Native tree seedling interactions with variations in edaphic properties in upland boreal forest restoration

by

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A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

Forest Biology and Management

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Abstract

The boreal forest is a vast circumpolar ecosystem covering approximately 11 % of terrestrial land globally. The Canadian boreal forest is rich in natural resources such as lumber, minerals, and oil, and therefore is heavily managed by humans in some regions. Surface mining for oil sands deposits in northern Alberta requires active forest reclamation which presents a unique challenge given the severity of this type of disturbance. I investigated the influence of organic- and mineral-dominated reclamation soils on native tree seedling establishment. Specifically I determined (1) the community recovery of important belowground mutualists, ectomycorrhizal fungi, and their influence on seedling growth in these soils using outplanted seedlings of *Populus tremuloides*, *Pinus banksiana*, and *Picea glauca*. In addition, I examined (2) the influence of low soil temperature during budflush for seedlings of *Populus tremuloides*. I found (1) the species of tree seedling was more important in determining ectomycorrhizal fungal community composition rather than reclamation soil type, and that (2) low soil temperature during budflush of *Populus tremuloides* seedlings results in lower growth. Based on these results I suggest that using a diversity of tree seedlings for outplanting onto reclamation areas may recover a more diverse ectomycorrhizal fungal community, and that efforts should be made to monitor the peat content and depth of reclamation soils to prevent the creation of low spring soil temperatures that may be detrimental to the establishment and growth of *Populus tremuloides* seedlings.

Acknowledgements

First and foremost I thank my two advisors, Justine Karst and Simon Landhäusser for their patience and guidance throughout every step of this process. Their advising fostered my development as an ecologist in many ways that have prepared and excited me to undertake important responsibilities in the future. Justine's support and collected nature was critical to the development of my writing skills, confidence, and patience. From Simon's energy and creativity, I have learned the art of critical thinking and the rewards of hard work. I also thank Derek MacKenzie for his help as a committee member. This research was supported by funding from the National Science and Engineering Research Council, Total E&P Canada Ltd., Imperial Oil Resources Ltd., Canadian Natural Resources Ltd., Syncrude Canada Ltd., and Shell Energy. I received foreign differential tuition offsets from the Department of Renewable Resources at the University of Alberta. I thank the Molecular Biology Services Unit at the University of Alberta for their support. Our research technicians, Fran Leishman and Pak Chow, were critical to success of these projects. Pak Chow performed the carbohydrate analysis and some of the soil nutrient analysis, in addition to helping with many problems encountered. Fran Leishman organized assistance these research projects. I thank many members of Simon Landhäusser's research group, particularly Patrick Lefebvre, Gauri Akolkar, Jake Gaster, Stefan Hupperts, Jana Bockstette, Ingo Siebert, Philipp Leberer, Caren Jones, and many others. I thank Erin Wiley for her stimulating conversations and discussion of research ideas. The friendship and support from fellow graduate students Alia Snively, Shaun Kulbaba, Alex Goeppel, and Sascha Bachmann was of great comfort throughout this process. Thanks to all of my family and friends for their support, and to my undergraduate professor, mentor, and friend, Christine Weilhoefer, for inspiring my interest in plant ecology.

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Chapter 1: General Introduction

1.1 Boreal Forests

The circumpolar boreal biome spans an area of over 14.7 million km^2 across North America and Eurasia with forests dominating the landscape (Bonan and Shugart, 1989). These forests are estimated to cover 11 % of terrestrial land globally (Bonan and Shugart, 1989). In northern Alberta, Canada, Central Mixedwood forests (Natural Regions Committee, 2006) dominate the landscape. Soils in this subregion originated from surface glacial deposits and are of sedimentary origin consisting of a complex mosaic of glaciolacustrine, glaciofluvial eolian sands, and fine textured glacial till materials (Macdonald *et al*., 2012). Erosion and deposition after the last deglaciation created topographic variation in this region where now exists defined forested uplands and lowlands. Upland forests occur on soils of the Luvisolic and Brunisolic orders with varying LFH thicknesses (Soil Classification Working Group, 1998). The forests are generally composed of pure or mixed stands of early successional *Populus tremuloides* Michx. and late successional *Picea glauca* (Moench) Voss, or dominated by *Pinus banksiana* Lamb. Forested lowlands occur on soils of the organic order and are composed of *Picea mariana* (Mill.) and/or *Larix laricina* (Du Roi). Common understory vegetation in upland forests are *Chamerion angustifolium* L., *Vaccinium myrtilloides* Michx., *Rubus idaeus* L., *Calamagrostis canadensis* (Michx.) Beauv., *Salix* spp., *Solidago* spp., *Cornus canadensis* L., *Linnea borealis* L., *Corylus cornuta* Marsh, and *Aralia nudicaulis* L. (Hart and Chen, 2006).

Natural disturbances in the boreal forest play a large role in shaping the occurrence and composition of vegetation upon the landscape. These natural disturbances include wildfires (Weber and Flannigan, 1997; Fauria and Johnson, 2008), drought, insect outbreaks (e.g., forest tent caterpillar and spruce budworm), and disease (i.e., fungal pathogens; Hogg *et al*., 2002). In addition to natural disturbances, logging, mining, and other anthropogenic activities also have a

substantial influence on the vegetation dynamics in these forests. For example, during the year 2008, over 9000 km^2 of forest in Alberta was harvested for lumber (Masek *et al*., 2011). In addition to forestry, *in-situ* extraction of oil sands deposits in northern Alberta has the potential to disturb over 138,000 km^2 of boreal forest (Schneider and Dyer, 2006), and surface mining for oil sands has the potential to disturb 4,800 km^2 (Government of Alberta). Although the potential area that may be disturbed for surface mining is relatively small in comparison with logging and *in-situ* oil extraction, the intensity of this disturbance (e.g., complete removal of soils and geologic materials) reduces the likelihood of rapid natural regeneration. Thus, to enhance the speed of ecosystem recovery from a primary successional stage in surface-mined areas, reclamation (i.e., re-engineering of the landscape) and ecosystem restoration (i.e., the re-initiation of ecological functions) efforts are required.

1.2 Reclamation and Boreal Forest Restoration after Surface Mining

The Land Surface Conservation and Reclamation Act of 1973 and the Environmental Protection and Enhancement Act of 1992 currently hold mine operators responsible for the reclamation of land disturbed for oil sands extraction. This legislation requires reclamation efforts to meet specific standards before certified as 'reclaimed'. These goals aim to restore self-sustaining ecosystems containing 'equal or greater land capability' than what previously existed through the re-establishment of native vegetation (Alberta Environment, 1999). Currently, an area of approximately 77 km^2 is undergoing reclamation, with only one certified reclaimed area of 1.04 km^2 (Poscente and Charette, 2012; Government of Alberta). Reclamation certification is difficult to achieve because surface mining creates a landscape level disturbance and reclamation of small areas is difficult in light that individual active mines can reach 50 or more square

kilometers. The reclamation of these areas not only requires the re-establishment of vegetation, but the re-engineering of whole landscapes and ecosystems (Johnson and Miyanishi, 2008).

Soils are completely removed in the mining process and are salvaged in a careful manner to be used in reclamation. After removal of vegetation, the surface organic layer is removed first, followed by B/C horizons, subsoil, and lastly overburden which consists of parent geologic materials that directly overlay the oil sands deposits. After soils are salvaged, they may be kept aside in stockpiles for reclamation. Ideally, reclamation efforts will become efficient enough to allow for direct soil transfers from intact forest (pre-mining) to reclamation areas, thus precluding stockpiling. Reclamation begins with soil profile recreation starting with placement of overburden materials, followed by subsoils (i.e., B and C materials), and lastly capped with mineral-organic materials such as salvaged forest floor material (FFM) or a peat, peat-mineral mixture. These surface horizon capping materials, or cover soils, provide organic materials important for ecosystem development through providing organic matter, nutrients, and biological propagules (Zipper *et al*., 2013; Rowland *et al*., 2009).

1.3 Selection of Cover Soils Used in Upland Forest Restoration

In effort to restore upland forests, salvaged FFM is an ideal cover soil because it originates from intact forests that are similar to the target ecosystem. However, the availability of this material is limited because the majority of surface mining occurs in bogs and fens, making peat the most abundant material. As such, salvaged peat is commonly used as an organic cover soil amendment in reclamation. Both FFM and peat cover soils contain desirable attributes for re-establishment of vegetation and surface soils, however these cover soils differ in their nutritional, biological, and physical properties. Peat and mixes thereof generally contain high

concentrations of nitrogen (N), primarily in the form of nitrate, while FFM contains less N, and primarily in the form of ammonium (MacKenzie & Quideau 2012; Hemstock *et al*., 2010). Peat has a lower bulk density, lower thermal conductivity, and greater water holding capacity compared with mineral-dominated upland soils, such as FFM and subsoil (Bonan and Shugart, 1989; Leatherdale *et al*., 2012). Though peat may have attractive nutritional and physical attributes, it is not considered a good native propagule source such as FFM; the direct placement of which has been shown to provide native vegetative propagules and nutrients to reclamation areas (Mackenzie and Naeth, 2010). Aside from native vegetation, cover soils may also serve as a propagule source for soil microorganisms critical to ecosystem development.

1.4 Plant-Soil Linkages

Soil microorganisms are recognized for their role in shaping aboveground plant communities (van der Heijden *et al.,* 2008). Of particular importance in boreal forests are symbiotic associations formed by tree roots and ectomycorrhizal fungi. Ectomycorrhizal (EM) fungi are critical to the growth and survival of boreal trees because they are able to access mineralized and organic nutrients otherwise unavailable to trees in exchange for photosynthetically-derived carbon. Ectomycorrhizal fungi are diverse in species and functioning (Gardes and Bruns, 1996; Lilleskov *et al*., 2002); with an estimated 700 species of EM fungi occurring in the boreal biome (Dahlberg *et al*., 2002). Community studies reveal EM fungal species to vary in presence and abundance according to soil properties (Dickie *et al*., 2002; Rosling *et al*., 2003; Tedersoo *et al*., 2003) and host species (Molina *et al*., 1992; Ishida *et al*., 2007; Tedersoo *et al*., 2008). For example, members of the Pinaceae family are known for their

specific EM associations (Molina *et al*., 1992; Horton and Bruns, 1998) upon which they are reliant for establishment and survival (Sousa *et al*., 2011).

Though shaped by below and aboveground properties, EM fungi are dependent on their host for survival in the event of disturbance. For example, if carbon supply belowground is constrained at the tree level, EM abundance rapidly declines (Lindahl *et al*., 2010). Additionally, intense wildfires that result in tree mortality and soil organic matter combustion result in the decline of EM fungal communities (richness and abundance). A decline in the abundance of EM sporocarps was found with mountain pine beetle attack in lodgepole pine (*Pinus contorta* Douglas ex Loudon) forests, a disturbance which leaves the soil intact but results in tree mortality (Treu *et al*., 2014). Clear-cut logging, a disturbance that also leaves the soil intact but results in aboveground tree removal, generally results in a change in EM fungal community composition (Jones *et al*., 2003). Abundance and community composition of EM fungi are influenced by disturbance; the type/severity of which will also define how EM fungi recover. One study investigated the recovery of EM fungi after a wildfire in jack pine stands and found an increase in species richness with time across six to 41 year old stands (Visser, 1995). Similarly, EM fungal diversity was found to increase with increasing stand age from five to 65 years after fire or logging disturbance (Twieg *et al*., 2007). Other studies of EM fungal succession on severely disturbed areas subjected to volcanic eruptions and deglaciation, for example, have found EM fungal richness and abundance to increase over time depending on the successful colonization of host plants (Jumpponen *et al*., 2002; Nara and Hogetsu, 2004). The species of EM fungi that recover after a disturbance may develop from the resistant EM fungal propagule bank in soils. Miller and others (1994) found both fungal spores and fungal sclerotia (mycelium mass) to remain viable in collected soils from burned and unburned forests after two years. Barr

and others (1999) found five of the seven most abundant colonizing EM fungi in a bishop pine (*Pinus muricata* D.Don.) forest to survive a wildfire (resistant sclerotia), while the uniform presence of the EM fungus *Rhizopogon* likely established from resistant spores. Other means of fungal presence in soils after disturbance, besides remaining resistant propagules, could be spores of EM fungi dispersed from nearby sources (Peay *et al*., 2012; Ashkannejhad and Horton, 2005; Frank *et al*., 2006). In addition to variation in the EM fungal propagule bank of various cover soils, the combination between a cold climate and variation in physical aspects of organic versus mineral-dominated cover soils may also have important implications for tree seedling establishment.

1.5 Variation in Reclamation Cover Soil Physical Properties

Differences in mean summer and winter air temperatures (>27 °C) in the boreal region are the largest of any ecosystem measured (Rumney, 1968). There are approximately six months of below-freezing air temperatures (the coldest of which average less than -12 °C), and three months of above 10 °C (average) summer temperatures. The greater water content associated with peat is likely beneficial for seedling growth, but may require more energy to warm in the spring compared with mineral dominated soils like FFM, which may limit the growth of outplanted seedlings if they are sensitive to cold temperatures during the growing season. Low soil temperatures are thought to be one of the main limitations of tree growth in the cool climate of the boreal biome (Bonan and Shugart, 1989), and treelines (Landhäusser *et al*., 1996; Körner and Paulsen, 2004). At the stand level, cold temperatures may drive community patterns. For example, the low solar angles at boreal latitudes create cool and moist north facing slopes, while relatively warm and dry conditions are characteristic of south facing slopes, which also

corresponds with differences in vegetation cover. At the tree level, low soil temperatures that occur during the early growing season and a short growing season may restrict the growth of trees, potentially through cold-induced limitations of physiology and water uptake. Aspen is a species particularly sensitive to cold temperatures compared with spruce (Landhäusser *et al*., 2001). Aspen seedlings are growth-limited by low soil temperatures at or below 5 °C; at this temperature seedlings were found to have reduced above- and belowground growth, in addition to reduced root water uptake and physiological processes compared with seedlings exposed to warmer temperatures (Wan *et al*., 1999; Wan *et al*., 2001; Landhäusser *et al*., 2001). Soils high in organic matter with a high water-holding capacity, such as peat, may remain cold during spring-thaw compared with FFM, resulting in poor native species regeneration and seedling growth (Hogg and Lieffers, 1991). Variation in the physical properties of peat and FFM cover soils may interact with temperature to influence the success of seedling establishment in reclamation areas. It is important to understand the relationship between organic and mineral-dominated cover soils and the performance of outplanted tree seedling on upland boreal forest reclamation areas occurring in northern Alberta, Canada.

1.6 Objectives

The objective of this thesis is to test the restoration of ecological processes through the investigation of cover soil biotic (structure of ectomycorrhizal fungal communities) and physical properties (soil temperature) in relation to growth of native boreal tree seedlings commonly used for outplanting onto upland boreal forest reclamation areas. To achieve this objective, this thesis contains two research chapters which each describe field and/or growth chamber experiments. The first research chapter investigates whether (1) cover soils (FFM, peat, and subsoil) retain EM

fungi, (2) ectomycorrhizal fungal communities display host (aspen, jack pine, and white spruce) and or cover soil preference, and (3) ectomycorrhizal fungal colonization and richness influences seedling growth. The second research chapter aims to determine whether variation in early seasonal soil temperatures between FFM and peat cover soils influence the growth of aspen seedlings.

Chapter 2: Influence of tree species and salvaged soils on the recovery of ectomycorrhizal propagules in upland boreal restoration after surface mining

2.1 Introduction

Surface mining, a relatively new disturbance in the Canadian boreal forest, involves the complete removal of vegetation and soils resulting in the local loss of biodiversity and ecosystem functioning. Prior to accessing the target resources, the organic and upper mineral soil horizons are selectively salvaged and either stockpiled or directly transferred to areas ready to be reclaimed. In Alberta, mine operators are required to reclaim areas of public forest to be self-sustaining and of equal or greater land capability than what previously existed (Alberta Environment, 1999). Towards this goal, restoration of vital ecosystem processes presents a unique challenge as many components (e.g. hydrology, soils, and vegetation) must be considered. In particular, restoring the biological linkages between aboveground vegetation and belowground soil organisms are important for re-establishing plant-soil feedbacks, critical to the functioning of the ecosystem as a whole (Kardol and Wardle, 2010).

In boreal forests, ectomycorrhizas are the interface between the roots of most trees and soils, aiding in nutrient acquisition, water uptake, and nutrient cycling (Högberg and Högberg, 2002; Read *et al*. 2004; van der Heijden *et al*. 2008; Courty *et al*. 2010). The inoculation of tree seedlings with ectomycorrhizal (EM) fungi to improve seedling growth and survival when planted onto barren strip-mined land has been performed since the 1970s (Marx, 1975), however the need for inoculation may be avoidable if extra effort is made to protect soils and the propagules within. Though the association between trees and fungi is severed as a result of surface mining, resistant propagules may survive in salvaged soils, depending on the time since soil disturbance and the soil handling techniques, which in turn will be important for the re-development of EM fungal communities. The factors affecting survival of soil organisms, such

as bacteria and fungi contained in salvaged materials, is poorly understood, however, recent investigations have shown direct forest floor placement (i.e., immediate transfer from intact forest to a reclaimed area) is an important source of native vegetative propagules and nutrients (MacKenzie and Naeth, 2010; Macdonald *et al*., 2012). Availability and viability of microbial propagules in soils is an important aspect of forest restoration because many microbes are commercially unavailable and critical to ecosystem function. Ectomycorrhizal fungal inoculum consists largely of dormant fungal spores, fungal hyphae, and sclerotia (EM tissue) able to survive in the absence of hosts. Inoculum potential is tested by collecting soils of interest and assaying them with seedlings known to host EM fungi. Typical assay measurements include levels of root colonization by EM fungi and the fungal species present.

There are four factors likely to affect the species of fungi along with their abundance comprising EM inoculum present in reclaimed soils: (i) initial differences in the fungal species present in salvaged soils, (ii) vegetation selected for re-establishment, (iii) abiotic conditions of reclaimed sites, and (iv) EM fungal immigration. Both initial stand composition and soils (i.e., the origin of the EM fungal inoculum) will influence the inoculum potential expressed by salvaged soils. Ectomycorrhizal fungal communities may be heterogeneous across forest stands differing in vegetative composition; this heterogeneity may in turn influence seedling growth. For instance, *Tsuga canadensis* (eastern hemlock) seedlings were found to have greater growth when inoculated with soil fungi from an eastern hemlock forest compared with a hardwood forest, and this was attributed to initial differences in EM community composition (O'Brien *et al*. 2011). In addition to stand level differences, variation in inoculum potential may also be driven by local-scale variation in soils. Some EM fungal taxa are common in coarse woody debris while others occupy mineral soil (Tedersoo *et al*., 2003). Ectomycorrhizal fungal communities can also

vertically partition soils reflecting interspecific differences in soil requirements (Dickie *et al*., 2002). Taken together, not only may differences in stand composition from which the EM fungal inoculum was retrieved influence the fungi present in salvaged soils, collection of particular microhabitats within those stands may also harbour unique EM fungal communities.

In addition to fungi present in salvaged soils, the species of tree host selected for re-establishment on reclaimed areas may also affect the expression of the EM fungal community. Some EM fungal taxa exhibit strong host preference (Molina *et al*., 1992), consequently the tree species used in forest reclamation (or in assays) can influence inoculum potential. Pronounced host preference was found by EM fungal taxa in Tasmanian sclerophyllous forests, mixed Japanese conifer-broadleaf forests, a greenhouse bioassay of mixed Sierra Nevada forest inoculum, and planted stands of southwestern Oregon conifers (Tedersoo *et al*., 2008; Ishida *et al.,* 2007; Izzo *et al*. 2006; Massicotte *et al.,* 1999). Ectomycorrhizal host preference may be driven by taxonomic distance, host successional status, and/or time since host establishment (Ishida *et al*., 2007; Twieg *et al*. 2007; Kranabetter *et al.,* 1999). Thus, not only can the substrate assayed give rise to differences in EM fungal inoculum potential, but the host species used may also select for particular fungi. Moreover, interactions between substrate and host may be indicative of the importance of particular combinations of each, giving rise to variation in inoculum potential.

Environmental stress often associated with reclaimed sites may also influence EM fungal inoculum potential once salvaged soils have been placed. Soils of reclaimed areas may have low moisture and low nutrient availability, high soil acidity and salinity (Bussler *et al*., 1984; Andersen *et al*., 1989; Cassleman *et al*., 2006). These stressful conditions may influence EM community development; species of EM fungi have been shown to vary in their sensitivity to soil

moisture, pH, nutrient availability, and temperature (Gehring *et al*., 1998; O'Dell *et al*., 1999; Kjøller and Clemmensen, 2009; Jones *et al*., 2012; Swaty *et al*., 1998). Following soil placement, immigration of propagules may also influence the development of EM fungal communities. Surrounding intact boreal forest may be a source of EM fungal spores capable of air (Peay *et al*., 2012) or animal dispersal (Ashkannejhad and Horton, 2005; Frank *et al*., 2006).

Of the factors influencing EM fungal inoculum potential of reclaimed sites, two stand out as being most easily controlled by restoration ecologists: host and substrate selection. Selecting host-substrate combinations capturing a range of EM fungal species may be important for promoting seedling establishment and survival in reclaimed areas. Towards this goal, the Aurora Soil Capping Study was constructed in 2012 to test the effects of substrate type, configuration, and depth on seedling performance. The study is a 36 hectare replicated field experiment located in the Athabasca oil sands region of northern Alberta designed to test these various reclamation protocols at an operational scale. In the current study, we tested the inoculum potential of three locally salvaged materials used as cover soils (peat, forest floor material, and subsoil) over one growing season in both field and growth chamber assays using seedlings of three common boreal tree species: *Populus tremuloides* Michx. (aspen), *Pinus banksiana* Lamb. (jack pine), and *Picea glauca* (Moench) Voss (white spruce). Across the field and greenhouse assays we asked: (1.) Are EM fungi present and viable in each of the cover soils? (2.) Do EM fungi exhibit structure that suggest the presence of host-specific taxa and similarly, do cover soils influence EM fungi in ways that suggest taxa with preferential affinities? (3.) How does colonization by EM fungi affect seedling growth?

2.2 Methods

2.2.1 Site description

The Aurora Soil Capping Study (denoted 'capping study' herein) is located within the Aurora North mine (57°19'20"N, 111°30'24"W) on the lease of Syncrude Canada Ltd., approximately 75 km north of Fort McMurray, Alberta. Oil sands surface mining in northern Alberta falls within the Central Mixedwood subregion of the Canadian boreal forest (Natural Regions Committee, 2006), composed of an undulating landscape, with upland forests in addition to bogs and fens. Upland forests in this region consist predominantly of white spruce, jack pine, and aspen and soils tend to be of the Luvisolic or Brunisolic orders (Soil Classification Working Group, 1998). Brunisols typically contain a thin eluvial A horizon, a well-defined Bm horizon, and an underlying C horizon, with Luvisols containing an eluvial A horizon and Bt horizon (Soil Classification Working Group, 1998). Bogs are dominated by *Picea mariana* Mill. (black spruce) and fens are dominated by black spruce and *Larix laricina* Du Roi (tamarack). The soils are poorly to very poorly drained and accumulate organic matter (peat). Salvaged peat is often used as an organic amendment in reclamation due to its abundance on this landscape.

The capping study (Fig. 2-1) covers an area of 36 hectares and was designed to test different cover configurations and depths to cap an overburden structure (representing mineral soils located directly above the oil sands deposits). The cover soils were all salvaged from within the Aurora North lease; specifically the upland materials were salvaged from forests dominated by jack pine. All cover soils were salvaged and directly placed (e.g. no stockpiling of materials) onto the research site during the winter prior to tree planting (which occurred in the spring of 2012 as described below). The directly placed soils were moved during the winter in effort to minimize disturbance to soil structure and biota such as EM fungal and plant propagules. Cover soils included peat salvage (surface to approximately 200 cm), upland forest

floor material (FFM) salvage (surface to approximately 15 cm), and blended mineral subsoil which included B and C soil horizons (approximately 50 to 100 cm soil depth; North Wind Land Resources Inc., 2013). Peat material was free of sand, silt, and clay and had an average pH of 7.4 (n=84; min: 5.0 max: 7.8) and an average electric conductivity (EC) of 1.2 dS/m (min: 0.4 max: 2.3). Forest floor material (n=48; 91.6 % sand/4.0 % silt/4.4 % clay) had an average pH of 5.6 (min: 4.9 max: 7.1) and an average EC of 0.2 dS/m (min: 0.1 max: 0.6). Blended B/C subsoil material (94.9 % sand/1.7 % silt/4.5 % clay; n=84) had an average pH of 7.2 (min: 6.2 max: 7.9) and an average EC of 0.2 dS/m (min: 0.1 max: 0.7; North Wind Land Resources Inc., 2013). Each cover soil treatment (1 ha each) was replicated three times; each replicate contained three 25 x 25 m single-species tree plots. In May 2012, single-species plots were planted with one-year-old seedlings of aspen with a plug diameter of 6 cm and depth of 15 cm, jack pine seedlings (4-12), or white spruce seedlings (6-15) obtained from Smoky Lake Forest Nursery (Smoky Lake, Alberta). Seeds for all species were collected from the Fort McMurray area. Based on a subsample (n=20) of seedlings collected for initial measurements prior to outplanting, average seedling height was 30 cm (±1.9 SE), 18 cm (±0.6), and 29 cm (±1.2) for aspen, jack pine, and white spruce, respectively. All tree plots were planted at a 1 m spacing (10,000 stems per hectare). Daily air temperatures at the capping study for 2012, the year of EM sampling, ranged between 34.6 °C and 6.3 °C for the growing season (June to August; high/low; data collected by O'Kane Consultants). Cumulative precipitation for the 2012 growing season (June-August) at the capping study was 90.1 mm, the majority of which occurred throughout June and the beginning of July (data collected by O'Kane Consultants).

2.2.2 Field assay of ectomycorrhizal fungi

To characterize the initial recovery of EM fungi present in soils following reclamation, we surveyed roots of planted seedlings for EM fungal associations in two stages. First, at the time of planting, we randomly selected 20 seedlings of each tree species to assess EM fungi present on roots, formed during nursery production. Second, in late August 2012, after a full growing season, we harvested a total of ten seedlings per species ranging from three to four subsamples from single-species plots within each replicate of three cover soil materials (peat, FFM, and subsoil; n=3). Seedlings were chosen systematically to ensure an even distribution in terms of visual size and health status. Once harvested, roots were separated from shoots and kept on ice until arrival at the University of Alberta where roots were stored at -20 °C until processing. The aspects of EM fungal community structure that were measured included frequency among hosts (i.e., the presence of each fungal OTU among host species), percent EM colonization per seedling (i.e., the proportion of root tips colonized of the 200 observed × 100), abundance of each OTU per seedling (i.e,. the proportion of root tips colonized by each OTU of the 200 observed root tips × 100) , and species richness (i.e., the number of fungal OTUs per cover soil replicate; see *Description of ectomycorrhizas* below*)*. Following assessment of EM fungi, roots (and shoots) were dried at 70 ºC for three days. In addition to dry weights, ten foliar samples were collected from three trees per species per cover soil. Foliar tissues were ground using a Wiley® Mill (Thomas Scientific, Swedesboro, New Jersey), and sent to Central Equipment Laboratory (University of Northern British Columbia, Prince George, British Columbia) for N analysis using combustion (ECS 4010 Elemental Combustion System), and P analysis using elemental (ICPMS) techniques.

2.2.3 Growth chamber assay of ectomycorrhizal fungi

We tested for EM fungal inoculum potential and possible EM-mediated effects of the three cover soils on seedling growth by manipulating the presence of EM fungi and the type of cover soil in which seedlings were grown. During the harvest of field seedlings described above, we also collected approximately 140 L of each cover soil from approximately the same locations within the capping study from which the field seedlings were sampled and immediately transported them to the University of Alberta where they were pooled and homogenized per cover soil. All subsamples of each cover soil were spread onto a tarp and mixed manually using shovels and rakes for approximately 10-15 minutes. Cover soils were stored at 4 °C for approximately two months until experimental setup. There were a total of 180 pots. We filled 90 2-L pots with the unsterilized cover soils; each cover soil type (unsterilized peat, FFM, subsoil) comprised 30 pots each. In addition to those 90 pots, another 90 were filled in the same manner using the cover soils that were sterilized (sterilized peat, FFM, subsoil). Cover soils for half of the seedlings were sterilized to eliminate viable soil organisms, including EM fungi and used as a control. Sterilization was achieved by heating materials to 121°C at 103.4 kPa twice for 120 minutes, with a 24 hour rest in between. All pots were sterilized with Kleengrow™ (Pace Chemicals Inc., Burnaby, British Columbia) disinfectant prior to potting and lined with window screen to prevent substrate loss. Subsamples (n=6) of the six cover soil treatments (peat, peat-sterilized, subsoil, subsoil-sterilized, FFM, FFM-sterilized) were analysed for total N, P, and available N (NO_3^- & NH_4^+). Available N was analysed from cover soil samples that were air dried and extracted in 20 ml of 0.5 M KCl by colorimetric methods (Keeney and Nelson, 1982; Miranda et al., 2001) using SmartChem Discrete Wet Chemistry Analyzer, Model 200 (Westco Scientific, Brookfield, Connecticut, 2007). Total N and P were analysed by Natural Resources Analytical Laboratory (University of Alberta, Edmonton, Alberta, Canada). Total N was

determined by Dumas combustion (Dumas Methods, 1996) using Costech Model EA 4010 Elemental Analyzer (Costech International Strumatzione, Florence, 2003), and total P was determined by acid digestion (Murphy and Riley, 1962).

We compared EM-mediated effects of cover soils across three host species which were also used in the capping study: aspen, jack pine and white spruce. Seeds were obtained from Smoky Lake Forest Nursery (Smoky Lake, Alberta) and were of the same seed collection as the outplanted field seedlings. Seeds of white spruce were soaked in water for 24 hours at 4°C and then cold stratified on moist filter paper at 4°C for three weeks. Jack pine seeds were soaked in water for 24 hours at 4°C. Aspen seeds did not require a stratification period. Seeds from all species were initially germinated in sterilized generic potting soil (Sunshine® Professional Growing Mix, Sun Gro Horticulture Canada Ltd., Vancouver). Aspen seedlings were transplanted into the experimental pots two weeks after germination; ten seedlings were planted individually into ten pots of each cover soil type. Jack pine and white spruce seedlings were transplanted in the same manner approximately three to four weeks after germination. Seedlings were arranged into five blocks that were rotated throughout the growth chamber every three weeks to expose each block to the range of air temperature present in the growth chamber we knew existed. There were 36 pots in each block in which each cover soil-tree species combination was represented twice. We randomly assigned the location of each seedling within a block (using a random number generator), and the locations were re-randomized with each rotation. All seedlings were fertilized (including those grown in sterilized cover soils) at low rates to promote seedling survival while minimizing negative effects on EM development. To promote establishment, fertilization occurred once immediately after transplanting with 1g L^{-1} of 10:52:10 (N:P:K) and all seedlings thereafter were fertilized monthly with 1g L^{-1} of 15:30:15

(N:P:K). Air temperature in the growth chamber was kept between 17 and 21°C throughout the experiment and photoperiod was set at 16 hours with a light intensity of 350 μm photons m^{-2} s^{-1}. Dormancy was induced after five months by reducing air temperature to 10-12°C and light to eight hours for an additional two weeks before seedlings were harvested (seedlings were approximately 22 weeks old at this time). At harvest, height of seedlings was measured. After which shoots were separated from roots and subsequently dried at 70°C for three days to yield measurements of seedling mass. Prior to drying, roots were stored at -20°C before they were assessed for EM associations (see below).

2.2.4 Description of ectomycorrhizas—morphotyping

All roots were thawed overnight at 4 °C. Adhering soil and debris was gently washed with tap water over a 1.2 mm sieve. Cleaned roots were cut into approximately 1 cm fragments, placed into a 10 x 10 x 5 cm container filled with water, and homogenized by hand using forceps for approximately 45 seconds. After homogenization, we subsampled, using forceps, from three different locations within the container and placed the selections onto a large petri dish filled with water for morphotyping. Entire root systems were subsampled from seedlings grown in the growth chamber; however, only lateral roots present outside the originally planted root plug were sampled from field seedlings to target those ectomycorrhizas formed after planting. To ascertain the number of EM root tips required to adequately characterize recovered EM fungi on seedlings, we did a preliminary survey on a subset of seedlings harvested from the field. We generated accumulation curves based on the observation of 750 of the randomly selected root tips for three seedlings per species per treatment. Across all species, we generally found morphotype richness

to plateau before examining 200 root tips, therefore we used this value for morphotyping the remaining seedlings (Appendix I).

Colonized root tips were morphotyped under a dissecting microscope on the basis of root tip texture and colour, followed by further examination under a compound microscope for variation in hyphal and mantle formations at 100X magnification (Agerer, 2001). If available, samples of four to five root tips from each morphotype per seedling were collected for DNA extraction.

2.2.5 Description of ectomycorrhizas—molecular analysis

Genomic DNA from two root tips representing each morphotype was individually extracted using Sigma Extraction Solution and Neutralization Solution B following the manufacturer's protocol (Sigma, Gillingham, Dorset, UK). Nested polymerase chain reaction (nested-PCR) amplification was performed using the fungal specific primer combinations NLC2/NSA3 and NSI1/NLB4 (Martin and Rygiewicz, 2005). An aliquot of 1.0 µL of extracted DNA was combined with PCR reactants (8.0 µL of Red Taq (Sigma-Aldrich, St. Louis), 5.4 µL sterile MilliQ H_20, and 1.6 µL of 10 mM primers) making up a 16 µL reaction. The first round of amplifications (NLC2/NSA3) were performed with an initial denaturation at 95 °C for 5 minutes followed by 30 cycles of 95 °C for 1.5 minutes, 67 °C for 1 minute, and 72 °C for 1.5 minutes, with a final extension of 72 °C for 10 minutes. The second round of amplifications (NSI1/NLB4) were performed with an initial denaturation at 95 °C for 5 minutes followed by 27 cycles of 95 °C for 1.5 minutes, 55 °C at 1 minutes, and 72 °C at 1.5 minutes, with a final extension of 72 °C for 10 minutes. Gel electrophoresis using a 1% agarose gel was used to visualize PCR products. Successful PCR products (55 % success rate) were purified using ExoSAP-IT (USB, Cleveland,

Ohio, USA). Cycle sequencing was conducted using BIGDYE v3.1 (Applied Biosystems, Foster City, California, USA) with NSI1 and NLB4 primers, and the resulting products were precipitated following the manufacturer's instructions for EDTA/ethanol. Bi-directional sequences were analyzed on an ABI Prism 3730 Genetic Analyzer (Applied Biosystems, Foster City, California, USA). Sequences were edited with Geneious (Biomatters, Auckland, New Zealand). Nucleotides were converted to Ns if they had phred scores below 15. The ends of sequences were trimmed using an error probability of 3%. In Geneious (Biomatters, Auckland, New Zealand), sequences across both experiments were aligned using MUSCLE (alignment software) into operational taxonomic units (OTUs). Consensus sequences were queried against the Genbank database using nBLAST. Sequences of all fungal OTUs identified were then submitted to Genbank for accession number assignment (Table 2-1). Most matches came back as 'uncultured ectomycorrhizal fungus', therefore sequences of fungal OTUs were queried against the UNITE database and given identities based distance to closest species hypothesis match given a 97-99 % similarity (Kõljalg *et al*., 2013).

2.2.6 Data analysis

In the field assay, seedlings collected within each of the single-species plots (three to four subsamples) per cover soil were pooled (n=3). Ectomycorrhizal fungal richness was summed across seedlings within each single-species plot, while EM fungal colonization rates and abundances were averaged. The growth chamber assay had a randomized complete block design with ten replicates. The experiment contained five blocks of seedlings which were rotated throughout the growth chamber every three weeks, removing the effect of blocking. There was no effect of block when added to the model for all analyses, therefore all analyses were run

without block as a factor. All statistical analyses were performed using R (R Development Core Team, 2008). The effects of host species, cover soil, and cover soil sterilization (growth chamber assay only) on total EM fungal percent colonization per seedling (i.e., the proportion of root tips colonized of the 200 observed × 100), abundance of each OTU per seedling (i.e,. the proportion of root tips colonized by each OTU of the 200 observed root tips × 100), species richness (i.e., the number of fungal OTUs per cover soil replicate, seedling height, total biomass, and foliar nutrient concentration (N and P; field assay only) were tested using analysis of variance (ANOVA) following a general linear model approach when assumptions were met, and a permutation ANOVA when assumptions were not met (Wheeler, 2010). If main effects were significant, post-hoc tests were performed using Tukey's HSD test. The effects of substrate sterilization on seedling mortality were analyzed by tree species using a chi-square test. Pearson's product-moment linear correlations were tested between EM attributes (richness and percent colonization) and seedling growth measurements in the field (height growth, total biomass, and foliar N & P concentrations), and growth chamber (height and total biomass). To visualize the expression of EM fungi in relationship with seedling host and/or cover soil, we used nonmetric multidimensional scaling (NMDS) using the metaMDS procedure from the vegan package in R (Oksanen, 2013), with a random starting configuration, a stability criterion of 0.0005, the Bray-Curtis distance measure, and two default standardizations: a square root transformation to deal with the large data counts, and the Wisconsin-style double standardization to normalize data into maximum percent abundance. The field dataset required five runs, while the growth chamber dataset required three. Graphs were made using the first two dimensions with species vectors added at a maximum *p*-value of 0.001. For inclusion of uncolonized seedlings in the NMDS, all EM fungal species abundance numbers were transformed by the

addition of a randomly selected positive value (+1.5) maintaining distance relationships among treatments.

2.3 Results

2.3.1 Host preference of EM fungi recovered from the field assay

2.3.1.1 Presence of ectomycorrhizal fungi on nursery seedlings prior to out-planting

Fifteen percent of aspen, jack pine, and 40 % of white spruce seedlings subsampled from the nursery stock were colonized by EM fungi. Two OTUs (operational taxonomic units) were identified from two observed morphotypes: *Amphinema byssoides* (Pers.) J. Erikss. and Thelephoraceae Chevall. Thelephoraceae occurred on roots of aspen and jack pine seedlings, while *Amphinema byssoides* occurred on roots of white spruce seedlings (Table 2-2). There was a significant effect of host species on percent EM fungal colonization of root tips (p=0.03). White spruce had marginally significant higher EM fungal colonization of root tips (mean=28 ±9.1 % SE) compared with aspen (7±4.6 %; p=0.07) and jack pine (8 ±5.1 %; p=0.09).

2.3.1.2 Presence of ectomycorrhizal fungi across cover soils

After seedlings grew for one season at the capping study, five OTUs were identified from seven observed morphotypes (two of which matched those found prior to outplanting; see Table 2-2): *Amphinema byssoides*, *Hebeloma hiemale* (uncertain naming status), *Rhizopogon rubescens* var *pallidimaculatus* A.H. Sm., Thelephoraceae, and *Wilcoxina mikolae* (Chin S. Yang & H.E. Wilcox) Chin S. Yang & Korf. One morphotype was identified as belonging to multiple species, typically *Cenococcum* spp. and a species of *Meliniomyces*; therefore both were disregarded from further analysis. Across all hosts and cover soils, *A. byssoides* was the most abundant EM fungus occurring on 100 % of white spruce seedlings. *Rhizopogon rubescens* occurred on roots of 33 % of aspen seedlings and 67 % of jack pine seedlings. *Thelephora*

terrestris was found on 78 % of jack pine seedlings, *Wilcoxina mikolae* on 44 % of jack pine seedlings, and 33 % of white spruce seedlings, and *H. hiemale* occurred on roots of all aspen seedlings. Average EM fungal colonization rates per seedling was 23 % (±16.9 %; SE) and were not influenced by host, cover soil, or their interaction (minimum p=0.16). Nonmetric multidimensional scaling indicated that abundances of EM fungi were grouped by host species rather than cover soil (Fig. 2-2a). Host identity drove the abundance of *A. byssoides*, Thelephoraceae, *H. hiemale*, while an interaction between host and cover soil influenced the abundance of *R. rubescens* (Table 2-3; Appendix II). *Amphinema byssoides*, Thelephoraceae, and *H. hiemale* were found only on one host species (white spruce, jack pine, and aspen, respectively), while *R. rubescens* was found on both jack pine and aspen seedlings, but was more abundant on jack pine seedlings grown in subsoil compared with all other seedlings colonized by *R. rubescens* (Table 2-3). There was no significant effect of host or cover soil on the abundance of *W. mikolae* (Appendix II) or on EM fungal richness (1.4 ±.012; effect of host p=0.91, effect of cover soil p=1.0, effect of cover soil × host p=0.46).

2.3.1.3 Seedling growth performance

Seedling height differed by species (p<0.01) and cover soil (p=0.07), but not their interaction (p=0.9). Aspen grew taller than jack pine (p<0.01) and white spruce (p<0.01); jack pine grew taller than white spruce (p<0.01). Seedlings planted in peat tended to be marginally taller than seedlings planted in subsoil (p=0.06). Seedling mass differed only by species (p<0.01). White spruce seedlings had greater total biomass than aspen (p=0.01) and jack pine (p<0.01), and aspen seedlings had greater total biomass than jack pine (p=0.02) seedlings. Species (p<0.01; p<0.01), cover soil (p<0.01; p<0.01), and their interaction (p=0.05; p=0.01) influenced foliar N and P concentrations, respectively. Aspen grown in FFM and peat contained similar foliar N concentrations, which were greater than those grown in subsoil (Table 2-4). Jack

pine grown in FFM had higher foliar N concentrations than those grown in peat and subsoil (Table 2-4). White spruce seedlings grown in FFM had higher foliar N concentration than those grown in subsoil but not peat (Table 2-4). Aspen grown in FFM had higher foliar P concentrations than seedlings grown in peat and subsoil and seedlings grown in subsoil had greater foliar P concentrations than those grown in peat (Table 2-4). Jack pine and white spruce seedlings grown in FFM had greater foliar P concentration than those grown in peat but not subsoil, and there was no difference between those grown in peat and subsoil (Table 2-4). There were significant positive correlations between EM fungal richness and foliar [N] for aspen seedlings, between EM fungal richness and foliar [P] for jack pine seedlings, and between EM fungal richness and height growth for white spruce seedlings (Table 2-5).

2.3.2 Host and cover soil preference of EM fungi recovered from the growth chamber assay

2.3.2.1 Presence of ectomycorrhizal fungi across cover soils

Sterilization substantially reduced the presence of EM fungi; after 22 weeks of growth only 5 % of seedlings grown in sterilized cover soils were colonized compared with 76 % of seedlings grown in unsterilized cover soils ($p<0.01$). Colonization rates were less than 1 % for the few seedlings colonized by EM fungi in sterilized cover soils compared with 23 % for seedlings grown in unsterilized cover soils. Ectomycorrhizal fungi colonizing seedlings in sterilized cover soils were identified as Thelephoraceae.

After seedlings were grown in unsterilized cover soils in the growth chamber for 22 weeks, a total of five OTUs were identified from seven observed morphotypes: *Hebeloma hiemale*, Thelephoraceae, *Amphinema byssoides*, *Tuber* spp., and *Cenococcum* spp. (Table 2-6). Two rare morphotypes (occurring on <2 % of seedlings and in low abundance) were unable to be identified and therefore disregarded from further analysis. Across all hosts and cover soils, *H. hiemale* was the most prevalent EM fungus occurring on 20 % of aspen, 20 % of jack pine, and

57 % of white spruce seedlings. The second most prevalent group of EM fungi was Thelephoraceae occurring on 73 % of aspen and 37 % of jack pine seedlings. *Amphinema byssoides* occurred on roots of 10 % of white spruce seedlings, and *Tuber* spp. occurred on 27 % of aspen, and 7 % of jack pine seedlings. *Cenococcum* spp. was found on 67 % of aspen and 3 % of white spruce seedlings. Average rates of EM colonization were not affected by host, cover soil, or their interaction (23 ±2.7 %; minimum p=0.24). Nonmetric multidimensional scaling indicated abundances of EM fungi were driven by host species and cover soil depending on the OTU (Fig. 2-2b). Cover soil affected the abundance of *Tuber* spp. (Appendix III); host and cover soil affected the abundance of *H. hiemale* and Thelephoraceae (Appendix III), and the abundance of *Cenococcum* spp. responded differently with an interaction between host and cover soil (Appendix III). More specifically, *Tuber* spp. and *H. hiemale* were more abundant on seedlings grown in peat compared with FFM and subsoil (Table 2-6), while Thelephoraceae and *Cenococcum* spp. were conversely more abundant on seedlings grown in FFM compared with peat (Table 2-6). *Cenococcum* spp. was found only on white spruce seedlings grown in FFM and also more abundant on seedlings grown in FFM compared with subsoil (Table 2-6). *Hebeloma hiemale* was more abundant on white spruce compared with jack pine or aspen seedlings (Table 2-6). The colonization rate of Thelephoraceae was similar for aspen and jack pine seedlings.

Host and cover soil also influenced EM fungal richness differently (p<0.01). Richness was significantly greater for aspen (2 ±0.2) compared with jack pine (1 ±0.1; p<0.01) and white spruce (1 ±0.1; p<0.01). Ectomycorrhizal fungal richness was greater for seedlings grown in peat (2 ±0.2) compared with FFM (1 ±0.2; p<0.01) or subsoil (1 ±0.1; p<0.01). The interaction between host and cover soil did not influence EM fungal richness (p=0.26).

2.3.2.2 Seedling growth response to cover soil sterilization

After 22 weeks of growth in sterilized and unsterilized cover soils, sterilization decreased the survival of jack pine seedlings (p=0.02). Twelve of 30 jack pine seedlings died (40 %) in the sterilized cover soils (of which six were in subsoil, four were in peat, and two were in FFM) compared with three deaths in unsterilized cover soils (of which all were in peat). Only one white spruce seedling grown in sterilized subsoil died, and there was no mortality of aspen seedlings. Seedling height and mass was affected by a three-way interaction among species, cover soil, and sterilization, therefore analyses were performed separately for each species of seedling. Height of aspen was affected by cover soil, sterilization, and their interaction, while height of jack pine and white spruce was affected by only cover soil and sterilization (Appendix IV). Aspen grew taller in sterilized peat compared with all other cover soils (Fig. 2-3). Jack pine and white spruce grew taller in FFM compared with peat or subsoil, and grew taller in unsterilized cover soils (Fig. 2-4). Trends were similar for seedling mass which was affected by cover soil, sterilization, and their interaction for all three hosts (Appendix IV; Fig. 2-5). In unsterilized cover soils, there was a significant positive correlation between EM fungal richness and height of aspen seedlings (Table 2-6).

2.3.2.3 Cover soil sterilization and nutrient availability

Cover soil sterilization affected the nutritional profiles of the cover soils. The availability of NH_4^+, NO_3^-, and PO_4^{-3} changed depending on cover soil type (Appendix V). Specifically, NH_4^+, NO_3^-, and PO_4^{-3} increased in availability in sterilized peat compared with unsterilized and the concentration of K^+ was generally greater in all sterilized cover soils than unsterilized cover soils (Table 2-7).

2.4 Discussion

This study investigated the ectomycorrhizal fungal inoculum potential of three directly placed, locally salvaged cover soils used in upland boreal forest reclamation following oil sands mining. We show that EM fungi are present and viable in salvaged and directly placed forest floor material (FFM), peat, and subsoil based on their ability to colonize three species of tree seedlings commonly occurring in the region: aspen, jack pine, and white spruce. Since seedlings from the field study show colonization by the nursery fungi before planting, interpretation of results is somewhat difficult. However given this caveat, in addition to their presence and viability, EM fungi also displayed a preference for host, the extent of which depended on the type of assay. Growth of both jack pine and white spruce generally decreased in response to autoclave sterilization of the cover soils, however, the response was less clear for aspen. This negative growth response exhibited by the conifers may have been from the lack of EM associations; however, nutritional changes to the cover soil materials due to autoclaving may have also had an influence on this response.

2.4.1 Expression of EM fungi in reclaimed soils

From both field and growth chamber inoculum potential assays, we identified EM fungi common to disturbed areas. *Rhizopogon* spp., *Wilcoxina* spp., also known as "E-strain fungi", and Thelephoracea have been found in early successional forests (Jones *et al*., 1997; Twieg *et al*., 2007; Visser, 1995), i.e., those with natural regeneration or planted within 1-6 years after disturbance by clearcut or fire. These types of EM fungi are considered 'pioneer' fungi (with the exception of *Rhizopogon* spp. that is better characterized as a 'multi-stage' fungus) because of prolific spore dispersal and fast root colonization (Newton, 1992). Additionally, *Amphinema byssoides*, Thelephoraceae, E-strain fungi, *Cenococcum* spp., and *Tuber* spp. have all been documented on nursery grown seedlings (Hunt, 1991; Smaill and Walbert, 2013) indicating their

ubiquitous dispersal and colonization abilities. Thelephoraceae was found on seedlings grown in sterilized cover soils in the growth chamber, potentially a growth chamber contaminant. Unexpectedly we found *R. rubescens* associating with aspen, which is very unusual due to the host specific association of *Rhizopogon* spp. with members of Pinaceae (Massicotte *et al*., 1994), and, to our knowledge, mycorrhizas of *Rhizopgon* spp. with aspen have not been documented. In our study, *Rhizopogon rubescens* was found in low abundance on aspen, and the root tips were not well colonized. Therefore we had difficulty amplifying DNA from these root tips with our nested PCR protocol. However, since the salvaged upland soil was from a pine dominated site, and pine is a well-known host for *Rhizopogon* spp., the nested amplification protocol might have amplified fragments of fungi attached to or near the colonized root tip. One possibility could have been that *R. rubescens* propagules were retained in the salvaged soil and while trying to amplify a weakly colonized aspen root tip, we may in fact amplified a fragment of *R. rubescens* which did not actually form an ectomycorrhiza. The other possibility is that *R. rubescens* did in fact form an ectomycorrhiza with aspen in a situation where there were no other hosts available.

Ectomycorrhizal fungus richness (at the plot or seedling level for the field and growth chamber assays, respectively) was generally less than two, which is slightly lower than what has been found in assays of other early successional ecosystems (Barker *et al*., 2013; Kranabetter, 2004; Durall *et al*., 1999). Field assays of previously logged areas using outplanted Douglas fir (*Pseudotsuga menziesii*) and paper birch (*Betula papyrifera*) seedlings (1 year old) by Jones *et al*. (1997) found averages of EM fungal richness between three and six per plot, and approximately two per seedling after four months. Kranabetter (2004) reported averages of EM fungal richness between three and five per seedling on transplanted naturally regenerated hybrid spruce (*Picea engelmannii* X *Picea glauca*) seedlings (~15 cm initial height) after three years.

Nara (2006) found an average of approximately three on individual root samples from naturally established saplings (estimated age of 7-22 years) of *Betula ermanii* and *Larix kaempferi* near *Salix reinii* patches in an early successional volcanic desert. Similarly, colonization rates of seedlings was lower in our study than that reported from studies of seedlings growing in early successional sites, which generally report greater than 50 % (hosts ranging from eight weeks old to root samples from mature forests; Pennanen *et al*., 2005; Nara *et al*., 2003; Visser 1995; Taylor and Bruns, 1999; Bois *et al*., 2005). A greenhouse bioassay of oil sands reclamation substrates (including peat, overburden, tailing sands, and composite tailings) collected in the same region as ours, identified (using DNA sequencing) a total of six EM fungal OTUs (including *Wilcoxina* sp., *Thelephora* sp., and *Tuber* spp.) and found <50 % EM fungal colonization on seedlings of jack pine and *Populus deltoids* X *Populus nigra* (Bois *et al*., 2005). Other greenhouse bioassays of inoculum potential of soils from intact mature forests report values of EM fungal richness as low as less than one per seedling (~9 months old; Karst *et al*. 2011). Relatively low values of EM richness and colonization in both field and growth chamber assays suggests that directly-placed reclamation cover soils have less inoculum potential than that expressed in early successional sites such as young clearcuts. Levels of colonization and richness of EM fungi may be lower in reclaimed soils compared with those from early successional systems because of the complete disruption (i.e., salvage, hauling, placement, and spreading) of the surface soil materials and the dilution of propagules in addition to the loss of pre-disturbance vegetation. In this experiment cover soils were directly placed, without stockpiling, which can reduce the viability of EM fungus propagules (Persson and Funke, 1988; Reddell and Milnes, 1992). Further, the capping study is young (< three years old) and isolated; distance to undisturbed forest edges at the capping study exceeds that of the average clearcut (> 1

km), thus, dispersal may be limited for many EM fungi (Peay *et al*., 2012). However, over time greater EM fungal immigration, germination of dormant spores, and host development may occur; all of which could increase EM richness and colonization rates.

2.4.2 Host Preference expressed by EM fungi

Composition of EM communities in the field assay was structured by host identity regardless of cover soil, while both host and cover soil influenced the expression of EM communities in the growth chamber assay. Of notable interest is the difference in host preference of *H. hiemale* between field and growth chamber assays. In the field assay, *H. hiemale* only associated with aspen seedlings; while in the growth chamber it associated with white spruce in greater abundance than aspen. This difference contributes to and complicates our conclusions on host preference. In each assay, *H. hiemale* was found primarily on one host (which supports our conclusions on host preference), however the host species was different between the two assays (complicating this conclusion). Since the EM fungal species found in our study tend to be classified as 'pioneer' and 'multi-stage' fungi, we do not expect to see strong host specificity, however in different circumstances these types of fungi may exhibit host preference (as shown in this study), but the species of host an EM fungus shows preference towards may be different for some species of EM fungi depending on the situation. General discrepancies in host and cover soil preference between the assays expressed by EM fungi could be due to (i) differences in abiotic condition (ii) the size and age of seedlings, and/or (iii) the mycorrhizal condition at the start of the experiment. For instance, the growth chamber seedlings were fertilized and watered consistently throughout the experiment to ensure survival, while the field seedlings relied on nutrients and moisture available in the cover soils which likely varied throughout the season. Air temperatures in the growth chamber were held constant throughout the experiment while seedlings in the field were exposed to variation in air and soil temperatures due to naturally

occurring seasonal and diurnal fluctuations. By design the Aurora Soil Capping Study removed many sources of natural variation, such as topography, soil reclamation material type and placement depth; however, completely eliminating all variation in large-scale soil placement operations is difficult. This means variation within the single 1 ha treatments exists which may preclude a consistent cover soil signal. Other studies on early successional systems such as outplanted seedlings on clearcut areas, and natural regeneration after wildfire or logging, have also found that EM fungi are host-specific and that substrate has little influence on shaping the fungal community. For instance, a recent study by Walker and Jones (2013) investigated the role of microhabitat (decayed wood and mineral substrate) in intact and clearcut forests in shaping EM fungal communities of planted hybrid spruce seedlings in interior British Columbia. They found EM fungal communities differed between intact and clearcut forests while homogeneous across microhabitats. Twieg *et al*. (2007) found a dominance of host-specific EM fungi in young forest stands (~5 years) compared with older stands in mixed temperate forests in interior British Columbia. Other studies of EM fungal communities in early successional systems, such as forest recovery following volcanic eruption (Nara *et al*., 2003) and deglaciation (Fujiyoshi *et al*., 2011), did not directly test for host/substrate specificity, making generalizations difficult. The degree of host specificity exhibited by pioneer fungi likely varies across ecosystems depending on environmental conditions. Some studies have shown less host specificity in EM fungi in stressful ecosystems. For example, EM fungal communities have been found to be homogenous across different hosts occurring at the arctic treeline; host specificity may be too costly for fungi under such extreme abiotic conditions (Botnen *et al*., 2014).

In addition to general differences in abiotic variables between the two assays, seedlings were also of different sizes. The growth chamber seedlings germinated from seed whereas the

field seedlings were nursery-grown and had been outplanted into the field. Growth chamber seedlings had lower biomass than those grown in the field, and combined with the relatively low light levels in the growth chamber field seedlings may have had greater potential to allocate carbon to supporting ectomycorrhizas. Consequently, potential carbon limitation in growth chamber seedlings may have hindered host-preference among EM fungi. Low host-specificity among EM fungi has also emerged in studies from stressful environments at ecosystem scales such as the arctic treeline (Botnen *et al*., 2014).

Aside from abiotic and seedling size/age variation between the two assays, initial EM status of seedlings prior to the start of both assays was different. Field seedlings prior to outplanting were colonized by either *Amphinema byssoides* or Thelephoraceae, while the growth chamber seedlings had zero initial colonization because they were germinated by seed first into a sterilized potting soil and later transplanted directly into cover soils. For the field seedlings, it is unclear whether the cover soils or the nursery was the source of Thelephoraceae and *Amphinema byssoides* (see Table 2-2). Since some of the seedlings grown in sterilized cover soils in the growth chamber were also colonized by Thelephoraceae, this EM fungus may therefore be a growth chamber contaminant. *Thelephora terrestris* (Thelephoraceae family) and *A. byssoides* have commonly been found to colonize seedlings grown in nurseries (Hunt, 1991), and *T. terrestris* is able to colonize roots from spore or small amounts of fungus mycelia (Visser, 1995; Jones *et al*., 1997; Kranabetter and Friesen, 2002), suggesting that these fungi are ubiquitous and were not necessarily a source of inoculum in the field assay.

2.4.3 Ectomycorrhizal associations and seedling growth

To manipulate EM fungal presence on seedlings in the growth chamber assay, we sterilized cover soils. The conifer seedlings and aspen differed in their response to sterilization

indicating different sensitivities to the outcome of soil sterilization, such as the presence of EM fungi (or other soil biota). Aspen grew more in sterilized peat compared with any other treatment, a response likely attributed to the large increase in available N as an outcome of sterilization, while the conifers collectively grew less with sterilization, regardless of substrate type. Different tree species vary in their ability to utilize different forms of N (Miller & Bowman 2002). For example, aspen has been found to be more efficient at nitrate uptake and has lower or similar uptake capabilities of ammonium compared to some coniferous species (Min *et al*., 2000; Shenoy *et al*., 2013). In an investigation of the biogeochemistry of forest floor material and peat reclamation materials, forest floor material was found to contain nitrogen (N) primarily in the form of ammonium, while peat mineral mix contained predominately nitrate (MacKenzie & Quideau 2012). We observed a large increase in the amount of available ammonium in the peat with sterilization. Consequently, we expected the conifers to increase in growth more than aspen. As this was not the case, other factors may be hindering growth of jack pine and white spruce in sterilized soils. For instance, uptake of soil nutrients may be completely mediated by the presence of microbes for conifer seedlings such as white spruce and jack pine (Trappe and Strand, 1969). Despite increases in nutrient availability with sterilization, without ectomycorrhizas both white spruce and jack pine may be unable to access the release of ammonium. Roots of aspen grow prolifically which may be a strategy coinciding with direct exploitation of soil resources, therefore requiring less dependence on EM fungi. The mortality of aspen and white spruce seedlings grown in sterilized cover soils provides more evidence that these conifer species may be more sensitive to the loss of EM associations than nutrient availability during seedling establishment. Although EM fungi may not be critical to aspen seedling establishment initially, they may be important for maintaining growth and survival in

older stands. Ectomycorrhizal fungi are common in mature aspen stands (Cripps and Miller, 1993) and of the few studies examining EM communities associated with aspen, EM fungal associations are generally reported as beneficial (Cripps, 2001; Quoreshi *et al.*, 2008; Landhäusser *et al*., 2002). Other outcomes of cover soil autoclave sterilization may have occurred including changes in pH, increases in dissolved organic carbon, and increases in available Mg, Mn, and Fe, (Mahmood *et al*., 2014 and references therein) which may be toxic to plant growth. This could potentially explain the significant mortality of jack pine seedlings in sterilized substrates. However, that aspen grew more in sterilized peat indicates toxicity likely was not a factor. In addition, the varied response to cover soil sterilization by the conifers compared with aspen highlights differences in growth strategies between tree species. Seedling performance in the field assay was influenced by cover soil rather than EM fungal richness or percent EM fungal root tip colonization. Overall, tree seedlings grew marginally taller when grown in peat compared with FFM and subsoil, but did not differ in total seedling mass. Greater height growth by seedlings grown in peat can be attributed to a greater soil moisture availability (0.5 ±0.01 cm^3/cm^3; volumetric water content) compared to the other two coarse textured cover soils (FFM & subsoil: 0.1 ±0.01 cm^3/cm^3; volumetric water content) (*data summarized from Syncrude Watershed Research Database from October 2012).* Although seedlings may have grown taller in peat, in general they acquired more N and P when grown in FFM.

2.4.4 Conclusions

The two factors likely to influence the species of fungi comprising EM inoculum in reclaimed sites that we investigated are: (i) initial differences in the species present in salvaged soils and (ii) host species selected for vegetation re-establishment. We tested for differences in EM fungal inoculum potential based on initial differences caused by cover soil origin and host-mediated effects. Of these, the primary factor found to influence the expression of EM fungi in the field

assay was host species selected for vegetation re-establishment, and in the growth chamber assay, both the type of salvaged soils and host species selected for vegetation re-establishment. The drivers of EM community composition between the two assays could be due to differences in abiotic conditions, the size and age of seedlings, and mycorrhizal condition at the start of the experiment. In addition, differences in belowground carbon allocation between seedlings from the two assays may have also contributed to variation in the expression of EM fungi. Despite these differences between the two assays, because both experiments were relatively short (three and five months for the field and growth chamber assays, respectively) the similarities between the outcomes of the two experiments likely tell us more than the differences. The extent of host preference of pioneer/multi-stage EM fungi in early successional ecosystems has important implications for ecosystem restoration. Choosing a range of tree species for establishment on upland boreal forest reclamation areas can be a strategy for recovering and potentially maintaining a greater diversity of EM fungi. Results from our growth chamber study suggest FFM is a favorable cover soil type for encouraging greater seedling height growth and biomass, which will likely promote the development of EM fungal associations. In addition to recovering EM fungi, cover soil type may also be critical for seedling survival due to factors such as nutrient cycling through microbial activity, moisture retention, nutrient availability, and soil temperature. The recovery of EM fungi in different reclamation scenarios should be monitored over time to better understand how different host-cover soil combinations govern community dynamics.

Tables

Table 2-1: Closest nucleotide BLAST and UNITE species hypothesis of consensus sequences submitted to GenBank.

Genbank Accession number	BLAST closest match	Hit accession number	Maximum score	Query cover	Percent identity	Query length	UNITE species hypothesis	UNITE species hypothesis number	Distance to closest species hypothesis
KJ938030	Uncultured ectomycorrhiza (*Amphinema*)	EF218741.1	1474	99 %	99 % 829/837	843	*Amphinema byssoides*	SH133496.06FU	1 %
KJ938039	Uncultured fungus	KC96595.1	1148	93 %	99 % 646/651	695	*Cenococcum*	SH196545.06FU	1.5 %
KJ938033	Uncultured *Rhizopogon* clone	FJ554251.1	1303	86 %	98 % 755/772	896	*Rhizopogon rubescens* var. *pallidimaculatus*	SH086837.06FU	1 %
KJ938031	Uncultured ectomycorrhiza (*Thelephora*)	EF218819.1	1566	95 %	99 % 872/876	921	Thelephoraceae	SH195956.06	1.5 %
KJ938040	Uncultured fungus clone	KC965209.1	1389	92 %	99 % 779/785	852	*Tuber*	SH204354.06FU	1.5 %
KJ938035	*Wilcoxina mikolae* voucher	GQ267499.1	1126	71 %	99 % 632/636	889	*Wilcoxina mikolae*	SH227976.06FU	1.5 %
KJ938032	Uncultured fungus clone	KF296921.1	1398	94 %	98 % 802/818	864	*Hebeloma hiemale*	SH200120.06FU	1.5 %
KJ938037	Uncultured fungus clone	KC966038.1	1234	96 %	95 % 747/787	811	Thelephoraceae	SH220161.06FU	1.5 %

Table 2-2: Occurrence of ectomycorrhizal fungi (i.e., percentage of seedlings colonized) on nursery grown seedlings of aspen, jack pine, and white spruce before (n=10) and after (three or four seedlings pooled by three experimental units) outplanting in three reclamation cover soils (FFM: forest floor material, peat, subsoil) in a field assay.

EM Taxon	Sample Period	Aspen			Jack pine			White spruce		
		FFM	Peat	Subsoil	FFM	Peat	Subsoil	FFM	Peat	Subsoil
Amphinema byssoides	Before	0%	0%	0%	0%	0%	0%	40%	0%	0%
	After	0%	0%	0%	0%	0%	0%	50%	60%	50%
Rhizopogen rubescens	Before	0%	0%	0%	0%	0%	0%	0%	0%	0%
	After	10%	10%	10%	60%	20%	100%	0%	0%	0%
Thelephoraceae	Before	15%	0%	0%	15%	0%	0%	0%	0%	0%
	After	0%	0%	0%	40%	40%	20%	0%	0%	0%
Wilcoxina mikolae	Before	0%	0%	0%	0%	0%	0%	0%	0%	0%
	After	0%	0%	0%	0%	10%	20%	10%	20%	0%
Hebeloma hiemale	Before	0%	0%	0%	0%	0%	0%	0%	0%	0%
	After	90%	100%	80%	0%	0%	0%	0%	0%	0%

Table 2-3: Mean ectomycorrhizal fungal abundance (± SE) measured as a percentage of root tip colonization of aspen, jack pine, and white spruce grown in three different reclamation cover soils (FFM: forest floor material, peat, subsoil) in a field assay. For each tree species, ten seedlings were sub-sampled across three replicates of each cover soil (n=3).

EM Fungi	Aspen			Jack pine			White spruce		
	FFM	Peat	Subsoil	FFM	Peat	Subsoil	FFM	Peat	Subsoil

Amphinema byssoides	0 ±0.0[a]	0 ±0.0[a]	0 ±0.0[a]	0 ±0.0[a]	0 ±0.0[a]	0 ±0.0[a]	21 ±6.9[a]	24 ±17.6[a]	30 ±18.9[a]
Rhizopogon rubescens	1 ±0.7[b]	2 ±1.8[b]	0 ±0.3[b]	12 ±6.8[b]	6 ±5.8[b]	35 ±7.3[a]	0 ±0.0[b]	0 ±0.0[b]	0 ±0.0[b]
Thelephoraceae	0 ±0.0[a]	0 ±0.0[a]	0 ±0.0[a]	6 ±3.7[a]	12 ±9.1[a]	1 ±0.9[a]	0 ±0.0[a]	0 ±0.0[a]	0 ±0.0[a]
Wilcoxina mikolae	0 ±0.0[a]	0 ±0.0[a]	0 ±0.0[a]	0 ±0.0[a]	1 ±0.6[a]	1 ±0.5[a]	6 ±5.7[a]	2 ±1.4[a]	0 ±0.0[a]
Hebeloma hiemale	10 ±1.9[a]	12 ±1.5[a]	8 ±1.1[a]	0 ±0.0[b]	0 ±0.0[b]	0 ±0.0[b]	0 ±0.0[b]	0 ±0.0[b]	0 ±0.0[b]

Table 2-4: Mean height growth (cm), total mass (g), foliar [N] (%), and foliar [P] (µg/g) (± 1 standard error) of aspen, jack pine, and white spruce grown in three different reclamation cover soils (FFM: forest floor material, peat, subsoil) in a field assay. For each tree species, ten seedlings were sampled across three replicates of each cover soil; measurements were pooled (averaged) within each replicate and then averaged across each replicate (n=3). Differences in lettering indicates significant (p<0.05) differences comparable within each host and across cover soils.

Host	Height Growth (cm)			Total Mass (g)			Foliar [N] (%)			Foliar [P] (µg/g)		
	FFM	Peat	Subsoil	FFM	Peat	Subsoil	FFM	Peat	Subsoil	FFM	Peat	Subsoil
Aspen	22.1 ±4.79[a]	24.3 ±1.06[a]	19.4 ±0.41[a]	16.6 ±2.36[a]	16.0 ±1.30[a]	13.2 ±1.72[a]	2.6 ±0.10[a]	2.2 ±0.09[a]	1.5 ±0.20[b]	2343.2 ±166.63[a]	969.6 ±15.78[b]	1678.3 ±167.54[c]
Jack pine	14.3 ±1.40[a]	17.8 ±1.19[a]	14.7 ±0.81[a]	12.5 ±1.7[a]	12.3 ±0.88[a]	10.5 ±0.91[a]	1.4 ±0.14[a]	0.7 ±0.05[b]	0.7 ±0.01[b]	1319.6 ±45.25[a]	692.7 ±3.10[b]	954.2 ±11.52[ab]
White spruce	9.2 ±0.63[a]	10.0 ±0.54[a]	6.9 ±0.44[b]	16.4 ±1.56[a]	21.2 ±1.24[a]	19.7 ±0.5[a]	1.3 ±0.20[a]	0.8 ±0.13[ab]	0.6 ±0.00[b]	1495.3 ±23.62[a]	969.2 ±91.65[b]	1092.9 ±138.74[ab]

Table 2-5: Pearson's product-moment linear correlation coefficients of EM fungal measurements (% root tip colonization and richness) correlated with seedling growth measurements (height, total seedling mass, foliar N concentration, and foliar P concentration) per seedling grown in three different reclamation cover soils in a field bioassay (n=30). Asterisk represents significant (p<0.05) correlations.

Host	EM Fungal Measurement	Height Growth (cm)	Total mass (g)	Foliar [N] (%)	Foliar [P] (µg/g)
Aspen	EM Fungal Richness	0.20	0.17	0.40*	0.08
	EM Fungal % Colonization	0.26	0.21	0.31	0.17
Jack pine	EM Fungal Richness	-0.15	-0.34	0.24	0.42*
	EM Fungal % Colonization	0.05	-0.08	-0.10	0.05
White spruce	EM Fungal Richness	0.44*	-0.22	0.30	0.14
	EM Fungal % Colonization	0.06	0.01	0.02	0.16

Table 2-6: Average ectomycorrhizal fungal abundance (± SE) as a percentage of root tip colonization of aspen, jack pine, and white spruce grown in three reclamation cover soils (FFM: forest floor material, peat, subsoil) in a growth chamber assay (n=10). *Note: values here are from unsterilized cover soils only.

EM Fungi	Aspen			Jack pine			White spruce		
	FFM	Peat	Subsoil	FFM	Peat	Subsoil	FFM	Peat	Subsoil
Hebeloma hiemale	0 ±0.0^{b}	5 ±2.0^{a}	0 ±0.0^{b}	0 ±0.0^{b}	17 ±6.6^{a}	0 ±0.0^{b}	13 ±6.6^{ab}	28 ±8.7^{a}	18 ±8.3^{ab}
Thelephoraceae	27 ±8.3^{a}	2 ±1.3^{b}	16 ±3.8^{ab}	21 ±9.4^{ab}	9 ±4.2^{ab}	15 ±10.0^{ab}	0 ±0.0^{b}	0 ±0.0^{b}	0 ±0.0^{b}

Amphinema byssoides	0 ±0.0[a]	0 ±0.0[a]	0 ±0.0[a]	0 ±0.0[a]	0 ±0.0[a]	0 ±0.0[a]	4 ±3.5[a]	0 ±0.0[a]	3 ±2.9[a]
Tuber spp.	0 ±0.2[a]	5 ±2.6[a]	0 ±0.1[a]	0 ±0.0[a]	6 ±3.9[a]	0 ±0.0[a]	0 ±0.0[a]	0 ±0.0[a]	0 ±0.0[a]
Cenococcum spp.	15 ±3.2[a]	2 ±0.5[b]	1 ±0.8[b]	0 ±0.0[b]	0 ±0.0[b]	0 ±0.0[b]	0 ±0.0[b]	0 ±0.0[b]	0 ±0.0[b]

Table 2-7: Pearson's product-moment linear correlation coefficients of EM fungal measurements (% root tip colonization and richness) correlated with seedling growth measurements (height, total seedling mass, foliar N concentration, and foliar P concentration) per seedling grown in three different reclamation cover soils in a growth chamber bioassay (n=30). Asterisk represents significant (p<0.05) correlations. *Note: values here are from unsterilized cover soils only.

Host	EM Fungal Measurement	Height (cm)	Total mass (g)
Aspen	EM Fungal Richness	0.50*	0.18
	EM Fungal % Colonization	0.12	0.33
Jack pine	EM Fungal Richness	-0.36	-0.25
	EM Fungal % Colonization	-0.27	-0.14
White spruce	EM Fungal Richness	0.10	-0.22
	EM Fungal % Colonization	-0.30	-0.33

Table 2-8: Effects of sterilization on average available nutrients (± 1 standard error) in different reclamation cover soils (FFM: forest floor material, peat, subsoil) in which seedlings of aspen, jack pine, and white spruce were grown (n=6). Differences in lettering indicates significant (p<0.05) differences comparable within each nutrient across sterilized and unsterilized cover soils separately.

Nutrients	Available Nutrients (mg/kg)					
	Unsterilized Cover Soil			Sterilized Cover Soil		
	FFM	Peat	Subsoil	FFM	Peat	Subsoil
NH_4^+	1.2 ±0.11[a]	0.5 ±0.07[a]	0.5 ±0.06[a]	1.4 ±0.09[a]	54.9 ±0.98[b]	0.5 ±0.08[a]
NO_3^+	2.0 ±0.33[a]	17.7 ±0.28[b]	1.3 ±0.15[a]	1.4 ±0.11[a]	18.9 ±0.13[c]	1.3 ±0.16[a]
PO_4^{-3}	14.1 ±0.28[a]	0.2 ±0.16[b]	5.2 ±0.37[c]	13.5 ±0.2[a]	2.3 ±0.2[d]	4.3 ±0.1[c]
K^+	43.7 ±1.21[a]	42.8 ±2.00[a]	22.0 ±0.20[b]	51.9 ±5.05[a]	49.2 ±1.01[a]	25.1 ±2.18[b]

Figures

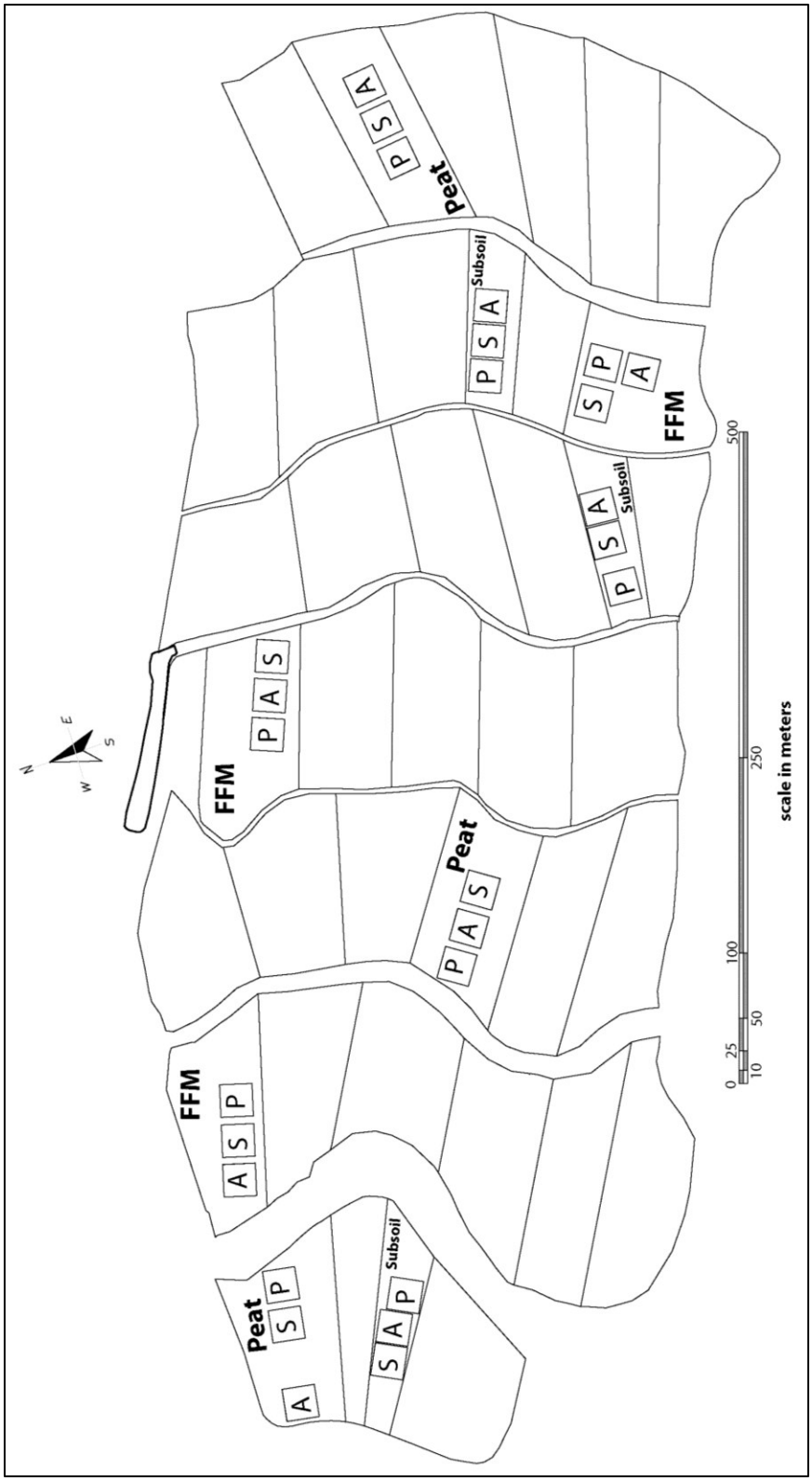

Figure 2-1: The Aurora Soils Capping Study consists of 13 treatments where cover soil type, configuration, and depth were manipulated (n=3). Map shows treatment cells used in this study:

"Peat": 30 cm cover of peat, "FFM": 20 cm cover of forest floor directly placed, and "subsoil": 150 cm cover of blended B/C subsoil soil horizons. Within each replicate, seedlings of *Populus tremuloides* (aspen), *Pinus banksiana* (jack pine), and *Picea glauca* (white spruce) were planted in single species 25 x 25 m vegetation plots at 10,000 stems per hectare. Vegetation plots are labeled as "A" (aspen), "P" (jack pine), or "S" (white spruce).

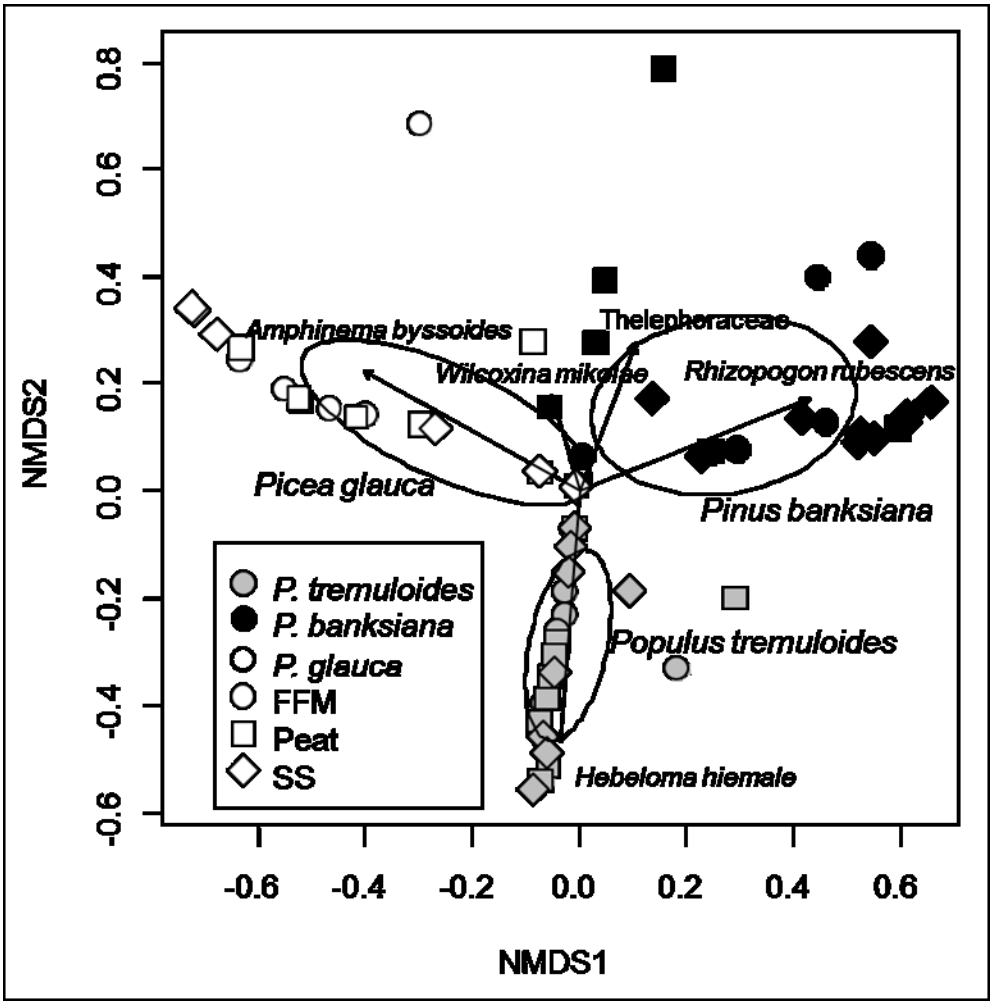

Figure 2-2a: Nonmetric multidimensional scaling ordination of ectomycorrhizal fungal species abundances measured as a percentage of root tip colonization per seedling for three host species (*Populus tremuloides*, *Pinus banksiana*, and *Picea glauca*) grown in three reclamation cover soils (FFM: forest floor material, peat, subsoil) in a field assay (stress = 0.120). Vectors are ectomycorrhizal fungal species; their direction and length represent their influence on the

ectomycorrhizal community composition. Ellipses were generated using the standard deviation of point scores to group seedlings by host species.

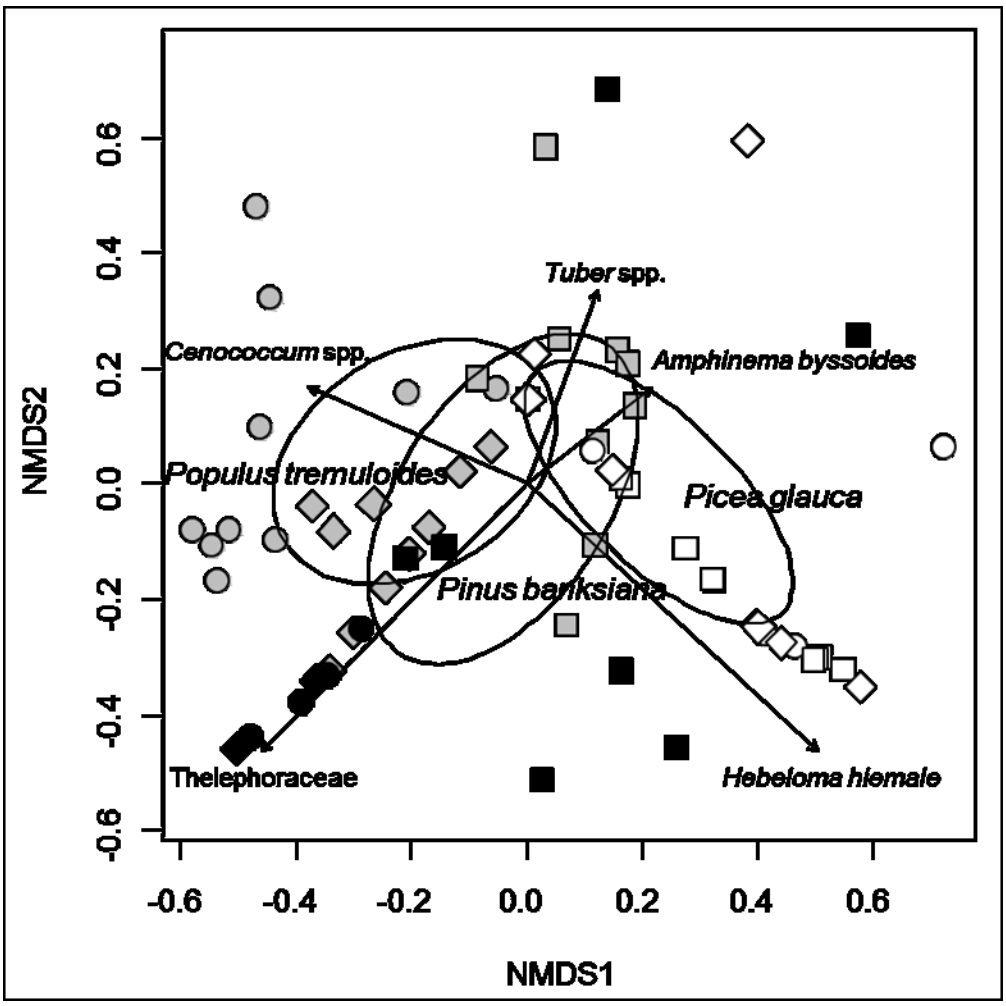

Figure 2-2b: (*Refer to Fig 2-2a legend*) Nonmetric multidimensional scaling ordination of ectomycorrhizal fungal species abundances measured as a percentage of root tip colonization per seedling for three host species (*Populus tremuloides, Pinus banksiana*, and *Picea glauca*) grown in three reclamation cover soils (FFM: forest floor material, peat, subsoil) in a growth chamber assay (stress = 0.100). Vectors are ectomycorrhizal fungal species; their direction and length represent their general influence on ectomycorrhizal community composition. Ellipses were generated using the standard deviation of point scores to group seedlings by host species. *Note: Only seedlings grown in unsterilized cover soils are represented.

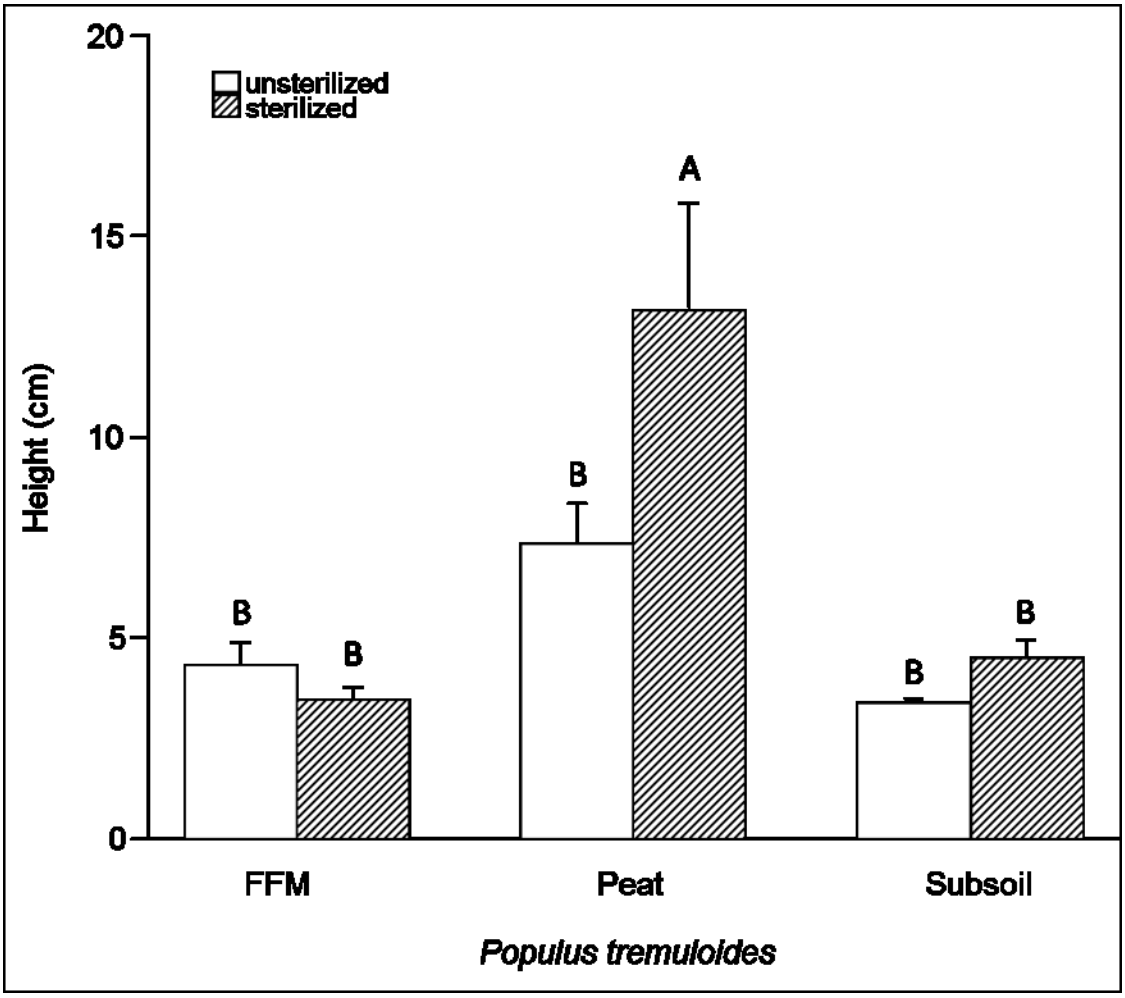

Figure 2-3: Height of *Populus tremuloides* seedlings grown in three different reclamation cover soils (FFM: forest floor material, peat, subsoil) sterilized or left unsterile (n=10). Differences in lettering represents a *p*-value of <0.05. Error bars represent one standard error.

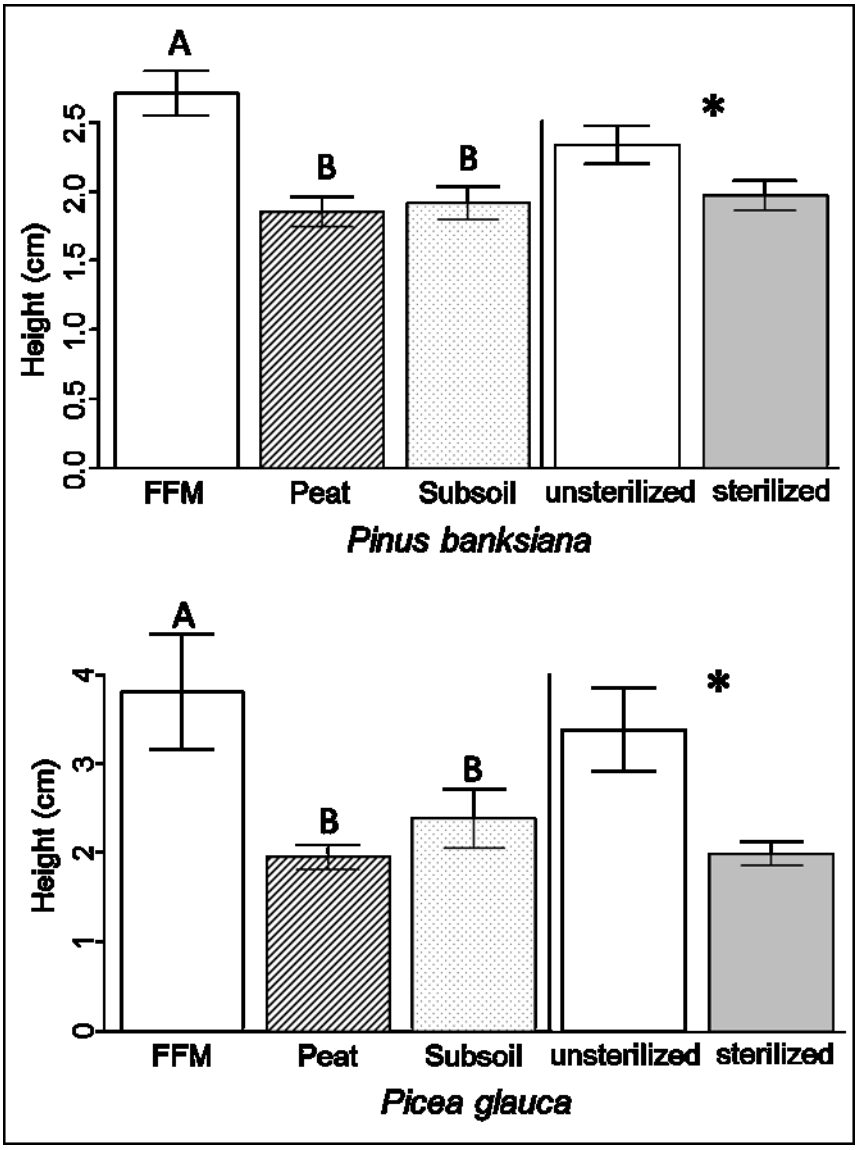

Figure 2-4: Height of *Pinus banksiana* and *Picea glauca* seedlings grown in three different reclamation cover soils (FFM: forest floor material, peat, subsoil) sterilized or left unsterilized (n=10). Asterisk or differences in lettering represent a *p*-value of <0.05. Error bars represent one standard error.

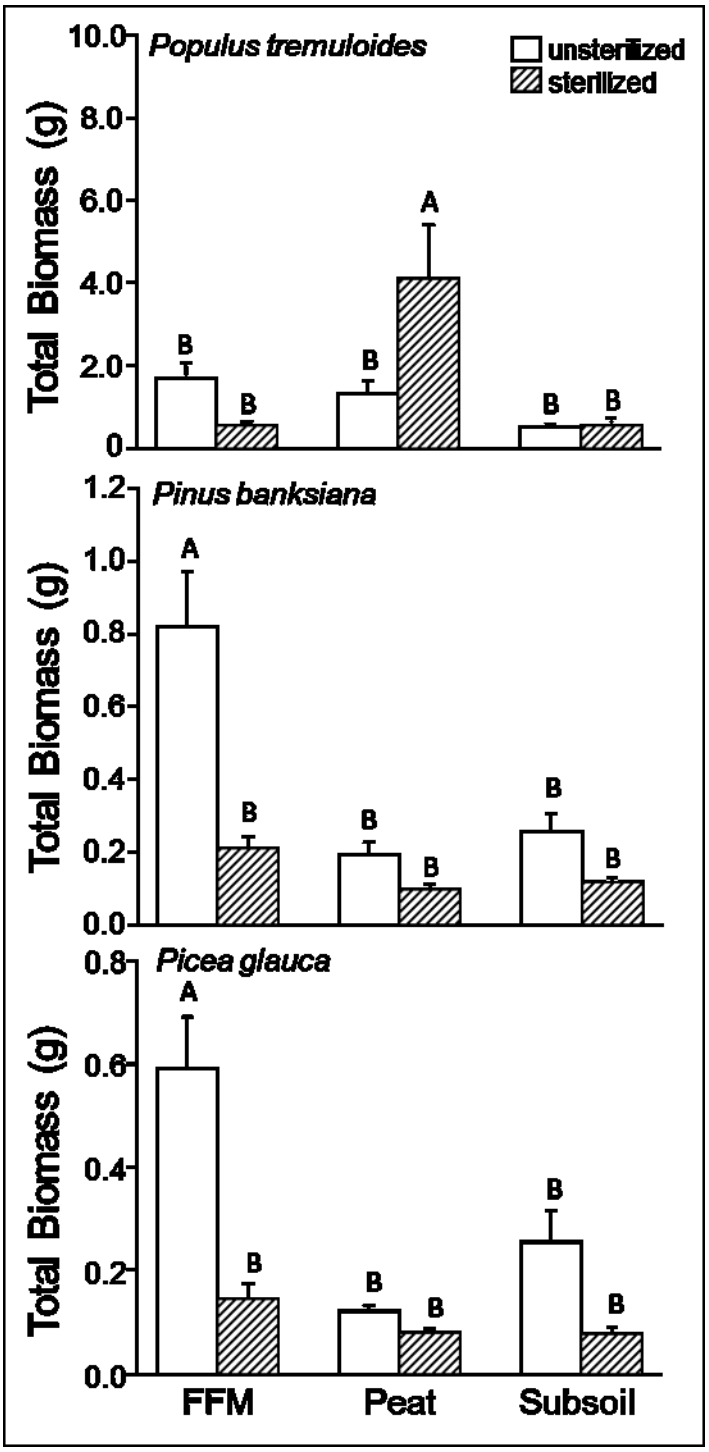

Figure 2-5: Total biomass of *Populus tremuloides*, *Pinus banksiana,* and *Picea glauca* seedlings grown in three different reclamation cover soils (FFM: forest floor material, peat, subsoil) sterilized or left unsterilized (n=10). Differences in lettering represents a *p*-value of <0.05 (comparisons were not made between host species). Error bars represent one standard error.

Chapter 3: Effects of soil temperature during budflush, and speed of soil warming on the growth of trembling aspen (*Populus tremuloides* Michx.) seedlings

3.1 Introduction

In the boreal biome, below-freezing temperatures can last for six or more months (Rumney, 1968) leaving a short window of time for tree growth to occur after soils warm in the spring. Low soil temperatures have a profound influence on tree growth (Bonan and Shugart, 1989) likely through limitations in soil nutrient availability (Van Cleve and Barney, 1980; Binkley *et al*., 1994; Chapin, 1995), plant physiological processes (Wan *et al*., 1999; Wan *et al*., 2001; Landhäusser *et al*., 2001), and/or cellular growth (Körner, 1998; Shi *et al*., 2008). However, the exact mechanism by which growth is reduced under cold soil temperatures is unknown and likely varies among ecosystem and species (Fajardo and Piper, 2014). *Populus tremuloides* Michx. (trembling aspen), a broadleaf tree common to the boreal forest and wide-spread in North America, is sensitive to cold soils; likely one of the reasons its ability to thrive at high altitudes or latitudes is restricted. Low soil temperature is also hypothesized as a factor driving the loss of aspen during late successional stages in boreal mixedwood forests. These late successional boreal forests are dominated by coniferous trees that, in comparison with early successional broadleaf forests, decrease the amount of sunlight reaching the soil and increase the accumulation of needle litter and/or the development of feather moss layers, which further insulates the soil (Landhäusser *et al*., 2001; Startsev *et al*., 2008). Aspen seedlings exposed to consistent soil temperatures of 5-10 °C have reduced growth, shoot water potential, and root water flow (Wan *et al*., 1999; Wan *et al*., 2001; Landhäusser *et al*., 2001). At or below 5 °C soil temperature, aspen seedlings exhibited complete inhibition of root growth and reductions in leaf gas exchange (Wan *et al*., 1999; Landhäusser *et al*., 2001). While seedlings showed reduced growth and suppressed photosynthesis in response to cold soils, concentrations of nonstructural carbohydrate (NSC) reserves increased (Landhäusser *et al*., 2001; Solfjeld and Johnsen, 2006).

Nonstructural carbohydrates are starches and water soluble sugars critical for growth, respiration, and osmotic regulation, which may be important for tolerating cold soils.

Aspen seedlings are more sensitive to cold soils than other coniferous trees that inhabit the boreal forest region. Peng and Dang (2003) have shown that early successional aspen and jack pine (*Pinus banksiana* Lamb.) seedlings grow best at soil temperatures of approximately 20 and 22 °C (respectively), whereas white spruce (*Picea glauca* Moench Voss.) and black spruce (*Picea mariana* Mill.) seedlings prefer lower temperatures (approximately 13.5 and 16.0 °C, respectively). In addition to the differences in growth, aspen seedlings had the lowest rates of photosynthesis at 5 °C soil temperature (Peng and Dang, 2004). While spruces tend to be more tolerant of low soil temperatures compared with aspen in terms of growth and physiology, most boreal tree seedlings responded similarly in the short-term with increased NSC concentrations. For example, Karst and Landhäusser (2014) found the NSC reserves in lodgepole pine (*Pinus contorta* Douglas ex Loudon) and black spruce increased in concentration when grown at 5 °C compared with 20 °C.

Though the boreal biome is known as a cold climate, the temperature of soils can be highly variable across the landscape and is determined by soil properties and an array of other environmental factors related to topography and vegetation. Soils in the Central Mixedwood boreal forest are dominated by Luvisolic and Brunisolic soil orders in the uplands which are composed of mineral soils with organic LFH soil horizons varying in thickness, while in lowland areas, soils are considered organic, dominated by peat of different stages of decomposition (Natural Regions Committee, 2006). Mineral and organic soils differ in physical properties that in-turn determine their temperature. Peat contains large amounts of organic matter, has a low bulk density, high water holding capacity, and low thermal conductivity compared with upland

forest soils (Bonan and Shugart, 1989; Leatherdale *et al*., 2012; Farnden *et al*., 2013). As a result, organic soils generally have lower average soil temperatures and slower warming rates (Bonan and Shugart, 1989). In addition to soil properties, differences in overstory vegetation may also have a profound influence on soil temperature. In the Central Mixedwood region, upland forests typically contain early successional aspen and jack pine stands, mid-successional mixedwood stands composed of aspen and white spruce, and late successional white spruce stands. Lowlands are dominated by black spruce and tamarack (*Larix laricina* Du Roi). Mature aspen forests have greater canopy cover, an easily decomposed litter layer, a thick mineral soil layer enriched with decomposed organic materials, a greater diversity of understory vegetation, and warmer soils compared with coniferous forests which often have moss dominated understories and thick poorly decomposed litter layers (Macdonald and Fenniak, 2006). Although broadleaved forests have greater canopy cover, light transmission to the understory is much greater compared with forests containing mature spruce (Constabel and Lieffers, 1996) resulting in warmer soils.

Natural or anthropogenic disturbances can also influence soil temperature regimes. Wildfires may combust soil organic matter, alter soil physical and chemical properties, and decrease the soil surface albedo, leading to increases in soil temperature (Certini, 2005; Nossov *et al*., 2013; Jafarov *et al*., 2013), potentially leaving seed dispersal as the only means of re-establishment after severe wildfires (Vacchiano *et al*., 2014). Wildfires can also result in lower soil temperatures in circumstances where much of the vegetation is removed and water levels rise, enabling paludification (Wein, 1983). Apart from wildfire, other natural disturbances that result in canopy gaps throughout the forest create warmer soils by allowing more light to reach the understory, encouraging the regeneration of early-successional understory vegetation such as

Calamagrostis canadensis and *Chamerion angustifolium* (Cháves and Macdonald, 2010). More recently, anthropogenic disturbances have increased significantly throughout the boreal forest. The disturbances range in scale and severity and have the capacity to alter vegetation recovery via alterations in soil temperature regimens. For instance, previously logged areas invaded by *Calamagrostis canadensis* led to a greater accumulation of organic matter (litter) on the soil surface which resulted in lower average soil temperatures (Hogg and Lieffers, 1991). The lower soil temperatures caused up to one month delay in spring soil thaw, which potentially inhibited the regeneration of native conifer seedlings (Hogg and Lieffers, 1991). Some anthropogenic disturbances are severe enough to require active reclamation. Oil sands surface mining in northern Alberta is a relatively new disturbance requiring forest reclamation, and given that this disturbance occurs in a region where cold soils are limiting to tree growth, it is critical to test whether reclamation practices influence soil temperature.

Salvaged peat and forest floor material (FFM) are often used as surface substrates (cover soils) in reclamation following oil sands mining. Both cover soils differ greatly in physical properties and soil biological activity (Moskal *et al*., 2001; McMillan *et al*., 2007; MacKenzie and Quideau, 2012). Landscape-scale forest reclamation research has revealed that spring soil temperatures of areas covered with pure peat remain lower than those of areas covered with FFM (Data collected by O'Kane Consultants; Fig. 3-1). This slower soil warming could have a profound effect on the establishment and growth of aspen seedlings in reclamation areas, which otherwise has been a promising species to use in reclamation for its rapid establishment and growth rate. After two growing seasons at a large-scale forest reclamation research site (i.e., the Aurora Soil Capping Study as described in Chapter 2), pronounced reductions in height growth were observed for aspen seedlings planted into peat compared with FFM (Fig. 3-2). It is not

clear, however, whether these observed differences were the result of delayed spring soil warming or due to other soil variables such as nutrient deficiencies. To test the effects of initial soil temperature during seedling budflush, and the rate of soil warming after budflush on subsequent growth, physiology and carbon storage of aspen seedlings, I set up a growth chamber experiment predicting that seedling growth would be reduced because (a) colder temperatures reduce stomatal conductance, photosynthesis, and shoot water potential, (b) nutrient availability decreases in cold soils, and (c) NSC reserves increase in concentration. A slow rate of soil warming results in seedlings exposed to cold temperatures for longer compared with those grown in soils with a faster warming rate, which will restrict their growth for reasons mentioned above. Lastly, I predict there to be no differences in growth of seedlings grown in peat and FFM should temperature be the single limiting factor.

3.2 Methods

3.2.1 Experimental design

The cover soils, peat and FFM, were collected from the Aurora Soil Capping Study field site in late August 2013 and kept at 4 °C for approximately eight months. Frozen, one- year- old, dormant, aspen 5-12 cm (diameter-depth of plug) seedlings were obtained from Woodmere Nursery Ltd. (Peace River, Alberta). Seedlings were thawed at 4 °C prior to planting. Seedlings of an even size distribution were hand-selected, and a subsample of 15 (for each of the two starting times; described below) were destructively sampled for initial morphological measurements. The experiment was a 3 × 2 factorial in which seedlings (n=10) were exposed to three soil temperature regimens emulating those observed in the field over a full growing season. Three periods were identified corresponding to different phenological stages of seedlings: (i) the *budflush period* where soil temperature was held constant for two weeks after the initiation of

budflush, (ii) the *warming period* where shoots of seedlings were expanding and the length of time required to reach a soil temperature of 20 °C was varied, and (iii) the *growth period* where soil temperature was maintained at approximately 20 °C for the remainder of the 70 day experiment (Fig. 3-3). Emulating spring and summer soil temperatures from FFM in the, the first group of seedlings (control) were (i) flushed at 8 °C soil temperature, (ii) warmed to 20 °C in eight days (by approximately 1.5 °C/day), and (iii) maintained at 20 °C for the remainder of the 70 day experiment. Emulating spring soil temperatures field regimes displayed in salvaged peat, the second group of seedlings were (i) flushed at 5 °C, (ii) warmed to 20 °C in eight days (by approximately 2 °C/day), and (iii) maintained at 20 °C for the remainder of the 70 day experiment. To test the influence of a slow rate of warming (observed in pure peat areas) on seedling growth, the third group of seedlings were (i) flushed at 5 °C, (ii) warmed to 20 °C in 26 days (by approximately 0.6 °C/day), and (iii) maintained at 20 °C for the remainder of the 70 day experiment (Fig. 3-3). Air temperature and relative humidity were maintained at an average of 15 °C and 40 %, respectively during the starting period, and increased to 20 °C and 55 % once the control treatment reached 20 °C soil temperature (Fig. 3-3). Air temperature in the growth chambers were monitored daily using data loggers (onset HOBO® pro series Data Logger). Light levels in the growth chamber were maintained between 350 and 400 nm, measured using a hand-held light meter (Decagon Sunfleck Ceptometer) and day length lasted 17 hours and decreased to 16 hours after 22 days. Seedlings were watered regularly to maintain near field capacity and received no fertilizer. The experiment was staggered in time due to limitations in cooling capacity; specifically, the group of seedlings flushed at 8 °C began 42 days after the other two groups.

3.2.2 Pot and water bath design

One hundred and thirty-two 20 × 10 cm (height × diameter) water-tight pots were constructed from 10 cm (diameter) polyvinyl chloride (PVC) sewer pipe fitted with couplings and sewer caps using PVC primer and glue (Fig. 3-4). In between the coupling and PVC pipe a piece of window screen was inserted allowing for water drainage into a false bottom. Before seedlings were planted into pots, 25 x 0.64 cm (length × diameter) clear vinyl tubing was inserted into the bottom of pots, passing through the window screen by a small cut hole. Through this tubing I was able to remove excess water that had drained into the false bottom after watering using a syringe. I constructed a water bath system using four water-tight 90 × 90 × 20 cm polyethylene containers in which the pots were placed to maintain soil temperatures (Fig. 3-4). Perlite was added to the top layer of the cover soil in each pot and Styrofoam peanuts were scattered across and between pots for insulation. Water bath temperatures were controlled using digital thermostats (Model A419, Johnson Controls Inc., Milwaukee, WI) and soil temperatures were measured daily throughout the experiment with buried data loggers (HOBO® Pendant Temperature Data Logger) into extra pots for each treatment (n=4) containing only cover soil (Fig. 3-5).

3.2.3 Seedling measurements

Seedlings (n=10) grown in groups one and two were sampled twice, first when soil temperature reached 20 °C, and second at the end of the 70 day experiment. Seedlings (n=10) grown in the third group were sampled once at the end of the 70 day experiment. In addition to these seedlings, a subsample (n=15) for each of the two start times were selected for initial measurements. Initial measurements included morphology (height, root volume, stem, and root dry mass), NSC (stem and root) concentration, and whole-seedling (stems, roots) nutrient (NPK)

concentration. Experimental seedlings were measured for morphological, physiological, and NSC and nutrient (NPK) tissue concentrations. Morphological measurements included height growth, stem, leaf and root dry mass, number of leaves, leaf area and root volume. Leaves were dried at 70 °C for three days for dry mass measurements. Roots were washed immediately after harvesting for root volume measurements, and then dried at 100 °C, along with shoots, for one hour and then at 70 °C for three days for dry mass measurements. Photosynthetic rate and stomatal conductance were measured on one newly formed, fully expanded leaf per seedling using a LI-6400 portable photosynthesis system (Li-Cor, Lincoln, NE) with a broadleaf cuvette. Light intensity and temperature in the cuvette were maintained at 530 μmol m^{-2} s^{-1} and 20 °C, respectively, while humidity was maintained at ambient conditions. Immediately after gas exchange measurements were taken, shoot water potential was measured by cutting seedlings mid-way up the main stem and inserting the whole upper shoot into a pressure bomb (Soilmoisture Equipment Corp., California, USA). Physiological measurements were taken only on seedlings sampled after the warming period due to leaf senescence at the end of the growing period. Dry roots, stems, and leaves of each seedling were ground by passing the tissue through #40 mesh (0.4 mm) using a Wiley Mini-Mill (Thomas Scientific, New Jersey, USA). Starch and soluble sugar concentration and content were analyzed on dry roots and stems following the methods described in Chow and Landhäusser (2004). Seedling nutrient (NPK) concentrations were measured for whole seedlings by combining the dried ground tissues in proportion relative to mass and pooling seedlings of each temperature treatment-cover soil combination (n=5). Total N was analyzed using Dumas combustion (Dumas Methods, 1996) using Costech Model EA 4010 Elemental Analyzer (Costech International Strumatzione, Florence, 2003), total P and K analyses followed US EPA digestion methods (US EPA, 2007) and concentrations were

analyzed using an iCAP6000 Series ICP-OES Spectrometer (Thermo Fisher Corporation, Cambridge, UK, 2012). The nutrient availability (NPK) for each cover soil was measured using plant root simulator (PRS™) probes (Western Ag Innovations, Saskatoon, SK) installed into extra pots without seedlings (n=4). Plant root simulator probes were inserted immediately before warming to capture soil nutrient availability at the beginning of the experiment when soils were still cold, and replaced by new PRS probes to measure nutrient availability once the soil temperature reached 20 °C to measure nutrient availability throughout the remainder of the experiment.

3.2.4 Data analysis

This 3 × 2 factorial experiment (three temperature regimens × two cover soils) was set up as a randomized complete block design. Each group of seedlings was divided into two blocks rotated once a week (pots were also repositioned within a block) to remove possible spatial environmental variation within the growth chamber; block was therefore not included in any statistical models. The warmer budflush temperature treatment was initiated approximately five weeks after the other two cold budflush treatments due to limitations in cooling capacity. This blocking in time was not included in any statistical models for several reasons: 1) the initial seedling measurements (morphology and NSC concentrations) did not vary between the two start times (Appendices VI & VII), 2) variation in environmental conditions between start times was unlikely to occur in a controlled growth chamber setting, and 3) the experiment was executed in the same manner for both start times. All statistical analyses were performed using R statistical programming language (R Development Core Team, 2008). The effects of soil temperature during budflush, and soil temperature warming speed, each by cover soil type on seedling morphology, physiology (measured only after the *warming period*), seedling nutrient concentration, root and shoot NSC concentrations and content, and cover soil nutrient

availability were separately tested using a two-way analysis of variance (ANOVA) following a general linear model approach when assumptions were met, and a permutation ANOVA when assumptions were not met (Wheeler, 2010). If main effects were significant (α=0.05), post-hoc tests were performed using Tukey's HSD test.

3.3 Results

3.3.1 Morphology

After the *warming period*, seedlings flushed at 8 °C had grown more stem mass (by 18 %) and leaf mass (by 15 %), and had marginally greater height growth (by 5 %) compared with seedlings which had flushed at 5 °C (Table 3-1; Appendix VIII). Cover soil affected the number of leaves produced per seedling regardless of budflush temperature, which was 21 % greater for those grown in FFM than peat (Table 3-1). After the *growing period*, the effect of soil temperature during budflush and cover soil on morphological seedling measurements became more pronounced (Appendix IX). At that time, seedlings flushed at 8 °C had 50 to >100 % increased height growth, root mass, and root volume (Fig. 3-6), in addition to having 16 % greater stem mass, 20 % greater leaf mass, and 25 % greater leaf area than those flushed at 5 °C (trends following those in Fig. 3-6). Stem mass was 10 % greater for seedlings grown in peat (1.9 ±0.06 g) compared with FFM (1.7 ±0.07 g), regardless of budflush temperature. Differences measured in leaf numbers earlier on were not detected due to leaf senescence at the end of the growing period; average number of leaves at this time was 30 (± 1.6). For seedlings flushed at 5 °C, seedlings grown in peat had 32 % greater root volume than those grown in FFM (Table 3-2; Appendix X). All seedlings started to set bud early on during the *warming period* and all seedlings had set bud by approximately day 40 of the experiment (Fig. 3-5). No seedlings re-flushed after setting bud nor was there any mortality of seedlings.

3.3.2 Physiology

After the *warming period*, stomatal conductance was twice as high in seedlings flushed at 8 °C compared with those flushed at 5 °C (Table 3-3; Appendix XI). However, photosynthetic rate and shoot water potential were not different between these two groups of seedlings (Table 3-3; Appendix XI). In all treatments leaf senescence had occurred after the end of the growing period, therefore physiology was not measured.

3.3.3 Seedling nutrition

After the *warming period*, tissue nutrient concentration of seedlings flushed at 5 °C had 11 % greater N concentration than those flushed at 8 °C (Table 3-4; Appendix XII). Seedlings grown in peat had 11 % greater N concentration than those grown in FFM. Seedlings grown in FFM had 10% greater K concentration than those grown in peat, and there were no differences in P concentration between cover soil and soil temperature during budflush (Table 3-4). After the *growing period* seedlings flushed at 5 °C had 14 % greater N concentration than those flushed at 8 °C (Fig. 3-7; Appendix XIII). Seedlings grown in FFM had 29 % greater P concentration than those grown in peat (Fig. 3-8). Seedling K concentration was not affected by soil temperature during budflush or cover soil type (5.2 % mean ±0.17 % SE). For seedlings flushed at 5 °C, seedlings grown in the fast warming treatment had 7 % greater N concentration than those grown in the slow warming treatment, however there were no differences in P or K concentration (Table 3-5; Appendix XIV). Seedlings grown in FFM had 29 % greater P concentration than those grown in peat and there were no differences in N or K concentration (Table 3-5).

3.3.4 Nonstructural carbohydrates

After the *warming period*, average stem sugar concentration and content were 23 and 16 % lower than initial conditions, respectively. Average stem starch concentration and content were over three times and 3 % greater than initial conditions, respectively. Total stem NSC

content was only influenced by cover soil type and total stem NSC concentration was not influenced by soil temperature during budflush or cover soil type. Seedlings grown in FFM had 23 % greater total stem NSC content than seedlings grown in peat. Stem starch concentration and content were also affected by cover soil type. Seedlings grown in FFM had 45 and 68 % greater stem starch concentration and content, respectively, than seedlings grown in peat (Fig. 3-9); there were no differences in stem sugar concentration or content (Table 3-6; Appendix XV). Average root sugar concentration and content were 45 and 64 % lower than initial conditions, respectively. Average root starch concentration and content were 70 and 43 % greater than initial conditions, respectively. Total root NSC concentration was influenced by soil temperature during budflush and there was an interaction between soil temperature during budflush and cover soil on total root NSC content (however, post-hoc tests do not show any significant differences). Seedlings flushed at 5 °C had 14 % greater total root NSC concentration than seedlings flushed at 8 °C. Specifically, seedlings flushed at 5 °C soil temperature had 16 % greater root sugar concentration than seedlings flushed at 8 °C, and there were no differences in root starch concentration (Fig. 3-10; Appendix XV). Seedlings flushed at 5 °C and grown in FFM had 27 % greater root sugar content than seedlings flushed at 8 °C, and there were no differences in root starch content between cover soil or budflush temperature (Table 3-6; Appendix XV).

After the *growing period*, average stem sugar concentration and content were 35 % lower and 24 % greater (respectively) than that of initial conditions and average stem starch concentration and content were over 12 times that of initial conditions. There were no differences in total stem NSC concentration or content (Appendix XVI). Stem and root sugar and starch concentration, and content were affected by cover soil, and/or soil temperature during budflush but no significant interactions were detected (Appendix XVI). Seedlings grown in FFM

had marginally greater (1 %) stem sugar concentration compared with seedlings grown in peat, and seedlings that were flushed at 8 °C had 15 % greater stem sugar content compared with seedlings flushed at 5 °C (Table 3-7). Seedlings grown in peat had marginally greater (14 %) stem starch content compared with seedlings grown in FFM (Fig. 3-9). Average root sugar concentration and content were twice as low and 2 % greater than initial conditions, respectively. Average root starch concentration and content were at least three times greater than initial conditions. Total root NSC concentration was greater for seedlings flushed at 5 °C, and total root NSC content was not affected by soil temperature during budflush or cover soil (Appendix XVI). Seedlings flushed at 5 °C soil temperature had 40 % greater sugar and 53 % greater starch concentrations compared with seedlings flushed at 8 °C (Fig. 3-10). Seedlings grown in peat had 17 % greater root starch content than seedlings grown in FFM, but there were no differences in root sugar content (Table 3-7).

For seedlings flushed at 5 °C, stem sugar concentration and content were 32 % lower and 19 % greater than initial conditions. Stem starch concentration and content were at least 15 times greater than initial conditions. Total stem and root NSC concentration and content were not affected by the speed of soil warming, the cover soil, or the interaction between the two (Appendix XVII). Stem starch content was affected by cover soil; seedlings grown in peat had 15 % greater stem starch content than those grown in FFM, however there were no differences in stem sugar content (Table 3-8). Average root sugar concentration and content were 71 % lower and 2 % greater than initial conditions, respectively. Average root starch concentration and content were at least three times greater than initial conditions. Root starch concentration was 10 % greater for seedlings grown in the fast warming treatment compared with those grown in the slow warming treatment; however there were no differences in root sugar concentration (Table

3-8). Conversely, root NSC content was not affected by the speed of soil warming, cover soil, or the interaction between the two (Appendix XVII).

3.3.5 Cover soil nutrient availability

After the *warming period*, soil nutrient availability differed between cover soils in response to soil temperature during budflush (N availability was affected by the interaction between soil temperature and cover soil; p=0.01; Appendix XVIII). Peat in the 8 °C budflush temperature treatment had the greatest total available N by at least 43 %, and peat in the 5 °C budflush temperature treatment had three times as much available N than FFM in the 5 °C budflush temperature treatment (Table 3-9). Forest floor material had four and a half times greater P and six times greater K concentration than peat, regardless of budflush temperature (Table 3-9). After the *growing period*, available N for cover soils in the 8 °C budflush soil temperature treatment was twice as high compared with 5 °C, and peat cover soils had twice as much available N than FFM cover soils regardless of budflush temperature (Fig. 3-7; Appendix XIX). Available P was over four times greater for FFM cover soils compared with peat, regardless of budflush temperature treatment (Fig. 3-8). Available K was at least three times greater for FFM cover soils in the 8 °C budflush soil temperature treatment (mean 112.5 μmol/10 cm^2, ±11.00 μmol/10 cm^2 SE) compared with all other cover soils, which were not different from each other. For those cover soils in the 5 °C budflush temperature treatment, available N was almost four times greater for peat compared with FFM, regardless of soil temperature warming speed (Table 3-10; Appendix XX). Available P was four times greater for FFM cover soils compared with peat, regardless of soil temperature warming speed (Table 3-10). Available K was at least three times greater for FFM cover soils in the slow warming treatment compared with all other cover soils (Table 3-10).

3.4 Discussion

Morphology, physiology, and NSC reserve status measured after both the *warming period* and the *growing period* indicate that aspen seedling growth was primarily limited by 5 °C soil temperature during budflush (as opposed to 8 °C) and less so by the rate of soil warming after budflush (slow or fast). The failure of seedlings to recover in growth after soils had warmed is surprising because other stresses, such as drought that initiate budset in aspen, lead to reflushing of these buds once conditions improved (Landhäusser *et al*. 2012). As predicted, nutritional differences between FFM and peat cover soils did not have a large influence on seedling growth compared with soil temperature. In addition, smaller differences between seedling nutrient status in response to soil temperature compared with the larger response observed in nutrient availability of cover soils indicates that seedling nutrient uptake may have been temperature limited. Seedling growth was not greatly influenced by the nutritional differences observed in cover soils, while stem NSC reserves showed a response to cover soil nutritional differences. On the other hand, root NSC reserves responded to low soil temperature during budflush. These main findings, limitations of this experiment, and suggestions for improvement are discussed below.

3.4.1 Low soil temperature during budflush limits growth after initial exposure and after temperatures have increased

Seedlings flushed at 8 °C exhibited 5-20 % greater aboveground growth than those flushed at 5 °C after the *warming period*, and substantially greater (>50 %) height growth, root mass and root volume after the *growing period*. One study found aspen seedlings exposed to 5 °C soil temperature for four months to show 7.5 times less root mass compared with those grown at 20 °C (Peng and Dang, 2003). Similarly, aspen seedlings flushed and exposed to 5 °C soil temperature for six weeks had only half the leader length compared with those grown at 15 °C

soil temperature (Landhäusser *et al*., 2001). In addition to cold-induced growth limitations, I found stomatal conductance (measured after the *warming period*) to be twice as low for seedlings flushed at 5 °C compared with 8 °C, but other physiological parameters (photosynthesis and stem water potential) did not differ between the two soil temperatures. A greater stomatal conductance observed for seedlings flushed at 8 °C compared with those flushed at 5 °C may have been an outcome of a greater (50 %; *data not shown*) relative humidity (RH) in the cuvette at the time of measurement, resulting in a 60 % (*data not shown*) lower vapor pressure deficit for these seedlings. A high RH at the time of measurement may have been an outcome of a greater number of leafed-out aspen seedlings in the growth chamber compared with when measurements were taken for the seedlings flushed at 5 °C, with a third less seedlings present. This difference in humidity could have been driven by greater transpiration (4 %; *data not shown*) of aspen seedlings flushed at the warmer soil temperature compared with seedlings flushed at 5 °C. Other studies have found larger differences in stomatal conductance, photosynthesis, and root water flow for seedlings grown at 5 and ~20 °C (Landhäusser *et al*., 2001; Wan *et al*., 1999), while finding no differences for seedlings grown at 5 and 10 °C soil temperature (Wan *et al*., 1999). Even though I observed lower stomatal conductance in seedlings flushed at 5 °C compared with 8 °C (which may not have been a response to cold but rather an outcome of differences in humidity), both groups of seedlings may have been experiencing physiological limitations from such low soil temperatures which may explain why photosynthetic and shoot water potential measurements were not different between the two groups of seedlings. Another explanation for why I did not observe strong cold-induced physiological limitations in seedlings flushed at 5 °C compared with 8 °C could have been because aspen is isohydric (Wan *et al*., 2004) allowing it to make active adjustments to maintain

xylem water potential during low water supply at a soil temperature of 5 °C (which has been shown to restrict root water flow; Wan *et al*., 1999). In my study, root sugar concentration increased for seedlings flushed at 5 °C after the *warming period,* a common response to cold soil temperatures (Sauter, 1988), possibly supporting this explanation.

While reductions in growth and physiological variables can be expected in aspen seedlings exposed to low soil temperature, given that aspen has an indeterminate growth strategy, it is surprising that these cold-stressed seedlings did not re-flush after soil temperature was increased to 20 °C for the remaining 45 days. This may suggest that stress induced by cold-during budflush restricted subsequent growth after warming. Speculatively, this may be the result of unrepaired damage to photosynthetic machinery and/or continuous hormonal stress signaling. Scots pine (*Pinus sylvestris* L.) seedlings that were overwintered followed by exposure to low spring soil temperatures (1 and -2 °C) showed reductions in photosynthetic recovery compared with seedlings grown at higher soil temperatures (15 and 10 °C; Ensminger *et al*., 2008). Consistent cold soil temperature treatment was more limiting to Scots pine photosynthetic capacity than exposure to intermittent freeze-thaw cycles, suggesting that consistent exposure to cold soil temperatures, even if not frozen, may have long lasting physiological effects (Ensminger *et al*., 2008). The authors conclude that consistent exposure to cold soil temperatures resulted in the suppression of electron transport between PSII and PSI in addition to the down regulation of Rubisco (reducing photosynthesis), both of which may be slow to recover (Ensminger *et al*., 2008).

In addition to damage to photosynthetic machinery, hormonal signaling from cold stress may also have played a role in reducing the growth of aspen seedlings exposed to low soil temperature during budflush. Increases in the concentration of stress hormones such as abscisic

acid (ABA) occurs in trees in times of cold stress and is thought to play a role in signaling between roots and shoots. Aspen seedlings exposed to 5 °C soil temperature have increased ABA concentration after eight hours of exposure, potentially playing a role in the signaling of stomatal closure (Wan *et al*., 2004; Wan and Zwiazek, 2001). Although concentrations of ABA have been shown to return to background levels after the removal of drought-induced stress in some plants (Harris and Outlaw, 1991; Zeevaart, 1980), other hormones may not be so transient and continuous hormonal signaling of stress may be an explanation as to why seedlings did not re-flush after warming. Important signaling elements such as Ca^{2+} are drawn into the cytosol from reserves during the onset of stress and are responsible for the initiation of signal transduction pathways to synthesize regulatory molecules, such as ABA and other important stress hormones (Knight, 2000). Therefore, reductions in dry-tissue calcium concentrations may be an indication of the occurrence of a stress-induced regulatory process. In our study, whole-seedling calcium concentrations were 50 and 73 % lower (*data not shown*) in seedlings flushed at 5 °C compared with those flushed at 8 °C after the *warming period* and after the *growing period*, respectively, which may suggest that stress hormones were being synthesized even after warming. It is also surprising that a slow rate of warming after budflush for seedlings flushed at 5 °C did not influence seedling growth given that exposure to cold soils during budflush had a profound influence on growth. This result could indicate that (1) the soil temperature during the budflush period is more influential on growth than the rate at which soils warm after budflush, and/or that (2) the chosen rate of 'fast' warming was not fast enough to result in additional growth. Rates of warming (slow vs. fast) were chosen to mimic field observations of peat versus FFM, which suggests that warming was not a factor in our study as soil temperatures of 8 °C had already such

negative effects on seedlings growth and physiology which could not have overcome by the following soil warming.

3.4.2 Cover soil nutrient availability in response to cold soil temperature and cover soil type showed greater differences compared with those of seedling nutrient status

After the warming period, NPK availability differed based on cover soil type, N being over four times greater in peat, while P and K were at least four times greater in FFM. Temperature during budflush only influenced N availability, which was greater in cover soils at 8 °C compared with 5 °C. After the *growing period*, trends in N availability remained similar, with K availability also being greater at 8 °C. It is surprising that initial soil temperature had a lasting effect on N and K nutrient availability in the cover soils even after warming had occurred. Perhaps the two different soil temperatures during budflush had differential influences on microbial activity which persisted throughout the experiment even after warming, although this process has not been tested to my knowledge. Whole-seedling (leaves, stems, and roots combined) nutrient concentrations after the *warming* and *growing periods*, moderately reflected cover soil nutrient availability patterns, but by a lower magnitude. Differences in NPK whole-seedling concentrations for seedlings grown in peat versus FFM were approximately 10 % across both sampling periods. Soil temperature during budflush only influenced seedling N concentration, which was approximately 10 % greater in seedlings exposed to 5 °C for both sampling periods, opposite of what was observed in cover soil nutrient availability. Lower N concentration in seedlings flushed at warmer soil temperatures was likely not a direct effect of soil temperature but rather a reflection of greater growth of seedlings exposed to 8 °C soil temperature. While whole-seedling N concentration may not have been influenced by soil temperature or cover soil type (even though cover soils differed greatly in available N), seedling P concentration was greater for seedlings flushed at 8 °C soil temperature, and for seedlings

grown in FFM compared with peat (reflecting cover soil P availability). Nutritional differences were not reflected in growth but they may have influenced the NSC levels in the stems. Stem starch concentration and content were greater for seedlings grown in FFM compared with peat after the *warming period*, while this trend faded after the *growing period*. Initial increases in stem NSC for seedlings grown in FFM compared with peat could have been due to the sensitivity of lower N availability in FFM cover soils. Lower N availability may limit growth, resulting in the accumulation of NSC, or active accumulation of NSC may have been a response to N limitation. The latter is more likely (Kabeya, 2010 *and references therein*) as there were not large growth differences observed between cover soils.

It is interesting that nutritional differences between cover soils were of a greater magnitude (~300 %) than what was observed in seedling tissue (~10 %), suggesting that seedling nutrient uptake is not a direct reflection of soil nutrient availability. Given that nutrient availability was substantially different between cover soils, but not reflected in seedling nutrition, seedling nutrient uptake may have been more restricted by low soil temperature than cover soil nutrient availability. Furthermore, cover soil type did not play a large role in driving seedling growth (compared with soil temperature during budflush) which also suggests that seedling nutrient uptake may have been limited by low temperature. Along with the small physiological differences observed between seedlings flushed at 5 and 8 °C, both groups of seedlings may also have been limited to some degree in their nutrient uptake capabilities, masking cold-induced reductions in whole-seedling nutrient concentration.

3.4.3 Conclusions

These results indicate that soil temperatures at or below 5 °C, which may occur in reclaimed areas using soils with high organic matter content, such as peat, during aspen seedling

budflush in the spring may limit seedling growth, and may be detrimental to seedling establishment on such reclamation areas. Overall low soil temperature during budflush ultimately decreased growth (mainly in height and root growth), stomatal conductance, and cover soil nutrient availability. Cold stress during budflush does not seem detrimental for NSC reserves since they increased by the end of the experiment compared with initial conditions, whereas other form of stress, such as drought, have been shown to deplete NSC reserves, potentially resulting in seedling mortality (Galvez *et al*., 2013). If seedlings are subjected to cold stress during budflush over multiple seasons, they may become preconditioned to deal with this stress which then may not be an issue for seedling establishment and survival, or if they continue to be growth-limited by the cold soil temperatures during budflush, they could be shaded out by other vegetation and/or become susceptible to disease/insect infestation, leaving them at risk to mortality.

Tables

Table 3-1: Morphological responses of *Populus tremuloides* seedlings to soil temperature at the time of budflush, and cover soil (peat or forest floor material (FFM)) after the soil *warming period* (n=10). Values represent means (± one standard error). Differences in lettering indicate statistically significant (α=0.05) differences among means for each response variable.

Response Variable	Soil temperature during aspen budflush			
	8 °C		5 °C	
	Peat	FFM	Peat	FFM
Height Growth (cm)	4.2 (±0.54)[a]	3.2 (±0.77)[a]	3.0 (±1.10)[a]	1.5 (±0.50)[a]

Stem Mass (g)	1.2 (±0.07)^{ab}	1.4 (±0.12)^{a}	1.0 (±0.04)^{b}	1.1 (±0.06)^{ab}
Root Volume (cm^3)	4.7 (±0.60)^{a}	4.4 (±0.70)^{a}	4.5 (±0.20)^{a}	5.5 (±0.44)^{a}
Root Mass (g)	1.5 (±0.10)^{a}	1.4 (±0.11)^{a}	1.3 (±0.05)^{a}	1.3 (±0.07)^{a}
Leaf Mass (g)	0.9 (±0.06)^{a}	0.8 (±0.04)^{a}	0.7 (±0.05)^{a}	0.7 (±0.05)^{a}
# Leaves	25 (±1.5)^{a}	27 (±2.5)^{a}	25 (±3.6)^{a}	33 (±2.1)^{a}
Leaf Area (mm^2)	138.8 (±8.83)^{a}	125.9 (±5.40)^{a}	127.2 (±10.00)^{a}	127.5 (±8.50)^{a}

Table 3-2: Morphological responses of *Populus tremuloides* seedlings to soil temperature warming speed (fast: 2.0 °C/day, slow: 0.5 °C/day), and cover soil (peat or forest floor material (FFM)) after the *growing period* (n=10). Values represent means (± one standard error). Differences in lettering indicate statistically significant (α=0.05) differences among means for each response variable.

Response variable	Rate of soil warming after aspen budflush			
	Fast		Slow	
	Peat	FFM	Peat	FFM
Height Growth (cm)	6.1 (±0.96)^{a}	3.5 (±1.05)^{a}	3.0 (±1.13)^{a}	4.4 (±1.16)^{a}
Stem Mass (g)	1.8 (±0.06)^{a}	1.6 (±0.08)^{a}	1.8 (±0.11)^{a}	1.7 (±0.09)^{a}
Root Volume (cm^3)	8.7 (±0.51)^{a}	7.0 (±1.03)^{a}	9.6 (±0.73)^{a}	6.9 (±0.87)^{a}
Root Mass (g)	2.7 (±0.14)^{a}	2.4 (±0.24)^{a}	2.8 (±0.13)^{a}	2.5 (±0.26)^{a}
Leaf Mass (g)	1.1 (±0.06)^{a}	1.0 (±0.09)^{a}	1.0 (±0.07)^{a}	1.0 (±0.06)^{a}
# Leaves	27 (±1.8)^{a}	28 (±4.3)^{a}	30 (±2.4)^{a}	28 (±1.3)^{a}
Leaf Area (mm^2)	130.6 (±7.70)^{a}	112.2 (±11.18)^{a}	125.2 (±11.00)^{a}	125.2 (8.01)^{a}

Table 3-3: Physiological responses of *Populus tremuloides* seedlings to soil temperature during budflush, and cover soil (peat or forest floor material (FFM)) after the *warming period* (n=10). Values represent means (± standard error). Differences in lettering indicate statistically significant (α=0.05) differences among means of each response variable.

Response Variable	Soil temperature during aspen budflush			
	8 °C		5 °C	
	Peat	FFM	Peat	FFM
Stomatal Conductance (mol H_2O m^{-2} sec^{-1})	0.2 ±0.01[a]	0.2 ±0.01[a]	0.1 ±0.01[b]	0.1 ±0.01[b]
Photosynthetic Rate (μmol CO_2 m^{-2} sec^{-1})	8.2 ±0.64[a]	8.5 ±0.46[a]	8.1 ±0.62[a]	7.1 ±0.36[a]
Shoot Water Potential (MPa)	-0.6 ±0.08[a]	-0.6 ±0.04[a]	-0.8 ±0.12[a]	-0.5 ±0.01[a]

Table 3-4: *Populus tremuloides* total seedling (root, stem, foliage combined) nutrient (NPK) concentration in response to soil temperature during budflush, and cover soil (peat or forest floor material (FFM)) after the *warming period* (n=4). Values represent means (± one standard error). Differences in lettering indicate statistically significant (α=0.05) differences among means of each response variable.

Response Variable	Soil temperature during aspen budflush			
	8 °C		5 °C	
	Peat	FFM	Peat	FFM
Nitrogen (%)	1.2 ±0.14[ab]	1.0 ±0.06[a]	1.3 ±0.11[b]	1.2 ±0.10[ab]
Phosphorus (%)	1.3 ±0.13[a]	1.2 ±0.08[a]	1.2 ±0.09[a]	1.3 ±0.13[a]
Potassium (%)	6.9 ±0.61[a]	7.6 ±0.77[a]	7.3 ±0.49[a]	7.9 ±0.62[a]

Table 3-5: *Populus tremuloides* total seedling (root, stem, foliage combined) nutrient (NPK) concentration in response to soil temperature warming speed (fast: 2.0 °C/day, slow: 0.5 °C/day), and cover soil (peat or forest floor material (FFM)) after the *growing period* (n=4). Values represent means (± one standard error). Differences in lettering indicate statistically significant (α=0.05) differences among means of each response variable.

Response Variable	Rate of soil warming after aspen budflush			
	Fast		Slow	
	Peat	FFM	Peat	FFM
Nitrogen (%)	0.8 ±0.05[a]	0.9 ±0.06[a]	0.8 ±0.03[a]	0.8 ±0.12[a]
Phosphorus (%)	0.7 ±0.02[b]	0.9 ±0.23[a]	0.8 ±0.11[ab]	0.9 ±0.07[a]
Potassium (%)	5.2 ±0.18[a]	5.2 ±0.59[a]	5.5 ±0.86[a]	5.3 ±0.60[a]

Table 3-6: Nonstructural carbohydrate (NSC) concentration and content of *Populus tremuloides* seedlings (n=10) in response to soil temperature during budflush, and cover soil (peat or forest floor material (FFM)) after the *warming period*. Values represent the mean (± one standard error). Differences in lettering indicate statistically significant (α=0.05) differences among means of each response variable.

Organ	Response Variable	Soil temperature during aspen budflush			
		8 °C		5 °C	
		Peat	FFM	Peat	FFM
Stem	[Sugar] (%)	10.70 ±0.278[a]	10.21 ±0.310[a]	10.59 ±0.416[a]	11.07 ±0.298[a]
	Sugar Content (g)	0.13 ±0.008[a]	0.15 ±0.015[a]	0.12 ±0.005[a]	0.13 ±0.007[a]
Root	Sugar Content (g)	0.13 ±0.010[ab]	0.11 ±0.004[a]	0.12 ±0.008[ab]	0.14 ±0.009[b]

	Starch Content (g)	0.10 ±0.012[a]	0.09 ± 0.005[a]	0.08 ±0.013[a]	0.11 ±0.011[a]

Table 3-7: Nonstructural carbohydrate (NSC) concentration and content of *Populus tremuloides* seedlings (n=10) in response to soil temperature during budflush, and cover soil (peat or forest floor material (FFM)) after the *growing period*. Values represent the mean (± one standard error). Differences in lowercase lettering indicate statistically significant (α=0.05) differences among means of each response variable.

Organ	Response Variable	Soil temperature during aspen budflush			
		8 °C		5 °C	
		Peat	FFM	Peat	FFM
Stem	[Sugar] (%)	9.42 ±0.313[a]	9.97 ±0.362[a]	9.44 ±0.322[a]	10.14 ±0.278[a]
	Sugar Content (g)	0.20 ±0.011[a]	0.20 ±0.009[a]	0.18 ±0.010[a]	0.17 ±0.009[a]
Root	Sugar Content (g)	0.23 ±0.017[a]	0.22 ±0.016[a]	0.21 ±0.017[a]	0.19 ±0.018[a]
	Starch Content (g)	0.46 ±0.027[a]	0.38 ±0.019[a]	0.44 ±0.030[a]	0.40 ±0.037[a]

Table 3-8: Nonstructural carbohydrate (NSC) concentration and content of *Populus tremuloides* seedlings (n=10) in response to soil temperature warming speed (fast: 2.0 °C/day, slow: 0.5 °C/day), and cover soil (peat or forest floor material (FFM)) after the *growing period*. Values represent the mean (± one standard error). Differences in lowercase lettering indicate statistically significant (α=0.05) differences among means of each response variable.

Organ	Response Variable	Rate of soil warming after aspen budflush			
		Fast		Slow	
		Peat	FFM	Peat	FFM

Stem	[Sugar] (%)	9.44 ±0.322[a]	10.14 ±0.278[a]	9.95 ±0.221[a]	10.22 ±0.388[a]
	Sugar Content (g)	0.18 ±0.010[a]	0.17 ±0.009[a]	0.19 ±0.014[a]	0.18 ±0.011[a]
	[Starch] (%)	9.24 ±0.426[a]	9.04 ±0.396[a]	9.59 ±0.278[a]	8.63 ±0.510[a]
	Starch Content (g)	0.16 ±0.006[a]	0.14 ±0.011[a]	0.17 ±0.008[a]	0.15 ±0.014[a]
Root	[Sugar] (%)	7.16 ±0.326[a]	7.69 ±0.539[a]	7.73 ±0.197[a]	7.96 ±0.510[a]
	Sugar Content (g)	0.21 ±0.017[a]	0.19 ±0.018[a]	0.23 ±0.011[a]	0.22 ±0.035[a]
	[Starch] (%)	16.42 ±0.679[a]	16.89 ±0.505[a]	15.42 ±0.688[a]	14.76 ±0.624[a]
	Starch Content (g)	0.44 ±0.030[a]	0.40 ±0.037[a]	0.43 ±0.021[a]	0.37 ±0.033[a]

Table 3-9: Cover soil nutrient (NPK) availability (n=4) in response to soil temperature during seedling budflush, and cover soil (peat and forest floor material (FFM)) after the *warming period*. Values represent the mean (± one standard error). Differences in lowercase lettering indicate statistically significant (α=0.05) differences among means of each response variable.

Response Variable	Soil temperature during aspen budflush			
	8 °C		5 °C	
	Peat	FFM	Peat	FFM
Total Nitrogen (μmol/10 cm^2)	123.5 (±21.86)[a]	16.7 (±3.22)[c]	86.2 (±20.30)[b]	27.5 (±9.24)[c]
Phosphorus (μmol/10 cm^2)	0.7 (±0.16)[a]	2.6 (±0.62)[a]	0.5 (±0.23)[a]	2.9 (±0.97)[a]
Potassium (μmol/10 cm^2)	16.9 (±1.72)[b]	110.4 (±10.19)[a]	19.2 (±0.95)[b]	103.9 (±7.30)[a]

Table 3-10: Cover soil nutrient (NPK) availability (n=4) in response to soil temperature warming speed (fast: 2.0 °C/day, slow: 0.5 °C/day), and cover soil (peat and forest floor material (FFM)) after the *growing period*. Values represent the mean (± one standard error). Differences in lowercase lettering indicate statistically significant (α=0.05) differences among means of each response variable.

Response Variable	Rate of soil warming after aspen budflush			
	Fast		Slow	
	Peat	FFM	Peat	FFM
Total Nitrogen (μmol/10 cm^2)	114.3 (±20.79)^a	23.1 (±9.80)^b	69.0 (±31.55)^{ab}	26.8 (±6.16)^b
Phosphorus (μmol/10 cm^2)	0.9 (±0.22)^b	3.6 (±0.79)^a	0.8 (±0.19)^b	3.3 (±0.74)^a
Potassium (μmol/10 cm^2)	14.7 (±4.75)^b	31.4 (±8.28)^b	15.6 (± 3.02)^b	106.3 (± 18.43)^a

Figures

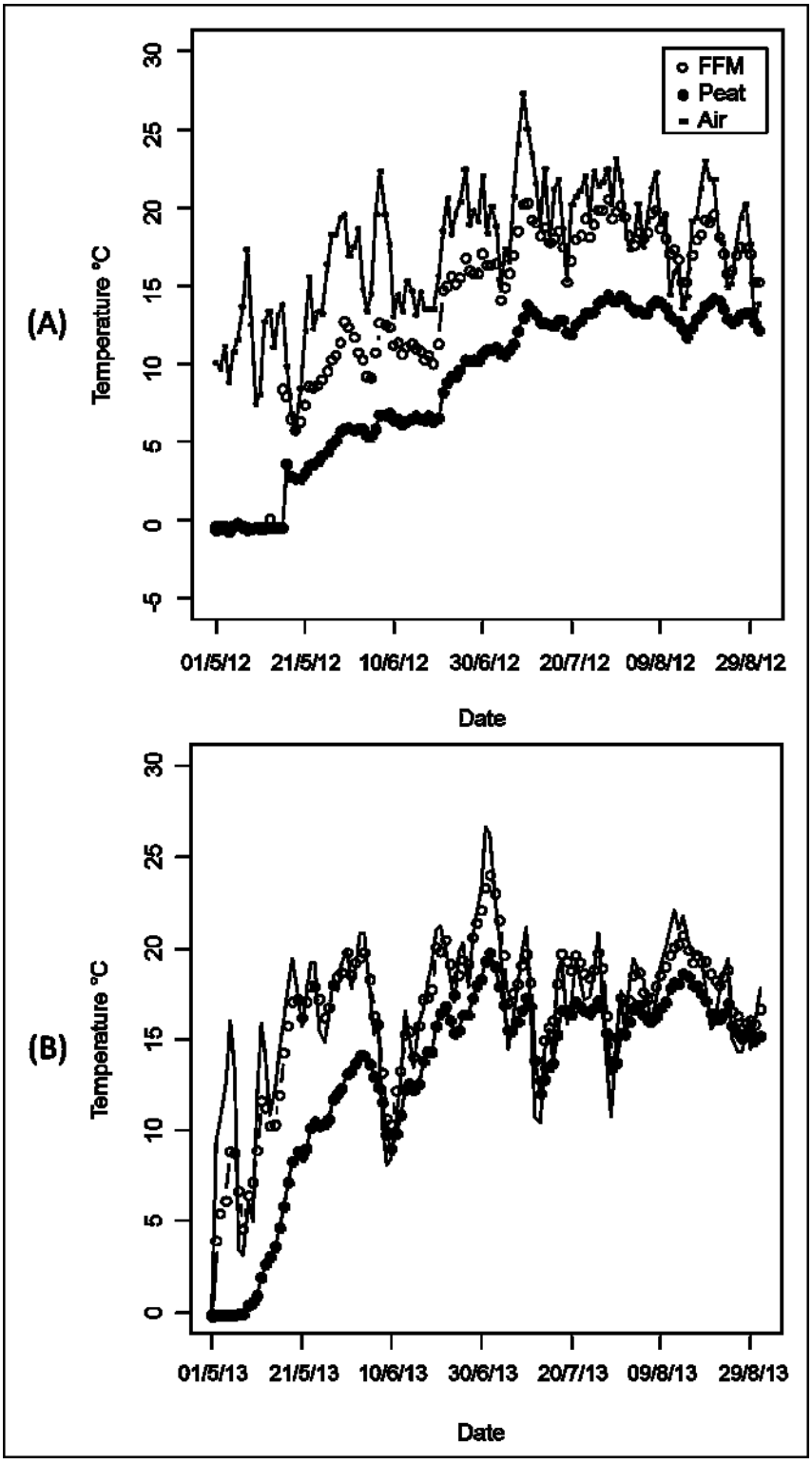

Figure 3-1: (A) 2012 and (B) 2013 seasonal air and soil temperatures (at 15 cm depth) for two reclamation cover soils (forest floor material (FFM), and peat) measured at the Aurora Soil Capping Study, Alberta, Canada.

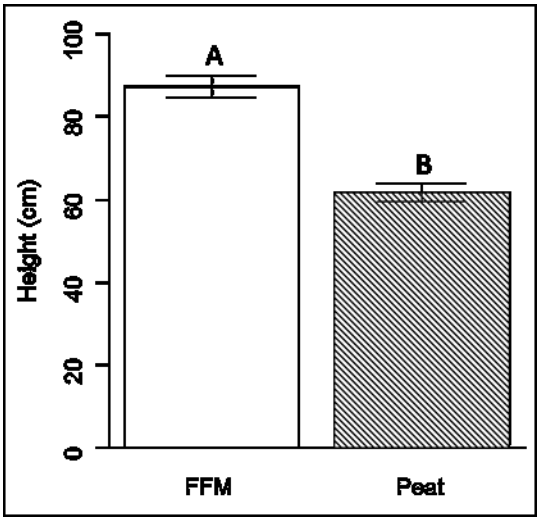

Figure 3-2: Height of nursery-grown *Populus tremuloides* seedlings after two growing seasons outplanted in either forest floor material (FFM) or peat reclamation materials at an upland boreal forest reclamation field experiment located in the Athabasca oil sands region of northern Alberta. Height measurements of seedlings were first pooled within tree plots and then averaged across tree plots per treatment (FFM: n=12; Peat: n=21) (*refer to Chapter 2.2.1 for experimental design*). Error bars represent one standard error and differences in lettering represent significant differences (p<0.05).

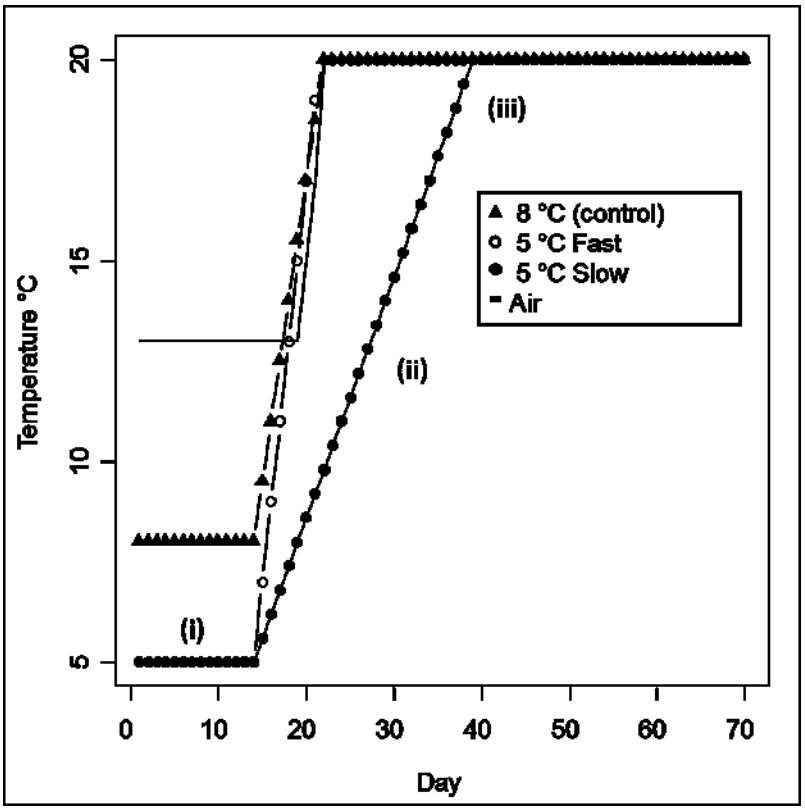

Figure 3-3: Manipulated air and soil temperature in a growth chamber simulating soil temperature and warming rates for three different groups of *Populus tremuloides* seedlings. See text for explanation of soil temperature periods (i): *budflush period*, (ii) *warming period*, and (iii) *growth period*.

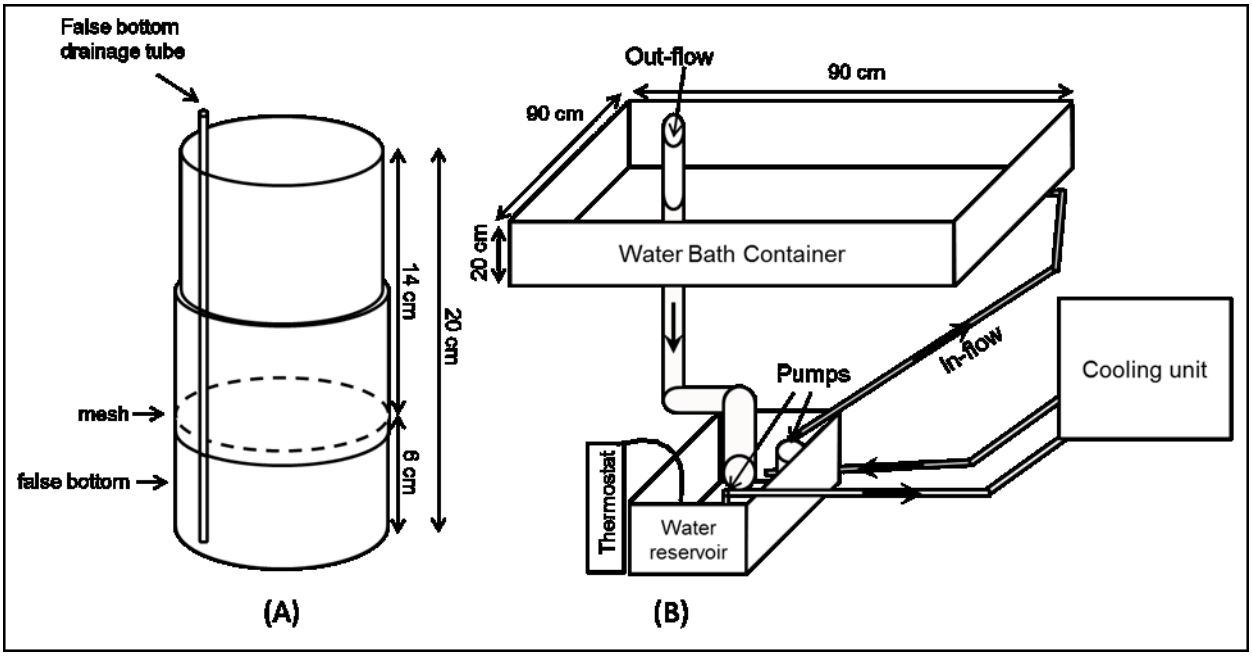

Figure 3-4: Design of (A) water-tight pots, and (B) water bath system used to control the soil temperature of *Populus tremuloides* seedlings.

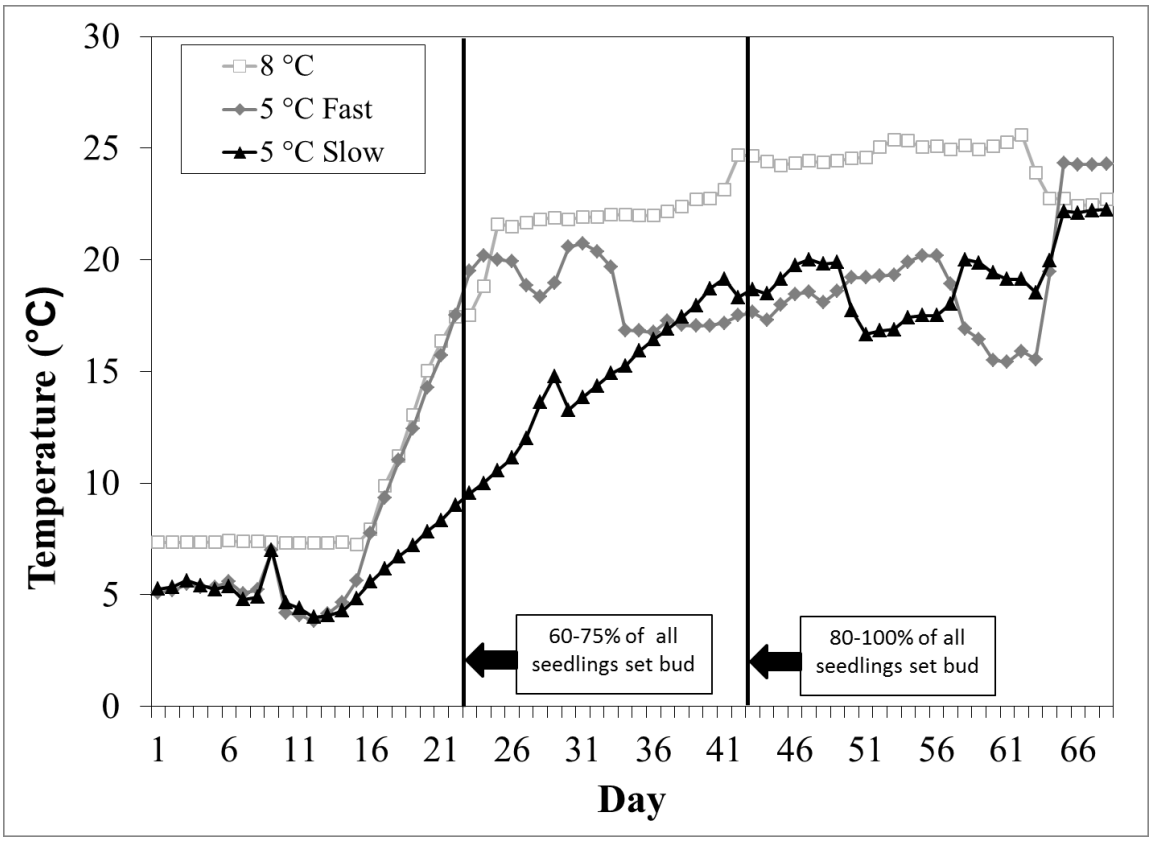

Figure 3-5: Soil temperature data recorded from data loggers buried in pots of soil throughout a growth chamber experiment manipulating soil temperature during budflush (8 or 5 °C) and warming speed (fast: 2.0 °C/day, slow: 0.5 °C/day) for *Populus tremuloides* seedlings grown in peat and forest floor material reclamation cover soils over 70 days. Temperature data for the two types of cover soils were pooled. Points in time when seedlings were setting bud are included.

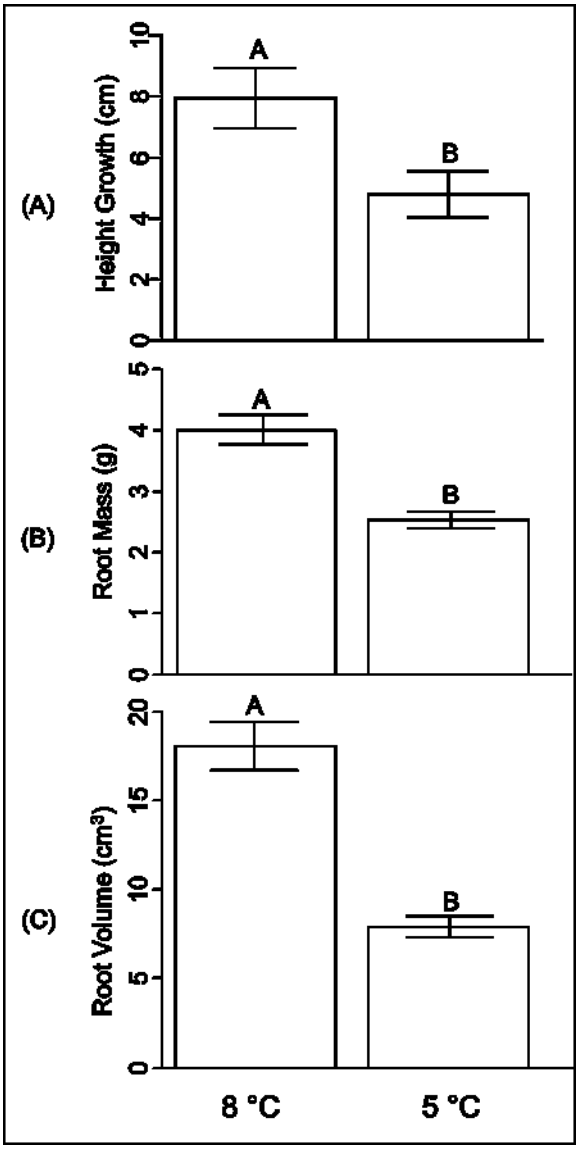

Figure 3-6: (A) Height growth, (B) root mass, and (C) root volume of *Populus tremuloides* seedlings (n=20) measured after the *growing period* (see Fig. 3-3) of a 70 day growth chamber experiment. Seedlings were subjected to either 8 or 5 °C soil temperature during budflush, followed by an increase to 20 °C for the remainder of the experiment. Differences in lettering indicates statistical significance ($\alpha = 0.05$) among means for each response variable.

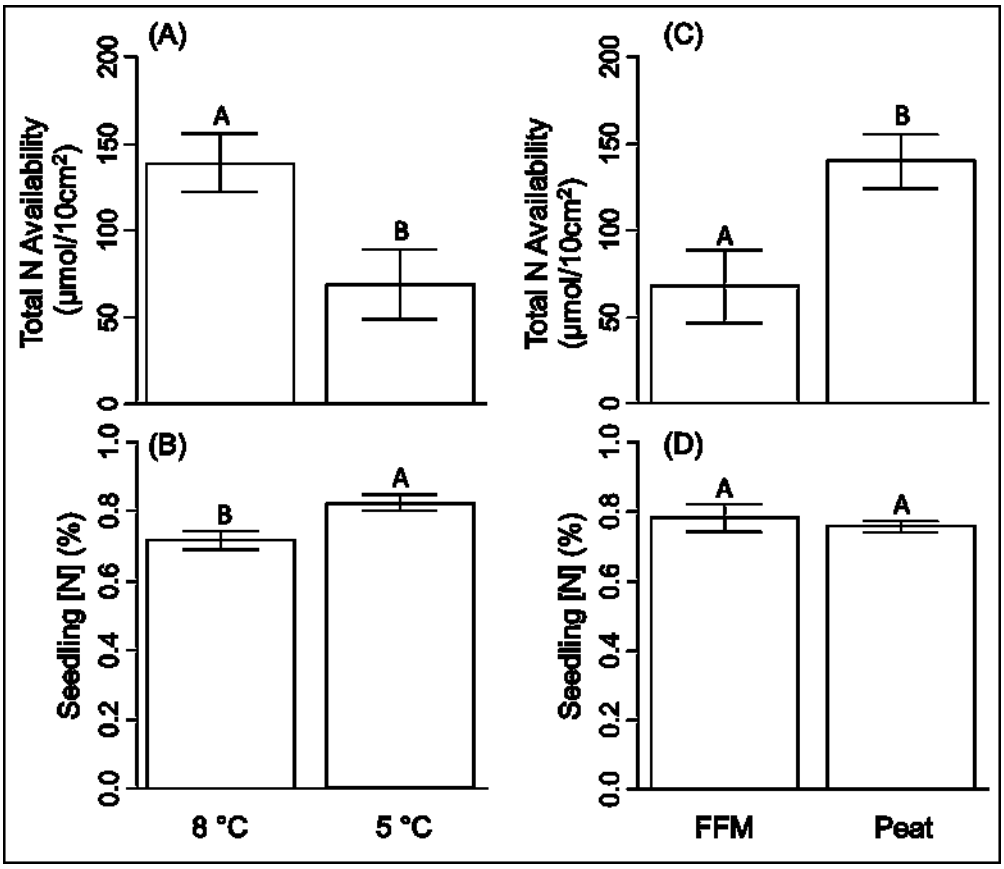

Figure 3-7: (A, C) Total cover soil N availability and (B, D) whole seedling (leaves, stem, roots combined) N concentration after the *growing period* (refer to Fig.3-3) for dormant *Populus tremuloides* seedlings flushed at a soil temperature of 8 or 5 °C, or grown in forest floor material (FFM) or peat reclamation cover soils. Differences in lettering indicates statistical significance ($\alpha = 0.05$) among means for each response variable.

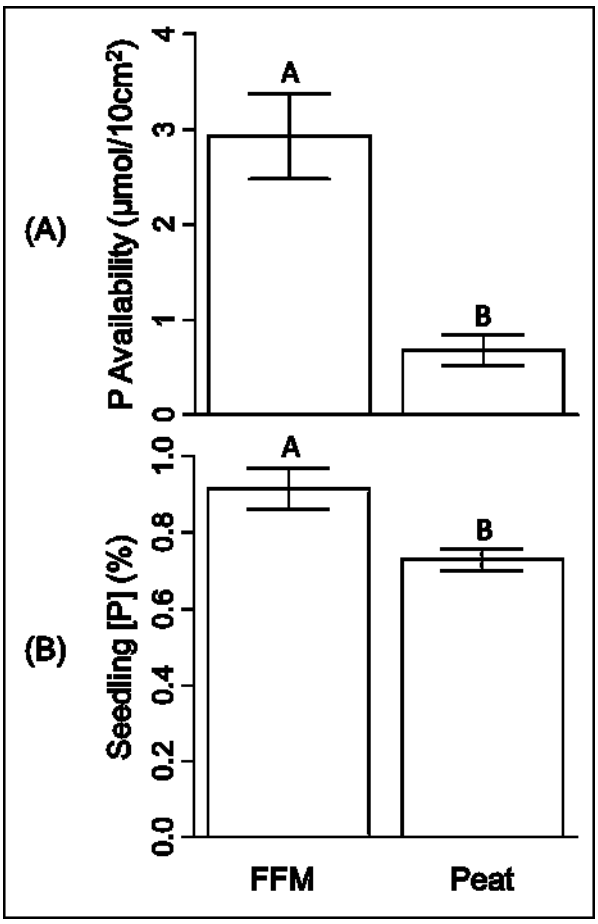

Figure 3-8: (A) Cover soil P availability and (B) whole seedling P concentration measured after the *growing period* (Fig. 3-3) for *Populus tremuloides* seedlings grown in forest floor material (FFM) or peat cover soils. Differences in lettering indicates statistical significance ($\alpha = 0.05$) among means for each response variable.

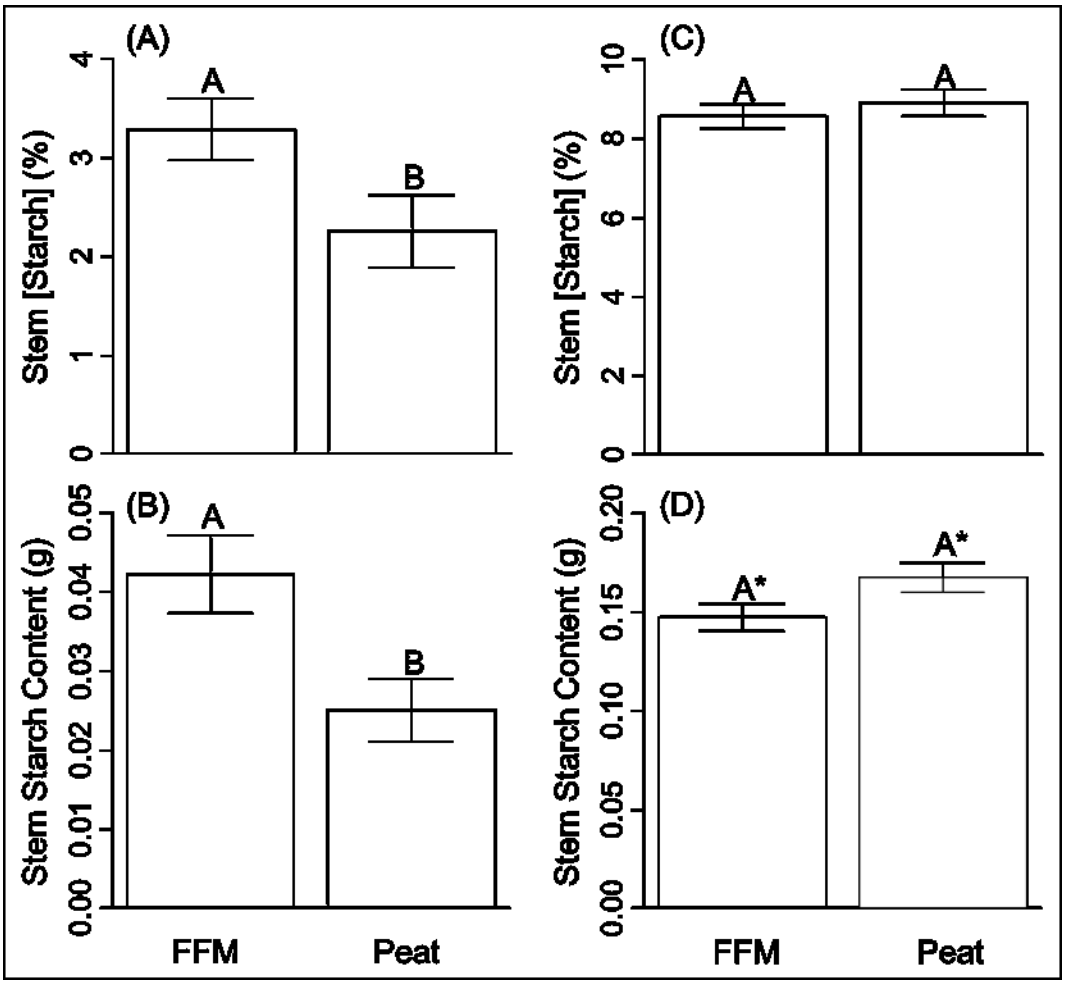

Figure 3-9: (A) Stem starch concentration and (B) starch content measured after the *warming period*, and (C) stem starch concentration, and (D) starch content measured after the *growing period* (refer to Fig. 3-3) for dormant *Populus tremuloides* seedlings flushed at either 8 or 5 °C soil temperature and grown in either forest floor material (FFM) or peat reclamation cover soils. Differences in lettering indicates statistical significance ($\alpha = 0.05$) among means of each response variable, and similar lettering with asterisks represent marginal differences ($\alpha = 0.06$).

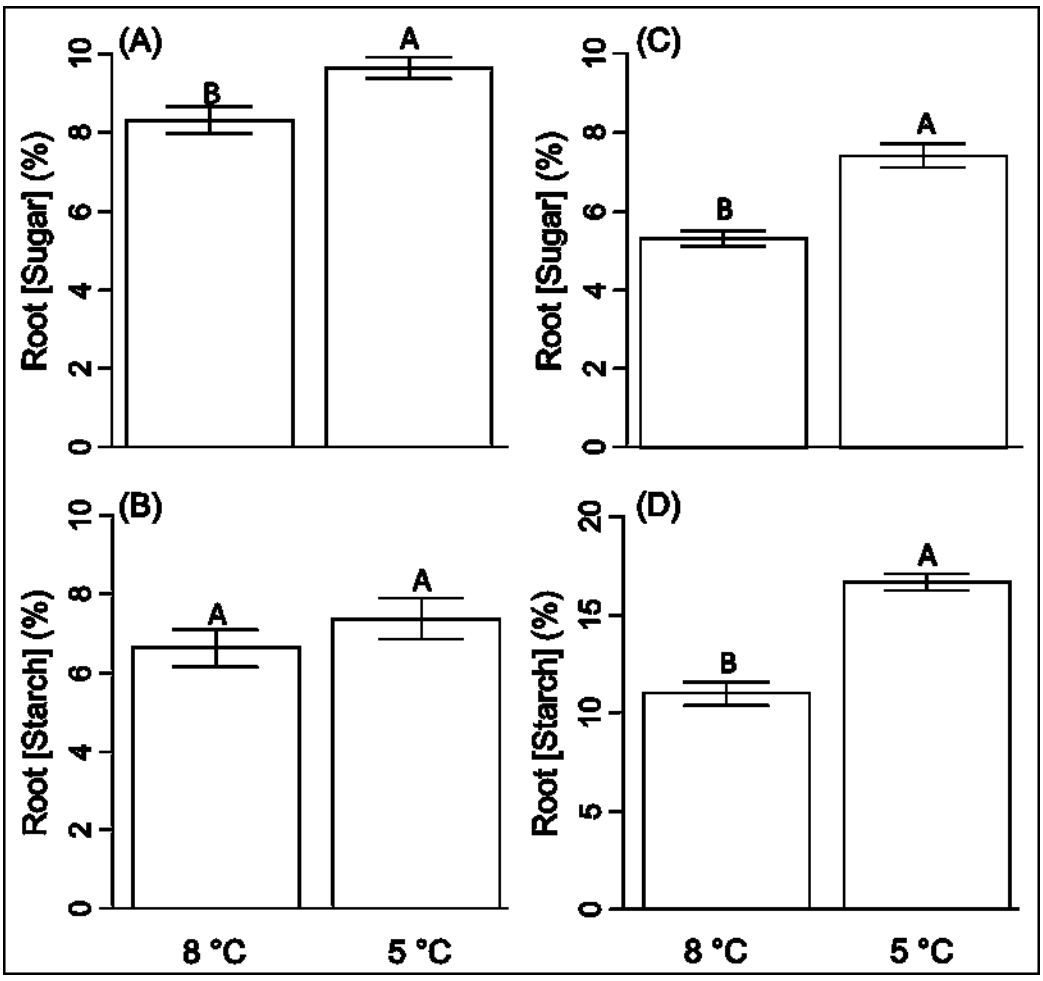

Figure 3-10: (A) Root sugar concentration and (B) starch concentration measured after the *warming period*, and (C) root sugar concentration, and (D) starch concentration measured after the *growing period* (refer to Fig. 3-3) for dormant *Populus tremuloides* seedlings flushed at either 8 or 5 °C soil temperature. Differences in lettering indicates statistical significance (α = 0.05) among means of each response variable.

Chapter 4: General Discussion and Conclusions

4.1 Research Summary

Restoration of forests and their functions necessitates considering the interactions among trees, other species, soils, and the physical environment. The objective of my thesis was to expand the scope of forest restoration following reclamation by accounting for interactions between vegetation and soils. I focused on interactions mediated by species, i.e., those which form between trees and ectomycorrhizal fungi, and physical attributes of soils (edaphic factors), i.e., the effects of soil temperature on seedling establishment. Towards this objective, I participated in an upland boreal forest reclamation research project designed by a collaboration of scientists to test the influence of substrate type and their configuration and depths on tree seedling establishment at an operational scale. The quality of the surface substrate (also referred to as cover soils) need to be suitable for vegetation growth and therefore is critical to further ecosystem development. Common reclamation cover soils used after oil sands surface mining are salvaged organic-dominated surface materials such as peat or salvaged mineral-dominated surface soils like forest floor material (FFM), and variations therein. Organic- and mineral-dominated soils differ in many biological and physical/abiotic qualities which affect seedling establishment. To determine whether belowground tree root symbionts, ectomycorrhizal (EM) fungi, which are important for seedling establishment, survival, and ecosystem development, persist and function in salvaged reclamation soils, I investigated EM fungi associated with aspen, jack pine, and white spruce seedlings planted into peat, FFM, and subsoil in both field and growth chamber experiments. Additionally, another growth chamber experiment was performed to determine if the cause for limitations in aspen height growth observed in seedlings planted into peat compared with FFM in the field was due to lower spring soil temperatures associated with peat, or differences in cover soil nutrition.

In the first research chapter I asked whether (1) FFM, peat, and subsoil cover soils retain EM fungi, (2) EM fungal communities display host (aspen, jack pine, or white spruce) and/or cover soil preference, and (3) EM fungal colonization and richness influences seedling growth. Through both field and growth chamber assays I found that FFM, peat, and subsoil contain EM fungi and that after one growing season EM fungal communities generally display host preference, regardless of cover soil type. When planted in the field, some EM fungal species that developed on seedlings during their time in the nursery may have been carried into the cover soils based on their presence on seedling roots prior to and after outplanting, however, these fungal species were also present on seed-germinated seedlings in the growth chamber assay, indicating these fungi could also have been contained in the cover soils. Air dispersal of EM spores may also have been a source of inoculum due to their ubiquitous presence in all three cover soils, and/or resistant EM fungal propagules may have been contained within the cover soils, based on their recovery from both the field and greenhouse studies. In the absence of EM fungal associations, which was tested through the removal of all soil biota by autoclave sterilization in the growth chamber assay, jack pine seedlings experienced 40 % mortality, spruce seedlings experienced 10 % mortality, and aspen experienced no mortality, indicating that EM fungi are critical to the establishment of coniferous seedlings, particularly jack pine. Aspen seedlings may not require EM fungi for their establishment since they experienced no mortality in sterilized cover soils and even exhibited a positive growth response in sterilized peat, although the interpretation and generalization of this result is difficult due to the substantial changes in available N with sterilization of peat. Regardless of EM-mediated effects on seedling growth, seedlings grown in unsterilized FFM in the growth chamber assay generally exhibited the greatest growth in terms of height and whole-seedling mass. Additionally, aspen seedling height

growth in the field after two growing seasons was observed to be reduced in those planted in pure peat compared with FFM, which led to the exploration of possible edaphic variables as drivers for seedling performance in our second investigation.

In the second research chapter I ask whether differences in early seasonal soil temperatures during budflush and differential soil warming between FFM and peat cover soils observed in the field influence growth of aspen seedlings. To address this question, I planted nursery grown aspen seedlings into either FFM or peat cover soils and exposed them to soil temperatures of either 5 or 8 °C for two weeks during budflush followed by an increase in soil temperature by a fast or slow rate to 20 °C for the remainder of the 70 day experiment. I found exposing aspen seedlings to 5 °C soil temperature during budflush resulted in lower growth, regardless of cover soil type, while rate of warming had no influence, indicating that soil temperature during budflush was likely the main driver for the observed variation in aspen seedling growth between the two cover soils in the field. Plant root simulator probes inserted into pots during the growth chamber experiment revealed differences in nutrient availability between cover soils, and lower nutrient availability in cover soils exposed to 5 °C compared with 8 °C. However, inconsistencies between cover soil nutrient availability and seedling nutrient concentration indicate that seedling nutrient uptake may have been limited by soil temperature, which could explain why there were no differences in seedling growth between cover soil type in the growth chamber experiment given that peat and FFM have different nutritional profiles.

Though not investigated in this thesis, EM associations may be important in the alleviation of physiological stress induced by cold temperatures for outplanted seedlings. For example, Landhäusser and others (2002) tested the influence of mycorrhizal associations on aspen and white spruce seedlings exposed to low soil temperatures (4 and 8 °C) and found

greater root hydraulic conductance and greater shoot water potential (only for aspen) in seedlings with mycorrhizal associations. In my field assay of EM fungi described in the first research chapter, host species drove the abundance of various EM fungi recovered across cover soils. However, the abundance of *Rhizopogon* associated with jack pine seedlings was significantly greater for those seedlings grown in subsoil compared with peat and FFM. Given that subsoil generally has lower water content than FFM and even more-so than peat in the field (*data not shown*), the greater abundance of *Rhizopogon* on roots of jack pine seedlings (~35 %) grown in subsoil could be an indication of water stress alleviation. Studies investigating the influence of mycorrhizal associations on alleviation of physiological stress report seedlings having at least 50 % EM colonization (Landhäusser *et al*., 2002); however, the degree of EM colonization required for stress-alleviation is unknown and may be variable across species of host and symbiont.

4.2 Applications for Upland Forest Reclamation

Results from these studies indicate several improvements can be made towards increasing establishment success of outplanted native tree seedlings on upland areas in the boreal region after surface mining. First, the recovery of EM fungi in directly-placed cover soils indicates that resistant EM fungal propagules remained in the cover soils and/or that the EM fungal propagule bank was replenished via air dispersed EM fungal spores. If the cover soil EM propagule bank was created solely from the latter, the proximity of reclamation areas to intact forests may be important for the re-establishment of EM communities and should be considered while planning for reclamation. Additionally, this research indicates that inoculation of seedlings with mycorrhizal fungi prior to outplanting may not be necessary, even when planted into subsoil materials. Although not directly tested, direct-placement and frozen-transfer of cover soils onto

the reclamation area may have protected against the loss of some EM fungal propagules. Substantial reductions in fungal propagule viability have been shown in soils stockpiled for as little as six months (Persson and Funke, 1988; Reddell and Milnes, 1992). Second, the host-preference exhibited by EM fungal communities indicates that increasing the number of tree species outplanted onto reclamation areas may increase the recovery of different species of EM fungi in directly-placed peat, FFM, and subsoil. The recovery of a diverse EM fungal community may facilitate ecosystem development through the establishment and survival of tree seedlings, and the cycling of soil nutrients and carbon (Read *et al*., 2004; Högberg and Högberg, 2002). Third, based on results from the greenhouse assay, regardless of the community composition of EM fungi, growing seedlings in FFM will likely result in greater growth. Forest floor material may be the most beneficial cover soil for outplanted seedlings because of the beneficial nutritional and biological content (MacKenzie and Naeth, 2010). Fourth, the peat content of reclamation cover soils should be considered. Using cover soils that have high peat content (or pure peat which was used in these experiments) at depths encompassing large portions of the root system may result in low spring soil temperatures reaching 5 °C or below in the field when seedlings are flushing, potentially detrimental to aspen seedling establishment. Some greenhouse studies which investigate the growth of tree seedlings in reclamation soils do so at soil temperatures that are similar to air temperatures and closer to summer temperatures (Renault *et al*., 2000; Khasa *et al*., 2005; Showalter *et al*., 2010; Vaario *et al*., 2011; Pinno *et al*., 2012; Pinno *et al*., 2014), which do not capture abiotic variations occuring in the field and potentially driving growth. Particularly, in boreal regions, the early growing season corresponds with a crucial phenological stage of the seedlings (e.g., budflush). During this time root systems are exposed to low soil temperatures, as soils are slower to warm than air temperatures, while shoots

are exposed to higher temperatures which regulate bud flush in aspen (Landhäusser *et al*. 1998). Therefore, in addition to greenhouse studies, more detailed field investigations could be useful in predicting seedling establishment success in various cover soils.

4.3 Experimental Limitations/Future Research Suggestions

In the first chapter, autoclave sterilization of cover soils for the greenhouse study was generally successful at removing biota present in the cover soil; however, interesting and challenging side effects occurred. Autoclave sterilization resulted in significant soil nutritional changes depending on cover soil type, which likely influenced seedling growth, confounding the interpretation of soil biota removal effects on seedling growth. A comparison of methods used to remove EM fungi could be tested (including fungicides, gamma radiation, and mesh bags) for common growth trends as a stronger indication of EM fungal absence. Autoclave sterilization is known to be the most effective at achieving sterilization (Alphei and Scheu, 1993) and the use of mesh bags surrounding seedlings has been shown to be an effective method at limiting EM fungal colonization by fungal hypha (Teste *et al*., 2006).

The potential for air dispersal of EM fungal spores on reclamation areas should be investigated as a way to distinguish between sources of re-established EM communities. The community composition and development of EM fungi on outplanted seedlings should be monitored over a longer period of time than one growing season to determine whether initial fungal associations are important for determining the future EM fungal community. Additionally, positive or negative effects of EM colonization on seedling growth/survival may become more apparent over time, therefore not only should EM fungal communities be assessed over longer periods of time, but their potential influence on seedling growth/health status should

also be monitored. The composition of ectomycorrhizal fungal communities tracked over longer periods of time can also be compared with those of intact forests and forests subjected to other types of disturbances as a factor in estimating the successional status of the restored ecosystem.

In the second research chapter, I identified some limitations to the growth chamber experiment. As an outcome of different start times, the two experimental groups of seedlings could not be kept at the exact same temperature throughout the *growing period* as planned (Fig. 3-5 versus Fig. 3-3), which may have contributed to variation in seedling growth between seedlings flushed at 5 and 8 °C. However, even given this inconsistency, there is evidence that supports our conclusion that low soil temperature during budflush limits subsequent growth. First, seedlings set bud early on during this period regardless of growth chamber temperature (Fig. 3-5) which indicates that aboveground growth was likely not influenced by differences in temperature between the two groups. Second, optimal soil temperature for aspen seedling growth has been shown to be ~20 °C (Pang and Dang, 2003). This indicates that temperatures reaching >20 °C (which occurred in one group of seedlings; Fig. 3-5) would not necessarily result in greater growth. Additionally, given these unexpected differences in temperature during the *growing period* between groups of seedlings, temperatures were never low enough to restrict re-flushing of the aspen seedlings. Given that aspen is a species with an indeterminate growth strategy, this indicates that there were other variables, potentially related to low soil temperature during budflush, which restricted re-flushing of aspen seedlings.

In order to better capture the effect of cold soil temperature during budflush on subsequent aspen seedling growth performance there are a number of improvements that can be made to this experiment. First, the difference in manipulated soil temperature treatments could be increased to potentially observe bigger differences in growth. Soil temperature treatments of 5

°C and 10 °C may show more pronounced differences whereas 8 °C may still have been growth-limiting to aspen. Second, gas exchange measurements could be made more frequently. Measurements taken after seedlings leaf-out while still exposed to low temperatures may better capture the effect of cold soil temperature on physiology. Additionally, photosynthetic acclimation can be measured as soils warm to 20 °C to assess photosynthetic recovery, if initially limited by cold temperatures. Third, destructively sampling before soil warming rather than after may better capture reductions in shoot water potential and changes in nonstructural carbohydrate (NSC) reserves due to cold soils. Fourth, experimental groups need to begin treatments at the same time and in the same growth chamber to reduce the introduction of random variation caused by differences in environmental conditions. It is also important that air and soil temperatures are monitored closely throughout the experiment to reduce the likelihood of seedlings experiencing temperature fluctuations; depending on the type of facility these variables may be difficult to control. In the second research chapter the quality of the aspen seedlings stock was not considered a factor; however, other research has shown that higher initial NSC reserves could result in a more pronounced growth response and potentially re-flushing of set buds (Martens *et al*., 2007; Landhäusser *et al*., 2012). Seedlings used in this study had relative low NSC reserves and root shoot ratios, characteristics that would be considered undesirable for high quality aspen seedlings for stressful site conditions and that could have also influenced the outcome and lack of response in this study.

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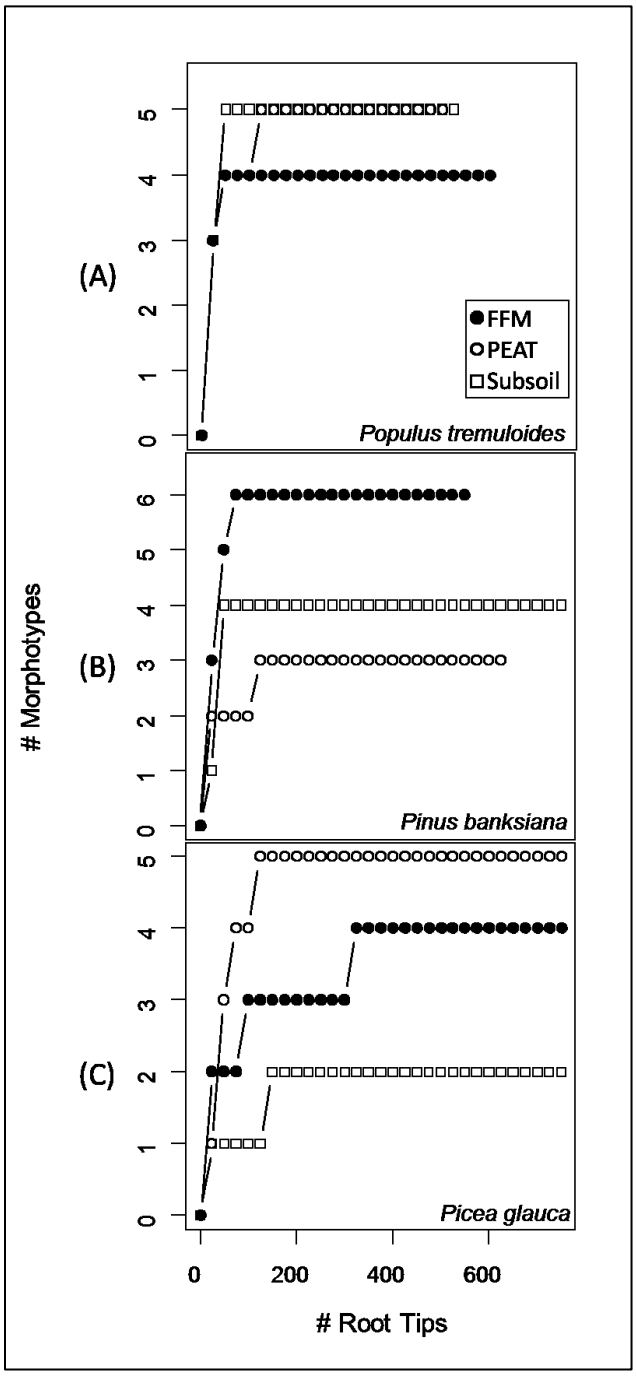
Appendices



Appendix I: Accumulation curves of number of ectomycorrhizal morphotypes per number of root tips counted for A) *Populus tremuloides*, B) *Pinus banksiana*, and C) *Picea glauca* grown forest floor material (FFM), peat, and subsoil.

Appendix II: Results of permutation ANOVAs from a field assay testing effects of host species and cover soil used in forest reclamation following oil sands mining on percent ectomycorrhizal fungal colonization of root tips. *P*-values bolded are significant at $\alpha<0.05$.

*Abbreviations: Df=Degrees of freedom; SS=Sums of Squares; MS=Mean Square Error; Iter=Number of Iterations

Amphinema byssoides	Statistics	*Df	*SS	*MS	*Iter	*p*-value
	Host	2	10953.6	5476.8	5000	**<0.01**
	cover soil	2	323.9	161.9	85	0.68
	host x cover soil	4	647.8	161.9	129	0.86
	Residuals	18	6930.5	385.0		
Thelephoraceae	Host	2	708.5	354.3	5000	**0.01**
	cover soil	2	140.1	70.0	252	0.38
	host x cover soil	4	280.1	70.0	735	0.41
	Residuals	18	1398.5	77.7		
Hebeloma hiemale	Host	2	733.2	366.6	5000	<**0.01**
	cover soil	2	2.4	1.2	51	0.75
	host x cover soil	4	4.9	1.2	72	0.82
	Residuals	18	105.1	5.8		
Rhizopogon rubescens	Host	2	2066.1	1033.1	5000	**<0.01**
	cover soil	2	252.1	126.0	361	0.32
	host x cover soil	4	861.9	215.5	2819	0.07

	Residuals	18	1625.3	90.3		
Wilcoxina mikolae	Host	2	429.6	214.8	1268	0.18
	cover soil	2	228.4	114.2	51	0.82
	host x cover soil	4	642.6	160.7	283	0.62
	residuals	18	3229.0	179.4		

Appendix III: Results of Permutation ANOVAs from a growth chamber assay testing effect of host species and cover soil used in forest reclamation following oil sands mining on percent ectomycorrhizal fungal colonization of root tips per host seedling. P-values bolded are significant at $\alpha<0.05$.

*Abbreviations: Df=Degrees of freedom; SS=Sums of Squares; MS=Mean Square Error; Iter=Number of Iterations

Tuber spp.	Statistics	*Df	*SS	*MS	*Iter	*p*-value
	host	2	78.4	39.2	1339	0.19
	cover soil	2	264.1	132.0	5000	**<0.01**
	host x cover soil	4	136.8	34.2	1266	0.18
	residuals	81	1982.6	24.5		
Amphinema byssoides	host	2	96.3	48.1	69	0.59
	cover soil	2	24.1	12.0	51	1.00
	host x cover soil	4	47.5	11.9	69	0.84
	residuals	81	1948.6	24.1		
Cenococcum spp.	host	2	722.5	361.2	5000	**< 0.01**
	cover soil	2	405.8	202.9	5000	**< 0.01**

	host x cover soil	4	802.3	200.6	5000	**< 0.01**
	residuals	81	1006.0	12.4		
Thelephoraceae	host	2	4682.3	2341.1	5000	**0.02**
	cover soil	2	2242.2	1121.1	4369	**0.05**
	host x cover soil	4	1593.6	398.4	5000	0.23
	residuals	81	25284.8	312.2		
Hebeloma hiemale	Host	2	5357.4	2678.7	5000	**<0.01**
	cover soil	2	2705.0	1352.5	5000	**0.01**
	host x cover soil	4	519.2	129.8	409	0.73
	residuals	81	21866.4	267.0		

Appendix IV: Results of Permutation ANOVAs from a growth chamber assay testing effect of host species and cover soil used in forest reclamation following oil sands mining on seedling height (cm) and mass (g). P-values bolded are significant at $\alpha<0.05$.

*Abbreviations: Df=Degrees of freedom; SS=Sums of Squares; MS=Mean Square Error; Iter=Number of Iterations

Height	Aspen	Statistics	*Df	*SS	*MS	*Iter	*p*-value
		cover soil	2	536.4	268.2	5000	**<0.01**
		sterilization	1	59.6	59.6	3162	**0.03**
		cover soil x sterilization	2	116.9	58.4	5000	**0.02**
		residuals	54	803.3	14.9		
	Jack pine	cover soil	2	9.1	4.5	5000	**<0.01**

		sterilization	1	1.9	1.9	5000	**0.02**
		cover soil x sterilization	2	0.6	0.3	254	0.50
		residuals	53	16.4	0.3		
	White spruce	cover soil	2	38.2	19.1	5000	**<0.01**
		sterilization	1	30.1	30.1	5000	**<0.01**
		cover soil x sterilization	2	14.5	7.3	949	0.10
		residuals	55	168.9	3.1		
Mass	Aspen	cover soil	2	490.8	245.4	5000	**<0.01**
		sterilization	1	71.5	71.5	5000	**0.01**
		cover soil x sterilization	2	319.3	159.7	5000	**<0.01**
		residuals	54	659.2	12.2		
	Jack pine	cover soil	2	1.6	0.8	5000	**<0.01**
		sterilization	1	1.2	1.2	5000	**<0.01**
		cover soil x sterilization	2	0.8	0.4	5000	**<0.01**
		residuals	53	2.4	0.05		
	White spruce	cover soil	2	0.8	0.4	5000	**<0.01**
		sterilization	1	0.7	0.7	5000	**<0.01**
		cover soil x sterilization	2	0.4	0.2	5000	**<0.01**
		residuals	55	1.5	0.03		

Appendix V: Results of Permutation ANOVAs testing effects of cover soil sterilization on available nutrient concentration.

*Abbreviations: Df=Degrees of freedom; SS=Sums of Squares; MS=Mean Square Error; Iter=Number of Iterations

	Statistics	*Df	*SS	*MS	*Iter	*p*-value
NH_4^+	cover soil	2	5748.7	2874.4	5000	**< 0.01**
	sterilization	1	2987.9	2987.9	5000	**< 0.01**
	cover soil x sterilization	2	5905.0	2952.5	5000	**< 0.01**
	residuals	30	30.2	1.0		
NO_3^-	cover soil	2	2272.1	1136.0	5000	**< 0.01**
	sterilization	1	0.4	0.4	146	0.41
	cover soil x sterilization	2	5.2	2.6	5000	**0.01**
	residuals	30	7.8	0.3		
PO_4^{-3}	cover soil	2	1001.78	500.9	5000	**< 0.01**
	sterilized	1	0.5	0.5	516	0.16
	cover soil x sterilized	2	16.2	8.1	5000	**< 0.01**
	residuals	30	9.2	0.3		
K^+	cover soil	2	4376.5	2188.3	5000	**< 0.01**
	sterilization	1	316.2	316.2	5000	**< 0.01**
	cover soil x sterilization	2	38.7	19.4	87	0.64
	residuals	30	1104.4	36.8		

Appendix VI: Initial morphological and non-structural carbohydrate (starches and sugars) measurements of *Populus tremuloides* nursery seedlings (n=15) prior to the start of the experiment. Experimental groups of seedlings differed in start times. Seedlings flushed at 8 °C

soil temperature and increased to 20 °C fast (control), flushed at 5 °C and increased to 20 °C fast, or flushed at 5 °C and increased to 20 °C slow. Seedlings were also grown in two different cover soils: peat and forest floor material (FFM). Height measurements were taken from seedlings used in the experiment, while all other measurements that required destructive sampling were taken from a sub-sample. Values represent means (± one standard error). Differences in lettering indicate statistically significant (α=0.05) differences among means for each response variable.

Experimental Group	First				Second	
Temperature Treatment	5 °C Fast		5 °C Slow		8 °C	
Cover soil	Peat	FFM	Peat	FFM	Peat	FFM
Height (cm)	35.7 (± 1.10)[a]	36.1 (±0.87)[a]	37.6 (±0.75)[a]	35.8 (±1.10)[a]	34.9 (± 1.39)[a]	36.3 (± 1.03)[a]
Stem Mass (g)	1.0 (±0.04)[a]				1.2 (±0.10)[a]	
Root Mass (g)	1.4 (±0.07)[a]				1.6 (±0.14)[a]	
Root Volume (cm^3)	6.3 (±0.44)[a]				6.5 (±0.60)[a]	
Stem [Sugar] (%)	12.93 (±0.277)[a]				13.30 (±0.209)[a]	
Stem [Starch] (%)	0.53 (±0.0727)[a]				0.65 (±0.088)[a]	
Root [Sugar] (%)	12.39 (±0.691)[a]				13.70 (±0.469)[a]	
Root [Starch] (%)	4.07 (±0.703)[a]				4.18 (±0.420)[a]	

Appendix VII: Results of Welch's Two Sample T-test testing for differences (α=0.05) in stem mass, root volume, root mass, and stem and root NSC concentrations between two groups of *Populus tremuloides* seedlings varying in experimental start times (n=15). The following are definitions for abbreviations: Adj. Df = adjusted degrees of freedom, and T = T value.

Response Variable	Effect	Adj. Df	T	*p*-value
Stem Mass (g)	Experimental Group	18.7	1.42	0.17
Root Volume (cm^3)	Experimental Group	25.6	0.24	0.81
Root Mass (cm)	Experimental Group	20.7	1.21	0.24
Stem Sugar Concentration (%)	Experimental Group	26.0	-1.09	0.29
Stem Starch Concentration (%)	Experimental Group	27.1	-1.04	0.31
Root Sugar Concentration (%)	Experimental Group	24.6	-1.56	0.13
Root Starch Concentration (%)	Experimental Group	22.9	-0.14	0.89

Appendix VIII: Results of ANOVA or permutation ANOVA testing for effects (α=0.05) of soil temperature during budflush, cover soil, and their interaction on *Populus tremuloides* seedling height growth, stem mass, root volume, root mass, leaf mass, number of leaves, and leaf area (n=10) after the *warming period* (see Fig. 3-3). The following are definitions for abbreviations: Df = degrees of freedom, SS = sums of squares, MS = mean square error, F (Iterations) = either F value if a regular ANOVA was performed, or (the number of iterations) if a permutation ANOVA was performed.

Response Variable	Effect	Df	SS	MS	F (Iterations)	*p*-value
Height Growth (cm)	Temperature	1	21.5	21.5	3.8	**0.06**$^+$
	Cover Soil	1	15.5	15.5	2.7	0.11
	Temperature*Cover soil	1	0.5	0.5	0.1	0.78
	Residuals	36	205.2	5.7		
Stem Mass (g)	Temperature	1	0.4	0.4	(4162)	**0.02***
	Cover Soil	1	0.2	0.2	(1354)	0.07

	Temperature*Cover soil	1	0	0	(51)	1.00
	Residuals	36	2.3	0.1		
Root Volume (cm^3)	Temperature	1	2.3	2.3	0.9	0.36
	Cover Soil	1	1.2	1.2	0.4	0.52
	Temperature*Cover soil	1	4.4	4.4	1.6	0.21
	Residuals	36	97.2	2.7		
Root Mass (g)	Temperature	1	0.1	0.1	1.3	0.26
	Cover Soil	1	0	0	0	0.97
	Temperature*Cover soil	1	0.1	0.1	1.1	0.30
	Residuals	36	2.8	0.1		
Leaf Mass (g)	Temperature	1	0.1	0.1	4.2	**0.05***
	Cover Soil	1	0	0	1.1	0.31
	Temperature*Cover soil	1	0	0	1.3	0.25
	Residuals	36	0.9	0		
# Leaves	Temperature	1	102.4	102.4	(156)	0.39
	Cover Soil	1	270.4	270.4	(2773)	**0.03***
	Temperature*Cover soil	1	115.6	115.6	(127)	0.44
	Residuals	36	2330	64.7		
Leaf Area (mm^2)	Temperature	1	253.6	253.6	(51)	0.88
	Cover Soil	1	402.4	402.4	(51)	0.88
	Temperature*Cover soil	1	435.5	435.5	(51)	0.67

	Residuals	36	25010.6	694.7		

Appendix IX: Results of ANOVA or permutation ANOVA testing for effects (α=0.05) of soil temperature at the time of budflush, cover soil, and their interaction on *Populus tremuloides* seedling height growth, stem mass, root volume, root mass, leaf mass, number of leaves, and leaf area (n=10) after the *growing period* (see Fig. 3-3). The following are definitions for abbreviations: Df = degrees of freedom, SS = sums of squares, MS = mean square error, F (Iterations) = either F value if a regular ANOVA was performed, or (the number of iterations) if a permutation ANOVA was performed.

Response Variable	Effect	Df	SS	MS	F (Iterations)	*p*-value
Height Growth (cm)	Temperature	1	99.2	99.2	6.5	**0.02***
	Cover Soil	1	19.3	19.3	1.3	0.27
	Temperature*Cover soil	1	15.6	15.6	1.0	0.32
	Residuals	36	551.1	15.3		
Stem Mass (g)	Temperature	1	0.7	0.7	12.0	**<0.01***
	Cover Soil	1	0.3	0.3	4.5	**0.04***
	Temperature*Cover soil	1	0.0	0.0	0.4	0.51
	Residuals	36	2.2	0.1		
Root Volume (cm^3)	Temperature	1	1036.3	1036.3	(5000)	**<0.01***
	Cover Soil	1	38.8	38.8	(221)	0.31
	Temperature*Cover soil	1	0.6	0.6	(51)	0.76
	Residuals	36	800.8	22.2		
Root Mass (g)	Temperature	1	22.0	22.0	26.6	**<0.01***

	Cover Soil	1	1.0	1.0	1.1	0.29
	Temperature*Cover soil	1	0.0	0.0	0.0	0.93
	Residuals	36	29.8	0.8		
Leaf Mass (g)	Temperature	1	0.4	0.4	7.6	**0.09***
	Cover Soil	1	0.1	0.1	1.1	0.30
	Temperature*Cover soil	1	0.0	0.0	0.3	0.59
	Residuals	36	2.0	0.1		
# Leaves	Temperature	1	302.5	302.5	(1048)	0.09
	Cover Soil	1	102.4	102.4	(84)	0.55
	Temperature*Cover soil	1	122.5	122.5	(343)	0.23
	Residuals	36	3379.0	93.9		
Leaf Area (mm^2)	Temperature	1	9055.0	9055.0	13.0	**<0.01***
	Cover Soil	1	1556.0	1556.0	2.2	0.14
	Temperature*Cover soil	1	352.0	352.0	0.5	0.48
	Residuals	36	25125.0	698.0		

Appendix X: Results of ANOVA or permutation ANOVA testing for effects (α=0.05) of soil temperature warming speed, cover soil, and their interaction on *Populus tremuloides* seedling height growth, stem mass, root volume, root mass, leaf mass, number of leaves, and leaf area (n=10) after the *growing period* (see Fig. 3-3). The following are definitions for abbreviations: Df = degrees of freedom, SS = sums of squares, MS = mean square error, F (Iterations) = either F value if a regular ANOVA was performed, or (the number of iterations) if a permutation ANOVA was performed.

Response Variable	Effect	Df	SS	MS	F (Iterations)	*p*-value
Height Growth (cm)	Speed	1	12.2	12.2	1.0	0.31
	Cover Soil	1	3.8	3.8	0.3	0.57
	Speed*Cover soil	1	41.0	41.0	3.5	0.07
	Residuals	36	420.4	11.7		
Stem Mass (g)	Speed	1	0.0	0.0	0.4	0.51
	Cover Soil	1	0.3	0.3	3.6	0.07
	Speed*Cover soil	1	0.0	0.0	0.4	0.55
	Residuals	36	2.7	0.1		
Root Volume (cm^3)	Speed	1	2.2	2.2	(141)	0.42
	Cover Soil	1	47.1	47.1	(5000)	**0.02***
	Speed*Cover soil	1	2.4	2.4	(51)	0.71
	Residuals	36	228.5	6.5		
Root Mass (g)	Speed	1	0.2	0.2	0.4	0.51
	Cover Soil	1	0.8	0.8	2.0	0.16
	Speed*Cover soil	1	0.0	0.0	0.1	0.80
	Residuals	36	14.3	0.4		
Leaf Mass (g)	Speed	1	0.0	0.0	0.0	0.92
	Cover Soil	1	0.1	0.1	1.0	0.32
	Speed*Cover soil	1	0.0	0.0	0.4	0.54
	Residuals	36	2.0	0.1		

# Leaves	Speed	1	15.6	15.6	(51)	0.86
	Cover Soil	1	11.0	11.0	(63)	0.62
	Speed*Cover soil	1	18.2	18.2	(51)	0.84
	Residuals	36	2653.9	73.7		
Leaf Area (mm^2)	Speed	1	142.0	142.0	0.2	0.70
	Cover Soil	1	854.0	854.0	0.9	0.34
	Speed*Cover soil	1	840.0	840.0	0.9	0.35
	Residuals	36	33171.0	921.4		

Appendix XI: Results of permutation ANOVA testing for effects (α=0.05) of soil temperature during budflush, cover soil, and their interaction on *Populus tremuloides* seedling stomatal conductance, photosynthetic rate, and shoot water potential (n=10) after the *warming period* (see Fig. 3-3). The following are definitions for abbreviations: Df = degrees of freedom, SS = sums of squares, MS = mean square error, Iterations = the number of iterations used.

Response Variable	Effect	Df	SS	MS	Iterations	*p*-value
Stomatal Conductance (mol $H_2O/m^2/sec$)	Temperature	1	0.1	0.1	5000	**<0.01***
	Cover Soil	1	0.0	0.0	583	0.15
	Temperature*Cover soil	1	0.0	0.0	87	0.54
	Residuals	36	0.0	0.0		
Photosynthetic Rate (μmol $CO_2/m^2/sec$)	Temperature	1	6.0	6.0	655	0.13
	Cover Soil	1	1.4	1.4	92	0.52
	Temperature*Cover soil	1	4.1	4.1	146	0.41

	Residuals	36	102.5	2.8		
Shoot Water Potential (MPa)	Temperature	1	0.0	0.0	71	0.46
	Cover Soil	1	0.2	0.2	1400	0.09
	Temperature*Cover soil	1	0.2	0.2	1745	0.09
	Residuals	36	2.1	0.1		

Appendix XII: Results of ANOVA or permutation ANOVA testing for effects (α=0.05) of soil temperature during budflush, cover soil, and their interaction on *Populus tremuloides* seedling nutrient (NPK) concentration (n=5) after the *warming period* (see Fig. 3-3). The following are definitions for abbreviations: Df = degrees of freedom, SS = sums of squares, MS = mean square error, F = F value.

Response Variable	Effect	Df	SS	MS	F	*p*-value
Total Nitrogen (%)	Temperature	1	0.1	0.1	7.1	**0.02***
	Cover Soil	1	0.1	0.1	7.8	**0.01***
	Temperature*Cover soil	1	0.0	0.0	0.2	0.69
	Residuals	16	0.2	0.0		
Phosphorus (%)	Temperature	1	0.1	0.0	0.0	0.90
	Cover Soil	1	0.1	0.0	0.2	0.64
	Temperature*Cover soil	1	0.0	0.0	3.4	0.08
	Residuals	16	0.2	0.0		
Potassium (%)	Temperature	1	0.4	0.4	1.1	0.31
	Cover Soil	1	2.3	2.3	5.7	**0.03***

	Temperature*Cover soil	1	0.0	0.0	0.1	0.80
	Residuals	16	6.4	0.4		

Appendix XIII: Results of ANOVA or permutation ANOVA testing for effects (α=0.05) of soil temperature during budflush, cover soil, and their interaction on *Populus tremuloides* seedling nutrient (NPK) concentration (n=5) after the *growing period* (see Fig. 3-3). The following are definitions for abbreviations: Df = degrees of freedom, SS = sums of squares, MS = mean square error, Iter = the number of iterations used.

Response Variable	Effect	Df	SS	MS	Iter	*p*-value
Total Nitrogen (%)	Temperature	1	0.1	0.1	5000	**<0.01***
	Cover Soil	1	0.0	0.0	156	0.39
	Temperature*Cover soil	1	0.0	0.0	624	0.14
	Residuals	16	0.1	0.0		
Phosphorus (%)	Temperature	1	0.0	0.0	127	0.44
	Cover Soil	1	0.2	0.2	5000	**0.01***
	Temperature*Cover soil	1	0.0	0.0	484	0.17
	Residuals	16	0.3	0.0		
Potassium (%)	Temperature	1	0.0	0.0	51	0.75
	Cover Soil	1	0.0	0.0	51	0.96
	Temperature*Cover soil	1	0.0	0.0	51	0.77
	Residuals	16	11.2	0.7		

Appendix XIV: Results of ANOVA or permutation ANOVA testing for effects (α=0.05) of soil temperature warming speed, cover soil, and their interaction on *Populus tremuloides* seedling nutrient (NPK) concentration (n=5) after the *growing period* (see Fig. 3-3). The following are definitions for abbreviations: Df = degrees of freedom, SS = sums of squares, MS = mean square error, Iter = the number of iterations used.

Response Variable	Effect	Df	SS	MS	Iter	*p*-value
Total Nitrogen (%)	Speed	1	0.0	0.0	1772	**0.05***
	Cover Soil	1	0.0	0.0	444	0.19
	Speed*Cover soil	1	0.0	0.0	241	0.30
	Residuals	16	0.1	0.0		
Phosphorus (%)	Speed	1	0.0	0.0	96	0.51
	Cover Soil	1	0.2	0.2	5000	**0.01***
	Speed*Cover soil	1	0.0	0.0	299	0.25
	Residuals	16	0.3	0.0		
Potassium (%)	Speed	1	0.2	0.2	119	0.46
	Cover Soil	1	0.1	0.1	51	0.82
	Speed*Cover soil	1	0.1	0.1	96	0.51
	Residuals	16	5.9	0.4		

Appendix XV: Results of ANOVA or permutation ANOVA testing for effects (α=0.05) of soil temperature during budflush, cover soil, and their interaction on *Populus tremuloides* seedling stem and root nonstructural carbohydrates (n=10) after the *warming period* (see Fig. 3-3). The following are definitions for abbreviations: Df = degrees of freedom, SS = sums of squares, MS = mean square error, F (Iterations) = either F value if a regular ANOVA was performed, or (the number of iterations) if a permutation ANOVA was performed.

Response Variable	Effect	Df	SS	MS	F (Iterations)	*p*-value
Total Stem NSC Concentration (%)	Temperature	1	7.2	7.2	1.6	0.21
	Cover Soil	1	10.4	10.4	2.4	0.13
	Temperature*Cover soil	1	3.5	3.5	0.8	0.34
	Residuals	36	157.6	4.4		
Total Stem NSC Content (g)	Temperature	1	0.0	0.0	1.5	0.23
	Cover Soil	1	0.0	0.0	5.3	**0.03***
	Temperature*Cover soil	1	0.0	0.0	0.0	0.83
	Residuals	36	0.1	0.0		
Stem Sugar Concentration (%)	Temperature	1	1.4	1.4	1.3	0.26
	Cover Soil	1	0.0	0.0	0.0	1.00
	Temperature*Cover soil	1	2.3	2.3	2.2	0.15
	Residuals	36	39.1	1.1		
Stem Sugar Content (g)	Temperature	1	0.0	0.0	3.2	0.08
	Cover Soil	1	0.0	0.0	3.2	0.08
	Temperature*Cover soil	1	0.0	0.0	0.0	0.87
	Residuals	36	0.0	0.0		
Stem Starch Concentration (%)	Temperature	1	2.2	2.2	1.0	0.33
	Cover Soil	1	10.5	10..5	4.5	**0.04***
	Temperature*Cover soil	1	0.1	0.1	0.1	0.82
	Residuals	36	83.7	2.3		

Stem Starch Content (g)	Temperature	1	0.0	0.0	0.0	0.89
	Cover Soil	1	0.0	0.0	6.9	**0.01***
	Temperature*Cover soil	1	0.0	0.0	0.1	0.81
	Residuals	36	0.0	0.0		
Total Root NSC Concentration (%)	Temperature	1	42.7	42.7	5.4	**0.03***
	Cover Soil	1	9.6	9.6	1.2	0.28
	Temperature*Cover soil	1	20.7	20.7	2.6	0.11
	Residuals	36	284.1	7.9		
Total Root NSC Content (g)	Temperature	1	0.0	0.0	1.2	0.28
	Cover Soil	1	0.0	0.0	0.4	0.54
	Temperature*Cover soil	1	0.0	0.0	5.5	**0.02***
	Residuals	36	0.1	0.0		
Root Sugar Concentration (%)	Temperature	1	17.6	17.6	9.8	**<0.01***
	Cover Soil	1	0.2	0.1	0.1	0.78
	Temperature*Cover soil	1	3.8	3.8	2.1	0.16
	Residuals	36	65.0	1.8		
Root Sugar Content (g)	Temperature	1	0.0	0.0	2.1	0.15
	Cover Soil	1	0.0	0.0	0.0	0.98
	Temperature*Cover soil	1	0.0	0.0	6.1	**0.02***
	Residuals	36	0.0	0.0		
Root Starch Concentration (%)	Temperature	1	5.5	5.5	(363)	0.22

	Cover Soil	1	7.3	7.3	(365)	0.22
	Temperature*Cover soil	1	6.8	6.8	(390)	0.21
	Residuals	36	168.7	4.7		
Root Starch Content (g)	Temperature	1	0.0	0.0	0.4	0.54
	Cover Soil	1	0.0	0.0	0.9	0.36
	Temperature*Cover soil	1	0.0	0.0	3.2	0.08
	Residuals	36	0.0	0.0		

Appendix XVI: Results of ANOVA testing for effects (α=0.05) of soil temperature during budflush, cover soil, and their interaction on *Populus tremuloides* stem and root nonstructural carbohydrates (n=10) after the *growing period* (see Fig. 3-3). The following are definitions for abbreviations: Df = degrees of freedom, SS = sums of squares, MS = mean square error, F = F value.

Response Variable	Effect	Df	SS	MS	F (Iterations)	*p*-value
Total Stem NSC Concentration (%)	Temperature	1	8.2	8.2	2.2	0.15
	Cover Soil	1	0.9	0.9	0.2	0.63
	Temperature*Cover soil	1	0.4	0.4	0.1	0.74
	Residuals	36	134.1	3.7		
Total Stem NSC Content (g)	Temperature	1	0.0	0.0	3.6	0.07
	Cover Soil	1	0.0	0.0	2.3	0.14
	Temperature*Cover soil	1	0.0	0.0	0.1	0.76
	Residuals	36	0.1	0.0		

Stem Sugar Concentration (%)	Temperature	1	0.1	0.1	0.1	0.77
	Cover Soil	1	4.0	4.0	3.9	**0.06**$^{+}$
	Temperature*Cover soil	1	0.1	0.1	0.1	0.82
	Residuals	36	36.9	1.0		
Stem Sugar Content (g)	Temperature	1	0.0	0.0	7.1	**0.01***
	Cover Soil	1	0.0	0.0	0.6	0.46
	Temperature*Cover soil	1	0.0	0.0	0.3	0.60
	Residuals	36	0.0	0.0		
Stem Starch Concentration (%)	Temperature	1	6.6	6.6	3.4	0.07
	Cover Soil	1	1.1	1.1	0.6	0.45
	Temperature*Cover soil	1	0.2	0.2	0.1	0.77
	Residuals	36	69.1	1.9		
Stem Starch Content (g)	Temperature	1	0.0	0.0	0.7	0.41
	Cover Soil	1	0.0	0.0	3.9	**0.06**$^{+}$
	Temperature*Cover soil	1	0.0	0.0	0.0	0.96
	Residuals	36	0.0	0.0		
Root NSC Concentration (%)	Temperature	1	614.0	614.0	66.1	**<0.01***
	Cover Soil	1	0.0	0.0	0.0	1.00
	Temperature*Cover soil	1	9.9	9.9	1.1	0.31
	Residuals	36	334.6	9.3		
Root NSC Content (g)	Temperature	1	0.0	0.0	0.3	0.56

	Cover Soil	1	0.1	0.1	3.2	0.08
	Temperature*Cover soil	1	0.0	0.0	0.1	0.81
	Residuals	36	0.7	0.0		
Root Sugar Concentration (%)	Temperature	1	44.0	44.0	(5000)	**<0.01***
	Cover Soil	1	1.5	1.5	(137)	0.42
	Temperature*Cover soil	1	0.2	0.2	(98)	0.51
	Residuals	36	52.0	1.4		
Root Sugar Content (g)	Temperature	1	0.0	0.0	2.8	0.10
	Cover Soil	1	0.0	0.0	0.6	0.45
	Temperature*Cover soil	1	0.0	0.0	0.1	0.70
	Residuals	36	0.1	0.0		
Root Starch Concentration (%)	Temperature	1	329.4	329.4	60.9	**<0.01***
	Cover Soil	1	1.5	1.5	0.3	0.60
	Temperature*Cover soil	1	7.3	7.3	1.4	0.25
	Residuals	36	194.6	5.4		
Root Starch Content (g)	Temperature	1	0.0	0.0	0.0	0.94
	Cover Soil	1	0.0	0.0	5.1	**0.03***
	Temperature*Cover soil	1	0.0	0.0	0.4	0.55
	Residuals	36	0.3	0.0		

Appendix XVII: Results of ANOVA testing for effects (α=0.05) of soil temperature warming speed, cover soil, and their interaction on *Populus tremuloides* stem and root nonstructural

carbohydrates (n=10) after the *growing period* (see Fig. 3-3). The following are definitions for abbreviations: Df = degrees of freedom, SS = sums of squares, MS = mean square error, F = F value.

Response Variable	Effect	Df	SS	MS	F	*p*-value
Stem NSC Concentration (%)	Speed	1	0.7	0.7	0.4	0.52
	Cover Soil	1	0.1	0.1	0.1	0.82
	Speed*Cover soil	1	3.6	3.6	2.1	0.15
	Residuals	36	60.5	1.7		
Stem NSC Content (g)	Speed	1	0.0	0.0	0.8	0.39
	Cover Soil	1	0.0	0.0	2.9	0.09
	Speed*Cover soil	1	0.0	0.0	0.0	0.98
	Residuals	36	0.1	0.0		
Stem Sugar Concentration (%)	Speed	1	0.9	0.9	0.9	0.35
	Cover Soil	1	2.4	2.4	2.5	0.12
	Speed*Cover soil	1	0.5	0.5	0.5	0.48
	Residuals	36	34.2	1.0		
Stem Sugar Content (g)	Speed	1	0.0	0.0	1.1	0.31
	Cover Soil	1	0.0	0.0	0.9	0.35
	Speed*Cover soil	1	0.0	0.0	0.0	0.87
	Residuals	36	0.0	0.0		
Stem Starch Concentration (%)	Speed	1	0.0	0.0	0.0	0.95
	Cover Soil	1	3.4	3.4	2.0	0.17

	Speed*Cover soil	1	1.4	1.4	0.9	0.36
	Residuals	36	60.8	1.7		
Stem Starch Content (g)	Speed	1	0.0	0.0	0.2	0.62
	Cover Soil	1	0.0	0.0	4.4	**0.04***
	Speed*Cover soil	1	0.0	0.0	0.0	0.89
	Residuals	36	0.0	0.0		
Root NSC Concentration (%)	Speed	1	13.0	13.0	2.0	0.17
	Cover Soil	1	0.8	0.8	0.1	0.73
	Speed*Cover soil	1	5.1	5.1	0.8	0.38
	Residuals	36	234.0	6.5		
Root NSC Content (g)	Speed	1	0.0	0.0	0.0	0.93
	Cover Soil	1	0.0	0.0	1.7	0.20
	Speed*Cover soil	1	0.0	0.0	0.0	0.96
	Residuals	36	0.9	0.0		
Root Sugar Concentration (%)	Speed	1	1.8	1.8	(148)	0.41
	Cover Soil	1	1.4	1.4	(274)	0.27
	Speed*Cover soil	1	0.2	0.2	(51)	0.77
	Residuals	36	62.6	1.7		
Root Sugar Content (g)	Speed	1	0.0	0.0	(468)	0.18
	Cover Soil	1	0.0	0.0	(56)	0.64
	Speed*Cover soil	1	0.0	0.0	(51)	0.75

	Residuals	36	0.2	0.0		
Root Starch Concentration (%)	Speed	1	24.4	24.4	6.2	**0.02***
	Cover Soil	1	0.1	0.1	0.0	0.88
	Speed*Cover soil	1	3.2	3.2	0.4	0.38
	Residuals	36	142.12	3.9		
Root Starch Content (g)	Speed	1	0.0	0.0	0.5	0.47
	Cover Soil	1	0.0	0.0	2.8	0.10
	Speed*Cover soil	1	0.0	0.0	0.0	0.90
	Residuals	36	0.3	0.0		

Appendix XVIII: Results of permutation ANOVA testing for effects (α=0.05) of soil temperature during budflush, cover soil, and their interaction on cover soil nutrient (NPK) availability (n=4) after the *warming period* (see Fig. 3-3). The following are definitions for abbreviations: Df = degrees of freedom, SS = sums of squares, MS = mean square error, F (Iterations) = the number of iterations performed.

Response Variable	Effect	Df	SS	MS	Iterations	*p*-value
Total Nitrogen (μmol/10 cm^2)	Temperature	1	705.7	705.7	1021	0.09
	Cover Soil	1	27401.8	27401.8	5000	**<0.01***
	Temperature*Cover soil	1	2317.9	2317.9	5000	**0.01***
	Residuals	12	2955.9	246.3		
Phosphorus (μmol/10 cm^2)	Temperature	1	0.0	0.0	51	0.92
	Cover Soil	1	17.8	17.8	5000	**0.01***

	Temperature*Cover soil	1	0.3	0.3	94	0.52
	Residuals	12	17.0	1.4		
Potassium (μmol/10 cm^2)	Temperature	1	18.0	18.0	51	0.82
	Cover Soil	1	31745.0	31745.0	5000	**<0.01***
	Temperature*Cover soil	1	78.0	78.0	129	0.44
	Residuals	12	1931.0	161.0		

Appendix XIX: Results of permutation ANOVA testing for effects (α=0.05) of soil temperature during budflush, cover soil, and their interaction on cover soil nutrient (NPK) availability (n=4) after the *growing period* (see Fig. 3-3). The following are definitions for abbreviations: Df = degrees of freedom, SS = sums of squares, MS = mean square error, Iterations = number of iterations used.

Response Variable	Effect	Df	SS	MS	Iterations	*p*-value
Total Nitrogen (μmol/10 cm^2)	Temperature	1	19789.5	19789.5	5000	**0.01***
	Cover Soil	1	20893.3	20893.3	5000	**<0.01***
	Temperature*Cover soil	1	1438.3	1438.3	197	0.34
	Residuals	12	16389.7	1365.8		
Phosphorus (μmol/10 cm^2)	Temperature	1	2.8	2.8	590	0.15
	Cover Soil	1	20.2	20.2	5000	**<0.01***
	Temperature*Cover soil	1	0.8	0.8	189	0.35
	Residuals	12	9.0	0.7		
Potassium (μmol/10 cm^2)	Temperature	1	6529.4	6529.4	5000	**<0.01***

	Cover Soil	1	13220.4	13220.4	5000	**<0.01***
	Temperature*Cover soil	1	2250.4	2250.4	5000	**<0.01***
	Residuals	12	2591.4	216.0		

Appendix XX: Results of ANOVA or permutation ANOVA testing for effects (α=0.05) of soil temperature warming speed, cover soil, and their interaction on cover soil nutrient (NPK) availability (n=4) after the *growing period* (see Fig. 3-3). The following are definitions for abbreviations: Df = degrees of freedom, SS = sums of squares, MS = mean square error, F (Iterations) = either F value if a regular ANOVA was performed, or (the number of iterations) if a permutation ANOVA was performed.

Response Variable	Effect	Df	SS	MS	F(Iterations)	*p*-value
Total Nitrogen (μmol/10 cm^2)	Speed	1	1726	1726	1.1	0.31
	Cover Soil	1	17782	17782	11.4	**0.01***
	Speed*Cover soil	1	2413	2413	1.5	0.24
	Residuals	12	18739.0	1562.0		
Phosphorus (μmol/10 cm^2)	Speed	1	0.1	0.1	(51)	1.00
	Cover Soil	1	27.0	27.0	(5000)	**<0.01***
	Speed*Cover soil	1	0.0	0.0	(51)	0.80
	Residuals	12	15.2	1.3		
Potassium (μmol/10 cm^2)	Speed	1	5761.6	5761.6	(5000)	**<0.01***
	Cover Soil	1	11533.7	11533.7	(5000)	**<0.01***
	Speed*Cover soil	1	5470.8	5470.8	(5000)	**<0.01***
	Residuals	1	5278.9	439.9		