed in this study.

When using FFM, I recommend a thinner cover soil thickness, as my findings showed that increasing FFM thickness did not result in better seedling growth. By using a thinner FFM layer this valuable material could be used to reclaim a larger area. Jones (2016) also did not find any additional benefits of using a thicker FFM cover with respect to the emergence of ground vegetation from the propagule bank. However, further research is needed to study the long-term effects of the different soil materials and thicknesses on tree growth. For example, the very high moisture content associated with the thick peat layer, may slow down growth initially, but may become a positive trait as the trees mature and water uptake increases.

I also recommend using foliar nutrient analysis as a tool to detect nutrient deficiencies, which could then be addressed through targeted fertilization.

When considering to use biochar as a soil amendment to improve seedling growth on unconsolidated mineral soil material I recommend a much higher application rate than we used in this study. Biochar has been shown to improve soil properties in a number of studies (Kammann et al. 2011; Robertson et al. 2012; Jones et al. 2012; Omil et al. 2013). However, in our case the application rate was likely too low to result in increased seedling growth. It is also important to take erosion into consideration when using biochar and to avoid using biochar on erosion prone slopes. Future studies on the effects of biochar and fertilizer could examine if the beneficial effect of added biochar might have been increased by presoaking the material in a fertilizer solution, or directly applying the fertilizer to the root system rather than broadcasting the fertilizer onto the soil surface.

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