Nitrogen Availability and Salinity as Limiting Factors for Lodgepole Pine (*Pinus contorta*) and White Spruce (*Picea glauca*) Growth on Reclaimed Sites in the Athabasca Oil Sands Region in Alberta, Canada

by

Min Duan

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

Doctor of Philosophy

in
SOIL SCIENCE

Department of Renewable Resources
University of Alberta

Abstract

Unfavorable conditions of reclaimed soils for vegetation establishment have been a concern for successful oil sands reclamation. Growth limitations of planted trees have been observed in the Athabasca Oil Sands Region (AOSR) in Alberta, Canada, but the factors limiting tree growth were poorly understood. This thesis research identified limiting factors for growth of lodgepole pine (*Pinus contorta*, Pl) and white spruce (*Picea glauca*, Sw) in reclaimed sites in the AOSR and tested these identified limiting factors through manipulative experiments. In a field study, lodgepole pine growth was found to be significantly correlated with water availability and Sw growth was positively correlated with soil nitrogen (N) concentrations and negatively correlated with salinity in the reclaimed soil, suggesting growth limitations of low water availability for Pl, and low N availability and high soil salinity for Sw. Both N (200 kg N ha^{-1}) and complete fertilizer (200 kg N, 20 kg P and 19 kg K per hectare plus micronutrients) similarly increased the height, diameter at breast height (DBH) and aboveground biomass growth for Sw, but not for Pl. Foliar N concentration, but not other nutrients, in needles of Sw increased after fertilization, confirming the N limitation for the growth of Sw. Among the unfavorable conditions, the effects of soil salinity on N cycling in a reclaimed soil were evaluated in a laboratory incubation study and the interactive effects of N and salinity on Pl and Sw seedling growth were studied in a greenhouse experiment. The short-term incubation experiment indicated that soil salinity significantly reduced gross N mineralization, nitrification, and N immobilization rates, but those rates were not completely inhibited even under high salinity level (with soil electric conductivity of 8 dS m^{-1}). Gross N immobilization rates in all salinity levels were greater than gross N mineralization rates, resulting in negative net N mineralization rates, which could partly explain low N availability in saline reclaimed soils in the studied sites. However, the greenhouse experiment demonstrated the effectiveness of N fertilization in improving the growth of seedlings in a saline growth media, in which N fertilization significantly increased net photosynthetic rates and N concentrations in needles and roots, resulting in improved seedling height and root collar diameter growth and total seedling biomass for both Pl and Sw seedlings. The N fertilization aggravated the inhibitory effects of soil salinity on seedlings treated with high salinity level (150 mmol L^{-1} NaCl). The research findings will help develop proper management practices to correct growth limiting factors in reclaimed sites with low nutrient availability, some of those sites may be affected by high salinity levels, and thus ensuring the success of reclaiming disturbed lands in the AOSR.

Preface

This thesis is an original work by Min Duan. Chapter 2 of this thesis has been published as Duan, M., House, J. and Chang, S.X., "Limiting factors for lodgepole pine (*Pinus contorta*) and white spruce (*Picea glauca*) growth differ in some reconstructed sites in the Athabasca oil sands region" Ecological Engineering, vol. 75, 323-331 (2015). I was responsible for the performance of the experiment, data collection and analysis as well as manuscript writing. House, J. assisted with the data collection. Chang, S.X. was the corresponding author and contributed to the design of the research and edited the manuscript.

Acknowledgements

I would like to express my sincere gratitude to my supervisor, Dr. Scott X. Chang. Without his support, my fantastic journey in the PhD program would not have been possible. The knowledge and skills I have gained from him during the last four and half years are great treasures and will benefit me for the rest of my career. I also express my deepest appreciation to the supervisory committee, Dr. Philip G. Comeau, Dr. Edward Bork and Dr. Janusz Zwiazek, for their encouragement and guidance in all stages of the program. The comments on the thesis from Dr. Zhihong Xu and Dr. Guillermo Hernandez Ramirez are appreciated.

The completion of this thesis would not have been possible without the assistance and support from professors, staff, friends, family, and colleagues in Canada and China.

I would like to thank Jason House, Ghulam Murtaza Jamro, Jin-Hyeob Kwak and Kangho Jung. I could not forget the time shared with them at school and in the bush. I would also like to extend my gratitude to our laboratory coordinators, Pak Chow and Shujie Ren, for their help and excellent suggestions on analytical methods. Thanks also go to colleagues Lei Sun, Xiaopeng Li, Qiting Chen, Abdelhafid Dugdug, Jenna Zee, Agathe Hery and Isabel Fodor for helping in the field and/or in the laboratory.

I thank Steven Williams at the University of Alberta for assistance with the greenhouse experiment. I am also thankful to Mr. Clarence Gilbertson at the Lethbridge Research Centre, Agriculture and Agri-Food Canada, Dr. Mingsheng Ma and Alvin Kwan at the University of

Alberta, and Chandelle Macdonald at the University of Wyoming for assistance in isotope analyses of samples. I thank Dr. Janusz Zwiazek for providing access to equipment in his laboratory and Wengqing Zhang and Feng Xu for suggestions on the measurement of seedling physiological properties.

Financial support was provided by the Environmental Reclamation Research Group (ERRG) of Canadian Oil Sands Network for Research and Development (CONRAD) with contributions from Suncor Energy Inc., Canadian Natural Resources Limited, and Shell Canada Energy. The Natural Science and Engineering Research Council of Canada (NSERC) also supported part of the thesis research. I would like to express my deepest appreciation to them. A special thanks to Suncor Energy Inc. for providing access to the study sites. I would particularly like to thank Drs. Francis Salifu and Carmela Arevalo, and Mrs. Lelaynia Wells for providing logistic support when the field work was conducted. Dr. Xiao Tan at Shell Canada Energy provided input at the site selection stage.

Finally, I especially would like to thank my parents and parents-in-law for all of their love and continued support over the past four and half years. I would also like to extend my sincerest thanks to my wife, Fan Xiao. Her unceasing dedication was the source of strength whenever I have a difficult time in the graduate program. It is my pleasure to thank my son for being the motive power of my life.

Table of Contents

List of Tables

List of Figures

List of Symbols and Abbreviations

$\delta^{13}C$: carbon isotope abundance

$\delta^{15}N$: nitrogen isotope abundance

Ψ_w: shoot water potential

AB: aboveground biomass

ABI: annual increment of aboveground biomass

AOSR: Athabasca oil sands region

Db: bulk density

DBH: diameter at breast height

DBHI: annual increment of diameter at breast height

DOC: dissolved organic carbon

DON: dissolved organic nitrogen

DW: dry weight

EC: electrical conductivity

g_s: stomatal conductance

HI: annual increment of height

HPFM: high pressure flow meter

LMM: LFH-mineral soil mix

L_p: root hydraulic conductivity

N/IA: ratio of gross nitrification to gross NH_4^+-N immobilization rate

OB: overburden

Pl: lodgepole pine

PMM: peat-mineral soil mix

P_n: net photosynthetic rate

RCD: root collar diameter

S: salinity

Sw: white spruce

T: transpiration rate

TS: tailings sand

VWC: volumetric water content

WHC: water holding capacity

Chapter 1 General introduction

1 Research background

There are 168.7 billion barrels of bitumen in Alberta’s oil sands deposits, which account for 99% of the province's total proven oil reserves (Alberta Energy, 2013), and about 98% of Canada's oil reserves. These Albertan oil sands deposits are primarily distributed in the watersheds of the Athabasca River, Peace River, and Cold Lake. Oil sands deposits within 75 m of the surface can be recovered by surface mining. The Athabasca oil sands region (AOSR) along the Athabasca River is the largest single bitumen deposit in the world, and is also the only one suitable for surface mining in Alberta (Mossop et al., 1982).

Prior to surface mining, the lands are drained; forests are clear-cut harvested, and the muskeg or peat material and surface mineral soils are stripped and either placed directly at reclamation sites or stockpiled for future reclamation purposes (Fung and Macyk, 2000). Below the surface soil and above the oil sands is the overburden (OB) material, which is physically removed and used for later reclamation efforts. The tailings sand (TS) material, one main byproduct of the extraction process, settles in tailings ponds; it is used as a reconstruction substrate in reclamation.

Surface mining can significantly disrupt the landscape and alter ecosystem structure and function. It is estimated that surface mining will disturb 4800 km^2 of lands, 65% of which were occupied by wetlands (Raine et al., 2002), while *in situ* operations will result in a potential loss of 6500 km^2 of boreal forest by direct disturbance (Dyer and Huot, 2010). To date, oil sands operation activities have disturbed about 715 km^2 of land, but only 10% of these disturbed lands are in the process of being reclaimed (Alberta Energy, 2013). According to the Alberta Environmental Protection and Enhancement Act (EPEA), oil sands mining operators are required to create reclaimed soils and landforms that are "capable of supporting and establishing a self-sustaining, locally common boreal forest, whose productivity is equivalent to or better than the pre-disturbance level" (Alberta Environment, 2010; Cumulative Environmental Management Association, 2009). However, the diverse boreal forest ecosystem has developed over millennia under the combined influence of parent material, topography, climate, and natural disturbance (Beckingham and Archibald, 1996). After surface mining, the ecosystem is totally disturbed, and it is not simple to restore oil sands disturbed boreal ecosystems to their previous state within a short period of time. It is estimated that it takes more than 200 years to develop an old growth forest in this region following surface mining (Thompson, 2009).

As reported by Fung and Macyk (2000), the general sequence of reclaiming upland forest after oil sands mining is: (i) surveying natural soils to delineate suitable reclamation materials; (ii) salvaging, placing, and preparing reclamation materials on sites; (iii) seeding of grass/legume mixture prior to tree planting to suppress soil erosion; (iv) planting trees; (v) maintaining and monitoring; and (vi) certifying the reclaimed land.

The important step of reclamation is to reconstruct functioning soil (Figure 1-1) (Macdonald et al., 2012), because soil provides the base upon which many other ecosystem services stem from, including anchorage support to vegetation, water storage and supply, habitat for microbial and faunal diversity, and nutrient supply (Dominati et al., 2010). In the oil sands region, overburden materials as well as tailings sand are used as substrates to rebuild landforms. Surface soils and near-surface geological materials (to a depth of 3 m) salvaged before mining activities are used as the reconstructed cover soil (Turchenek and Lindsay, 1982). Peat (due to its availability across large portions of the mined area) and/or the forest floor (LFH) salvaged from pre-mining upland areas as the soil amendment (20-50 cm) is usually mixed with the cover soil to increase the nutrient availability and water-holding capacity (Mackenzie and Naeth, 2010).

After the reconstruction of landscapes, native tree species, primarily trembling aspen (*Populus tremuloides* Michx.), balsam poplar (*Populus balsamifera* L.), white spruce (*Picea glauca* (Moench) Voss), black spruce (*Picea mariana* (P. Mill.) B.S.P.), and jack pine (*Pinus banksiana* Lamb.), are planted back into the harsher boreal reclamation sites according to the guidelines for reclamation to forest vegetation in the Athabasca oil sands region (Alberta Environment, 2010). The rapid establishment of a closed tree canopy is expected to suppress the establishment of weedy forb and grass species (Maundrell and Hawkins, 2004), and encourage the production of litter and forest floor layers, which will facilitate soil redevelopment (Klinka et al., 1990). Apart from the establishment of overstory tree species, understory plant communities are restored as well. The most common shrubs planted in reclaimed sites include nitrogen (N)-fixing shrubs such as green alder (*Alnus crispa* (Ait.) Pursh) and Canadian buffaloberry

(*Shepherdia canadensis* (L.) Nutt.), and other shrubs such as red osier dogwood (*Cornus stolonifera* Michx.), wild roses (*Rosa acicularis* Lindl. and *Rosa woodsii* Lindl.), pin cherry (*Prunus pensylvanica* L.f.), choke cherry (*Prunus virginiana* L.), and saskatoonberry (*Amelanchier alnifolla* (Nutt.) Nutt.) (Fung and Macyk, 2000).

Soil construction using peat and/or LFH, mineral soil, OB and/or TS forms an artificial soil profile, with abrupt changes between those materials, so it takes time to develop the new hydrogeological cycle, nutrient cycles, vegetation succession patterns, and establish plant, animal, insect, and microorganism communities in the soil (Macdonald et al., 2012). In addition, reclaimed soils can have extreme pH, high soluble salt concentrations (Howat 2000), and extensive soil compaction and hostile temperature regimes (Moffat and McNeill, 1994). Because of the nature of reclaimed soils, particularly when the reconstructed soil cover is peat over tailings sand or peat-mineral mix over tailings sand, those soils may have low nutrient supply capacity, low nutrient availability, and low nutrient and water holding capacity. Those factors may negatively affect vegetation growth (Fung and Macyk, 2000). Among the nutrients required by trees and other plants that establish on a reclaimed site, N is often the most limiting nutrient. Other nutrients such as phosphorus (P) and micronutrients can also be limiting (Lanoue, 2003). Besides the harsh rooting medium in reclaimed landscapes, the vegetation has to endure unfavorable climatic conditions. The short growing season in the boreal forest region leaves little time for species to become established on a site during the first growing season and limited water and nutrient uptake makes seedling survival rates extremely low (Radoglou and Raftoyannis, 2002). Topographic factors, such as slope and aspect, also influence vegetation establishment and growth by affecting energy input, soil and air temperature, soil moisture content, evapotranspiration, and other processes (Kumar et al., 1997; Nicolau et al., 2005; Badia et al., 2007).

Whether those limiting factors act alone or in combination, the productivity of regenerated forest ecosystems can be severely limited. Under such stressful conditions, plants may exhibit various visual symptoms indicating growth limitation (Fisher and Binkley, 2000). One such example is the foliar discoloration of planted trees observed in the reclaimed area in the Suncor lease north of Fort McMurray. Because of the potential multiple number of growth limiting factors involved, it is essential to identify the factor (or factors) limiting tree growth in those reclaimed sites, so that proper management practices can be applied to correct those problems. It is also necessary to evaluate the interactive effects among limiting factors to further clarify the underlying reasons. If those problems are not identified and corrected, the eventual success of reclamation can be significantly affected.

2 Review of limiting factors for tree growth in reclaimed landscapes

In this section, I review the research that has been conducted in the last several decades on land reclamation, and provide detailed information about limiting factors in different reclaimed sites. Further, I introduce feasible methods to alleviate these limiting factors in different reclaimed landscapes.

Forest growth depends on environmental conditions such as temperature, light, the supply of water and nutrients (Peace and Grubb, 1982). Only when these growth requirements are met, can trees grow well. However, these requirements are often hard to meet in reclaimed sites because of environmental conditions such as restricted rooting depths, high soluble salt concentrations, extreme pH, poor drainage, and soil compaction, some of the common limiting factors that negatively affect tree growth on reclaimed lands (Torbert et al., 1988). Showalter et al. (2010) studied the hardwood forest of the Appalachian mountains on reclaimed surface mine sites, and found that better growth of *F. americana* and *Q. rubra* was attributed to the lower percentage of coarse fragments, higher water retention, and lower pH, whereas *L. tulipifera* grew poorly in all growth media. Better growth was also due to greater nutrient availability and microbial activity in the soil.

2.1 Physical factors affecting tree growth

After reclamation, soil properties may be greatly changed. Some adverse soil physical properties, such as high bulk density and rock fragments, poor structure, low porosity and low water-holding capacity, of reclaimed mine soils may inhibit soil-forming processes and adversely affect plant growth.

2.1.1 Water

Soil water storage and transmission characteristics were influenced by morphological and physical properties of soils (Barnhisel and Hower, 1997). Reclaimed soil and spoil that were compacted lacked a continuous macro-pore network, which impeded root development and aeration, and decreased water retention and transmission (Indorante et al., 1981). Some studies were conducted on reclaimed soil alone (Underwood and Smeck, 2002), while others compared soil development in reclaimed and unmined sites for soil water retention and water infiltration (Pedersen et al., 1980). A slow water infiltration rate was reported from newly reclaimed mining surface soils mainly due to the compaction and sorting that took place during the reclamation process (Chong and Cowsert, 1997; Harms and Chanasyk, 2000; Guebert and Gardner, 2001).

Limited soil moisture availability also affects growth of vesicular-arbuscular fungi within mine soils in arid regions, which could adversely affect fungal spore germination and levels of colonization attained in plants, in turn retarding initial establishment of vegetation during reclamation (Reid and Bowen, 1979; Tommerup, 1984).

Data on water infiltration, movement, redistribution, and storage within the soil profile were important (Chong et al., 1986) to identify the best management practices for tree growth on reclaimed surface soils. Also, studies on soil water transmission properties including measurements of soil moisture characteristic curves, unsaturated hydraulic conductivity, saturated hydraulic conductivity, and water infiltration tests in the field could be used to determine water movement and availability in reclaimed sites (Gorman and Sencindiver, 1999; Barnhisel and Gray, 2000; Thomas et al., 2001).

2.1.2 Soil compaction

Soil compaction of reclaimed mined lands is very common due to the use of large equipment during reclamation. Soil compaction brings about the following problems: (i) reduced soil porosity and associated aeration, (ii) loss of soil structure, (iii) reduced hydraulic and gas conductivity, (iv) changes in thermal properties, (v) increased strength of dry soil, (vi) greater depth of soil freezing, and (vii) reduced growth of soil organisms (Omi, 1986; Chong and Cowsert, 1997). Compaction reduced root growth, which limited the tree's ability to explore the soil volume for nutrients and moisture. Any of these changes could contribute to short-term reductions in tree growth or long-term reductions in site productivity (Ministry of Forests, 1993).

Planting trees into dense, herbaceous ground cover and compacted soils generally results in high tree mortality. Compaction of topsoil and establishment of thick ground cover were found to be counterproductive in producing hardwood forests (Ashby, 1991; Chaney et al., 1995; Torbert, 1995; Rodrigue and Burger, 2004). Potter et al. (1988) showed that material handling operations for restoration (e.g., land forming and spreading topsoil material) exacerbated soil compaction, altered physical and structural characteristics, and restricted root development. Tyner et al. (1948) reported that compacting surface layers down to 45 cm created survival problems for tree seedlings. Research also showed that compaction restricted infiltration, and increased runoff and erosion (Hatchell et al., 1970). Less intensive grading left looser, albeit rougher, surface which enhanced moisture retention and root penetration, thereby promoting tree growth (Phillips, 2006).

2.1.3 Soil aggregate

Previous studies reported that soil disturbance caused by mining led to loss of aggregation, decline of soil organic carbon, loss of soil structure, increase of bulk density and reduce of soil porosity (Shukla et al., 2004b). Such soils had markedly lower productive capacity for plant growth. However, coarse-textured soils were utilized as a growth medium in reclamation following disturbances of surface coal mining because they were often present in areas with mining activities (Wick et al., 2008). These kinds of growth medium greatly influence the end land use such as forestry, grassland, cropping land, or wildlife habitats. Armstrong and Bragg (1984) reported that drainage problems often occurred because aggregate stability and soil porosity tended to be low compared to similar undisturbed soil. Angel (2008) showed that most of the mid- to late-successional species that occurred in the mixed mesophytic forest did not volunteer on compacted mined spoils with variable chemistry and fertility, and if they were planted, they did not grow well because they could not tolerate raw mine spoil conditions.

2.1.4 Slope

The slope of reclaimed sites was reported to be important in reclamation processes to ensure geotechnical and ecological stability for the restored ecosystem. The degree of the slope affected different processes, such as solar radiation flux, soil and air temperature, surface runoff, soil moisture content, evapotranspiration, and thus influenced the growth of plants (Kumar et al., 1997; Nicolau et al., 2005). Badia et al. (2007) studied the slope effect on the growth and survival of five woody species in central Spain, and found that flatter slopes optimized soil water conservation and favored vegetation development. A slope increase from 11.4 to 15.5 degrees significantly reduced height growth in10-year-old woody pine species. This slope increase also reduced basal diameter growth of juniper and pine. Much of the variability in the establishment and development of afforestation in semi-arid conditions was related to the volume of useful soil for root systems and its water storage capacity.

2.2 Chemical factors affecting tree growth

Reclaimed soils usually contain low concentrations of soil organic carbon (C), N, and P, and have unfavorable soil pH, which could influence tree performance in reclaimed sites (Hearing et al., 2000; Burger, 2004).

2.2.1 Nutrient availabilities

It is generally recognized that availability of N and water are two of the main factors limiting plant growth in reclaimed sites. One of the most important issues for restoration of functional ecosystems in post-mining landscapes was reconstruction of soil, in which accumulation of organic C in surface soil layers was of crucial importance (Jochimsen, 1996; Bradshaw, 1997). Mining and related activities led to severe loss of soil organic C because of topsoil loss during removal, and mechanical mixing of A horizon with B and C horizons during removal, handling and replacement (Lal et al., 1998).

Foliage chlorosis with different intensities was often observed in tree species in the reclaimed sites. Mercuri et al. (2006) showed that plantation forest growing after ore excavation was usually limited by poor nutrient availability in the mine substrates. Deficiency of plant available N was often a limiting factor in the re-vegetation and maintenance of disturbed lands, such as mine soils (Matson and Boone, 1984). Mineralization of organic N was slower in mine soil than in natural soil, and the slow N mineralization in reclaimed soils may be caused by small microbial population size, reduced enzymatic activities, low microbial diversity and poor N adsorption by plants (Reeder and Sabey, 1987). Vimmerstedt et al. (1989) observed that a long time period was needed (more than 30 years) to increase total N concentrations to favorable levels in the surface horizon of afforested coal mine barren soils. Accretion of C and N on mine soils was influenced by both overburden strata (placed on the surface as parent material of the new soil) and tree species (Vimmerstedt et al., 1989).

McMillan et al. (2007) found that net ammonification was significantly higher at natural sites compared to reclaimed sites. The importance of net nitrification at reclaimed sites was further evidenced by higher relative nitrification index values than those found at natural sites, in particular for the reclamation treatments that used LFH as an organic amendment. Low nitrification rates in boreal soils have been linked to low pH (Ste-Marie and Paré, 1999), anaerobic conditions, low substrate (ammonium) availability, low nitrifying bacterial populations (Davidson et al., 1992), and chemical inhibition (Wardle et al., 1998).

Plant-available P was low in mine soils due to high P fixation capacity of the spoil (Roberts et al., 1988). Increased rates of mineralization in disturbed mine soil improved soil quality, increased soil organic C concentration, and enhanced biomass productivity (Lal et al., 1998). Incorporating organic amendments into reconstructed strip mine soil provided an alternative to topsoil replacement (Olsen and Jones, 1989). The addition of organic material to the soil increased fertility and stimulated microbial activity, promoted N transformation and nutrient cycling, and accelerated ecosystem recovery (Coyne et al., 1998). Moreover, providing organic C as an energy source for soil biota induced decomposition and mineralization of plant residues, thereby releasing soil nutrients. Accumulation of organic C resulted in changes to physical and chemical properties of the mine soil, such as water retention and sorption capacities, nutrient content and availability, soil bulk density, and buffering capacity (Herrick and Wander, 1998).

2.2.2 Acidity and salinity

Haynes and Naidu (1998) reported that soil pH values greater than 5.5 were favorable for root development and biomass production in all land uses. However, reclaimed landscapes often had low or high soil pH, which was detrimental to tree growth. The pH of mine soils was largely determined by their parent material and weathering conditions. Haering et al. (2004) found that soil materials consisting of weathered brown sandstone tended to be more acidic (4.0-5.5) while unweathered gray sandstone materials were generally much more alkaline (7.5-8.0) on a surface mine in West Virginia. Growth-limiting acidity in the surface mined soils was caused by deposition of low-base-content overburden, and acidic conditions limited root growth and establishment of plants (Mays et al., 2000).

Elevated electrical conductivity (EC) values indicated either highly alkaline or highly acidic soils (Jurinak et al., 1987; Sobek et al., 2000). Soluble salts were influential variables in the survival and growth of trees (McFee et al., 1981; Davidson et al., 1984) and site productivity decreased with an increase of soluble salt concentration (Andrews et al., 1998; Rodrigue and Burger, 2004). Soluble salts with EC of more than 3 dS m^{-1} were found to be unfavorable for plant growth and tree survival (McFee et al., 1981). Also, several studies indicated that the decrease of saturated hydraulic conductivity was related to an increase of Na concentration in soils (Frenkel et al., 1978; Suarez et al., 1984; Shainberg and Singer, 2011).

Higher pH of reclaimed material has been observed in many reclamation studies (Mummey et al., 2002). McMillan et al. (2007) showed that pH values were significantly higher in reclaimed sites compared to natural sites. Salvaged mineral materials of the Athabasca oil sands region were typically associated with a high pH (Fung and Macyk, 2000). Mixing this alkaline material with surface organic material during over-stripping produced a more alkaline reclaimed material compared to that found in natural boreal soils.

2.2.3 Heavy metals

After reclamation, the reclaimed soil may be affected by heavy metals, which may accumulate in tissue of growing plants, and affect plant growth. The main toxic effects of heavy metals are disruptions of photosynthesis, functions of the stomata, respiration, and biomass production (Fouroughi et al., 1982; Larcher, 1984), which may lead to substantial growth inhibition. Brams and Fiskell (1971) reported that copper (Cu) tended to be very strongly bound to soil organic matter; although it was nontoxic to plants, it hindered the uptake of essential nutrients and thus retarded plant growth when it accumulated in plant roots.

Different decontamination or reclamation procedures were suggested to restore the soil fertility for plants grown in reclaimed sites containing heavy metals. One method consisted of immobilizing accumulated heavy metals through the addition of a nontoxic compound such as lime, iron sulphate, cation exchangers, organic matter, or clays (Czupyrna et al., 1989). Another method attempted to dilute contaminants in the uppermost soil layer using a deep plow down to

80 cm (Koster and Merkel, 1985).

2.3 Biological factors affecting tree growth

2.3.1 Vegetation competition

Another hindrance for tree growth was competition from herbaceous vegetation (Torbert, 1995). For example, according to the Surface Mining Control and Reclamation Act in the U.S., coal operators must establish “a diverse, effective, and permanent vegetative cover of the same seasonal variety native to the area…that is capable of self-regeneration and plant succession…” (Section 816.111 of SMCRA). To comply with these standards, coal operators generally establish an aggressive, dense ground cover. The resulting cover is very dense and competitive, which in turn impedes the establishment of seedlings of trees and shrubs (Burger and Torbert, 1990; Ashby, 1997; Holl, 2002). These grasses and legumes competed with tree seedlings for light, water and nutrients. Some annual or perennial grasses can grow much taller than the young seedlings in the early stages of development and smothered seedlings when the aerial portions died back in winter. Consequently, survival and growth of trees planted on reclaimed mine sites is often poor. Weed control usually increased survival and growth of trees planted on these reclaimed mine sites (Chaney et al., 1995).

However, there are different opinions about vegetation competition in reclaimed sites. In northeast Wyoming, coarse-textured soils were used in reclamation following surface coal mining and shrubs or grasses were then planted for reclamation. Although reestablished post-mining plant communities were dominated by invasive annual forbs (such as *Salsola iberica auct.*) for the first 2 years of reclamation, the "seeded" plant communities (grasses or shrubs) were usually well established after 2 years and started to influence aggregate formation through microbial associations, root phenology and development, the quality and quantity of organic matter inputs, and wet-dry cycles (Chen and Stark, 2000; Ehrenfeld et al., 2005; McClaran et al., 2008).

2.3.2 Microbial activities

Soil microbial biomass typically decreased following disturbance. Mummey et al. (2002) found that microbial C was 56% lower in reclaimed surface mined sites than in adjacent undisturbed soils 20 years after land reclamation. They explained that soil stockpiling was extremely detrimental to fungi, in particular mycorrhizal fungi, and stated that reclaimed soils are not able to provide any inoculant for regenerating plants. This was potentially a large problem in the boreal forest, where most tree species rely on mycorrhizal associations to secure nutrients (Jonsson et al., 1999). McMillan et al. (2007) reported that lower microbial C and N in reclaimed sites compared to natural forest was the result of lower moisture content and/or differences in organic matter composition.

The return of microbial populations led to improvements in the establishment of the native forest vegetation on reclaimed surface mine sites by mineralization of nutrients, N fixation, and symbiotic relationships with tree roots (Showalter et al., 2010). Establishment of vesicular-arbuscular fungi within roots of plants growing on mining disturbed lands was beneficial for enhancing P uptake by vegetation. However, vesicular-arbuscular fungal establishment has not been readily achieved during reclamation in arid regions (Alien and Alien, 1980) as compared to regions with mesic moisture regimes (Medve, 1984). Studies show that vesicular-arbuscular fungal establishment on disturbed lands in semiarid regions required 6 years or more (Biondini et al., 1985; Loree and Williams, 1987). In addition to P, vesicular-arbuscular fungal colonization in plants may provide nutrients such as N, Ca, S, Mg, Mn, Fe, Zn, and K (Stribley, 1987). Reclamation with relatively infertile mine soils required rapid rates of vesicular-arbuscular fungal colonization to satisfy the nutritional requirements of dependent plant species as well as more obligate species (White et al., 1992).

2.3.3 Insects and disease

In some reclaimed sites, disease and insects were problems for plant growth. Skousen et al. (2007) reported that a heavy groundcover of grasses and legumes during reclamation provided cover for small mammals, which fed on the bark and girdled trees. Tyner et al. (1948) found that compacted surface layers down to 45 cm created survival problems for tree seedlings and hardwoods like oaks (*Quercus spp.*) and cherry (*Prunus spp.*) did not grow well because of rodent damage.

2.4 Potential treatments to alleviate limiting factors

The main objective of reclamation is to reclaim disturbed land to a stable, biologically self-sustaining state as soon as possible. Restoration of soil quality and ecosystem function of reclaimed mine soils depended on the reclamation methods and re-establishment of vegetation, which could enhance and sustain plant growth (Shrestha and Lal, 2006). Successful establishment of a vegetative cover on reclaimed mine soils depended on understanding the chemical and physical properties of disturbed soils. Successful reforestation of surface mined land required reconstructed sites with suitable soil characteristics for good establishment and growth of trees.

Understanding the relationship between soil properties in reclaimed sites and tree growth was useful in matching tree species to their use on specific sites. The interactions among these factors determined the success or failure of reforestation efforts. In land reclamation, information on soil-site-tree growth relationships can be useful in constructing mine soils best suited to the tree species of interest. Activities such as spoil selection, grading practices, and fertilization and soil amendments can have a profound effect on long-term soil productivity and tree growth (Torbert et al., 1988; Burger and Torbert, 1990). Andrews et al. (1998) showed that moderate to light grading, use of tree-compatible herbaceous ground cover, and selection of a fertile, slightly acid mine soil with low concentrations of soluble salts, can result in a productive site that will allow for good early survival and growth of trees.

Since limiting factors varied in different reclaimed sites, the responding treatments would need to be varied. Take coal mining as an example, after mining the reconstructed landscape was severely compacted due to heavy equipment use for stockpiling soil and combining topsoil and subsoil (Grigal, 2000; Mummey et al., 2002). Tree survival and growth on compacted mine soils was invariably poor. Tillage practices could be used to ameliorate the detrimental effects of compaction and improve plant growth. Also, soil could be loosened by other methods, including the use of rippers, winged rippers, winged subsoilers, excavators with bucket, or excavators with fork. James et al. (2010) studied the effects of ripping on tree survival, height, and diameter of sycamore (*Platanus occidentialis*), sweetgum (*Liquidambar styraciflua*), yellow poplar (*Liriodendron tulipifera*), loblolly pine (*Pinus taeda*), and white pine (*Pinus strobus*) on mine land after it was graded and reclaimed, and reported that ripping significantly increased average tree survival and improved the growth of all species except white pine.

Disturbance during mining generally results in the loss of soil organic C and N. Fertilization could offset nutrient loss from the soil, stimulate biological activity, speed up the process of soil structure restoration, and accelerate ground cover development. Regardless of the overburden type used, plant available N and P tended to be low on mine soils. Consequently, fertilization at some point during the rotation was usually needed to obtain good growth of trees planted on reclaimed mine sites (Kost et al., 1998). Fertilizer application could help to offset an anticipated decline in productivity. Studies showed that a yearly rate of 140 to 170 kg N ha^{-1} provided economical yield increases of planted trees in reclaimed soils (Underwood et al., 2006). Shukla et al. (2004a) showed that fertilizer application also improved soil aggregation, structure, and water transmission properties in the reclaimed soils, and reclamation with topsoil and fertilizer application doubled the soil organic C concentration in the 0-10 cm layer in southeast Ohio.

For salinity problems in reclaimed sites, gypsum has been used extensively to improve infiltration rates. Application of gypsum to sodic soils significantly improves soil physical conditions by promoting flocculation, enhancing aggregate stability and increasing the infiltration rate (Lebron et al., 2002). The use of amendment could also decrease the detrimental effects of salinity. In central Ohio, Kost et al. (1998) reported that survival rates of *F. pennsylvanica*, *P. nigra*, and *Acer saccharinum* increased by 10, 62, and 171%, respectively, when 30 cm of topsoil was applied over alkaline cast overburden spoil compared with the overburden alone, and tree height of these species increased 33, 69, and 26%, respectively, with the topsoil addition. As for low water availability, irrigation practices could be used to improve seed germination and seedling establishment on arid mined lands as well as influence vegetation composition and productivity (DePuit et al., 1982; Powell et al., 1990).

3 Thesis structure

The overall objective of this thesis research was to identify the limiting factor/factors of two tree species planted in reclaimed sites for oil sands reclamation, and to provide recommendations of proper management practices to correct growth limiting factors in some reclaimed sites in the AOSR. The specific objectives were to determine: 1) whether growth of lodgepole pine and white spruce would be affected by nutrient and water availabilities and soil salinity, 2) how lodgepole pine and white spruce would respond to fertilization in reclaimed sites, 3) how soil salinity would influence N cycling and availability, and 4) whether N fertilization could alleviate salt stress and improve growth of lodgepole pine and white spruce seedlings.

Four experiments were conducted in this thesis research to test the following four hypotheses (Figure 1-2): 1) the growth of lodgepole pine planted in PMM over TS in the AOSR is affected by low nutrient availability, while white spruce planted in PMM over OB are limited by low water availability due to coarse textured tailings sand as a substrate, 2) fertilization effects on Pl and Sw growth vary due to different dominant limitations in different reclaimed sites, 3) soil salinity inhibits N transformations, resulting in low N availability in saline reclaimed sites, and 4) the application of N fertilization may improve salt-stressed seedling growth due to improved physiological properties.

This thesis includes six chapters. Chapter 1 describes background information and reviews literature on limiting factors in different reclaimed sites. Each of the data chapters (2 to 5) constitutes a manuscript that has already been under review or will be submitted for publication:

Chapter 2 “Limiting factors for lodgepole pine (*Pinus contorta*) and white spruce (*Picea* *glauca*) growth differ in some reconstructed sites in the Athabasca oil sands region". This chapter has been published in Ecological Engineering.

Chapter 3 "Responses of lodgepole pine (*Pinus contorta*) and white spruce (*Picea glauca*) to fertilization in reconstructed boreal forest soils in the oil sands region". This chapter is under review in Ecological Engineering.

Chapter 4 "Salinity affects gross nitrogen transformation rates in a reclaimed boreal forest soil in Alberta, Canada". This chapter is to be submitted for publication.

Chapter 5 "Nitrogen fertilization increased lodgepole pine and white spruce seedling growth in a reclaimed saline boreal forest soil". This chapter is to be submitted for publication.

Chapter 6 provides a summary of key findings, makes general conclusions and recommends some directions for future research.

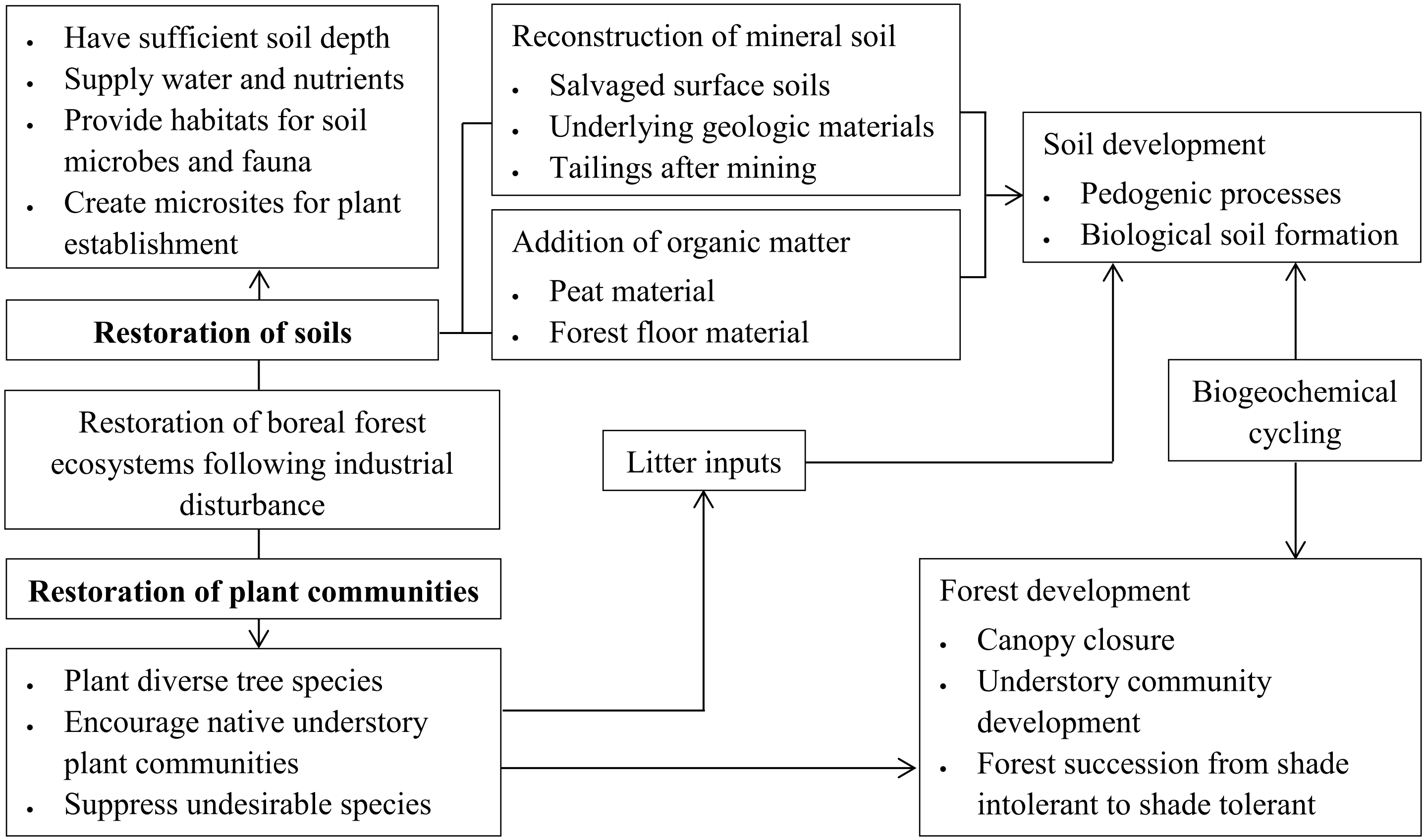

Figure 1-1 Key steps and processes in restoration of boreal forest ecosystems following industrial disturbance (Macdonald et al., 2012).

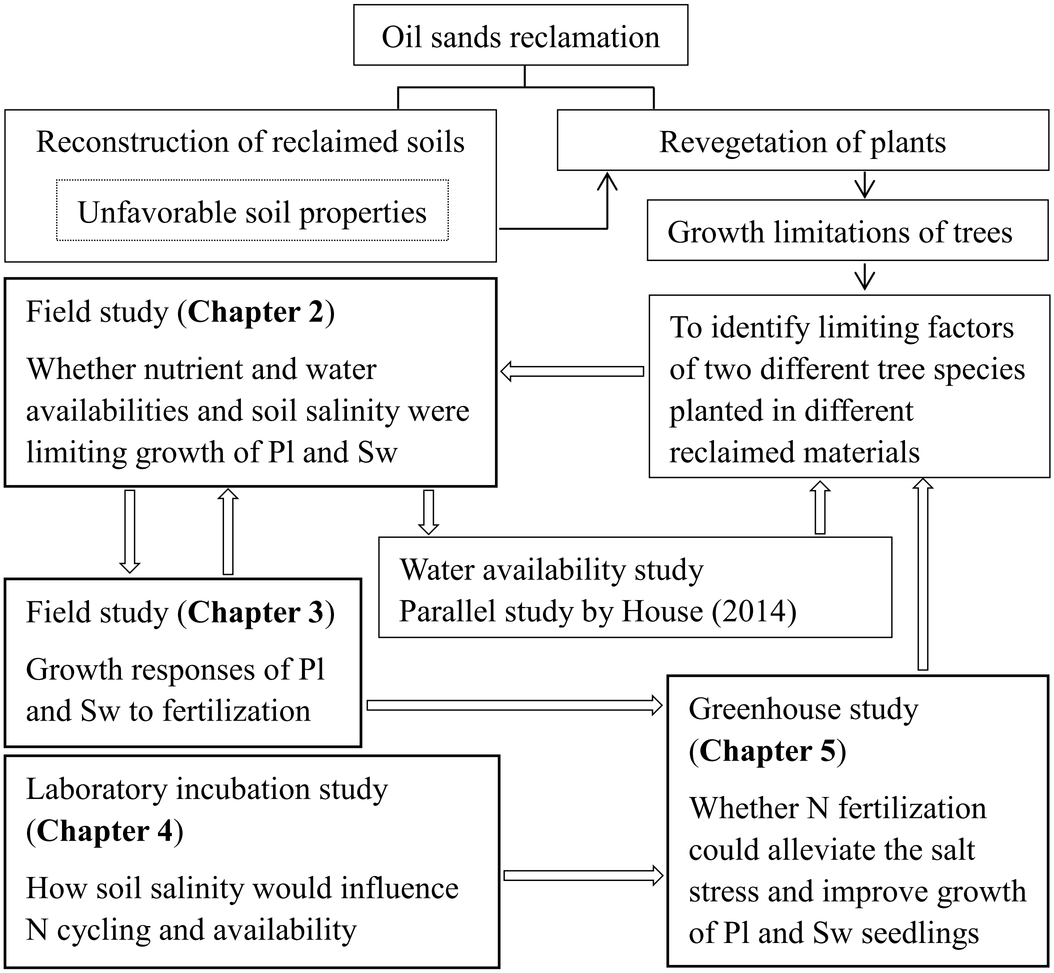

Figure 1-2 Flow chart of the research questions in the thesis research.

References

Alberta Energy. 2013. Facts and Statistics. http://www.energy.alberta.ca/OilSands/791.asp (Accessed 15 October 2013).

Alberta Environment. 2010. Guidelines for Reclamation to Forest Vegetation in the Athabasca Oil Sands Region, 2nd edition. Edmonton, Alberta.

Alberta Government. 2005. Government of Alberta Minable Oil Sands Strategy. http://www.energy.gov.ab.ca/docs/oilsands/pdfs/MOSS_Policy2005.pdf (Accessed 3 July 2007).

Allen, E.B., Allen, M.F. 1980. Natural re-establishment of vesicular-arbuscular mycorrhizae following strip mine reclamation in Wyoming. J. Appl. Ecol. 17:137-147.

Andrews, J.A., Johnson, J.E., Torbert, J.L., Burger, J.A., Kelting D.L. 1998. Minesoil and site properties associated with early height growth of eastern white pine. J. Environ. Qual. 27:192-199.

Angel, P.N. 2008. Forest establishment and water quality characteristics as influenced by spoil type on a loose graded surface mine in eastern Kentucky. Ph.D. dissertation. Department of Forestry, University of Kentucky, Lexington, KY.

Armstrong, M.J., Bragg, N.C. 1984. Soil physical parameters and earthworm populations associated with opencast coal working and land restoration. Agric. Ecosyst. Environ. 11:131-143.

Ashby, W.C. 1991. Surface mine tree planting in the midwest pre- and post-public law 95-87. Proceedings of the American Society for Surface Mining and Reclamation Meeting. American Society for Surface Mining and Reclamation. Durango, CO.

Ashby, W.C. 1997. Soil ripping and herbicides enhance tree and shrub restoration on stripmines. Restor. Ecol. 5:169-177.

Badia,D., Valero, R., Gracia, A., Marti, C. 2007. Ten-year growth of woody species planted in reclaimed mined banks with different slopes. Arid Land Res. Manag. 21:67-79.

Barnhisel, R.I., Hower, J.M. 1997. Coal surface mine reclamation in the eastern United States: The revegetation of disturbed lands to hayland/pasture or cropland. Adv. Agron. 61:233-275.

Barnhisel, R.I., Gray, R.B. 2000. Changes in morphological properties of a prime land soil reclaimed in 1979. Proceedings of the American Society for Surface Mining and Reclamation Meeting, American Society for Surface Mining and Reclamation. Tampa, FL.

Beckingham, J.D., Archibald. J.H. 1996. Field Guide to Ecosites of Northern Alberta. Special report 5. Canadian Forest Service, Northern Forestry Centre, Edmonton, Alberta.

Biondini, M.E., Bonham, C.D., Redente, E.F. 1985. Secondary successional patterns in a sagebrush (*Artemisia tridentata*) community as they relate to soil disturbance and soil biological activity. Vegetatio 60:25-36.

Bradshaw, A. 1997. Restoration of mined lands-using natural processes. Ecol. Eng. 8:255-269.

Brams, E.A., Fiskell, J.G.A. 1971. Copper accumulation in citrus roots and desorption with acid.

Soil Sci. Soc. Am. Proc. 35:772-775.

Burger, J.A. 2004. Restoring forests on mined land in the Appalachians: Results and outcomes of a 20-year research program. Proceedings of a Joint Meetings of the American Society of Surface Mining and Reclamation and the 25th West Virginia Surface Mine Drainage Task Force. American Society of Surface Mining and Reclamation. Morgantown, WV.

Burger, J.A., Torbert, J.L. 1990. Mined land reclamation for wood production in the Appalachian region. Proceedings of the 1990 Mining and Reclamation Conference and Exhibition. American Society of Mining and Reclamation. Lexington, KY.

Chaney, W.R., Pope, P.E., Byrnes, W.R. 1995. Tree survival and growth on land reclaimed in accord with public law 95-87. J. Environ. Qual. 24:630-634.

Chen, J., Stark, J.M. 2000. Plant species effects and carbon and nitrogen cycling in a sagebrush-crested wheatgrass soil. Soil Biol. Biochem. 32:47-57.

Chong, S.K., Cowsert, P.T. 1997. Infiltration in reclaimed mine land ameliorated with deep tillage treatments. Soil Tillage Res. 44:255-264.

Coyne, M.S., Zhai, Q., Mackown, C.T., Barnhisel, R.I. 1998. Gross nitrogen transformation rates in soil at a surface coal mine site reclaimed for prime farmland use. Soil Biol. Biochem. 30:1099-1106.

Cumulative Environmental Management Association. 2009. A framework for reclamation certification criteria and indicators for mineable oil sands. Fort McMurray, Alberta.

Czupyrna, G., Levy, R.D., MacLean, A.I., Gold, H. 1992. *In situ* immobilization of heavy metal-contaminated soils. Soil Sci. 154:338.

Davidson, E.A., Hart, S.C., Firestone, M.K. 1992. Internal cycling of nitrate in soils of a mature coniferous forest. Ecology 73:1148-1156.

Davidson, W.H., Hutnik, R.J., Parr, D.E. 1984. Reforestation of mined land in the northeastern and north-central US. North. J. Appl. For. 1:7-12.

DePuit, E.J., Skilbred, C.L., Coenenberg, J.G. 1982. Effects of two years of irrigation on revegetation of coal surface-mined land in southeastern Montana. J. Range Manage. 35:67-74.

Dominati, E., Patterson, M., Mackay, A. 2010. A framework for classifying and quantifying the natural capital and ecosystem services of soils. Ecol. Econ. 69:1858-1968.

Dyer, S., Huot, M. 2010. Mining vs. *in situ*: What is the highest environmental impact oil? http://www.pembina.org/pub/2017 (Accessed 20 May 2011).

Ehrenfeld, J.G., Ravit, B., Elgersma, K. 2005. Feedback in the plant-soil system. Annu. Rev. Environ. Resour. 30:75-115.

Fisher, R.F., Binkley, D. 2000. Nutrition management: Nutrient limitations. In: Fisher, R.F., Binkley, D. (Eds.), Ecology and Management of Forest Soils, 3rd edition. John Wiley & Sons, Inc., New York, NY. p. 282-310.

Frenkel, H., Goertzen, J.O., Rhoades, J.D. 1978. Effects of clay type and content, exchangeable sodium percentage, and electrolyte concentration on clay dispersion and soil hydraulic conductivity. Soil Sci. Soc. Am. J. 42:32-39.

Fung, M.Y.P., Macyk, T.M. 2000. Reclamation of oil sands mining areas. In: Barnhisel, R.I., Darmody, R.G., Daniels, W.L. (Eds.), Reclamation of Drastically Disturbed Lands. ASA, CSSA, and SSSA, Madison, WI. p. 755-774.

Fyles, J.W., McGill, W.B. 1986. Nitrogen mineralization in forest soil profiles from central Alberta. Can. J. For. Res. 17:242-249.

Gorman, J.M., Sencindiver, J.C. 1999. Changes in minesoil physical properties over a nine-year period. Proceedings of the American Society for Surface Mining and Reclamation Meeting. American Society for Surface Mining and Reclamation. Scottsdale, AZ. p. 245-253.

Granstrom, A. 1982. Seed banks in five boreal forest stands originating between 1810 and 1963. Can. J. Bot. 60:1815-1821.

Grigal, D.F. 2000. Effects of extensive forest management on soil productivity. For. Ecol. Manage. 138:167-185.

Guebert M.D., Gardner, T.W. 2001. Macropore flow on a reclaimed surface mine: Infiltration and hill slope hydrology. Geomorphology 39:151-169.

Harms, T.E., Chanasyk, D.S. 2000. Plot and small-watershed scale runoff from two reclaimed surface-mined watersheds in Alberta. Hydrol. Process. 14:1327-1339.

Hatchell, G.E., Ralston, C.W., Foil, R.R. 1970. Soil disturbances in logging: Effects on soil characteristics and growth of loblolly pine in the Atlantic Coastal Plain. J. For. 68:772-775.

Haynes, R.J., Naidu, R. 1998. Influence of lime, fertilizer, and manure applications on soil organic matter content and soil physical conditions: A review. Nutr. Cycl. Agroecosyst.

51:123-137.

Hearing, K.C., Daniels, W.L., Feagley, S.E. 2000. Reclaiming mined lands with biosolids, manures, and papermill sludges. In: Barnhisel, R.I., Darmody, R.G., Daniels, W.L. (Eds.), Reclamation of Drastically Disturbed Lands. ASA, CSSA, and SSSA, Madison, WI. p. 615-644.

Hearing, K.C., Daniels, W.L., Galbraith, J.M. 2004. Appalachian mine soil morphology and properties: Effects of weathering and mining method. Soil Sci. Soc. Am. J. 68:1315-1325.

Herrick, J.E., Wander, M.M. 1998. Relationship between soil organic carbon and soil quality in cropped and rangeland soils: The importance of distribution, composition, and soil biological activity. In: Lal, R., Kimble, J.M., Follett, R.F., Stewart, B.A. (Eds.), Soil Processes and the Carbon Cycle. CRC Press Inc., Boca Raton, FL. p. 405-425.

Holl, K.D. 2002. Long-term vegetation recovery on reclaimed coal surface mines in the eastern USA. J. Appl. Ecol. 39:960-970.

Howat, D. 2000. Acceptable Salinity, Sodicity and pH Values for Boreal Forest Reclamation. Envionmental Sciences Division, Edmonton, Alberta.

James, A.B., Evans, D.M. 2010. Ripping compacted mine soils improved tree growth 18 years after planting. Proceedings of the Meeting of the American Society of Mining and Reclamation. American Society of Mining and Reclamation. Pittsburgh, PA.

Jochimsen, M.E.A. 1996. Reclamation of colliery mine spoil founded on natural succession. Water Air Soil Pollut. 91: 99-108.

Jonsson, L., Dahlberg, A., Nilsson, M.C., Zackrisson, O., Karen, O. 1999. Ectomycorrhizal fungal communities in late successional Swedish boreal forests, and their composition following wildfire. Mol. Ecol. 8:205-215.

Jurinak, J.J., Bowden, J., Samson, F., Portal, T. 1987. Electrical conductivity. In: Williams, R.D., Schuman, G.E. (Eds.), Reclaiming Mine Soils and Overburden in the Western United States. Soil Conservation Society of America, Ankeny, IA. p. 27-34.

Klinka, K., Wang, Q., Carter, R.E. 1990. Relationships among hunus forms, forest floor nutrient properties, and understory vegetation. For. Sci. 36:564-581.

Kost, D.A., Vimmerstedt, J.P., Brown, J.H. 1998. Topsoiling, ripping and fertilizing effects on tree growth and nutrition on calcareous mine soils. For. Ecol. Manage. 103:307-319.

Kumar, L., Skidmore, A. K., Knowles, E. 1997. Modeling topographic variation in solar radiation in a GIS environment. Int. J. Geogr. Inf. Sci. 11:475-497.

Lal, R., Kimble, J.M., Follett, R.F. 1998. Land use and soil C pools in terrestrial ecosystem. In: Lal, R., Kimble, J.M., Follett, R.F., Stewart, B.A. (Eds.), Management of Carbon Sequestration in Soil. CRC Press Inc., Boca Raton, FL. p. 1-8.

Lanoue, A. 2003. Phosphorus content and accumulation of carbon and nitrogen in boreal forest soils. M.Sc. thesis, University of Alberta, Edmonton, Alberta.

Lebron, I., Suarez, D.L., Yoshida, T. 2002. Gypsum effect on the aggregate size and geometry of three sodic soils under reclamation. Soil Sci. Soc. Am. J. 66:92-98.

Macdonald, E., Quideau, S., Landhäusser, S. 2012. Rebuilding boreal forest ecosystems after industrial disturbance. In: Vitt, D.H., Nhatti, J.S. (Eds.), Restoration and Reclamation of Boreal Ecosystems: Attaining Sustainable Development. Cambridge University Press, NY. p. 123-160.

Mackenzie, D.D., Naeth, M.A. 2010. The role of the forest soil propagule bank in assisted natural recovery after oil sands mining. Restor. Ecol. 18:418-427.

Matson, P. A., Boone, R. D. 1984. Natural disturbance and nitrogen mineralization: Wave-form dieback of mountain hemlock in the Oregon Cascades. Ecology 65:1511-1516.

Maundrell, C., Hawkins, C. 2004. Use of an aspen overstory to control understory herbaceous species, bluejoint grass (*Calamagrotis canadensis*), and Fireweed (*Epilobium angustifolium*). North. J. Appl. For. 21:74-79.

Mays, D.A., Sistani, K.R., Soileau, J.M. 2000. Lime and fertilizer needs for land reclamation. In: Barnhisel, R.I., Darmody, R.G., Daniels, W.L. (Eds.), Reclamation of Drastically Disturbed Lands. ASA, CSSA, and SSSA, Madison, WI. p. 217-271.

McClaran, M.P., Moore-Kucera, J., Martens, D.A., van Haren, J., Marsh, S.E. 2008. Soil carbon and nitrogen in relation to shrub size and death in a semi-arid grassland. Geoderma 145:60-68.

McFee, W.W., Byrnes, W.R., Stockton, J.G. 1981. Characteristics of coal mine overburden important to plant growth. J. Environ. Qual. 10:300-308.

McMillan, R., Quideau, S. A., MacKenzie, M. D., Biryukova, O. 2007. Nitrogen mineralization and microbial activity in oil sands reclaimed boreal forest soils. J. Environ. Qual.

36:1470-1478.

Medve, R.M. 1984. The mycorrhizae of pioneer species in disturbed ecosystems in western Pennsylvania. Am. J. Bot. 71:787-794.

Mercuri, A.M., Duggin, J.A., Daniel, H., Lockwood, P.V., Grant, C.D. 2006. Estimating the unknown components of nutrient mass balances for forestry plantations in mine rehabilitation, Upper Hunter Valley, New South Wales, Australia. Environ. Manage. 37:496-512.

Ministry of forests. 1993. Site disturbance and reclamation. Williams Lake, BC. http://www.for.gov.bc.ca/cariboo/research/research.htm. (Accessed 16 July 2012).

Moffat, A.J., Bending, N.A.D., Roberts, C.J. 1991. The use of sewage sludge as a fertilizer in the afforestation of opencast coal spoils in South Wales. In: Davies, M.C.R. (Ed.), Land Reclamation: An end to dereliction? CRC Press Inc., Boca Raton, FL. p. 347-356.

Mossop, G.D., Flach, P.D., Pemberton, S.G., Hopkins, J.C. 1982. Field excursion 22: Athabasca oil sands, sedimentology and development technology. 11th International Congress on Sedimentology, Hamilton, ON.

Mummey, D.L., Stahl, P.D., Buyer, J.S. 2002. Soil microbiological properties 20 years after surface mine reclamation: Spatial analysis of reclaimed and undisturbed sites. Soil Biol. Biochem. 34:1717-1725.

Nicolau, J.M., Moreno, M., Espigares, T. 2005. Ecohydrology of rilled slopes derived from opencast mining reclamation in a semiarid area. Geophysical Research. Abstract 7:85-95.

Olsen, S.R, Sommers, I.E. 1982. Phosphorus. In: Page, A.L. (Ed.), Methods of Soil Analysis, Part 2: Chemical and Microbiological Properties. ASA, CSSA and SSSA, Madison, WI. p. 403-430.

Omi, S.K. 1986. Soil compaction: Effects on seedling growth. Report M-125. USDA Forest Service.

Peace, W.J.H., Grubb, P. 1982. Interaction of light and mineral nutrient supply in the growth of *Impatiens parviflora*. New Phytol. 90:127-150.

Phillips, M. 2006. Procedures to insure reclamation success. Proceedings of the American Society for Surface Mining and Reclamation Meeting. American Society for Surface Mining and Reclamation. Billings, MT. p. 682-685.

Powell, K.B., Vincent, R.B., DePuit, E.J., Smith, J.L., Parady, F.E. 1990. Role of irrigation and fertilization in revegetation of cold desert mined lands. J. Range Manage. 43:449-455.

Qi, M., Scarratt, J.B. 1998. Effect of harvesting method on seed bank dynamics in a boreal mixedwood forest in northwestern Ontario. Can. J. Bot. 76:872-883.

Radoglou, K., Raftoyannis, Y. 2002. The impact of storage, desiccation and planting date on seedling quality and survival of woody plant species. Forestry 75:179-190.

Raine, M., Mackenzie, I., Gilchrist, I. 2002. Terrestrial vegetation, wetlands and forest resources baseline. CNRL Horizon Project Environmental Impact Assessment Volume 6, Appendix B. Report #012-2220. Golder Associates, Alberta.

Reeder, J.D., Sabey, B. 1987. Nitrogen. In: Williams, R.D., Schuman, G.E. (Eds.), Reclaiming

Mine Soils and Overburden in the Western United States. Soil Conservation Society of America, Ankeny, IA. p.155-184.

Reid, C.P.P., Bowen, G.D. 1979. Effects of soil moisture on v/a mycorrhiza formation and root development in *Medicago.* In: Harley, J.L., Scott-Russel, R. (Eds.), The Soil-Root Interface. Academic Press Inc., London, UK. p. 211-219.

Roberts, J.A., Daniels, W.L., Bell, J.C., Burger, J.A. 1988. Early stages of mine soil genesis in a southwest Virginia spoil lithosequence. Soil Sci. Soc. Am. J. 52:716-723.

Rodrigue, J.A., Burger, J.A. 2004. Forest soil productivity of mined land in the mid-western and eastern coalfield regions. Soil Sci. Soc. Am. J. 68: 833-844.

Shainberg, I., Singer, M. 2011. Soils response to saline and sodic conditions. In: Wesley, W.W., Kenneth, K.T. (Eds.), Agricultural Salinity Assessment and Management, 2nd edition. American Society of Civil Engineers, Reston, VA. p. 139-167.

Showalter, J.M., Burger, J.A., Zipper, C.E. 2010. Hardwood seedling growth on different mine spoil types with and without topsoil amendment. J. Environ. Qual. 39:483-491.

Shrestha, R.K., Lal, R. 2006. Ecosystem carbon budgeting and soil carbon sequestration in reclaimed mine soil. Environ. Int. 32:781-796.

Shukla, M.K., Lal, R., Ebinger, M. 2004a. Soil quality indicators for reclaimed minesoils in southeastern Ohio. Soil Sci. 169:133-142.

Shukla, M.K., Lal, R., Underwood, J., Ebinger, M. 2004b. Physical and hydrological characteristics of reclaimed minesoils in southeastern Ohio. Soil Sci. Soc. Am. J.

68:1352-1359.

Skousen, J.G., Gorman, J., Emerson, P. 2007. Survival of commercial hardwoods on reclaimed minesoils in West Virginia: 6th year results. Proceedings of the American Society for Surface Mining and Reclamation Meeting. American Society for Surface Mining and Reclamation. Gillette, WY. p. 229-237.

Sobek, A.A., Skousen, J.G., Fisher, S.E. 2000. Chemical and physical properties of overburdens and minesoils. In: Barnhisel, R.I., Darmody, R.G., Daniels, W.L. (Eds.), Reclamation of Drastically Disturbed Lands. ASA, CSSA, and SSSA, Madison, WI. p. 77-104.

Ste-Marie, C., Paré, D. 1999. Soil, pH, and N availability effects on net nitrification in the forest floor of a range of boreal forest stands. Soil Biol. Biochem. 31:1579-1589.

Stribley, D.P. 1987. Mineral nutrition. In: Safir, G.R. (Ed.), Ecophysiology of v/a Mycorrhizal Plants. CRC Press Inc., Boca Raton, FL. p. 59-70.

Suarez, D.L., Rhoades, J.D., Lavado, R., Grieve, C.M. 1984. Effect of pH on saturated hydraulic conductivity and soil dispersion. Soil Sci. Soc. Am. J. 48:50-55.

Thomas, K.A., Sencindiver, J.C., Skousen, J.G., Gorman, J.M. 2001. Chemical properties of minesoils on a mountaintop removal mine in southern West Virginia. Proceedings of the American Society for Surface Mining and Reclamation Meeting. American Society for Surface Mining and Reclamation. Albuquerque, NM. p. 448-455.

Thompson, D. 2009. The facts on oil sands reclamation. The oil sands developers group. Fort McMurray, Alberta.

Tommerup, I.C. 1984. Development of infection by a vesicular-arbuscular mycorrhizal fungus in *Brassica napus* L. and *Trifolium subterraneum* L. New Phytol. 98:487-495.

Torbert, J.L. 1995. Reclamation of surface-mined forestland in the southern Appalachians. Ph.D. Dissertation. Virginia Polytechnical Institute and State University, Blacksburg, VA.

Torbert, J.L., Tuladhar, A.R., Burger, J.A., Bell, J.C. 1988. Mine soil property effects on the height of ten-year-old white pine. J. Environ. Qual. 17:189-192.

Turchenek, L.W., Lindsay, J.D. 1982. Soils inventory of the Alberta oil sands environmental research program study area. Alberta Research Council Report 122. Edmonton, Alberta.

Tyner, E.H., Smith, R.M., Galpin, S.L. 1948. Reclamation of strip-mined areas in West Virginia. Agron. J. 40:313-323.

Underwood, J.F., Smeck, N.E. 2002. Soil development in two Ohio minesoils under continuous grass cover for twenty-five years following reclamation. Proceedings of the American Society for Surface Mining and Reclamation Meeting. American Society for Surface Mining and Reclamation. Lexington, KY. p. 1158-1172.

Underwood, J., Sutton, P., Mark S.R. 2006. Forage production on reclaimed surface mined land. Ohio State University Extension Fact Sheet, Horticulture and Crop Science Department. Ohio State Univiversity, Columbus, OH. http://ohioline.osu.edu/agf-fact/0025.html (Accessed 9 August 2007).

Wardle, D.A., Nilsson, M., Gallet, C., Zackrisson, O. 1998. An ecosystem-level perspective of allelopathy. Biol. Rev. 73:305-319.

White, J.A., DePuit, E.J., Smith, J.L., Williams, S.E. 1992. Vesicular-arbuscular mycorrhizal fungi and irrigated mined land reclamation in southwestern Wyoming. Soil Sci. Soc. Am. J. 56:1464-1469.

Wick, A.F., Stahl, P.D., Ingram, L.J. 2008. Aggregate-associated carbon and nitrogen in reclaimed sandy loam soils. Soil Sci. Soc. Am. J. 73:1852-1860.

Chapter 2 Limiting factors for lodgepole pine (*Pinus contorta*) and white spruce (*Picea glauca*) growth differ in some reconstructed sites in the Athabasca oil sands region*

1 Introduction

Oil sand deposits in northern Alberta, Canada, contain about 168 billion barrels of bitumen, making it the third largest crude oil reserve in the world (Alberta Energy, 2014). The Athabasca oil sands region (AOSR) is the largest oil sands deposit in the province, in which crude oil can be extracted by open-pit mining (Fung and Macyk, 2000). Large scale open-pit mining in Alberta has disturbed approximately 767 km^2 of land, which represents 0.2% of Alberta's boreal forest (Government of Alberta, 2014a). Current legislation requires that disturbed mine land should be returned to equivalent capability similar to that existed pre-disturbance (Government of Alberta, 2014b). The success of reclamation to upland forest after open-pit mining in the AOSR largely depends on the construction of landform and the placement of reconstructed soils. Reclaimed soils are reconstructed using peat-mineral soil mix (PMM) or LFH-mineral soil mix as capping soils overlying substrates such as overburden (OB) or tailings sand (TS), both of which are byproducts of the surface mining and oil sands extraction processes (Rowland et al., 2009; Naeth et al., 2013). However, reconstructed soils may not provide conditions required for sustainable

---

* A version of this chapter has been published:
Duan, M., House, J., Chang, S.X. 2015. Limiting factors for lodgepole pine (*Pinus contorta*) and white spruce (*Picea glauca*) growth differ in some reconstructed sites in the Athabasca oil sands region. Ecological Engineering. 75, 323-331.

development of reestablishing vegetation.

Reconstruction of multi-layered soils can markedly change soil water availability for plant growth (Li et al., 2014). For example, the interface between the reclamation capping soil and the substrate restricts water movement between layers because of low capillary action of TS or low permeability of OB that can be highly-compacted (Naeth et al., 2011; Jung et al., 2014). The dynamics of soil water content can greatly change during the growing season, making periodic soil water content measurement restricted for assessing soil water availability. However, foliar ^{13}C (carbon) isotope abundance ($\delta^{13}C$) has been shown to be a good indicator of long-term soil water availability, as foliar $\delta^{13}C$ integrates soil water availability over time and is closely related to plant water use efficiency (Sun et al., 1996). The closure of stomata under water limitation increases the fixation of $^{13}CO_2$ and reduces the discrimination against ^{13}C during photosynthesis, resulting in less negative $\delta^{13}C$ in plant tissues (Choi et al., 2005; Matsushima and Chang, 2007). The same tree species under different moisture regimes may have different $\delta^{13}C$ patterns (Sun et al., 1996; Zhang et al., 1997). We therefore employed the ^{13}C technique to study if water stress is limiting tree growth in these reconstructed landscapes.

Nutrient availability, especially nitrogen (N) availability, can be another major factor that can restrict the reestablishment of plant communities. For example, planted trees growing on reconstructed mine sites were limited by low N availability in the substrates (Farnden et al., 2013). Mining and related activities may lead to severe loss of soil organic C and N because of the loss of topsoil and mechanical mixing of the A with B and C horizon soils (Ussiri and Lal, 2005; Shrestha and Lal, 2011). Although capping soils such as PMM and LFH-mineral soil mix have high organic N concentrations, the slow-release of organic N due to small microbial population sizes and reduced microbial activities may affect N availability (McMillan et al., 2007; Hemstock et al., 2010) and limit plant growth in environment of the boreal forest (Reeder and Sabey, 1987; Farnden et al., 2013). Low plant available N often limits the reestablishment and maintenance of vegetation on disturbed lands (Reeder and Sabey, 1987). Farnden et al. (2013) reported a positive relationship between tree height and soil organic matter content in a 21-year old jack pine stand in a reclaimed oil sands area, and they attributed this relationship to the contribution of organic matter to increased soil N availability. Although emissions of nitrogen oxides resulting from oil sands development in the last 40 years increased in the area surrounding oil sands mining, it was estimated that total N deposition into the ecosystems only ranged from 2.45 to 3.80 kg N ha^{-1} yr^{-1} in the AOSR (Laxton et al., 2010), which was lower than those in other parts of Canada and the United States (Watmough et al., 2005; Pardo et al., 2006), and many parts of Europe (Dise and Wright, 1995). In addition, N concentration and net N mineralization rates in soils were not affected by increased N emission (Laxton et al., 2010). Therefore, N deposition may not offer substantial relief for the high demand for nutrients by the reestablished vegetation during oil sands reclamation.

Reconstructed soils in the AOSR are often affected by high salinity as well (Fung and Macyk, 2000). The salts, mainly composed of sodium, sulfate and chloride ions, normally migrate from the underlying processed TS or marine shale OB (Barbour et al., 2007). Upward movement of salts from the OB into the cover soil can increase the electrical conductivity (EC) up to 6.0 dS m^{-1} in the lower part of the cover soil (Kessler et al., 2010). Boreal tree species have considerable variability in salt tolerance between and within species (Allen et al., 1994; Khasa et al., 2002). High salinity can affect water availability and nutrient uptake, and thus may have detrimental effects on plant survival and growth and ultimately, site productivity (McFee et al., 1981; Allen et al., 1994; Andrews et al., 1998; Rodrigue and Burger, 2004).

Reconstructed soils often possess other properties such as increased soil pH (Howat, 2000), extensive soil compaction, and high concentrations of residual bitumen (Fung and Macyk, 2000); those properties also can negatively affect the growth of trees. Poor tree growth can be identified by foliar discoloration, stunted growth, or other symptoms. The objective of this study was to identify the limiting factors for growth of two tree species planted for oil sands reclamation, including lodgepole pine (*Pinus contorta*, Pl) planted in PMM over TS and white spruce (*Picea glauca*, Sw) planted in PMM over OB on reconstructed sites in the AOSR, Alberta. I hypothesized that the growth of Pl would be likely limited by water availability due to the coarse-textured TS substrate, while nutrient availability and salinity would be major limiting factors for the growth of Sw because of the saline OB substrate with low nutrient availability.

2 Materials and methods

2.1 Site description

The study area, located within the Suncor Energy Inc. Lease 86/17 (56°59′N and 111°32′W), is 22 km north of Fort McMurray in northeastern Alberta, Canada. The site is characterized by a continental boreal climate with short and cool summers, and long and cold winters. The mean annual temperature is 0.7 °C, with mean daily temperatures ranging from -18.8 °C in January to 16.8 °C in July; the mean annual precipitation is 455.7 mm, with an average of 342.2 mm falling as rainfall during the growing season (Environment Canada, 2013). In the study area, twelve sites were set up in June 2011 (six Pl sites, site no. 1-6; six Sw sites, site no. 10-15) and six sites were set up in April 2012 (three Pl sites, site no. 7-9; three Sw sites, site no. 16-18), for a total of 18 sites, with nine Pl sites on PMM over TS and nine Sw sites on PMM over OB (Figure 2-1). On each site, a 10 × 10 m plot was set up. The thickness of PMM and tree age varied from site to site (Table 2-1). The nine sites for each tree species made up a site productivity gradient from low to high based on visual inspection of tree growth performance and the gradient was later confirmed by tree growth measurements. Understory plant communities on Pl sites were dominated by prickly rose (*Rosa acicularis*), raspberry (*Rubus idaeus*), sweet clover (*Melilotus* sp.), dandelion (*Taraxacum officinale*), and slender wheat grass (*Agropyron trachycaulum*), while willow (*Salix* sp.), green alder (*Alnus crispa*), sweet clover, dandelion, and bluejoint grass (*Calamagrostis canadensis*) were the dominant understory species on Sw sites.

2.2 Tree growth and understory plant community measurements

Trees within each plot were tagged. Tree size was measured in September 2011 and 2012 for the first twelve established sites, and in May and September 2012 for the additional six established sites, assuming there was minimal growth during the winter season. Tree height (H) and diameter at breast height (DBH, 1.3 m above the ground) were measured using a height pole and a diameter tape, respectively. Aboveground biomass (AB) of trees on each site was calculated with DBH and height-based allometric equations described in Lambert et al. (2005) and Ung et al. (2008). Mean annual growth of height (HG), diameter at breast height (DBHG), and aboveground biomass (ABG) were calculated using the H, DBH and AB measured in 2012 divided by tree age. The differences in H, DBH, and AB of trees between 2011 and 2012 for sites established in June 2011, between May and September 2012 for sites established in April 2012, were calculated as the current annual increment of height (HI), diameter at breast height (DBHI), and aboveground biomass (ABI).

Five 1 x 1 m quadrats on each site were randomly established in August 2012 along the perimeter of the site to avoid sampling and other disturbance to understory plant community. Understory plant species including shrubs, herbs, grasses and mosses/lichens were identified, and the total cover for each category was visually estimated by the same person to minimize variation among operators. Total cover for understory community in each quadrat was calculated by summing total cover for shrubs, herbs, grasses and mosses/lichens.

2.3 Soil and plant sampling

Soil samples were collected by layer in each site in July 2012. The top 20 cm PMM and the upper 20 cm substrate were collected using an auger in five randomly selected locations within each plot to obtain a composite PMM or substrate sample. If the thickness of the PMM was less than 20 cm, the entire PMM layer was collected. All soil samples were placed in plastic bags immediately after sampling, stored in a cooler and taken back to the laboratory. Bulk density (Db) of both PMM and the substrate (OB or TS) was determined by sampling soils from PMM or substrate layers in two soil pits in each site with a 100 cm^3 stainless steel ring sampler. In October 2012, five healthy trees were randomly selected in each plot, and one branch from the upper one third of the live crown of each tree was collected to form a composite branch sample. Branch samples were placed in paper bags and taken back to the laboratory.

2.4 Soil and plant analyses

Upon returning to the laboratory, fresh soil samples were passed through a 2 mm sieve and manually homogenized after removing coarse fragments and roots. Samples were then analyzed for water content, NH_4^+-N, NO_3^--N, and dissolved organic carbon (DOC) and dissolved organic nitrogen (DON). A subsample of each soil sample was air-dried for other chemical and physical analyses as described below.

The gravimetric water content of each soil sample was measured after oven-drying a subsample at 105 °C for 24 h and the volumetric water content (VWC) was calculated from gravimetric water content and bulk density. Soil texture was determined using the hydrometer method (Gee and Or, 2002). Soil pH was measured in deionized water using a digital DMP-2 mV/pH meter (Thermo Fisher Scientific Inc., Waltham, MA) with a soil to water ratio of 1:2 (w:v*)*. Soil EC was measured using an AP75 portable waterproof conductivity/TDS meter (Thermo Fisher Scientific Inc., Waltham, MA) using 30 g of air-dried soil in 60 mL deionized water after shaking for 1 h at 25 °C and filtration through Whatman No. 42 filter papers. Concentrations of soluble cations including K^+, Ca^{2+}, Mg^{2+}, Na^+, and Al^{3+} were analyzed using a Perkin Elmer Optima 3000 DV inductively coupled plasma mass spectrometer (ICP-MS) (PerkinElmer Inc., Shelton, CT) after extraction with deionized water at 1:2 of soil to water ratio (w:v) and filtration through Whatman No. 42 filter papers; a 1:4 ratio was used for the PMM from site 15 due to the high organic matter content in this site (Table 2-1).

Fresh soil samples were extracted with 0.5 mol L^{-1} KCl at 1:3 soil to solution ratio (w:v). After being shaken at 250 rpm on a mechanical shaker for 1 h, samples were filtered through Whatman No. 42 filter papers. The KCl extracts were steam-distilled with MgO to determine NH_4^+-N concentrations using a Kjeldahl distillation system (Vapodest 20, C. Gerhardt, Königswinter, Germany). The extracts were distilled again after addition of Devarda's alloy to determine NO_3^--N concentrations. A portion of each KCl extract was used for analysis of dissolved C and N concentrations with a TOC-Vcsn analyzer with TNM-1 (Shimadzu Corp., Kyoto, Japan). The total dissolved C concentration was the same as DOC concentration as no inorganic C was found in the soil. The DON concentration was calculated by subtracting NH_4^+-N and NO_3^--N concentrations from the total dissolved N concentration. Each air-dried soil sample was ground with a ball mill and used to analyze total C and N concentrations with a Carlo Erba NA 1500 elemental analyzer (Carlo Erba Instruments, Milano, Italy) at the Lethbridge Research Centre of Agriculture and Agri-Food Canada.

Foliage from each branch was separated into current-year and 1-year-old needles. After being quickly rinsed twice using distilled water, needles were oven-dried at 65 °C for 24 h or until constant weight. Three hundred needles were randomly selected and weighed. Needles were ground to pass through a 0.15 mm sieve in preparation for nutrient analyses. Concentrations of total C and N and ^{13}C in current-year and 1-year-old needles were determined with a stable isotope ratio mass spectrometer (IRMS) (Optima-EA, Micromass Ltd., Manchester, UK) linked to a Carlo Erba NA 1500 elemental analyzer (Carlo Erba Instruments, Milano, Italy). The ^{13}C isotope abundance was expressed as a relative deviation from the international standard of Pee Dee Belemite (PDB). Other total macro- (P, K, Ca and Mg) and micronutrients (Fe, Mn, Cu, Zn, B and Mo) in current-year needles were analyzed with the ICP-MS described above after digestion using concentrated HNO_3 and 30% H_2O_2 on a digestion block at 125 °C for 4 h (Campbell and Plank, 1998).

2.5 Statistical analyses

Correlation analyses were performed for HG, DBHG, ABG and foliar nutrients and soil properties, including soil VWC, pH, EC, Db, NH_4^+-N, NO_3^--N, DOC, DON, and PMM thickness. The nine sites for each tree species were classified into low, medium, and high productivity sites based on visual inspection of tree growth performance before tree size measurements. The productivity classification was later confirmed based on HG and DBHG. A one-way analysis of variance (ANOVA) and Tukey's multiple comparison were applied to examine the statistical significance of a variable that was significantly correlated with tree growth along the productivity gradient. A regression analysis with stepwise selection for variables to be included in the model was conducted to determine the relationship between HI, DBHI, ABI and all soil variables. The significance level of both variables entering and removed from the model was set at 0.05. The stepwise regression equation for the HI, DBHI, and ABI of Pl on PMM over TS or Sw on PMM over OB was set up as follows:

$$y = \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_j x_j + \beta_0$$

Where y is HI, DBHI or ABI for Pl or Sw; x_j is one of the soil properties; β_j is the coefficient of an estimate. An α value of 0.05 was chosen to indicate statistical significance in all analyses. All statistical analyses were conducted using the SAS software (SAS 9.2, SAS Institute Inc., Cary, NC).

3 Results

3.1 Lodgepole pine and white spruce growth and plant community understory cover along a productivity gradient

The DBHG ranged from 0.27 to 0.45 and 0.15 to 0.41 cm yr^{-1} on Pl and Sw sites, respectively. The ABG ranged from 0.70 to 1.88 and 0.58 to 1.76 Mg ha^{-1} yr^{-1} on Pl and Sw sites, respectively (data not shown). The ABI of high productivity sites were 5.18 and 4.00 Mg ha^{-1} $yr^{-1}$for Pl and Sw, respectively, both of which were two times greater than those on low and medium productivity sites (Figure 2-2 A). Total understory plant community covers were greater in low and medium than in high productivity sites for Pl ($p = 0.042$), but not different for Sw (Figure 2-2 B).

3.2 Relationships between Pl growth and VWC and foliar $\delta^{13}C$

The VWC were greater in high and medium than in low productivity sites (Figure 2-2 C). On Pl sites, HG, DBHG and ABG were all positively correlated with VWC (p = 0.042, 0.045 and 0.033, respectively) (Table 2-2). Foliar $\delta^{13}C$ in current-year and 1-year-old needles ranged from -28.2 to -25.4 and -27.9 to -25.2‰, respectively, along the site productivity gradient. The Pl sites with the highest productivity had the lowest foliar $\delta^{13}C$ in current-year needles, followed by those in medium and low productivity sites ($p = 0.027$) (Figure 2-2 D), while there was no significant difference in foliar $\delta^{13}C$ of 1-year-old needles among the productivity levels (p = 0.640). The HG and ABG were negatively correlated with foliar $\delta^{13}C$ in current-year needles (p = 0.036 and 0.044, respectively) (Table 2-2). Based on the regression analysis, VWC and DOC together explained 80% of the variation in both HI and ABI (p = 0.007 and 0.008, respectively) (Table 2-3), while there was no relationship between DBHI and any soil variables.

3.3 Relationships between Sw growth and foliar and soil properties

In Sw sites, HG, DBHG and ABG were all positively correlated with foliar N, foliar K, and soil inorganic N in PMM, and negatively correlated with Db. Only ABG was negatively correlated with soil pH in PMM (p = 0.047), and positively correlated with soil NH_4^+-N in PMM (p = 0.042) (Table 2-2). The Db and soil inorganic N in PMM explained 67% of the variation in HI (p = 0.037); the pH, EC and inorganic N in PMM explained 86% of the variation in DBHI (p = 0.013), and the EC and Db in PMM explained 79% of the variation in ABI (p = 0.009) (Table 2-3).

Foliar N and K concentrations in current-year needles in high productivity sites were greater than those in low and medium productivity sites (p = 0.038 and <0.001, respectively) (Figure 2-3 A and B). Foliar N concentrations in current-year needles in all sites were less than the critical value of 13 mg g^{-1} that indicates N deficiency (Ballard and Carter, 1986) except for the high productivity sites, while foliar K concentrations in current-year needles in all sites were greater than the critical value of 3.0 mg g^{-1} for K deficiency (Ballard and Carter, 1986).

High and medium productivity sites had greater soil NH_4^+-N, inorganic N and DON concentrations in PMM than low productivity sites (p = 0.001, 0.001 and 0.046, respectively) (Figure 2-4 A), but NO_3^--N concentrations in PMM were not significantly different among the three productivity levels. There was no difference between high and medium productivity sites in NH_4^+-N, inorganic N and DON concentrations in PMM (Figure 2-4 A). The K concentrations in PMM were greater in high and medium than in low productivity sites (p = 0.047) (Figure 2-4 B), while not different between high and medium productivity sites.

There were significant differences in soil EC and Db among the productivity levels. High productivity sites had the lowest EC in PMM and OB, followed by medium and low productivity sites (Figure 2-5 A). The EC in PMM or in OB in low productivity sites was two times greater than that in high productivity sites, but EC in all sites were less than the salinity threshold of 4 dS m^{-1} for tree growth in the AOSR except for the EC in OB in low productivity sites, which was 4.71 dS m^{-1}. High productivity sites had the lowest Db in PMM, followed by medium and low productivity sites (Figure 2-5 B). The Db of OB in low and medium productivity sites was 1.46 and 1.55 Mg m^{-3}, respectively; which was greater than in high productivity sites, but there was no significant difference between low and medium productivity sites.

4 Discussion

4.1 Limiting factors for lodgepole pine growth on PMM over TS sites

The significant positive correlation between VWC in PMM and Pl growth on PMM over TS sites suggested that water limitation was a factor affecting tree growth on those sites (Table 2-2). The total understory plant community cover was greater in low than in high productivity sites (Figure 2-2 B), suggesting that soil water availability may have been reduced due to high evapotranspiration under high vegetation cover. The understory vegetation could be a strong competitor with trees for the water stored in the soil, which could significantly decrease water availability for tree growth (Nambiar and Sands, 1993). Root distribution may also play a critical role in accessing plant available water in soils (Jung et al., 2014). In layered soils with distinct textural differences, the majority of roots in lodgepole pine sites were distributed in the fine-textured cover soil with good moisture availability and fertility, and the penetration of roots was inhibited by the coarse-textured soil layer (Horton, 1958). In a study conducted on the same sites as this study, fine root biomass significantly decreased in the tailings sand substrate compared to that in fine-textured peat-mineral soil mix (Jung et al., 2014). The main distribution of roots in the shallow peat-mineral soil mix layer could decrease the accessibility to available water in deep soil layers and induce water stress under dry conditions.

Soil water storage and transmission characteristics are influenced by soil structure and texture (Barnhisel and Hower, 1997). Coarse-textured TS is generally a poor plant growth medium because of its low water and nutrient holding capacities and undesirable soil chemical properties that may be influenced by tailings water (Fung and Macyk, 2000); therefore it must be capped with organic materials such as PMM to improve water holding capacity and nutrient availability and to minimize the risk of contaminants contained in the substrates from affecting the establishment of vegetation on those reclaimed sites (Rowland et al., 2009). Although there is no standard prescription for reclamation, and oil companies adopt different capping depths based on the substrates used (Rowland et al., 2009), previous studies showed that placement of a thick capping soil layer with finer materials over coarser-textured TS layers can enhance water holding capacity due to the nature of the peat material and the capillary barrier effect (Moskal, 1999; Barbour et al., 2007; Li et al., 2014). The thickness of the PMM layer ranged from 12 to 30 cm, and was not different among low, medium and high productivity levels in this study, indicating that the PMM thickness was not the most limiting factor for the growth of Pl trees. Soil water storage is also influenced to a large extent by soil organic carbon content in capping soils. Positive relationships between soil water storage and PMM thickness or soil organic carbon content in PMM layer suggested that a thick capping soil layer with high organic matter content could increase plant available water and reduce water stress in reclaimed soils (House et al., unpublished).

The significant difference in foliar δ^{13}C among productivity levels and significant correlation between the foliar δ^{13}C and tree growth parameters indicated water limitation in Pl sites (Table 2-2 and Figure 2-2 D). The low soil water availability in TS would limit fine root growth in the layer (Jung et al., 2014), thus restricting the ability of roots to absorb water and nutrients from the soil. High precipitation could increase soil water availability, resulting in increased stomatal conductance of leaves during photosynthesis, thus the plant could fix more $^{12}CO_2$ instead of $^{13}CO_2$, resulting in higher discrimination against ^{13}C and more negative foliar $\delta^{13}C$ (Matsushima and Chang, 2007; Peri et al., 2012). Bowling et al. (2002) examined the variation in C isotopic composition in plant leaves in six coniferous forests across a moisture gradient (precipitation ranging from 227 to 2,760 mm) in western Oregon, USA, and found that sites with greater precipitation had more negative $\delta^{13}C$ than sites with less precipitation. However, in this study, although the mean monthly precipitation during the 2011 growing season was 20 mm lower than that during the 2012 growing season (House et al., unpublished), lack of significant differences in foliar $\delta^{13}C$ between the current-year and 1-year-old needles reflected that the extra 20 mm precipitation did not significantly improve soil water availability.

The lack of difference in foliar N and K concentrations of current-year needles among productivity levels suggested that nutrient availability was not a limiting factor for Pl growth. Foliar N concentration in current-year needles ranged from 13.9 to 14.5 mg g^{-1} (data not shown), and foliar K concentration in current-year needles ranged from 4.4 to 4.5 mg g^{-1} (data not shown). Both of those concentrations were greater than the critical value of 12 mg g^{-1} for N and 4.0 mg g^{-1} for K that indicate nutrient deficiency (Ballard and Carter, 1986). The moderate inorganic N concentration in PMM could contribute to sufficient N availability in Pl sites. In addition, the root systems of Pl trees were shallow and favored nutrient uptake from nutrient-rich surface organic layers (Jung et al., 2014). However, nitrogen availability has been reported as a limiting factor for jack pine growth on PMM over TS site in the oil sands region (Farnden et al., 2013). This can be explained by different preferences of NH_4^+-N and NO_3^--N uptake by tree species and different dominant inorganic N forms in the soil (Lavoie et al., 1992; Hawkins et al., 2008).

4.2 Limiting factors for white spruce growth on PMM over OB sites

Positive relationships between Sw growth and foliar N and K and the higher soil inorganic N and K concentrations in the high productivity sites indicated that N and K availabilities in the soil were limiting Sw growth on PMM over OB sites (Table 2-2 and Figure 2-4). Foliar N concentration for optimal Sw growth has been reported to be between 15 and 25 mg g^{-1} (Nienstaedt and Zasada, 1990). However, foliar N concentration in this study was only 13 mg g^{-1} even in the high productivity sites, indicating that the Sw sites were N deficient. Greater NH_4^+-N concentrations in medium and high than in low productivity sites and lack of differences in NO_3^--N concentrations contributed to foliar N differences among the productivity classes, as previous study reported that Sw had preference of NH_4^+-N over NO_3^--N (Kronzucker, et al., 1997). The dominant form of available N was NH_4^+-N in this study, as compared to NO_3^--N as the dominant form of available N in reconstructed soils (Hemstock et al., 2010). The high salinity in the PMM in this study might have reduced net N mineralization (McClung and Frankenberger, 1987; Pathak and Rao, 1998) and inhibited net nitrification (Darrah et al., 1987; Inubushi et al., 1999), decreasing N availability and NO_3^--N concentrations in the soil. In addition, dramatic changes in soil physical and chemical properties after reclamation would influence microbial activity, organic matter decomposition, and lower net N transformation rates in reconstructed soils as compared to those in natural boreal forest soils (McMillan et al., 2007; Hemstock et al., 2010).

The average EC was 2.12 dS m^{-1} in the PMM layer and 3.05 dS m^{-1} in the OB layer (Figure 2-5 A). The average sodium adsorption ratio was 7.1 and 12.3 in PMM and OB layer, respectively (data not shown). The increased salinity in PMM layer may be caused by salt migration through convective flow and diffusion from OB to PMM layers (Merrill et al., 1980, 1983; Kessler et al., 2010). Although the EC and sodium adsorption ratio in the PMM in all sites were less than the critical values of 4 dS m^{-1} and 15 for causing salt stress in tree growth in the AOSR (Howat, 2000), the roots still would be affected by high salinity levels, particularly when available water was limited in the thin PMM layer. High salt concentrations, especially sodium, could reduce K uptake by plants (Renault et al., 1999), causing the lower foliar K concentrations on sites with higher salinity levels in this study. Munns and Tester (2008) reported that plants could increase the uptake of sodium and chloride ions in saline soils, which led to ion toxicity to plants and affected plant growth.

Soil compaction may also influence Sw growth. Previous studies showed that compaction decreased soil porosity, increased soil bulk density, reduced soil water holding capacity, restricted water infiltration, resisted root penetration to deep soil layers, changed nutrient cycling, and affected tree growth (Greacen and Sands, 1980; Corns, 1988; Cullen et al., 1991; Kozlowski, 1999; Startsev and McNabb, 2001; Tan et al., 2005). However, compaction of sub-layers could reduce water infiltration and increase water storage in soil layers above, increasing the amount of available water for plant uptake (Jung et al., 2014). This may explain the lack of a relationship between soil water content in PMM and Sw growth on OB sites, with the high bulk density in the OB layer and increased water availability in the PMM layer in this study. Although the Db in the TS layer was not significantly different from that in the OB layer, the high Db in TS was due to the sandy texture of TS, while the high Db in OB was from soil compaction by heavy equipment or from highly compacted reconstructed materials; the same Db in TS and OB had different pore size distributions, which would influence the expansion of root systems in different ways. Roots could penetrate deeply into the TS layer but not the OB layer because of greater macro porosity in TS than in OB.

5 Conclusions

The growth of lodgelole pine on sites reconstructed using the PMM as a capping soil over the TS substrate was mainly affected by water availability, while N availability, salinity and soil compaction combined limited Sw growth on reconstructed sites using the PMM over the OB substrate. Effects of water and nutrient availabilities on tree growth and effects of salinity on N availability should be taken into consideration when planning reclamation prescriptions, which require appropriate capping depth over substrates to reduce the upward movement of salts to the rooting zone and at the same time increase water storage for tree growth. Fertilization may be used to increase nutrient availability and improve tree growth performance in some of the low productivity sites. Therefore, reclamation of disturbed oil sands sites should consider multiple factors that may limit the success of land reclamation to ensure the sustainable development of reconstructed oil sands sites.

Table 2-1 Characteristics of lodgepole pine (*Pinus contorta*, Pl) and white spruce (*Picea glauca*, Sw) sites selected for this study in the Athabasca oil sands region, Alberta.

Site type[a]	Site no.	Year[b]	Density[c]	Peat-mineral soil mix					Substrate		
			(stem ha^{-1})	Thickness (cm)	Bulk density (Mg m^{-3})	Total C (g kg^{-1})	Total N (g kg^{-1})	Texture[d]	Bulk density (Mg m^{-3})	Total C (g kg^{-1})	Texture
Pl	1	1996	1900	17	0.77	67.1	2.7	SCL	1.50	0.9	S
(TS)	2	1991	2300	14	1.09	64.9	2.1	SCL	1.11	1.7	S
	3	1996	2300	14	1.04	50.2	1.3	SL	1.37	2.5	S
	4	1992	2700	30	0.84	78.8	2.9	SCL	1.47	2.2	S
	5	1991	2300	18	0.92	61.7	3.9	SCL	1.43	2.6	S
	6	1991	2100	24	0.64	159.6	3.7	SCL	1.58	2.9	S
	7	1992	2200	12	1.21	15.1	0.6	SCL	1.22	2.0	S
	8	1991	2100	22	1.42	84.9	3.6	SCL	1.61	1.6	S
	9	1996	2600	30	1.03	51.1	1.7	SCL	1.28	0.6	S
Sw	10	1982	1900	20	0.90	49.2	2.4	SCL	1.57	29.0	SCL
(OB)	11	1991	2300	11	1.24	44.9	1.7	SCL	1.48	42.4	SCL
	12	1991	3100	27	1.18	48.1	1.6	SCL	1.38	45.1	SCL
	13	1992	2800	16	0.76	73.5	2.6	SCL	1.06	38.9	SCL
	14	1992	1900	14	0.92	59.3	3.5	SCL	1.35	44.8	SCL
	15	1984	2500	25	0.32	235.1	10.5	N/A[e]	1.36	34.0	CL
	16	1991	2600	48	0.94	56.0	1.8	SCL	1.28	36.4	SCL
	17	1992	2300	26	1.03	170.5	9.3	SCL	1.46	43.3	SCL
	18	1992	2100	20	1.21	53.1	1.5	SCL	1.63	41.0	SCL

[a] TS stands for a site reconstructed using tailings sand as a substrate below the peat-mineral soil mix and OB stands for a site reconstructed using overburden as a substrate below the peat-mineral soil mix.

[b] Year is when Pl and Sw trees were planted after soil reconstruction.

[c] Density at the time of the study.
[d] S, SL, SCL and CL stand for sand, sandy loam, sandy clay loam and clay, respectively.
[e] N/A stands for not applicable because of high organic matter content in this site.

Table 2-2 Pearson correlation coefficients (*r*) and probability (*p*) for relationships between mean annual growth of height (HG), diameter at breast height (DBHG), aboveground biomass (ABG) and foliar nutrients and soil properties in lodgepole pine (*Pinus contorta*, Pl) and white spruce (*Picea glauca*, Sw) sites.

			Foliar δ^{13}C	Foliar N	Foliar P	Foliar K	Foliar Ca	Foliar Mg	VWC[a]	Soil pH	Soil EC	Bulk density	PMM[b] thickness	NH_4^+-N	NO_3^--N	Inorganic N	DON[c]	DOC[d]
Pl	HG	*r*	**-0.70**[e]	-0.10	0.22	0.32	0.27	0.36	**0.80**	0.03	-0.25	-0.38	0.09	0.26	0.38	0.35	0.35	0.09
		p	**0.04**	0.81	0.57	0.41	0.48	0.34	**0.04**	0.94	0.53	0.32	0.82	0.50	0.32	0.35	0.35	0.81
	DBHG	*r*	-0.59	-0.09	0.22	0.29	0.31	0.41	**0.80**	-0.04	-0.22	-0.44	0.02	0.41	0.56	0.53	0.49	<0.01
		p	0.10	0.82	0.58	0.45	0.41	0.27	**0.05**	0.92	0.56	0.24	0.96	0.28	0.11	0.14	0.18	1.00
	ABG	*r*	**-0.67**	-0.11	0.31	0.18	0.19	0.23	**0.93**	0.03	-0.35	-0.43	-0.01	0.21	0.37	0.32	0.35	-0.05
		p	**0.04**	0.78	0.42	0.65	0.63	0.55	**0.03**	0.94	0.36	0.25	0.99	0.59	0.32	0.39	0.35	0.91
Sw	HG	*r*	-0.04	**0.81**	0.46	**0.80**	-0.16	-0.51	-0.20	-0.65	-0.61	**-0.78**	-0.03	0.64	0.55	**0.72**	0.56	-0.28
		p	0.93	**0.01**	0.21	**0.01**	0.68	0.16	0.61	0.06	0.08	**0.01**	0.94	0.07	0.12	**0.03**	0.11	0.47
	DBHG	*r*	-0.08	**0.77**	0.40	**0.72**	-0.22	-0.49	-0.13	-0.51	-0.63	**-0.68**	-0.06	0.63	0.58	**0.73**	0.48	-0.23
		p	0.85	**0.02**	0.28	**0.03**	0.56	0.18	0.74	0.10	0.07	**0.05**	0.89	0.07	0.11	**0.03**	0.19	0.56
	ABG	*r*	-0.05	**0.85**	0.54	**0.84**	-0.25	-0.49	-0.17	**-0.67**	-0.65	**-0.79**	-0.17	**0.69**	0.53	**0.75**	0.64	-0.23
		p	0.90	**<0.01**	0.13	**0.01**	0.52	0.19	0.66	**0.05**	0.06	**0.01**	0.66	**0.04**	0.15	**0.02**	0.06	0.55

[a] VWC, volumetric water content.

[b] PMM, peat-mineral soil mix.

[c] DON, dissolved organic nitrogen.

[d] DOC, dissolved organic carbon.

[e] Values in bold indicate significant correlation at $p < 0.05$.

Table 2-3 Regression equations developed based on the relationship between increments of height (HI), diameter at breast height (DBHI) and aboveground biomass (ABI) from 2011 to 2012 and soil variables through stepwise multiple regression analyses.

Site type	Growth parameter	Regression equation[a]
Pl	HI	HI = 0.025 VWC + 0.001 DOC - 0.278 ($R^2 = 0.80$, $p = 0.007$)
	ABI	ABI = 0.445 VWC + 0.014 DOC - 4.645 ($R^2 = 0.80$, $p = 0.008$)
Sw	HI	HI = - 0.511 Db + 0.112 Inor.N + 0.610 ($R^2 = 0.67$, $p = 0.037$)
	DBHI	DBHI = - 0.185 pH - 0.071 EC + 0.023 Inor.N + 1.750 ($R^2 = 0.86$, $p = 0.013$)
	ABI	ABI = - 0.453 EC - 2.007 Db + 5.690 ($R^2 = 0.79$, $p = 0.009$)

[a] VWC, volumetric water content; DOC, dissolved organic carbon; EC, electrical conductivity; Db, bulk density and Inor.N, inorganic N.

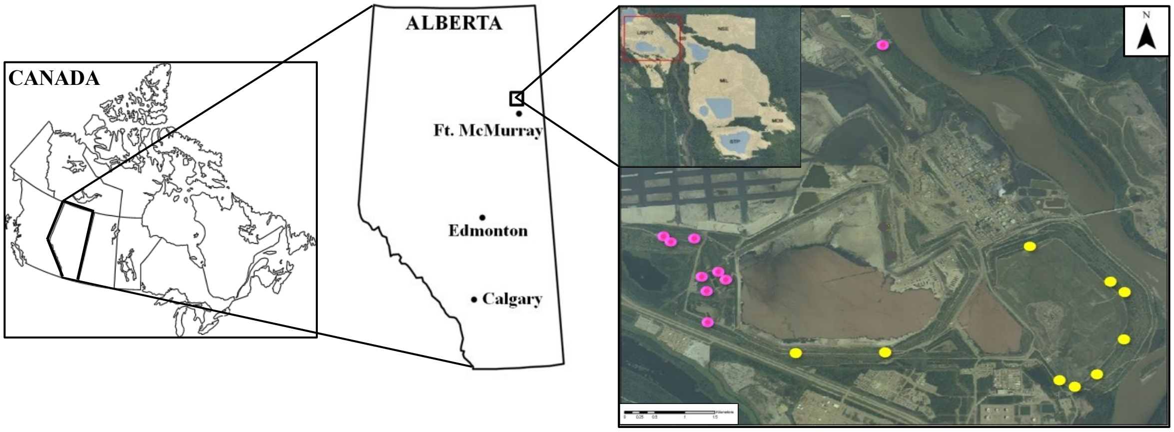

Figure 2-1 Distribution of the lodgepole pine (*Pinus contorta*, Pl) and white spruce (*Picea glauca*, Sw) study sites in Suncor Energy Inc. Lease 86/17 in the Athabasca oil sands region, Alberta, Canada. The yellow dots indicate Pl sites, and the purple dots indicate Sw sites.

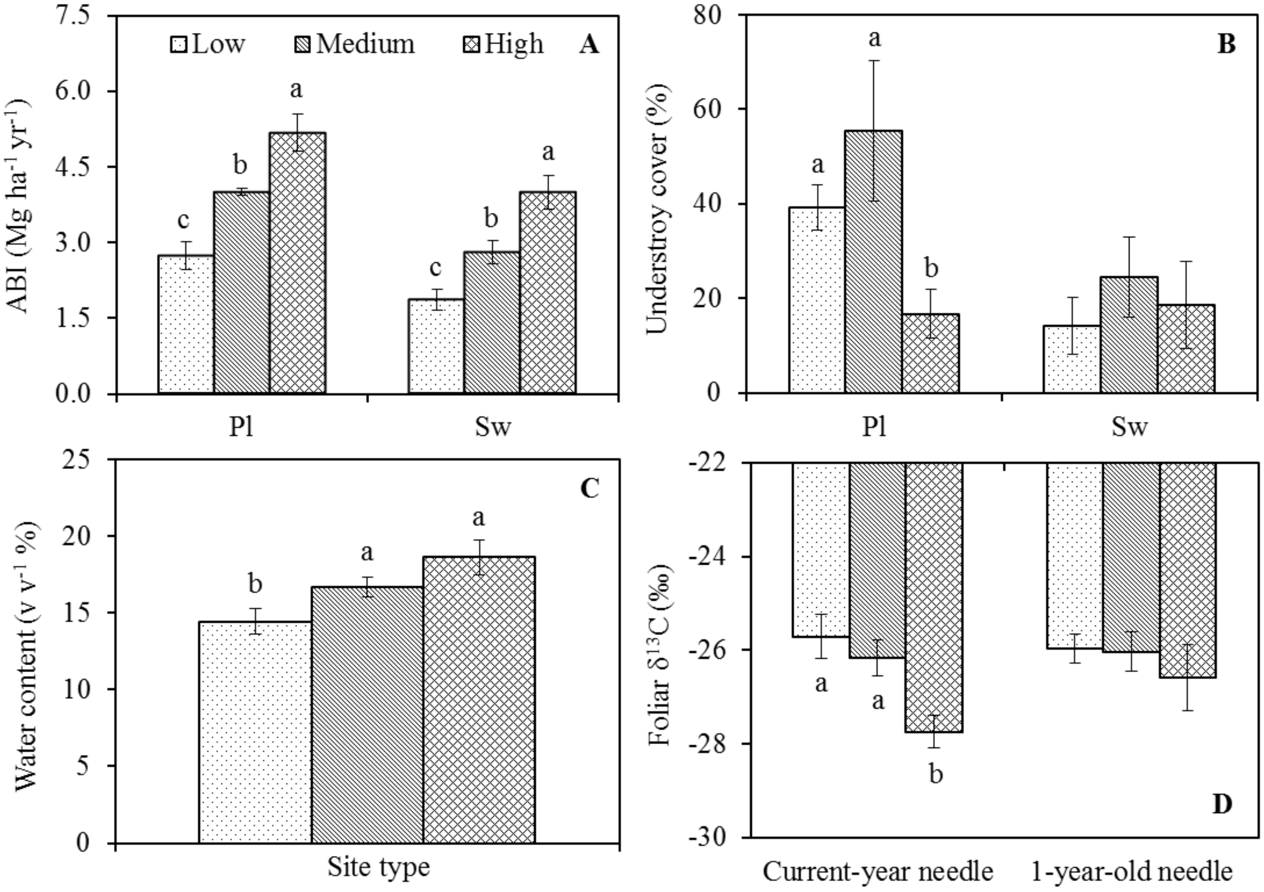

Figure 2-2 A) Increment of aboveground biomass (ABI) from 2011 to 2012 in lodgepole pine (*Pinus contorta*, Pl) and white spruce (*Picea glauca*, Sw) sites, B) total understory plant community cover in Pl and Sw sites, C) volumetric water content of peat-mineral soil mix (PMM) in Pl sites and D) foliar δ^{13}C of current-year (2012) and 1-year-old needles of Pl. Different letters represent significant differences among different productivity levels at $p < 0.05$. Vertical bars are standard errors of means (n = 3).

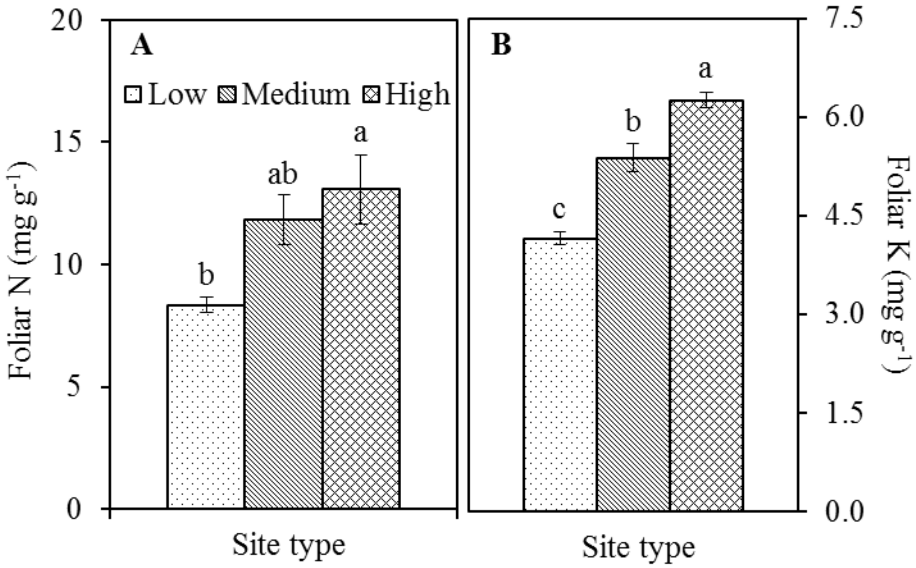

Figure 2-3 A) Foliar N and B) K concentrations of current-year needles of white spruce (*Picea glauca*, Sw). Different letters represent significant differences among different productivity levels at $p < 0.05$. Vertical bars are standard errors of means (n = 3).

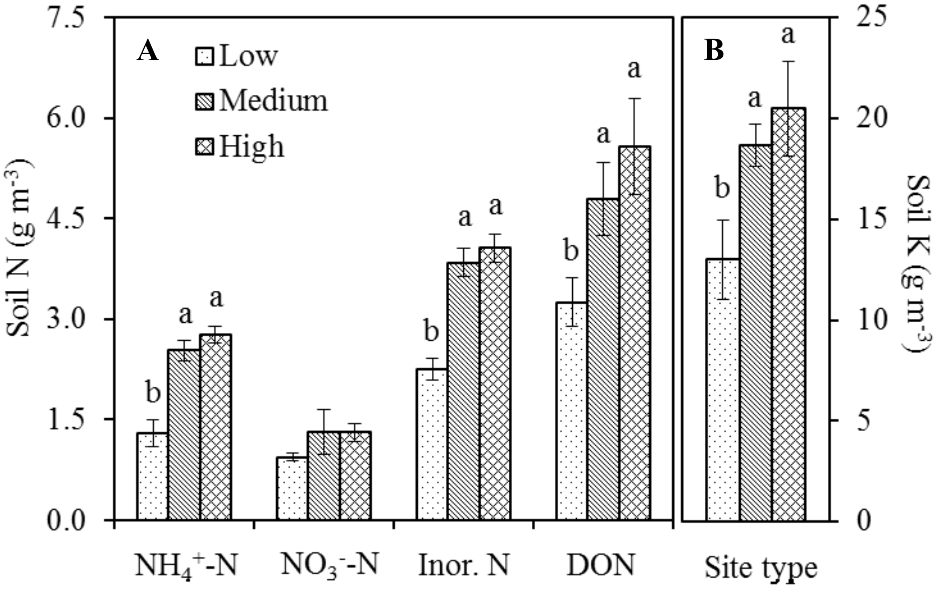

Figure 2-4 A) Soil N and B) K concentrations of peat-mineral soil mix (PMM) in white spruce (*Picea glauca*, Sw) sites. Different letters represent significant differences among different productivity levels at $p < 0.05$. Vertical bars are standard errors of means (n = 3).

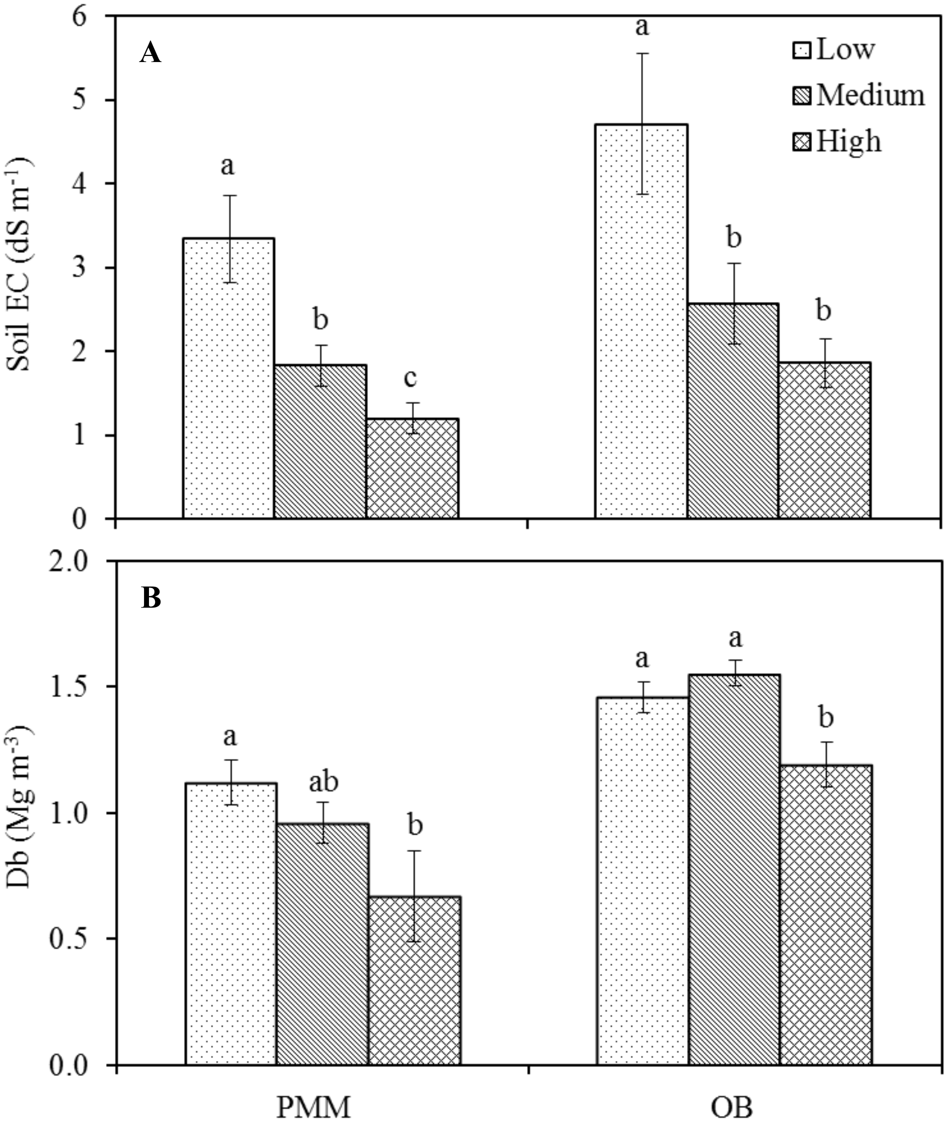

Figure 2-5 A) Soil electrical conductivity (EC) and B) bulk density (Db) of peat-mineral soil mix (PMM) and overburden (OB) in white spruce (*Picea glauca*, Sw) sites. Different letters represent significant differences among different productivity levels at $p < 0.05$. Vertical bars are standard errors of means (n = 3).

References

Alberta Energy. 2014. Facts and Statistics. http://www.energy.alberta.ca/OilSands/791.asp (Accessed on August 30, 2014).

Allen, J.A., Chambers, J.L., Stine, M. 1994. Prospects for increasing the salt tolerance of forest trees: a review. Tree Physiol. 14:843-853.

Andrews, J.A., Johnson, J.E., Torbert, J.L., Burger, J.A., Kelting, D.L. 1998. Mine soil and site properties associated with early height growth of eastern white pine. J. Environ. Qual. 27:192-199.

Ballard, T.M., Carter, R.E. 1986. Evaluating Forest Stand Nutrient Status. Ministry of Forests, Victoria, British Columbia. p. 33.

Barbour, S.L., Chanasyk, D., Hendry, J., Leskiw, L., Macyk, T., Mendoza, C., Naeth, A., Nichol, C., O'Kane, M., Purdy, B., Qualizza, C., Quideau, S., Welham, C. 2007. Soil Capping Research in the Athabasca Oil Sands Region. Volume 1: Technology Synthesis. Syncrude Canada Ltd., FortMcMurray, Alberta. p. 20-88.

Barnhisel, R.I., Hower, J.M. 1997. Coal surface mine reclamation in the eastern United States: the revegetation of disturbed lands to hay land/pasture or cropland. Adv. Agron. 61:233-275.

Bowling, D.R., McDowell, N.G., Bond, B.J., Law, B.E., Ehleringer, J.R. 2002. ^{13}C content of ecosystem respiration is linked to precipitation and vapor pressure deficit. Oecologia 131:113-124.

Campbell, C.R., Plank, C.O. 1998. Preparation of plant tissue for laboratory analysis. In: Kalra, Y.P. (Ed.), Handbook of Reference Methods for Plant Analysis. CRC Press, Boca Raton, Florida. p. 37-50.

Choi, W.J., Chang, S.X., Curran, M.P., Ro, H.M., Kamaluddin, M., Zwiazek, J.J. 2005. Foliar $\delta^{13}C$ and $\delta^{15}N$ response of lodgepole pine and Douglas-fir seedlings to soil compaction and forest floor removal. For. Sci. 51:546-555.

Corns, I.G.W. 1988. Compaction by forestry equipment and effects on coniferous seedling growth on four soils in the Alberta foothills. Can. J. For. Res. 18:75-84.

Cullen, S.J., Montagne, C., Ferguson, H. 1991. Timber harvest trafficking and soil compaction in western Montana. Soil Sci. Soc. Am. J. 55:1416-1421.

Darrah, P.R., Nye, P.H., White, R.E. 1987. The effect of high solute concentrations on nitrification rates in soil. Plant Soil 97:37-45.

Dise, N.B., Wright, R.F. 1995. Nitrogen leaching from European forests in relation to nitrogen deposition. For. Ecol. Manage. 71:153-171.

Environment Canada. 2013. Canadian Climate Normals 1971-2000 Station Data. http://climate.weather.gc.ca/climate_normals/results_e.html?stnID=2519&lang=e&dCodd=1&province=ALTA&provBut=Go&month1=0&month2=12 (Accessed on June 12, 2013).

Farnden, C., Vassov, R.J., Yarmuch, M., Larson, B.C. 2013. Soil reclamation amendments affect long term growth of jack pine following oil sands mining. New For. 44:799-810.

Fung, M.Y.P., Macyk, T.M. 2000. Reclamation of oil sands mining areas. In: Barnhisel, R.I., Darmody, R.G., Daniels, W.L. (Eds.), Reclamation of Drastically Disturbed Lands. ASA, CSSA and SSSA, Madison, Wisconsin. p. 755-774.

Gee, G.W., Or, D. 2002. Particle size analysis. In: Dane, J.H., Topp, G.C. (Eds.), Methods of Soil Analysis. Part 4: Physical Methods. SSSA, Madison, Wisconsin. p. 255-293.

Government of Alberta. 2014a. Alberta's Oil Sands: Reclamation. http://www.oilsands.alberta.ca/reclamation.html (Accessed on August 30, 2014).

Government of Alberta. 2014b. Environmental Protection and Enhancement Act. Alberta Queen's Printer, Edmonton, Alberta. p. 97.

Greacen, E.L., Sands, R. 1980. Compaction of forest soils: a review. Aust. J. Soil Res. 18:163-189.

Hawkins, B.J., Boukcim, H., Plassard, C. 2008. A comparison of ammonium, nitrate and proton net fluxes along seedling roots of douglas-fir and lodgepole pine grown and measured with different inorganic nitrogen sources. Plant Cell Environ. 31:278-287.

Hemstock, S.S., Quideau, S.A., Chanasyk, D.S. 2010. Nitrogen availability from peat amendments used in boreal oil sands reclamation. Can. J. Soil Sci. 90:165-175.

Horton, K.W. 1958. Rooting Habits of Lodgepole Pine. Forest Research Division Technical Note no. 67. Northern Affairs and National Resources, Ottawa, Ontario. p. 5-26.

House, J., Duan, M., Chang, S.X. Forest productivity related to water availability on reclaimed upland forest sites in the Athabasca oil sands region. (Under preparation)

Howat, D.R. 2000. Acceptable Salinity, Sodicity and pH Values for Boreal Forest Reclamation.

Alberta Environment, Edmonton, Alberta.

Inubushi, K., Barahona, M.A., Yamakawa, K. 1999. Effects of salts and moisture content on N_2O emission and nitrogen dynamics in yellow soil and andosol in model experiments. Biol. Fertil. Soils 29:401-407.

Jung, K., Duan, M., House, J., Chang, S.X. 2014. Textural interfaces affected the distribution of roots, water, and nutrients in some reconstructed forest soils in the Athabasca oil sands region. Ecol. Eng. 64:240-249.

Kessler, S., Barbour, S.L., van Rees, K.C.J., Dobchuk, B.S. 2010. Salinization of soil over saline-sodic overburden from the oil sands in Alberta. Can. J. Soil Sci. 90:637-647.

Khasa, P.D., Hambling, B., Kernaghan, G., Fung, M., Ngimbi, E. 2002. Genetic variability in salt tolerance of selected boreal woody seedlings. For. Ecol. Manage. 165:257-269.

Kozlowski, T.T. 1999. Soil compaction and growth of woody plants. Scand. J. For. Res. 14:596-619.

Kronzucker, H.J., Siddiqi, M.Y., Glass, A.D.M. 1997. Conifer root discrimination against soil nitrate and the ecology of forest succession. Nature 385:59-61.

Lambert, M.C., Ung, C.H., Raulier, F. 2005. Canadian national tree aboveground biomass equations. Can. J. For. Res. 35:1996-2018.

Lavoie, N., Vezina, L.P., Margolis, H.A. 1992. Absorption and assimilation of nitrate and ammonium ions by jack pine seedlings. Tree Physiol. 11:171-183.

Laxton, D.L., Watmough, S.A., Aherne, J., Straker, J. 2010. An assessment of nitrogen saturation in *Pinus banksiana* plots in the Athabasca oil sands region, Alberta. J. Limnol. 69 (Suppl. 1):171-180.

Li, X.P., Chang, S.X., Salifu, K.F. 2014. Soil texture and layering effects on water and salt dynamics in the presence of a water table: a review. Environ. Rev. 22:41-50.

Matsushima, M., Chang, S.X. 2007. Nitrogen and water availabilities and competitiveness of bluejoint: spruce growth and foliar carbon-13 and nitrogen-15 abundance. Soil Sci. Soc. Am. J. 71:1547-1554.

McClung, G., Frankenberger, W.T. 1987. Nitrogen mineralization rates in saline vs salt-amended soils. Plant Soil 104:13-21.

McFee, W.W., Byrnes, W.R., Stockton, J.G. 1981. Characteristics of coal mine overburden important to plant growth. J. Environ. Qual. 10:300-308.

McMillan, R., Quideau, S.A., MacKenzie, M.D., Biryukova, O. 2007. Nitrogen mineralization and microbial activity in oil sands reclaimed boreal forest soils. J. Environ. Qual. 36:1470-1478.

Merrill, S.D., Doering, E.J., Power, J.F. 1980. Changes of sodicity and salinity in soils reconstructed on strip-mined lands. N. D. Farm Res. 37:13-16.

Merrill, S.D., Doering, E.J., Power, J.F., Sandoval, F.M. 1983. Sodium movement in soil-mine spoil profiles: diffusion and convection. Soil Sci. 136:308-316.

Moskal, T. 1999. Moisture Characteristics of Coarse Textured Soils and Peat: Mineral Mixtures. M.Sc. thesis. Department of Renewable Resources, University of Alberta, Edmonton, Alberta.

Munns, R., Tester. M. 2008. Mechanisms of salinity tolerance. Annu. Rev. Plant Biol. 59:651-681.

Naeth, M.A., Chanasyk, D.S., Burgers, T.D. 2011. Vegetation and soil water interactions on a tailings sand storage facility in the Athabasca oil sands region of Alberta, Canada. Phys. Chem. Earth. 36:19-30.

Naeth, M.A., Wilkinson, S.R., Mackenzie, D.D., Archibald, H.A., Powter, C.B. 2013. Potential of LFH Mineral Soil Mixes for Land Reclamation in Alberta. Oil Sands Research and Information Network, Edmonton, Alberta. p. 1-64.

Nambiar, E.K.S., Sands, R. 1993. Competition for water and nutrients in forests. Can. J. For. Res. 23:1955-1968.

Nienstaedt, H., Zasada, J.C. 1990. *Picea glauca* (Moench) Voss: white spruce. In: Burns, R.M., Honkala, B.H. (Eds.), Silvics of North America. Volume 1: Conifers. USDA Forest Service, Washington, DC. p. 204-226.

Pardo, L.H., Templer, P.H., Goodale, C.L., Duke, S., Groffman, P.M., Adams, M.B., Boeckx, P., Boggs, J., Campbell, J., Colman, B., Compton, J., Emmett, B., Gundersen, P., Kjønaas, J., Lovett, G., Mack, M., Magill, A., Ollinger, S., Ross, D., Rueth, H., Rustad, L., Schaberg, P., Schiff, S., Schleppi, P., Spoelstra, J., Wessel, W. 2006. Regional assessment of N saturation using foliar and root δ^{15}N. Biogeochemistry 80:143-171.

Pathak, H., Rao, D.L.N. 1998. Carbon and nitrogen mineralization from added organic matter in saline and alkali soils. Soil Biol. Biochem. 30:695-702.

Peri, P.L., Ladd, B., Pepper, D.A., Bonser, S.P., Laffan, S.W., Amelung, W. 2012. Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope composition in plant and soil in Southern Patagonia's native forests. Global Change Biol. 18:311-321.

Reeder, J.D., Sabey, B. 1987. Nitrogen. In: Williams, R.D., Schuman, G.E. (Eds.), Reclaiming Mine Soils and Overburden in the Western United States: Analytic Parameters and Procedures. Soil Conservation Society of America, Ankeny, Iowa. p.155-184.

Renault, S., Paton, E., Nilsson, G., Zwiazek, J.J., MacKinnon, M.D. 1999. Responses of boreal plants to high salinity oil sands tailings water. J. Environ. Qual. 28:1957-1962.

Rodrigue, J.A., Burger, J.A. 2004. Forest soil productivity of mined land in the mid-western and eastern coalfield regions. Soil Sci. Soc. Am. J. 68:833-844.

Rowland, S.M., Prescott, C.E., Grayston, S.J., Quideau, S.A., Bradfield, G.E. 2009. Recreating a functioning forest soil in reclaimed oil sands in northern Alberta: an approach for measuring success in ecological restoration. J. Environ. Qual. 38:1580-1590.

Schulze, E.D., Mooney, H.A., Sala, O.E., Jobbagy, E., Buchmann, N., Bauer, G., Canadell, J., Jackson, R.B., Loreti, J., Oesterheld, M., Ehleringer, J.R. 1996. Rooting depth, water availability, and vegetation cover along an aridity gradient in Patagonia. Oecologia 108:503-511.

Shrestha, R.K., Lal, R. 2011. Changes in physical and chemical properties of soil after surface mining and reclamation. Geoderma 161:168-176.

Startsev, A.D., Mcnabb, D.H. 2001. Skidder traffic effects on water retention, pore-size distribution, and van Genuchten parameters of boreal forest soils. Soil Sci. Soc. Am. J. 65:224-231.

Sun, Z.J., Livingston, N.J., Guy, R.D., Ethier, G.J. 1996. Stable carbon isotopes as indicators of increased water use efficiency and productivity in white spruce (*Picea glauca* (Moench) Voss) seedlings. Plant Cell Environ. 19:887-894.

Tan, X., Chang, S.X., Kabzems, R. 2005. Effects of soil compaction and forest floor removal on soil microbial properties and N transformations in a boreal forest long-term soil productivity study. For. Ecol. Manage. 217:158-170.

Ung, C.H., Bernier, P., Guo, X.J. 2008. Canadian national biomass equations: new parameter estimates that include British Columbia data. Can. J. For. Res. 38:1123-1132.

Ussiri, D.A.N., Lal, R. 2005. Carbon sequestration in reclaimed mine soils. Crit. Rev. Plant Sci. 24:151-165.

Watmough, S.A., Aherne, J., Alewell, C., Arp, P., Bailey, S., Clair, T., Dillon, P., Duschesne, L., Eimers, C., Fernandez, I., Foster, N., Larssen, T., Miller, E., Mitchell M., Page, S. 2005. Sulphate, nitrogen and base cation budgets at 21 forested catchments in Canada, the United States and Europe. Environ. Monit. Assess. 109:1-36.

Zhang, J.W., Feng, Z., Cregg, B.M., Schumann, C.M. 1997. Carbon isotopic composition, gas exchange, and growth of three populations of ponderosa pine differing in drought tolerance. Tree Physiol. 17:461-466.

Chapter 3 Responses of lodgepole pine (*Pinus contorta*) and white spruce (*Picea glauca*) to fertilization in reconstructed boreal forest soils in the oil sands region

1 Introduction

Reconstructed soils after disturbance by surface mining in the oil sands region usually possess some unfavorable properties such as increased soil pH (Howat, 2000), high salinity (Barbour et al., 2007), extensive soil compaction, high concentrations of residual bitumen, and low availability of nutrients, nitrogen (N) in particular (Fung and Macyk, 2000). Those factors can negatively affect the early growth of trees planted during the land reclamation process. Understanding what factor or factors affect the reestablishment of plant communities and designing strategies to deal with factors limiting plant growth will help to ensure the success of reclamation to upland forest in the oil sands region (Macdonald et al., 2012).

Among those potential limiting factors, N availability may be the most limiting one for tree growth. Organic amendments including peat-mineral soil mix (PMM) or forest floor-mineral soil mix (FMM) are common cover soils used in oil sands reclamation. These are incorporated into reconstructed capping layer over either overburden (OB) or tailings sand (TS) (by-products of oil sands mining) substrates to improve nutrient availability. As the decomposition rate of organic matter is slow due to small microbial population sizes and reduced microbial activities in the reclaimed soil, it may restrain the supply of N to plants (McMillan et al., 2007; Hemstock et al., 2010). To overcome low N availability, planted seedlings are usually fertilized in the initial 5 years after planting (Fung and Macyk, 2000). As the planted stands develop, the demand for nutrients may increase; therefore, the need for fertilization in older stands should be continually assessed. For example, planted trees in some reconstructed soils in the Athabasca oil sands region (AOSR) showed yellowish foliar color, short needles and stunted growth (personal observation), which indicate problems that need to be dealt with to insure the success of reclamation.

Fertilization is an important management practice to supply nutrients for plant and enhance tree growth and stand productivity (Yang, 1985a; 1985b; 1998), but effects of fertilization on tree growth vary, which depend on nutrient demands of different tree species, nutrient reserves in soils, fertilizer types and fertilizer application rates and methods (Thomas and Mead, 1992a; Preston and Mead, 1994; Munson et al., 1995; Kishchuk et al., 2002; Khasa et al., 2005; Santiago et al., 2012; Sloan and Jacobs, 2013). Therefore, the effect of fertilization on tree growth may be positive, negative, or non-significant (Sutton, 1992; Yang, 1998; Kishchuk et al., 2002; Bennett et al., 2003; Brockley, 2007). Sutton (1992) reported that N fertilization at 56, 112, or 168 kg N ha^{-1} did not increase white spruce (*Picea glauca*, Sw) growth in growth-limited boreal plantations. Nitrogen fertilization at the rate of 360 kg N ha^{-1} significantly increased tree height, diameter at breast height (DBH, 1.3 m above the ground) and total stem volume by 19, 34 and 28%, respectively, compared to the no fertilization treatment in lodgepole pine (*Pinus contorta*, Pl) stands (Yang, 1998). However, N fertilizer applications at 540 kg N ha^{-1} resulted in decreased height growth in a 40-year-old Pl stand (Yang, 1998). Improved growth in response to fertilization may be attributed to increased shoot length, leaf area index (Albaugh et al., 2004; Amponsah et al., 2005), photosynthetic rate (Chandler and Dale, 1995; Murthy et al., 1996), and shifts in carbon (C) allocation between roots to shoots (Haynes and Gower, 1995).

Nitrogen fertilization not only affects tree growth but also influences soil N dynamics. Repeated fertilization could affect the size of the microbial community that mediates ammonia oxidation, nitrate reduction and denitrification in soils, thus affecting N cycling (Hallin et al., 2009). Nemergut et al. (2008) reported that the activities of leucine amino peptidase and urease, two important enzymes directly involved in N transformations in soils, significantly decreased and increased, respectively, in response to N fertilization, and demonstrated that high levels of N fertilization could markedly change soil N availability and the chemical composition of soil organic matter pools. Such changes would be reflected in foliar chemistry, such as nutrient concentrations and foliar ^{15}N isotope abundance. The ^{15}N natural isotope abundance (δ^{15}N) of foliage integrates isotopic composition of external N and ^{15}N/^{14}N fractionations during N uptake, assimilation and retranslocation within plants (Högberg, 1997), and therefore can provide insights into effects of management practices in forests, such as forest floor removal, irrigation and fertilization, on plant and soil N dynamics (Choi et al., 2003; 2005a; 2005b). For example, application of ^{15}N-enriched compost (organic fertilizer) significantly increased δ^{15}N in plants and soil NO_3^--N pool compared to inorganic fertilizer (Choi et al., 2003).

Many studies have been conducted to test the effect of fertilization on seedling establishment and growth in oil sands reclamation (Khasa et al., 2005; Pinno et al., 2012; Sloan and Jacobs, 2013). However, no results have been reported on growth response of established trees to fertilization in reconstructed soils in the oil sands region. Fertilization effects in reclaimed sites may be different from those in natural forest sites because of dramatic disturbance and changes in soil physical, chemical, and biological properties during soil reconstruction.

A two-year field study conducted in the reclaimed sites in the AOSR has identified that N availability was limiting Sw growth on reconstructed sites using the PMM as a cover soil over the OB substrate, while the growth of Pl in sites reconstructed using the PMM over the TS substrate was mainly affected by water availability (Duan et al., 2015). The above results from a retrospective study need to be experimentally tested to confirm what factors limit tree growth in reclaimed sites. The objective of this study was to test the growth responses of Pl and Sw to fertilization in reconstructed boreal forest soils in the AOSR in Alberta, Canada. Since testing tree species differences was not the objective of the study and both species belong to conifers; they should have very similar biological properties, the different responses to fertilization may reflect different prescriptions used in soil reconstruction. Therefore I hypothesized that 1) fertilization would increase nutrient uptake, and thus improve tree growth, and 2) growth responses of Pl and Sw would differ due to different substrates used in soil reconstruction.

2 Materials and methods

2.1 Site description

A detailed description of the study area can be found in Duan et al. (2015). Briefly, the research sites are located within the Suncor Energy Inc. Lease 86/17, which is 22 km north of Fort McMurray in northeastern Alberta, Canada. The mean annual temperature from 1971 to 2000 was 0.7 °C, and the mean annual precipitation was 455.7 mm, with an average of 342.2 mm occurring as rainfall during the growing season (Environment Canada, 2013). The research sites were reclaimed by placing PMM on underlying TS or OB substrates following oil sands mining. Lodgepole pine seedlings were planted on soils with TS as a substrate and Sw seedlings on soils with OB material as a substrate two years after soil reconstruction. Trees in the Pl and Sw sites in this study ranged from 18 to 23 years old. Understory plant communities on Pl sites were dominated by prickly rose (*Rosa acicularis*), raspberry (*Rubus idaeus*), sweet clover (*Melilotus* sp.), dandelion (*Taraxacum officinale*), and slender wheat grass (*Agropyron trachycaulum*), while willow (*Salix* sp.), green alder (*Alnus crispa*), sweet clover, dandelion, and bluejoint grass (*Calamagrostis canadensis*) were the dominant understory species on Sw sites.

2.2 Experimental design

The experiment used a randomized complete block design for each of the two species studied. Three Pl sites and three Sw sites with poor tree growth were selected (Table 3-1) and fifteen trees with similar height and DBH growth (based on observations and measurements of the trees) were randomly chosen in each site and tagged to receive the treatments. The selected trees were at least 10 meters apart to minimize interference between treatments. Nitrogen (Nitrogen, 200 kg N ha^{-1}, applied as urea) or complete fertilizer (Complete, 15-30-15 ($N:P_2O_5:K_2O$) plus micronutrients, at a rate of 200 kg N, 20 kg P and 19 kg K per hectare, urea was used to provide the additional N) was applied to five randomly selected trees in each site in June 2012. No fertilizer was applied to the remaining five trees (Control). Fertilizers were broadcast over an area within a radius of two meters around the tree. The three sites selected for this study within each species represented three replications.

2.3 Tree growth measurements

Tree growth was measured before and two growing seasons after the fertilizer application. Tree height and DBH were measured using a height pole and a diameter tape, respectively. Aboveground biomass of trees was calculated with DBH and height-based allometric equations (Lambert et al., 2005; Ung et al., 2008). Annual increments of height, DBH and aboveground biomass were calculated based on the differences of height, DBH and aboveground biomass of trees between June 2012 and October 2013.

2.4 Soil and plant samplings

Soil samples in the capping layer were collected before fertilization in June 2012 from the top 20 cm using an auger in five randomly selected locations in each site (three sites per species) to obtain basic soil properties. Soil samples were collected again in October 2013 from the top 20 cm in three randomly selected locations two meters around the stem of each tree and mixed. If the thickness of the capping layer was less than 20 cm, the entire capping layer was collected. All soil samples were placed in plastic bags immediately after sampling, stored in a cooler and taken back to the laboratory for analyses. Before analyses, soil samples from the same treatments in each site were completely mixed again as a replicate for a treatment. Three branches from the upper one third of the live crown of each tree were collected in October of 2012 and 2013. Branch samples from the five trees in each treatment in each site formed a composite branch sample, and were placed in paper bags and taken back to the laboratory.

2.5 Soil and plant analyses

Fresh soil samples were passed through a 2 mm sieve and homogenized to determine water content, pH, electrical conductivity (EC) and nutrient concentrations, including NH_4^+-N, NO_3^--N, dissolved organic N (DON), extractable P, K, Ca, Mg and other micronutrients. The detailed descriptions of the analyses are described below.

The water content of each soil sample was measured by weighing a portion of the sample before and after oven-drying at 105 °C for 24 h. Soil pH was measured in deionized water using an Orion 3-Star portable pH meter (Thermo Fisher Scientific Inc., Waltham, MA) with a soil to water ratio of 1:2 (w:v). Soil EC was measured by an AP75 portable waterproof conductivity/TDS meter (Thermo Fisher Scientific Inc., Waltham, MA) using fresh soil (equivalent to 30 g oven-dried soil) in 60 mL deionized water after shaking for 1 h at 25 °C and filtration through Whatman No. 42 filter papers.

Soil samples were extracted with 0.5 mol L^{-1} KCl at 1:3 soil to solution ratio (w:v). After being shaken at 250 rpm on a mechanical shaker for 1 h, samples were filtered through Whatman No. 42 filter papers. Concentrations of NH_4^+-N and NO_3^--N in the extracts were analyzed using the steam distillation method. The KCl extracts were steam-distilled with MgO to determine NH_4^+-N concentrations using a Kjeldahl distillation system (Vapodest 20, C. Gerhardt, Königswinter, Germany). The extracts were distilled again after addition of Devarda's alloy to determine NO_3^--N concentrations. A portion of each KCl extract was used for the analysis of total dissolved N concentration with a TOC-Vcsn analyzer with TNM-1 for N analysis (Shimadzu Corp., Kyoto, Japan). The DON concentration was calculated by subtracting NH_4^+-N and NO_3^--N concentrations from the total dissolved N concentration. The distillate was acidified to pH 3 using 0.1 mol L^{-1} H_2SO_4, and oven-dried at 65 °C to powder. The ^{15}N isotope abundance in the dried distillate was determined with an elemental analyzer (EuroEA3028-HT, EuroVector SpA., Tortona, Italy) coupled to a continuous-flow isotope ratio mass spectrometer (IRMS) (IsoPrime, GV Instruments Ltd., Manchester, UK). The ^{15}N isotope abundance was expressed as the deviation from the international standard of atmospheric N_2. Each oven-dried soil sample was ground with a ball mill and used to analyze total C and N concentrations with an elemental analyzer (CE440, Exeter Analytical Inc., North Chelmsford, MA). Concentrations of water dissolved nutrients, including P, K, Ca, Mg and other micronutrients, were analyzed using a Perkin Elmer Optima 3000 DV inductively coupled plasma mass spectrometer (ICP-MS) (PerkinElmer Inc., Shelton, CT) after extraction with deionized water at 1:2 of soil to water ratio (w:v) and filtration.

Branches from each treatment were separated into current-year and one-year-old shoots. After being washed twice using distilled water, shoots were oven-dried at 65 °C until constant weight and needles were removed from the shoots and separated into current-year and one-year-old needles. Three hundred needles were randomly selected from each sample and weighed. The needles were ground to pass through a 0.15 mm sieve for nutrient analyses. Concentrations of total C and N, and ^{15}N isotope abundance were analyzed as described above. Other total macro- (P, K, Ca and Mg) and micronutrients (Fe, Mn, Cu, Zn, B, and Mo) were analyzed with the ICP-MS as described above after digestion using concentrated HNO_3 and 30% H_2O_2 on a digestion block at 125 °C for 4 h (Campbell and Plank, 1998). All results are reported on an oven-dry basis.

2.6 Statistical analyses

A one-way analysis of variance (ANOVA) and Tukey's multiple comparison were applied to examine the statistical significance of responses of tree growth to fertilization, and to evaluate effects of fertilization on foliar and soil nutrient concentrations at an α value of 0.05, for each species. Before performing the ANOVA analysis, the normality of distribution and homogeneity of variance were tested. All statistical analyses were conducted with the SAS software (SAS 9.2, SAS Institute Inc., Cary, NC).

3 Results

3.1 Tree growth responses to fertilization

Increments of height, DBH and aboveground biomass of Pl were 9.2 cm yr^{-1}, 0.5 cm yr^{-1} and 2.13 kg tree^{-1} yr^{-1}, respectively, in the Control, and there were no differences among the treatments (Figure 3-1 A, B and C). For Sw, increments of height, DBH and aboveground biomass were greater in the fertilization than in the Control treatment (p = 0.005, 0.009 and 0.016, respectively), but there were no differences between the two fertilization treatments in height and DBH increments. The Complete treatment had the larger increments of aboveground biomass (1.36 kg tree^{-1} yr^{-1}) compared to the Control (Figure 3-1 C).

Two growing seasons after fertilization, the length of current-year or 1-year-old shoots was not affected by the fertilization in Pl (Table 3-2). However, in Sw sites the length of current-year shoots in the fertilization treatments was higher than in the Control treatment (*p* = 0.004), but no treatment effect was found for the 1-year-old shoots. The mass of 1-year-old needles was higher in the Nitrogen and Complete than in the Control treatment in Pl sites (*p* = 0.035), but there were no differences in current-year needle mass among the three treatments. In Sw sites, the masses of current-year and 1-year-old needles were both higher in the fertilization than in the Control treatment (*p* = 0.035 and 0.001, respectively), indicating that fertilization enhanced foliage production.

3.2 Foliar nutrient concentrations and contents

There were no differences in N concentrations in the current-year and 1-year-old needles among the three treatments for Pl in 2012, but foliar N concentration of 1-year-old needles was higher in the fertilization than in the Control treatment in 2013 (*p* = 0.047) (Figure 3-2). No differences of foliar N contents were found in either current-year or 1-year-old needles in both 2012 and 2013 (Table 3-3). In Sw sites, foliar N concentrations in current-year and 1-year-old needles in the fertilization treatments were higher than in the Control in 2012 (*p* < 0.001 for both), but a significant difference was only found in 1-year-old needles in 2013 (*p* = 0.013). Foliar N contents in current-year needles were higher in the fertilization treatments compared to the Control in 2012 (*p* < 0.001), but not in 2013. However, foliar N contents in 1-year-old needles were higher in the fertilization treatments than in the Control in both 2012 (*p* < 0.001) and 2013 ($p = 0.002$), indicating a short-lived effect of fertilization on Sw foliage growth.

In Pl sites, there were no differences in foliar P, Ca and Mg contents in current-year and 1-year-old needles among the treatments in 2012 except that foliar K in current-year needles was higher in the fertilization treatments than in the Control ($p = 0.007$) (Table 3-3). Foliar Ca contents in both current-year and 1-year-old needles were higher in the Complete than in the other two treatments ($p = 0.021$ and 0.048, respectively). No differences were found for other nutrient contents in 2013. In Sw sites, fertilization did not change foliar P contents in both 2012 and 2013. Foliar K contents in current-year and 1-year-old needles significantly increased after fertilization in both 2012 ($p < 0.001$ for both) and 2013 ($p = 0.032$ and 0.004, respectively). Contents of foliar Ca and Mg were higher in the Control and Nitrogen than in the Complete treatment for current-year needles ($p = 0.017$ and 0.050, respectively).

3.3 Soil nutrient concentrations

Two growing seasons after fertilization, concentrations of NH_4^+-N, NO_3^--N and DON were not significantly different among the treatments in both Pl and Sw sites except for the concentration of DON in Pl sites, which was significantly higher in the fertilization treatments than in the Control ($p = 0.009$) (Figure 3-3). Soil K concentration was higher in the Complete than in the other two treatments in both Pl and Sw sites ($p = 0.011$ and < 0.001, respectively). No differences in soil K concentration were found between the Control and Nitrogen treatments in Pl sites, but soil K concentration was higher in the Nitrogen than in the Control treatment in Sw sites. Soil P, Ca, Mg and micronutrient concentrations were not affected by fertilization (data not shown).

3.4 Foliar and soil δ^{15}N

In Pl sites, foliar δ^{15}N in current-year and 1-year-old needles was not significantly different among the three treatments in 2012, but foliar δ^{15}N in 1-year-old needles in fertilization treatments was higher than in the Control in 2013 ($p = 0.038$) (Figure 3-4). In Sw sites, fertilization increased foliar δ^{15}N in current-year and 1-year-old needles as compared to the Control treatment in both 2012 ($p < 0.001$ and $p = 0.014$, respectively) and 2013 ($p < 0.001$ for both), corresponding to the increase of δ^{15}N in soil NO_3^--N ($p = 0.005$) (Figure 3-5 A). However, δ^{15}N in soil NH_4^+-N was higher in the Control than in the fertilization treatments in both Pl and Sw sites ($p = 0.001$ and 0.012, respectively) (Figure 3-5 B).

4 Discussion

Reconstruction of multi-layered soils using different substrates resulted in different distributions of soil nutrients and water, thus leading to varied root production and tree growth (Jung et al., 2014). For example, TS as a substrate with low water holding capacity and availability likely limited tree fine root production, while highly compacted OB as a substrate with less macro porosity potentially restricted root penetration into deeper soil layers (Jung et al., 2014). A previous study in the same area identified that N availability was affecting Sw growth in reclaimed sites using OB as a substrate, while Pl growth in reclaimed sites using TS as a substrate was mainly limited by low water availability (Duan et al., 2015). The improved Sw growth, including increased increments of height, DBH, and aboveground biomass two growing seasons after fertilization, accompanied with increased needle mass, foliar N concentrations in both current-year and 1-year-old needles, partially supported previous findings. This also partially supported the first hypothesis that fertilization would improve tree growth on reconstructed soils. Fertilization effects on tree growth could be attributed to increased growth of needle length and surface area and enhanced photosynthetic rate (Chandler and Dale, 1995; Murthy et al., 1996; Albaugh et al., 2004; Amponsah et al., 2005). The same growth responses of Sw to N and complete fertilizer applications in both 2012 and 2013 in this study suggested that N availability was the dominant growth limiting factor and other nutrients contained in the complete fertilizer did not significantly enhance tree growth in the study sites. However, a study conducted in a young Pl stand in British Columbia found that two applications of complete fertilizers within 12 years significantly increased basal area and volume increments compared to application of only N and boron (Brockley, 2007). Enhanced tree growth could be attributed to improved nutrient balance in the soil resulting from application of multiple nutrients.

Lodgepole pine is often reported to be able to grow on infertile soils (Lotan and Critchfield, 1990). Previous studies showed that foliar N concentration for optimal Sw growth was between 15 and 25 mg g^{-1} (Nienstaedt and Zasada, 1990), while foliar N concentration in the Control treatment in this study was only around 10 mg g^{-1} in both 2012 and 2013, indicating severe N deficiency in the reclaimed soil. However, both N and complete fertilizer applications significantly increased foliar N concentrations above the critical value of N deficiency. The low foliar N concentration of Sw may be attributed to low N availability in the reconstructed soil in Sw sites and high N demand by Sw trees. The relatively high salinity in the PMM after reclamation could further influence N cycling in Sw sites (Table 3-1). Previous studies showed that nitrification and N mineralization rates were partly or completely inhibited in soils with high salinity, leading to reduced N availability (Pathak and Rao, 1998; Inubushi et al., 1999; Cheng et al., 2013).

The lack of differences in N concentrations in current-year needles and soils in Sw sites two years after fertilization partially indicated the short-lived nature of the fertilization effect. Yarie et al. (1995) reported that significant growth responses to fertilization only lasted for two years in a Sw stand in Interior Alaska. Yang (1998) also showed that no differences in needle length and mass were detected two years after N fertilization in a semi-mature Pl stand in Alberta. However, Bennett et al. (2003) found that fertilization increased tree growth and nutrient supply in salal-dominated cedar hemlock stands even 10 to 13 years after fertilization on Vancouver Island. Variation in the durations of fertilization effects depended on different abilities of plant to take up increased nutrient, original nutrient reserves in soils, and fertilizer loss following application, such as nitrate leaching or denitrification (DiTommaso and Aarssen, 1989; Thomas and Mead, 1992b; Preston and Mead, 1994).

Lodgepole pine is commonly associated with a large range of habitats, and has remarkable edaphic and climatic tolerances (Wheeler and Critchfield, 1985). In contrast to results of most fertilization studies in Pl stands showing that fertilizer applications could improve tree growth (Preston and Mead, 1994; Kishchuk et al., 2002; Brockley, 2007), the lack of growth response to either N or complete fertilizer application in Pl sites in this study suggested that other factors such as water rather than nutrient availability may be limiting tree growth in reconstructed boreal forest soils with PMM as a cover soil over a TS substrate. The lack of growth response may also be due to N leaching loss (Thomas and Mead, 1992b; Preston and Mead, 1994), which would decrease N uptake by trees. Splitting the application of N fertilizer has been shown to increase fertilizer N retention by more than 15% over a one-time application (Thomas and Mead, 1992b). However, such effects depend on soil texture. The coarse-textured TS in Pl sites in this study could greatly increase the risk of N leaching regardless of fertilizer application methods.

Coarse-textured TS is generally a poor plant growth medium because of its low water and nutrient holding capacity, which would often cause trees to be under water stress, especially in dry years. In the AOSR, oil companies use different capping depths depending on the substrates placed (Rowland et al., 2009) because there is no standard prescription for soil reconstruction. Previous studies reported that placing thick cover soil layers with fine materials over coarse-textured TS layers could increase water holding capacity due to high organic matter content of peat material as a cover soil and decreased infiltration induced by the capillary barrier between layers (Moskal, 1999; Barbour et al., 2007; Li et al., 2014). However, the thickness of the capping layer ranged from 13 to 21 cm in the Pl sites studied, suggesting limited capacity to increase water availability in the soil.

The lack of differences in soil N concentrations before and two growing seasons after fertilization in both Pl and Sw sites were probably due to N leaching loss and plant uptake, respectively. Although Pl and Sw sites were both capped with PMM, concentrations of total C, total N and inorganic N were slightly greater in Pl than in Sw sites before fertilization, which could result in lower foliar N concentrations of needles in Sw than in Pl without fertilization. The foliar N concentration in Sw needles was also lower that the critical value of 13 mg g^{-1} that indicates N deficiency for Sw (Ballard and Carter, 1986). In contract, foliar N concentration in Pl needles was higher than the critical value of 12 mg g^{-1} (Ballard and Carter, 1986) even in the Control treatment, indicating no N deficiency in Pl sites.

Application of N fertilizer changed N cycling and availability in soils, which induced the changes of $\delta^{15}N$ in foliage and soils. The different patterns of $\delta^{15}N$ in soil NH_4^+-N and NO_3^--N after fertilization reflected $^{15}N/^{14}N$ fractionations during N mineralization, nitrification, NO_3^--N leaching and plant uptake. Previous studies showed that processes leading to loss of N would discriminate against the heavier ^{15}N and result in greater $\delta^{15}N$ in soils (Högberg et al., 1997). Changes of $\delta^{15}N$ in soils were also closely related to the enrichment of ^{15}N in the fertilizers applied. For example, ^{15}N-enriched compost significantly increased $\delta^{15}N$ of soil total N and

NO_3^--N, but had no effect on that of NH_4^+-N (Choi et al., 2003). However, in this study, the $\delta^{15}N$ of N and complete fertilizers was 1.0 ± 0.7‰ (n = 5) and 1.5 ± 0.9‰ (n = 5), respectively; the applications of ^{15}N-depleted fertilizers decreased $\delta^{15}N$ in soil NH_4^+-N in both sites and increased $\delta^{15}N$ in soil NO_3^--N only in Sw site, which may result from different dominant NO_3^--N consumption processes in the reclaimed soils reconstructed using different substrates. The inconsistent response of soil $\delta^{15}N$ to fertilization in the two studies was probably because different fertilizers used (organic fertilizer and inorganic fertilizer) had influences on different N cycling processes. On the other hand, foliar $\delta^{15}N$ increased after fertilization in Sw sites in this study, which was consistent with previous studies reporting that trees with high loads of N have a greater foliar $\delta^{15}N$, while trees with low loads of N developed a smaller foliar $\delta^{15}N$ (Högberg et al., 1992; Matsushima et al., 2012). Although conifers could take up both NH_4^+-N and NO_3^--N from soils, the same patterns of $\delta^{15}N$ in foliage and soil NO_3^--N in both sites indicated N uptake preference of NO_3^--N over NH_4^+-N for Pl and Sw in this study. However, Choi et al. (2005b) reported that foliar $\delta^{15}N$ was positively correlated with the $\delta^{15}N$ in soil NH_4^+-N, and indicated that tree uptake of NH_4^+-N affected foliar $\delta^{15}N$. The differences could be attributed to N uptake preference of NO_3^--N and NH_4^+-N in different tree species.

5 Conclusions

We conclude that Sw and Pl had different growth responses to fertilization because of the species grown on sites with different land reclamation prescriptions used. Applications of N and complete fertilizers both improved the growth of Sw, but not that of Pl; therefore, N availability was one of the main limiting factors for Sw growth in the reconstructed soil with PMM as a cover soil over overburden and other factors instead of N availability influenced Pl growth in soils with PMM over tailings sand substrate. The short-lived fertilization effects on Sw growth suggested the limitations of fertilizer applications for improving tree growth in the reclaimed soils. In the future, studies on the optimum thickness of a cover soil layer above TS or OB substrates should be conducted to improve nutrient availability for tree growth and speed up nutrient cycling within reclaimed sites. Studies testing techniques for correcting growth-limiting factors for Pl planted on soils with tailings sand as a substrate should be carried out to ensure the long-term success of oil sands reclamation.

Table 3-1 Characteristics of lodgepole pine (Pl) and white spruce (Sw) sites selected for this study in the Athabasca oil sands region, Alberta, Canada.

Site	Location		Year[a]	Density	Tree size		Soil properties before fertilization					
no.	Latitude	Longitude		(stem ha^{-1})	Height (m)	DBH[b] (cm)	Total C (g kg^{-1})	Total N (g kg^{-1})	NH_4^+-N (mg kg^{-1})	NO_3^--N (mg kg^{-1})	pH	EC[b] (dS m^{-1})
Pl-1	56°59′02″	111°27′04″	1996	1900	2.97±0.13[c]	4.1±0.3	67.1±7.1	2.7±0.2	2.3±0.2	3.3±0.3	7.8±0.02	1.3±0.1
Pl-2	56°58′38″	111°27′39″	1991	2300	3.32±0.11	5.2±0.4	64.9±3.9	2.1±0.4	1.7±0.2	1.3±0.1	7.8±0.02	1.1±0.2
Pl-9	56°59′25″	111°27′04″	1996	2600	3.17±0.12	4.4±0.3	51.1±5.9	1.7±0.2	1.3±0.1	1.2±0.1	7.8±0.04	1.1±0.1
Sw-11	56°59′24″	111°32′09″	1991	2300	3.59±0.14	4.4±0.2	44.9±4.3	1.7±0.2	1.5±0.4	0.8±0.1	8.1±0.06	2.5±0.2
Sw-12	56°59′51″	111°32′40″	1991	3100	3.39±0.09	4.1±0.2	48.1±6.8	1.6±0.1	1.5±0.3	1.0±0.2	7.7±0.03	4.3±0.3
Sw-16	56°59′31″	111°32′13″	1991	2600	3.47±0.06	4.2±0.1	56.0±2.5	1.8±0.3	0.9±0.1	1.0±0.1	7.8±0.02	3.2±0.1

[a] Year is when Pl and Sw trees were planted after soil reconstruction.

[b] DBH stands for diameter at breast height and EC stands for electrical conductivity.

[c] Mean ± standard error of the mean (n = 15 for tree size and n = 5 for soil properties).

Table 3-2 Branch length and needle mass of lodgepole pine (Pl) and white spruce (Sw) trees in the three treatments two growing seasons after fertilization.

Tree species	Treatment	Branch length (cm)		Needle mass (g 100 needles^{-1})	
		Current-year	1-year-old	Current-year	1-year-old
Pl	Control	12.1±1.8[a]	7.5±0.3	2.04±0.03	1.91±0.044 b[c]
	Nitrogen	13.2±1.4	7.5±0.5	2.16±0.02	2.07±0.022 a
	Complete[b]	15.0±1.8	7.5±0.6	2.09±0.04	2.01±0.023 ab
Sw	Control	4.0±0.2 b	4.3±0.3	0.24±0.01 b	0.26±0.005 b
	Nitrogen	8.0±0.5 a	4.8±0.6	0.27±0.01 a	0.29±0.002 a
	Complete	9.1±1.1 a	5.7±0.2	0.25±0.01 ab	0.29±0.003 a

[a] Mean ± standard error of the mean (n = 3).

[b] Complete stands for complete fertilizer treatment.

[c] Different letters after numbers represent significant differences among different fertilization treatments within each tree species at $p < 0.05$ (n = 3).

Table 3-3 Nutrient contents of current-year and 1-year-old needles of lodgepole pine (Pl) and white spruce (Sw) sampled in both 2012 and 2013 in different fertilization treatments.

	Year	Treatment	Foliar N[a]		Foliar P		Foliar K		Foliar Ca		Foliar Mg	
			Current-year	1-year-old	Current-year	1-year-old	Current-year	1-year-old	Current-year	1-year-old	Current-year	1-year-old
Pl	2012	Control	30.34±0.90[b]	28.75±1.32	2.52±0.05	2.18±0.06	9.04±0.07 b[d]	8.36±0.14	7.27±0.19	10.00±0.62	3.25±0.15	2.94±0.03
		Nitrogen	33.73±0.24	31.65±1.27	2.60±0.09	2.26±0.13	9.52±0.10 ab	8.90±0.13	7.29±0.38	9.49±0.19	3.64±0.08	3.24±0.15
		Complete[c]	31.87±1.57	30.12±1.07	2.57±0.09	2.25±0.12	10.03±0.21 a	8.11±0.24	7.56±0.29	10.81±0.40	3.37±0.12	3.25±0.01
	2013	Control	32.17±1.10	28.32±0.37	2.63±0.04	2.15±0.01	11.63±0.38	8.62±0.06	8.25±0.22 b	11.81±0.50 b	3.45±0.12 b	3.35±0.06
		Nitrogen	34.84±1.73	32.11±1.13	2.71±0.18	2.28±0.17	11.61±0.11	8.97±0.28	8.22±0.34 b	11.53±0.53 b	3.77±0.04 ab	3.62±0.05
		Complete	32.51±0.51	32.05±1.09	2.81±0.08	2.27±0.05	11.46±0.10	8.48±0.17	9.10±0.28 a	13.16±0.36 a	4.02±0.10 a	3.78±0.15
Sw	2012	Control	2.18±0.11 c	2.21±0.09 b	0.28±0.01	0.25±0.01	1.10±0.05 b	1.11±0.03 b	2.88±0.03 a	4.05±0.10	0.33±0.01	0.33±0.01
		Nitrogen	3.86±0.04 a	3.47±0.02 a	0.32±0.01	0.29±0.01	1.44±0.01 a	1.37±0.02 a	2.61±0.05 b	4.37±0.16	0.36±0.01	0.37±0.01
		Complete	3.43±0.04 b	3.41±0.04 a	0.30±0.01	0.28±0.01	1.32±0.01 a	1.31±0.02 a	2.58±0.12 b	4.11±0.13	0.35±0.01	0.35±0.01
	2013	Control	2.59±0.13	2.64±0.13 b	0.33±0.02	0.31±0.02	1.25±0.07 b	1.24±0.02 b	3.17±0.21 a	4.66±0.20	0.42±0.03 a	0.44±0.03
		Nitrogen	2.99±0.16	3.31±0.07 a	0.30±0.01	0.29±0.01	1.46±0.05 ab	1.50±0.06 a	2.89±0.09 ab	4.99±0.04	0.36±0.01 ab	0.39±0.03
		Complete	2.64±0.04	3.42±0.07 a	0.29±0.01	0.28±0.01	1.48±0.02 a	1.50±0.02 a	2.39±0.04 b	4.42±0.08	0.32±0.01 b	0.34±0.01

[a] The unit of nutrient contents for foliar N, P, K, Ca and Mg is mg N 100 needles^{-1}.

[b] Mean ± standard error of the mean (n = 3).

[c] Complete stands for complete fertilizer treatment.

[d] Different letters after numbers represent significant differences among different fertilization treatments within each tree species at $p < 0.05$ in either 2012 or 2013 (n = 3).

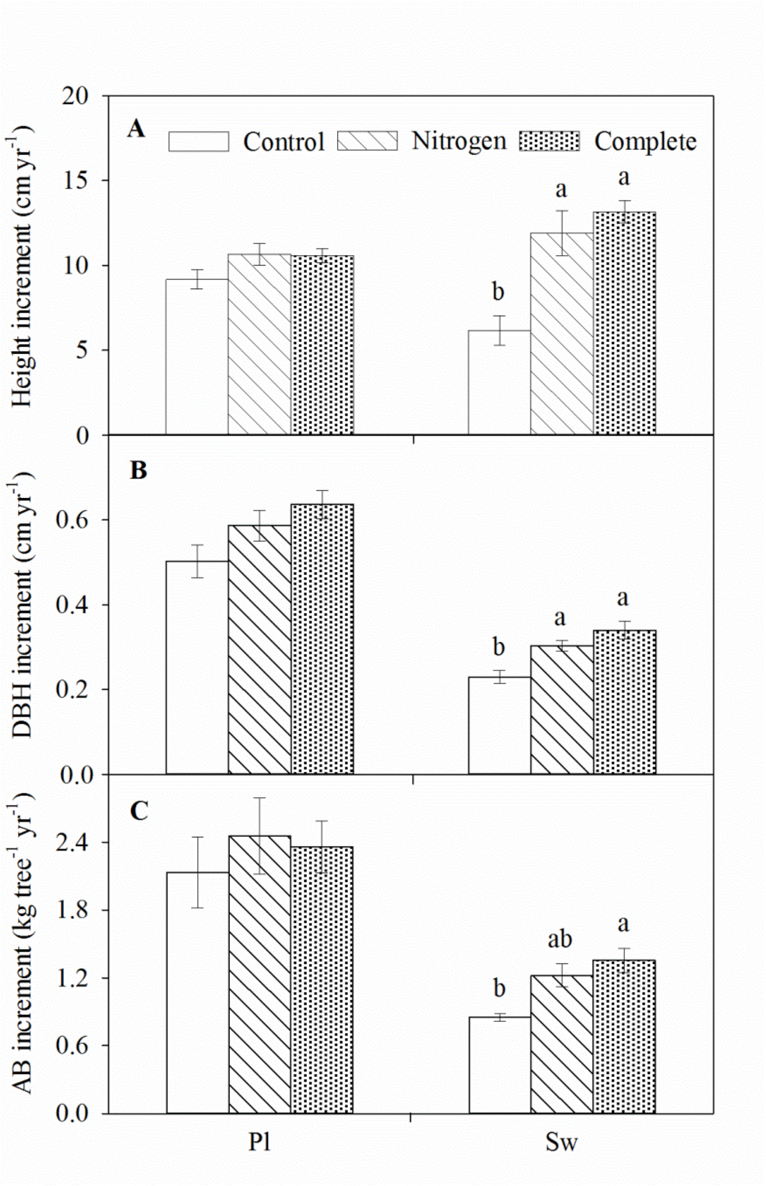

Figure 3-1 Increments of A) height, B) DBH and C) aboveground biomass (AB) from 2012 to 2013 of lodgepole pine (Pl) and white spruce (Sw) trees. Different letters represent significant differences among different fertilization treatments within each tree species at $p < 0.05$. Vertical bars are standard errors of means (n = 3).

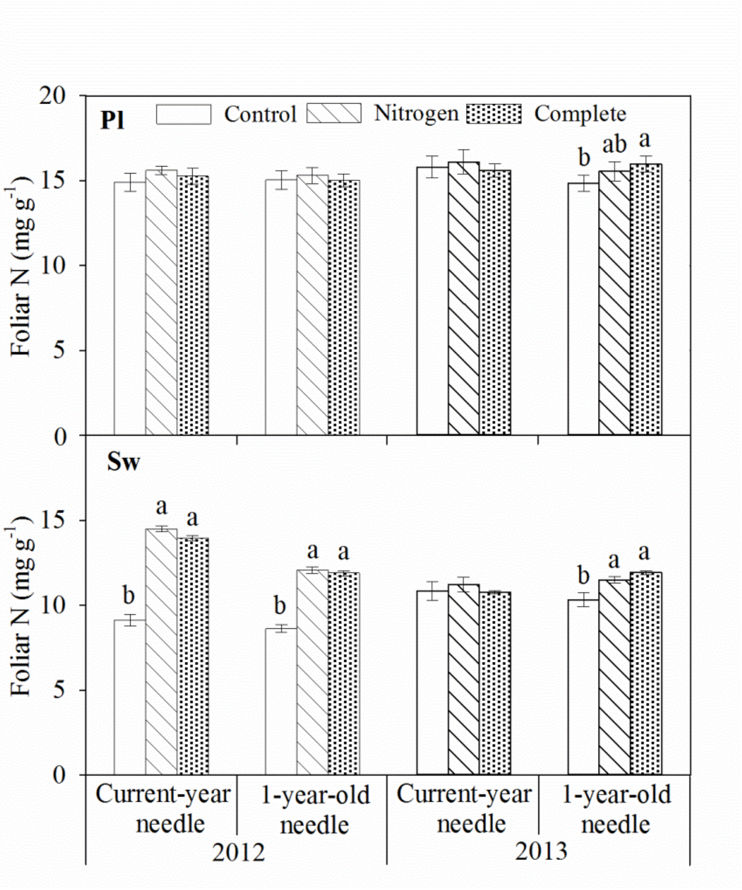

Figure 3-2 Foliar N concentrations of current-year and 1-year-old needles of lodgepole pine (Pl) and white spruce (Sw) sampled in 2012 and 2013. Different letters represent significant differences among different fertilization treatments within each needle type, year and tree species at $p < 0.05$. Vertical bars are standard errors of means (n = 3).

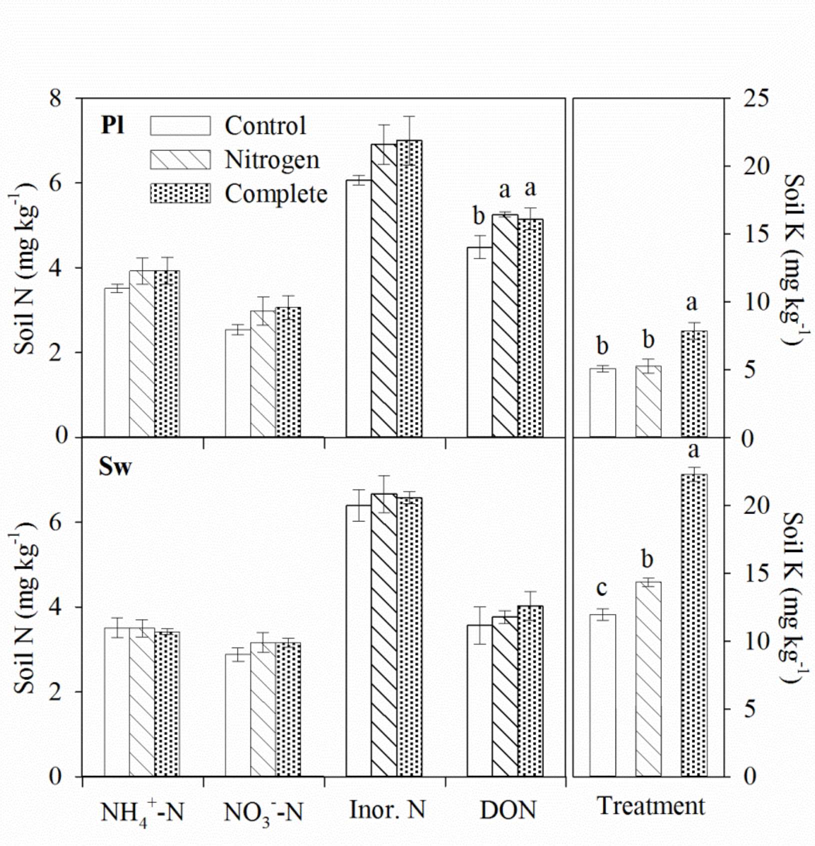

Figure 3-3 Soil N and K concentrations in lodgepole pine (Pl) and white spruce (Sw) sites. Different letters represent significant differences among different fertilization treatments within each N form and tree species at $p < 0.05$. Vertical bars are standard errors of means (n = 3).

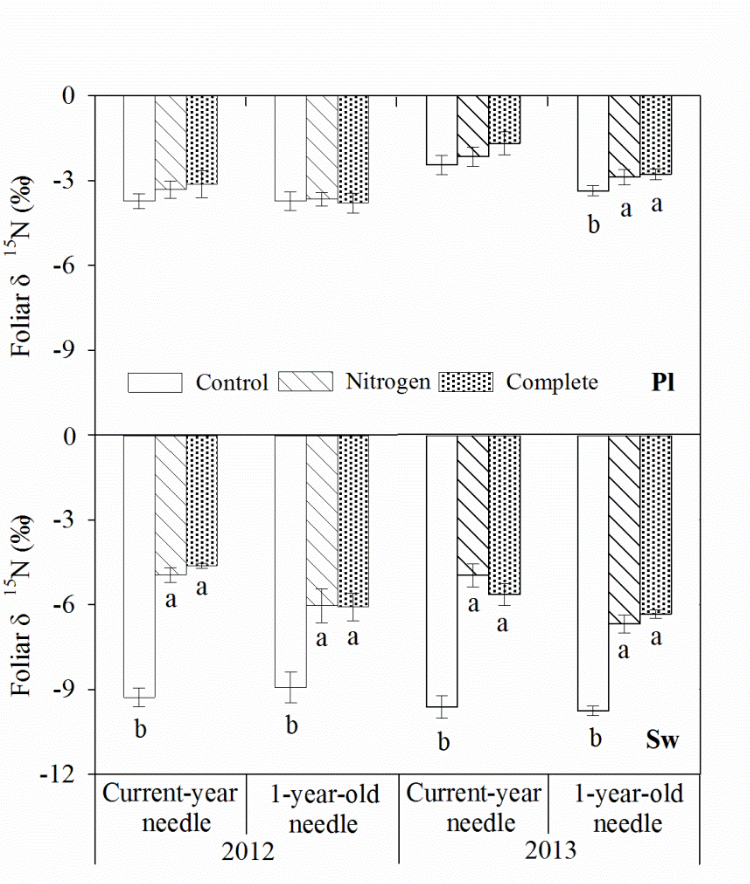

Figure 3-4 Foliar δ^{15}N of current-year and 1-year-old needles of lodgepole pine (Pl) and white spruce (Sw) sampled in 2012 and 2013. Different letters represent significant differences among different fertilization treatments within each needle type, year and tree species at $p < 0.05$. Vertical bars are standard errors of means (n = 3).

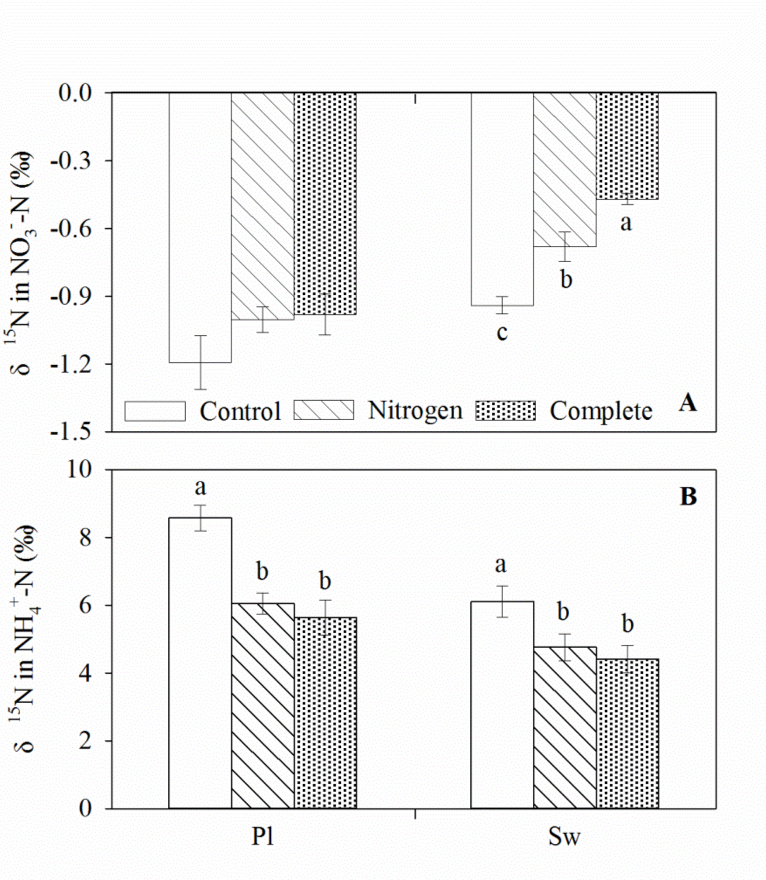

Figure 3-5 δ^{15}N in A) NO_3^--N and B) NH_4^+-N in soils of lodgepole pine (Pl) and white spruce (Sw) sites. Different letters represent significant differences among different fertilization treatments within each tree species at $p < 0.05$. Vertical bars are standard errors of means (n = 3).

References

Albaugh, T.J., Allen, H.L., Dougherty, P.M., Johnsen, K.H. 2004. Long term growth responses of loblolly pine to optimal nutrient and water resource availability. For. Ecol. Manage. 192:3-19.

Amponsah, I.G., Comeau, P.G., Brockley, R.P., Lieffers, V.J. 2005. Effects of repeated fertilization on needle longevity, foliar nutrition, effective leaf area index, and growth characteristics of lodgepole pine in interior British Columbia, Canada. Can. J. For. Res. 35:440-451.

Ballard, T.M., Carter, R.E. 1986. Evaluating Forest Stand Nutrient Status. Ministry of Forests, Victoria, British Columbia. p. 33.

Barbour, S.L., Chanasyk, D., Hendry, J., Leskiw, L., Macyk, T., Mendoza, C., Naeth, A., Nichol, C., O'Kane, M., Purdy, B., Qualizza, C., Quideau, S., Welham, C. 2007. Soil Capping Research in the Athabasca Oil Sands Region. Volume 1: Technology Synthesis. Syncrude Canada Ltd., FortMcMurray, Alberta. p. 20-88.

Bennett, J.N., Blevins, L.L., Barker, J.E., Blevins, D.P., Prescott, C.E. 2003. Increases in tree growth and nutrient supply still apparent 10 to 13 years following fertilization and vegetation control of salal-dominated cedar hemlock stands on Vancouver Island. Can. J. For. Res. 33:1516-1524.

Brockley, R.P. 2007. Effects of 12 years of repeated fertilization on the foliar nutrition and growth of young lodgepole pine in the central interior of British Columbia. Can. J. For. Res. 37:2115-2129.

Campbell, C.R., Plank, C.O. 1998. Preparation of plant tissue for laboratory analysis. In: Kalra, Y.P. (Ed.), Handbook of Reference Methods for Plant Analysis. CRC press, Boca Raton, Florida. p. 37-50.

Chandler, J.W., Dale, J.E. 1995. Nitrogen deficiency and fertilization effects on needle growth and photosynthesis in Sitka spruce (*Picea sitchensis*). Tree Physiol. 15:813-817.

Cheng, Y., Wang, J., Mary, B., Zhang, J.B., Cai, Z.C., Chang, S.X. 2013. Soil pH has contrasting effects on gross and net nitrogen mineralizations in adjacent forest and grassland soils in central Alberta, Canada. Soil Biol. Biochem. 57:848-857.

Choi, W.J., Chang, S.X., Allen, H.L., Kelting, D.L., Ro, H.M. 2005a. Irrigation and fertilization effects on foliar and soil carbon and nitrogen isotope ratios in a loblolly pine stand. For. Ecol. Manage. 213:90-101.

Choi, W.J., Chang, S.X., Curran, M.P., Ro, H.M., Kamaluddin, M., Zwiazek, J.J. 2005b. Foliar $\delta^{13}C$ and $\delta^{15}N$ response of lodgepole pine and douglas-fir seedlings to soil compaction and forest floor removal. For. Sci. 51:546-555.

Choi, W.J., Ro, H.M., Hobbie, E.A. 2003. Patterns of natural ^{15}N in soils and plants from chemically and organically fertilized uplands. Soil Biol. Biochem. 35:1493-1500.

DiTommaso, A., Aarssen, L.W. 1989. Resource manipulations in natural vegetation: a review. Vegetatio 84:9-29.

Duan, M., House, J., Chang, S.X. 2015. Limiting factors for lodgepole pine (*Pinus contorta*) and white spruce (*Picea glauca*) growth differ in some reconstructed sites in the Athabasca oil sands region. Ecol. Eng. 75:323-331.

Environment Canada. 2013. Canadian Climate Normals 1971-2000 Station Data. http://climate.weather.gc.ca/climate_normals/results_e.html?stnID=2519&lang=e&dCodd=1&province=ALTA&provBut=Go&month1=0&month2=12 (accessed 12 Jun. 2013).

Fung, M.Y.P., Macyk, T.M. 2000. Reclamation of oil sands mining areas. In: Barnhisel, R.I., Darmody, R.G., Daniels, W.L. (Eds.), Reclamation of Drastically Disturbed Lands. ASA, CSSA and SSSA, Madison, Wisconsin. p. 755-774.

Hallin, S., Jones, C.M., Schloter, M., Philippot, L. 2009. Relationship between N-cycling communities and ecosystem functioning in a 50-year-old fertilization experiment. ISME J. 3:597-605.

Haynes, B.E., Gower, S.T. 1995. Belowground carbon allocation in unfertilized and fertilized red pine plantations in northern Wisconsin. Tree Physiol. 15:317-325.

Hemstock, S.S., Quideau, S.A., Chanasyk, D.S. 2010. Nitrogen availability from peat amendments used in boreal oil sands reclamation. Can. J. Soil Sci. 90:165-175.

Högberg, P. 1997. ^{15}N natural abundance in soil-plant systems. New Phytol. 137:179-203.

Högberg, P., Tamm, C.O., Högberg, M. 1992. Variations in ^{15}N abundance in a forest fertilization trial: critical loads of N, N saturation, contamination and effects of revitalization fertilization. Plant Soil 142:211-219.

Howat, D.R. 2000. Acceptable Salinity, Sodicity and pH Values for Boreal Forest Reclamation. Alberta Environment, Edmonton, Alberta.

Inubushi, K., Barahona, M.A., Yamakawa, K. 1999. Effects of salts and moisture content on N_2O emission and nitrogen dynamics in Yellow soil and Andosol in model experiments. Biol. Fertil. Soils 29:401-407.

Jung, K., M. Duan, J. House, Chang, S.X. 2014. Textural interfaces affected the distribution of roots, water, and nutrients in some reconstructed forest soils in the Athabasca oil sands region. Ecol. Eng. 64:240-249.

Khasa, D.P., Fung, M., Logan, B. 2005. Early growth response of container-grown selected woody boreal seedlings in amended composite tailings and tailings sand. Bioresour. Technol. 96:857-864.

Kishchuk, B.E., Weetman, G.F., Brockley, R.P., Prescott, C.E. 2002. Fourteen-year growth response of young lodgepole pine to repeated fertilization. Can. J. For. Res. 32:153-160.

Lambert, M.C., Ung, C.H., Raulier, F. 2005. Canadian national tree aboveground biomass equations. Can. J. For. Res. 35:1996-2018.

Li, X.P., Chang, S.X., Salifu, K.F. 2014. Soil texture and layering effects on water and salt dynamics in the presence of a water table: a review. Environ. Rev. 22:41-50.

Lotan, J.E., Critchfield, W.B. 1990. *Pinus contorta* Dougl. ex. Loud.: Lodgepole pine. In: Burns, R.M., Honkala, B.H. (Eds.), Silvics of North America: Volume 1. Conifers. USDA Forest Service, Washington, DC. p. 302-315.

Macdonald, E., Quideau, S., Landhäusser, S. 2012. Rebuilding boreal forest ecosystems after industrial disturbance. In: Vitt, D.H., Bhatti, J.S. (Eds.), Restoration and Reclamation of Boreal Ecosystems: Attaining Sustainable Development. Cambridge University Press, New York City, New York. p.123-160.

Matsushima, M., Choi, W.J., Chang, S.X. 2012. White spruce foliar δ^{13}C and δ^{15}N indicate changed soil N availability by understory removal and N fertilization in a 13-year-old boreal plantation. Plant Soil 361:375-384.

McMillan, R., Quideau, S.A., MacKenzie, M.D., Biryukova, O. 2007. Nitrogen mineralization and microbial activity in oil sands reclaimed boreal forest soils. J. Environ. Qual. 36:1470-1478.

Moskal, T. 1999. Moisture Characteristics of Coarse Textured Soils and Peat: Mineral Mixtures. M.Sc. thesis. Department of Renewable Resources, University of Alberta, Edmonton, Alberta.

Munson, A.D., Margolis, H.A., Brand, D.G. 1995. Seasonal nutrient dynamics in white pine and white spruce in response to environmental manipulation. Tree Physiol. 15:141-149.

Murthy, R., Dougherty, P.M., Zarnoch, S.J., Allen, H.L. 1996. Effects of carbon dioxide, fertilization, and irrigation on photosynthetic capacity of loblolly pine trees. Tree Physiol. 16:537-546.

Nemergut, D.R., Townsend, A.R., Sattin, S.R., Freeman, K.R., Fierer, N., Neff, J.C., Bowman, W.D., Schadt, C.W., Weintraub, M.N., Schmidt, S.K. 2008. The effects of chronic nitrogen fertilization on alpine tundra soil microbial communities: implications for carbon and nitrogen cycling. Environ. Microbiol. 10:3093-3105.

Nienstaedt, H., Zasada, J.C. 1990. *Picea glauca* (Moench) Voss: White spruce. In: Burns R.M., Honkala, B.H. (Eds.), Silvics of North America: Volume 1. Conifers. USDA Forest Service, Washington, DC. p. 204-226.

Pathak, H., Rao, D.L.N. 1998. Carbon and nitrogen mineralization from added organic matter in saline and alkali soils. Soil Biol. Biochem. 30:695-702.

Pinno, B.D., Landhäusser, S.M., MacKenzie, M.D., Quideau, S.A., Chow, P.S. 2012. Trembling aspen seedling establishment, growth and response to fertilization on contrasting soils used in soil sands reclamation. Can. J. Soil Sci. 92:143-151.

Preston, C.M, Mead, D.J. 1994. Growth response and recovery of ^{15}N-fertilizer one and eight growing seasons after application to lodgepole pine in British Columbia. For. Ecol. Manage. 65:219-229.

Rowland, S.M., Prescott, C.E., Grayston, S.J., Quideau, S.A., Bradfield, G.E. 2009. Recreating a functioning forest soil in reclaimed oil sands in northern Alberta: an approach for measuring success in ecological restoration. J. Environ. Qual. 38:1580-1590.

Santiago, L.S., Wright, S.J., Harms, K.E., Yavitt, J.B., Korine, C., Garcia, M.N., Turner, B.L. 2012. Tropical tree seedling growth responses to nitrogen, phosphorus and potassium addition. J. Ecol. 100:309-316.

Sloan. J.L., Jacobs, D.F. 2013. Fertilization at planting influences seedling growth and vegetative competition on a post-mining boreal reclamation site. New For. 44:687-701.

Sutton, R.F. 1992. White spruce (*Picea glauca* [Moench] Voss): stagnating boreal old-field plantations unresponsive to fertilization and weed control. For. Ecol. Manage. 68:249-258.

Thomas, R.C., Mead, D.J. 1992a. Uptake of nitrogen by *Pinus radiata* and retention within the soil after applying ^{15}N-labelled urea at different frequencies. 1. Growth response and nitrogen budgets. For. Ecol. Manage. 53:131-151.

Thomas, R.C., Mead, D.J. 1992b. Uptake of nitrogen by *Pinus radiata* and retention within the soil after applying ^{15}N-labelled urea at different frequencies. 2. Seasonal trends and processes. For. Ecol. Manage. 53:153-174.

Ung, C.H., Bernier, P., Guo, X.J. 2008. Canadian national biomass equations: new parameter estimates that include British Columbia data. Can. J. For. Res. 38:1123-1132.

Wheeler, N.C., Critchfield, W.B. 1985. The distribution and botanical characteristics of lodgepole pine: biogeographical and management implications. In: Baumgartner, D.M., Krebill, R.G., Arnott, J.T., Weetman, G.F. (Eds.), Proceedings of Lodgepole Pine-the Species and Its Management: Symposium Proceedings. May 14-16, Vancouver, British Columbia. p. 1-13.

Yang, R.C. 1985a. Ten-year growth response of 70-year-old lodgepole pine to fertilization in Alberta. Canadian Forestry Service, Northern Forest Research Centre, Edmonton, Alberta. Information Report NOR-X-266.

Yang, R.C. 1985b. Effects of fertilization on growth of 30-year-old lodgepole pine in

West-central Alberta. Canadian Forestry Service, Northern Forest Research Centre, Edmonton, Alberta. Information Report NOR-X-268.

Yang, R.C. 1998. Foliage and stand growth responses of semi-mature lodgepole pine to thinning and fertilization. Can. J. For. Res. 28:1794-1804.

Yarie, J., van Cleve, K., Schlentner, R. 1990. Interaction between moisture, nutrients and growth of white spruce in interior Alaska. For. Ecol. Manage. 30:73-89.

Chapter 4 Salinity affected gross nitrogen transformation rates in a reclaimed boreal forest soil in Alberta, Canada

1 Introduction

Soil reconstruction after oil sands surface mining in the Athabasca oil sands region (AOSR) in northeastern Alberta, Canada, usually uses a peat-mineral soil mix (PMM) as an organic amendment and cover soil over substrates of tailings sand (TS) or overburden (OB) materials because of high availability of peat material in the mined area (Fung and Macyk, 2000). However, reclaimed soils are often affected by salinity in the AOSR. Salts, mainly sodium chloride (NaCl) and sodium sulfate (Na_2SO_4), may migrate from the underlying saline processed TS or marine shale OB to PMM layers (Renault et al., 1999; Barbour et al., 2007). Occasionally, salts are introduced into the cover soil when saline OB material is mixed with the cover soil. The upward movement of salts from saline OB layers into the cover soil could raise the electrical conductivity (EC) level up to 6.0 dS m^{-1} in the lower part of the PMM (Kessler et al., 2010).

High salinity can affect water availability and nutrient uptake (Kayama et al., 2003), and thus can have detrimental effects on plant survival and growth, and consequently, site productivity (McFee et al., 1981; Andrews et al., 1998; Rodrigue and Burger, 2004). High salinity may also influence nitrogen (N) cycling, which largely determines N availability in soils. Reclaimed soils are often reported to have low availability of nutrients, especially N. The reason is that surface mining and related activities result in severe loss of soil organic carbon (C) and N due to the loss of topsoil and mechanical mixing of the A with the B and C horizon soils (Ussiri and Lal, 2005; Shrestha and Lal, 2011). Although PMM materials were added to improve nutrient and water availabilities, the release of organic N from PMM is slow due to small microbial population sizes, reduced enzymatic activities and low microbial diversity in reclaimed soils (McMillan et al., 2007; Hemstock et al., 2010). In addition, boreal forests are typically reported to be N-limited (Binkley and Hart, 1989; Reich et al., 1997), and the negative effects of salinity on N transformations make N availability even lower in the mined area in the boreal forest region.

Although many studies in the literature have reported effects of salinity on net N transformation, the results have been inconsistent. Some studies showed that high salinity increased net N mineralization rates (McClung and Frankenberger, 1987; Pathak and Rao, 1998; Irshad et al., 2005), while others indicated that net N mineralization rates were decreased by high salinity (Green and Cresser, 2008; Compton and Church, 2011). Some studies observed an enhanced effect (Agarwal et al., 1971), while others observed an inhibitory effect (McClung and Frankenberger, 1987; Pathak and Rao, 1998), or no effect (Laura, 1974) of salinity on ammonification rates. Net nitrification was reported to be more sensitive to salts than net N mineralization (McCormick and Wolf, 1980; Cheng et al., 2013). Salt additions could partially or completely inhibit net nitrification rates in both agricultural soils (Darrah et al., 1987; Inubushi et al., 1999) and forest soils (Cheng et al., 2013), and the inhibition on nitrification was attributed to decreased soil pH and osmotic potential (Martikainen, 1985; Chowdhury et al., 2011).

Previous studies mainly focused on the influence of soil salinity on net N transformation rates. It is becoming increasingly recognized that net N transformation represents the outcome of several concurrent N cycling processes such as N mineralization, nitrification and immobilization, and thus does not quantify the rates of individual N transformation processes (Davidson et al., 1991; Hart et al., 1994). In addition, it has been reported that net N transformation rates *in situ* were often an order of magnitude lower than gross N transformation rates (Hart et al., 1994; Verchot et al., 2001). Differences between net and gross N transformation rates were probably attributed to immobilization of NH_4^+-N or NO_3^--N by microbes (Cheng et al., 2013). To provide detailed information about the responses of individual N cycling processes to soil salinity, gross N transformation rates need to be determined.

However, N cycling responses to salinity in oil sands reclaimed soils have been little addressed despite the importance of gross N transformations in explaining N availability in soils. Therefore, the objective of this study was to evaluate the short-term effects of salinity (NaCl) level on gross N transformation rates in a reclaimed soil using the ^{15}N tracing technique. I hypothesize that 1) low to medium salinity would reduce gross N mineralization and nitrification rates, thus decreasing available N in reclaimed soils; and 2) high salinity would completely inhibit gross N transformation due to detrimental effects of salinity on microbial activities.

2 Materials and methods

2.1 Site description and soil sampling

Three sampling sites (10 × 10 m) were selected from 18 existing study sites (Chapter 2) in the reclaimed area on the Suncor Energy Inc. Lease 86/17 in Fort McMurray (56°59′N and 111°32′W) in the AOSR in northern Alberta, Canada. The area has a mean annual temperature of 0.7 ℃ and a mean annual precipitation of 455.7 mm, with an average of 342.2 mm as rainfall during the growing season from 1971 to 2000 (Environment Canada, 2010). All three sites selected for sampling for this study were established in 1991 and planted with lodgepole pine (*Pinus contorta*) one year after soil reconstruction.

After removal of the litter and moss layer, surface PMM was collected from the top 20 cm in five soil cores in each site and composited. The PMM samples from the three sites were mixed again to obtain a composite soil sample. Upon returning to the laboratory, the PMM was passed through a 4 mm sieve, manually homogenized and stored in sealed plastic bags at 4 ℃ in the dark until use. A subsample of the PMM was used for soil analyses as described below. The PMM had a pH of 7.42 (1:2 soil to water ratio w:v) and an EC of 0.91 dS m^{-1} (1:2 soil to water ratio w:v), with a total carbon (C) and N concentrations of 658.7 and 24.8 g kg^{-1} soil, respectively.

2.2 Incubation procedures and soil N analyses

The experiment used a completely randomized design with five soil salinity levels with the EC of 2, 4, 6, and 8 dS m^{-1}(1:2 soil to water ratio w:v). A preliminary experiment was conducted to determine the exact amounts of NaCl required for obtaining the desired soil salinity levels. Homogenized PMM samples were placed into flasks (30 g PMM per flask, oven-dry weight basis) and subjected to five different salinity levels: EC - A (ambient) - addition of 3.6 mL deionized water; EC2 - addition of 0.6 mL 0.5 mol L^{-1} NaCl solution and 3.0 mL deionized water; EC4 - addition of 1.6 mL 0.5 mol L^{-1} NaCl solution and 2.0 mL deionized water; EC6 - addition of 2.6 mL 0.5 mol L^{-1} NaCl solution and 1.0 mL deionized water; EC8 - addition of 3.6 mL 0.5 mol L^{-1} NaCl solution. The NaCl solution and deionized water mix was evenly applied over the soil surface with a pipette. Soils were rewetted to 50% of water holding capacity (WHC) by adding additional deionized water. Flasks were covered with parafilm to prevent water loss and to allow gas exchange between the flask and ambient air. Flasks were then the incubated at 25 ℃ in the dark for 7 days to allow soil salinity to reach equilibrium.

After pre-incubation, each soil sample in the flasks received 2 mL of either a $^{15}NH_4NO_3$ (10 atom%) or $NH_4{}^{15}NO_3$ (10 atom%) solution evenly applied over the soil surface at a rate of 20 mg N kg^{-1} soil. Subsequently, soil water contents were adjusted to 65% of WHC by adding deionized water. During the incubation, deionized water was added to the soil samples every 3 days to compensate for water loss through evaporation. Another set of soil samples incubated with the same treatments was used for analyses of soil pH and EC changes during the incubation.

After 0.5 h, or 1, 2, 3 and 4 days of incubation at 25 °C in the dark, three replicates of either $^{15}NH_4NO_3$- or $NH_4{}^{15}NO_3$-labeled soils were destructively sampled for N analyses. Soil samples were extracted with 0.5 mol L^{-1} KCl solution at a soil to solution ratio of 1:3 (w:v). After being shaken at 250 rpm for 1 h on a mechanical shaker, the slurries were filtered through Whatman No. 42 filter papers. Extracts were stored at 4 °C before analyses within 1 week. The extracted soils were subsequently washed with 150 mL deionized water 3 times and oven-dried at 60 °C until at a constant weight. Oven-dried soils were ground to pass through a 0.15 mm sieve for ^{15}N abundance analysis of soil insoluble N.

A steam distillation system (Vapodest 20s, C. Gerhardt GmbH & Co. KG, Königswinter, Germany) was used to determine concentrations of NH_4^+-N and NO_3^--N in KCl extracts. A portion of the KCl extract was distilled after adding MgO to determine NH_4^+-N concentration. The liberated NH_3 during distillation was absorbed in a 0.005 mol L^{-1} H_2SO_4 solution, and then the H_2SO_4 solution was titrated with a 0.01 mol L^{-1} NaOH solution to determine the concentration of NH_4^+-N using an automatic potentiometric titrator (719 S Titrino, Metrohm AG, Herisau, Switzerland). The extract was distilled again after adding Devarda's alloy to determine NO_3^--N concentration following the same procedure for NH_4^+-N described above. The distillates for NH_4^+-N and NO_3^--N samples were acidified to pH 3 using 0.1 mol L^{-1} H_2SO_4 and oven-dried at 60 °C to determine ^{15}N abundances of NH_4^+-N and NO_3^--N. The ^{15}N abundances of NH_4^+-N, NO_3^--N and insoluble N were analyzed using a stable isotope ratio mass spectrometer (Optima-EA, Micromass UK Ltd., Manchester, UK).

2.3 Analyses of other soil chemical properties

Soil pH was measured in deionized water with a soil to water ratio of 1:2 (w:v*)* using an Orion 3-Star portable pH meter (Thermo Fisher Scientific Inc., Waltham, MA). Soil EC was measured by an AP75 portable waterproof conductivity/TDS meter (Thermo Fisher Scientific Inc., Waltham, MA) using 30 g of oven-dried soil in 60 mL deionized water after shaking at 25 ℃ for 1 h and filtration through Whatman No. 42 filter papers. Total N and total C concentrations were determined using a Carlo Erba NA 1500 elemental analyzer (Carlo Erba Instruments Ltd., Milano, Italy).

2.4 Calculation and statistical analysis

Gross N mineralization, nitrification and N immobilization rates were estimated by the ^{15}N isotopic pool dilution method using equations of Kirkham and Bartholomew (1954) and Hart et al. (1994). The gross NH_4^+-N immobilization rate was calculated by subtracting the gross nitrification rate from the gross NH_4^+-N consumption rate, and the gross NO_3^--N immobilization rate was equivalent to the gross NO_3^--N consumption rate on the assumption that NO_3^--N consumption through the denitrification was negligible under an aerobic incubation (Murphy et al., 2003). Net N mineralization and nitrification rates were calculated as the difference between final and initial inorganic N (NH_4^+-N and NO_3^--N) or NO_3^--N pool sizes, respectively, divided by incubation intervals.

A one-way analysis of variance (ANOVA) and Tukey's multiple comparison were used to evaluate the statistical significance of different salinity levels on gross and net N transformation rates of the PMM during the 4-day incubation at an α value of 0.05. Before performing the ANOVA analysis, the normality of distribution and homogeneity of variance were tested and net nitrification rate data were logarithmically transformed to meet the assumption of normal distribution and homogeneous variance for ANOVA. The non-transformed data were presented in this paper. The statistical analysis was performed using the SAS software (SAS 9.2, SAS Institute Inc., Cary, NC).

3 Results

3.1 Soil pH and EC

After addition of different amounts of NaCl solutions and pre-incubated at 25 ℃ for 7 days, the targeted soil salinity levels were achieved (Figure 4-1 a). The salinity levels in the EC2 and EC4 were exactly 2 and 4 dS m^{-1}, respectively, but salinity levels in the EC6 and EC8 were slightly lower than the targeted 6 and 8 dS m^{-1}, respectively. In addition, the salinity levels remained stable in all treatments during the 4-day incubation. On the other hand, soil pH in all treatments did not change following NaCl addition, even when EC level was 8 dS m^{-1}. Soil pH remained around 7.4 during the incubation in all treatments (Figure 4-1 b).

3.2 Nitrogen concentrations and ^{15}N abundances

The NH_4^+-N concentrations decreased quickly in the first 2 days, and then remained stable in all treatments, except for EC-A and EC2, in which NH_4^+-N concentrations continuously declined till the end of the incubation (Figure 4-2 a). After a 4-day incubation, the decrease of NH_4^+-N concentrations in low salinity levels (EC-A and EC2) were greater than those in high salinity levels (EC4, EC6, and EC8) ($p < 0.001$), indicating that high salinity suppressed NH_4^+-N consumption by microorganisms. In contrast, NO_3^--N concentrations remained relatively stable or slightly increased with the incubation time in all treatments (Figure 4-2 b).

In the $^{15}NH_4NO_3$ labeled samples, the % ^{15}N isotopic excess in NH_4^+-N declined gradually over the incubation time in all treatments due to the dilution from mineralization of soil organic N (Figure 4-3 a), while the % ^{15}N isotopic excess in NO_3^--N and organic N increased gradually due to nitrification and immobilization of $^{15}NH_4^+$-N, respectively (Figure 4-3 b and c). The % ^{15}N isotopic excess in NH_4^+-N under high salinity (EC4, EC6, and EC8) were higher than those in low salinity (EC-A and EC2) in each incubation interval ($p < 0.001$), except for the first incubation interval ($p = 0.106$). In contrast, the % ^{15}N isotopic excess in NO_3^--N and organic N had the opposite trends.

In the $NH_4{}^{15}NO_3$ labeled samples, the % ^{15}N isotopic excess in NO_3^--N declined gradually during the 4-day incubation in all treatments, except for the EC8, in which it increased in the last incubation interval (Figure 4-3 d). There were no differences in the % ^{15}N isotopic excess in NO_3^--N among all treatments in each incubation interval, except for the last incubation interval, in which it was higher in the EC8 than those in other salinity levels ($p = 0.001$). In contrast, the % ^{15}N isotopic excess in NH_4^+-N increased gradually in the EC-A, EC2 and EC4, and kept stable in the EC6 and EC8, whereas the % ^{15}N isotopic excess in organic N remained unchanged during the 4-day incubation in all treatments (Figure 4-3 e and f). Greater changes in $^{15}NH_4NO_3$ labeled samples indicated that NH_4^+-N was the preferable N source for microbial consumption in the soil studied here.

3.3 Recovery of ^{15}N

In the $^{15}NH_4NO_3$ labeled samples, ^{15}N recovery rates of NH_4^+-N and total N pools increased as salinity levels increased (Figure 4-4 a), while ^{15}N recovery of NO_3^--N pools had the opposite trend, where ^{15}N recovery rates decreased with salinity levels. Although the EC8 had the highest ^{15}N recovery rate of total N, only 25% of added ^{15}N was recovered. However, in the $NH_4{}^{15}NO_3$ labeled samples, ^{15}N recovery of NO_3^--N and total N pools increased as the salinity levels increased (Figure 4-4 b), and more than 60% of the added ^{15}N was recovered in total N. There were no differences in ^{15}N recovery rates in NH_4^+-N pools among all treatments, except for EC6 and EC8, where ^{15}N recovery rates were lower than in the other treatments. This indicated that NH_4^+-N was more active than NO_3^--N in microorganism-mediated N cycling processes. We assumed that the loss of added ^{15}N was due to the N immobilization by microbes. Because the changes of ^{15}N were less sensitive in organic N pools compared to those in inorganic N pools due to high background organic N concentrations, we could not detect significant changes of ^{15}N recovery rates in organic N pools. When salts were added to the soil, microorganism-mediated N immobilization would decrease; thus less ^{15}N was recovered in organic N pools after the 4-day incubation (Figure 4-3 c).

3.4 Nitrogen transformation rates

Gross N mineralization and immobilization rates were the highest in the first incubation interval in all treatments (Table 4-1), gradually decreased in the following two incubation intervals, and then increased in the last interval, except for the immobilization rates in EC-A, EC6 and EC8, in which they decreased over time. In contrast to gross N mineralization and immobilization rates, gross nitrification rates in all treatments increased over incubation time, peaking in the third interval, and then decreasing. The NH_4^+ immobilization rates were higher than NO_3^--N immobilization rates in all treatments, indicating that microbes had a preference for NH_4^+-N to that of NO_3^--N. Salinity significantly suppressed the weighted-average gross N mineralization, nitrification, and N immobilization rates (Figure 4-5). Gross N immobilization rates were greater than gross N mineralization rates, which resulted in negative net N mineralization and ammonification rates. There were no differences in net nitrification rates in all treatments after the 4-day incubation.

4 Discussion

The decreased gross N mineralization rates with increasing salinity found here was samilar to decreased net N mineralization rates reported in many previous studies (Laura, 1974; McClung and Frankenberger, 1987; Pathak and Rao, 1998). They were attributed to decreased osmotic water potential, changed soil pH, and toxicity of the chloride ion (Heilman, 1975; Martikainen, 1985; Omar et al., 1994; Low et al., 1997; Chowdhury et al., 2011). Decreased soil osmatic potential from increasing EC has been found to decrease water availability to microbes and reduce microbial activity and biomass (Yuan et al., 2007; Elmajdoub et al., 2014), thus influencing N mineralization in soils (Chowdhury et al., 2011). Fungi were reported to be less tolerant to salinity than bacteria, and fungi abundance decreased at low osmatic potential in saline soils (Badran, 1994; Omar et al., 1994). As fungi is important in decomposing lignin and cellulose of plant residues (Harper and Lynch, 1985); when bacteria dominated in saline soils, decomposition of recalcitrant organic compounds and N mineralization would be inhibited (Badran, 1994). Salt addition would increase or decrease soil pH depending on salt type, and change of microbial community. Cheng et al. (2013) reported that increased soil pH enhanced gross N mineralization rates, suppressed net N mineralization rates in a forest soil grown with native aspen (*Populus tremuloides*) in central Alberta, Canada. However, salt addition did not significantly change soil pH in this study, even in the high salinity level (EC8) during the 4-day incubation, suggesting that decreased N mineralization rates were probably attributed to toxicity of the chloride or sodium ion or a decrease of soil osmotic potential, rather than changes in soil pH.

Inhibitory effects of ammonium sulphate ($(NH_4)_2SO_4$) or potassium sulphate (K_2SO_4) addition on net nitrification have been reported in a soil from the organic horizon of a pine forest stand in southern Finland (Martikainen, 1985). Cheng et al. (2013) found that low or high amounts of potassium chloride (KCl) addition partly or completely inhibited gross nitrification rates, respectively, in an aspen forest soil in Alberta, Canada. However, high soil salinity suppressed but did not completely inhibit gross nitrification rates in this study. This was probably due to different salt tolerances of microbial communities in the two soils studied. Salinity levels in natural forest soils were normally lower than those in reclaimed forest soils that were affected by salinity (Fung and Macyk, 2000). Microbes in saline reclaimed soils may adapt to saline conditions more quickly than those in non-saline natural soils when salts were added. Previous studies have reported that effects of salinity on soil microbes were more detrimental when salts were added to non-saline soils compared to saline soils because the rapid increase of salinity in non-saline soils may not allow soil microbial communities to adapt to salinity (Khan et al., 2008; Setia et al., 2011). However, Yan and Marschner (2013) found that an abrupt increase in soil salinity did not result in less microbial respiration and biomass than a gradual increase. The contrasting findings could be attributed to differences in microbial community composition and the initial microbial biomass in the soils studied (Yan and Marschner, 2013). As members of a microbial community differed in their abilities to decompose organic compounds in different soils, nitrification could be affected differently because of changes of microbial community composition induced by salt addition. For example, high salinity could decrease the abundance and activity of nitrifiers; although greater substrate (NH_4^+-N) was available, only parts of them could be used for nitrification.

Negative net N mineralization and nitrification rates were observed in this study, suggesting decreased N availability in the soil studied. The negative rates also indicated both low NH_4^+-N production and high NO_3^--N consumption. Previous studies attributed them to high C: N ratio in soils (Christenson et al., 2009; Goodale and Aber, 2001; Lovett et al., 2002). This may also be the reason in this study, where C: N ratio as high as 30 was observed in the reclaimed soil. High C: N ratios enhance microbial immobilization of N due to high demand for inorganic N for microbial growth. Microorganisms are responsible for both the release and immobilization of nutrients (Booth et al., 2005). The different extent of changes in gross N mineralization and NH_4^+-N immobilization rates resulted in different responses of net N mineralization rates.

The ratio of gross nitrification to gross NH_4^+-N immobilization rate (N/IA) has been used as an indicator to assess the dominant consumption process of NH_4^+-N in a soil (Hoyle et al., 2006). The N/IA ratios were less than 1 in all treatments, indicating that the majority of NH_4^+-N was immobilized rather than nitrified by microbes in this study. Salinity did not affect N/IA in all salinity levels except in EC8, in which N/IA was lower than the other treatments (Figure 4-6), because high salinity affected microbial immobilization of NH_4^+-N less than nitrification (Table 4-1). The similar N/IA ratios were probably because the effect of high C: N ratio in PMM muted the salinity effect on N/IA in the soil studied. In contrast to the findings of Westerma and Tucker (1974) and Allen et al. (1994), gross N immobilization rates were inhibited by high salinity levels in this study. This could be attributed to different tolerance in microbes to low osmotic potential induced by high salinity (Mandeel, 2006; Chowdhury et al., 2011; Baumann and Marschner, 2013). Tolerant bacteria and fungi could accumulate compatible solutes in cells to deal with low osmotic stress (Killham and Firestone, 1984; Schimel et al., 1989; Beales, 2004). However, synthesis and transport of osmolytes required large amounts of energy, and less energy was available for microbial growth, which posed a significant metabolic burden on microorganisms (Oren, 1999).

5 Conclusions

Reclaimed soils with high salinity significantly decreased N availability because of reduced gross N mineralization and nitrification rates. Although soil salinity with the EC of 8 dS m^{-1} was not common, EC values greater than 4 dS m^{-1} were often reported in the AOSR region, which suggested the risk of N deficiency for tree growth in saline soils. Greater gross N immobilization than N mineralization rates when N was supplied should be considered when applying N fertilizer to improve N availability in reclaimed soils because a portion of the added N would be immobilized by microbes to make them unavailable to plants in a short term, which would pose a big challenge to improve N availability in reclaimed saline soils in the oil sands region. However, our data on soil salinity effects on gross N transformations should be interpreted with caution, since it is difficult to extrapolate our findings to long-term field studies where soil salinity changes gradually, thus microbes would have time to adjust. In addition, we did not follow the N transformation long enough. It would be interesting to evaluate the long-term fate of the ^{15}N response to salinity. Nevertheless, this laboratory study provides a mechanistic understanding of the dynamics of soil N cycling as a response to soil salinity.

Table 4-1 Gross rates of N mineralization (m), nitrification (n), N immobilization (i), NH_4^+-N immobilization (i_a), and NO_3^--N immobilization (i_n) in treatments with different levels of salinity in peat-mineral soil mix at the four incubation intervals. Values in brackets are standard errors of means (n = 3).

Treatment	Interval	Gross N transformation rates (mg N kg^{-1} soil d^{-1})				
		m	n	i	i_a	i_n
EC-A	d_o-d_1	4.32 (0.11)	0.50 (0.09)	5.99 (0.22)	5.40 (0.23)	0.59 (0.45)
	d_1-d_2	3.49 (0.16)	0.67 (0.05)	4.83 (0.13)	4.50 (0.21)	0.33 (0.23)
	d_2-d_3	3.38 (0.14)	1.26 (0.05)	4.68 (0.04)	3.63 (0.18)	1.06 (0.22)
	d_3-d_4	3.49 (0.19)	1.22 (0.10)	4.43 (0.27)	3.58 (0.36)	0.85 (0.12)
EC2	d_o-d_1	3.79 (0.08)	0.55 (0.07)	5.40 (0.09)	4.97 (0.06)	0.43 (0.05)
	d_1-d_2	3.29 (0.09)	0.69 (0.01)	4.72 (0.09)	4.32 (0.10)	0.40 (0.01)
	d_2-d_3	2.83 (0.11)	1.22 (0.09)	4.02 (0.17)	3.06 (0.09)	0.97 (0.11)
	d_3-d_4	3.40 (0.31)	1.00 (0.06)	4.26 (0.20)	3.53 (0.19)	0.73 (0.03)
EC4	d_o-d_1	3.15 (0.24)	0.36 (0.02)	4.85 (0.80)	4.55 (0.53)	0.29 (0.57)
	d_1-d_2	2.60 (0.06)	0.57 (0.05)	4.16 (0.26)	3.61 (0.23)	0.54 (0.08)
	d_2-d_3	2.69 (0.20)	1.14 (0.06)	3.53 (0.21)	2.74 (0.27)	0.78 (0.12)
	d_3-d_4	3.16 (0.21)	0.86 (0.04)	3.65 (0.24)	3.16 (0.24)	0.49 (0.07)
EC6	d_o-d_1	2.77 (0.17)	0.26 (0.06)	4.38 (0.30)	4.29 (0.37)	0.09 (0.15)
	d_1-d_2	2.57 (0.16)	0.46 (0.05)	3.68 (0.12)	3.39 (0.13)	0.29 (0.24)
	d_2-d_3	2.59 (0.08)	1.05 (0.05)	3.18 (0.04)	2.41 (0.07)	0.77 (0.11)
	d_3-d_4	2.63 (0.06)	0.77 (0.07)	2.92 (0.05)	2.48 (0.13)	0.44 (0.13)
EC8	d_o-d_1	2.88 (0.19)	0.03 (0.08)	3.60 (0.26)	3.78 (0.30)	-0.18 (0.55)
	d_1-d_2	2.38 (0.01)	0.38 (0.05)	3.22 (0.10)	3.11 (0.15)	0.11 (0.23)
	d_2-d_3	2.25 (0.09)	0.86 (0.06)	2.64 (0.08)	2.05 (0.17)	0.59 (0.23)
	d_3-d_4	2.44 (0.12)	0.49 (0.04)	2.60 (0.12)	2.41 (0.15)	0.18 (0.19)

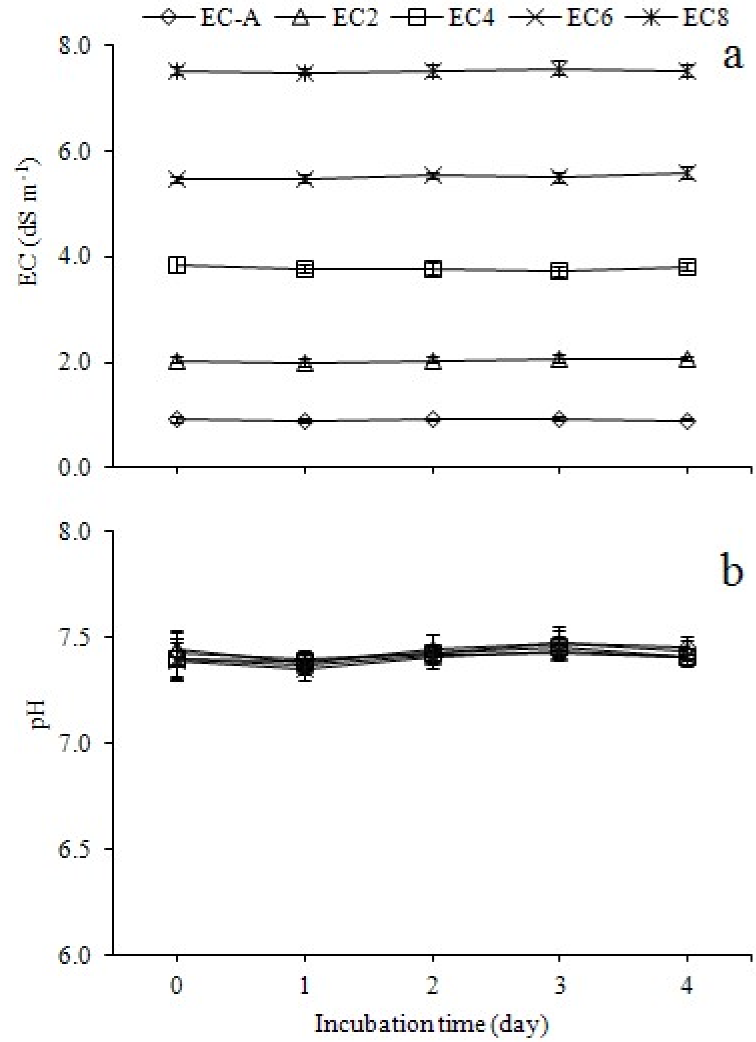

Figure 4-1 Changes in a) soil electrical conductivity (EC, dS m^{-1}) and b) soil pH over a 4-day period incubated with NH_4NO_3 in treatments with different levels of salinity in peat-mineral soil mix. Vertical bars are standard errors of means ($n = 6$).

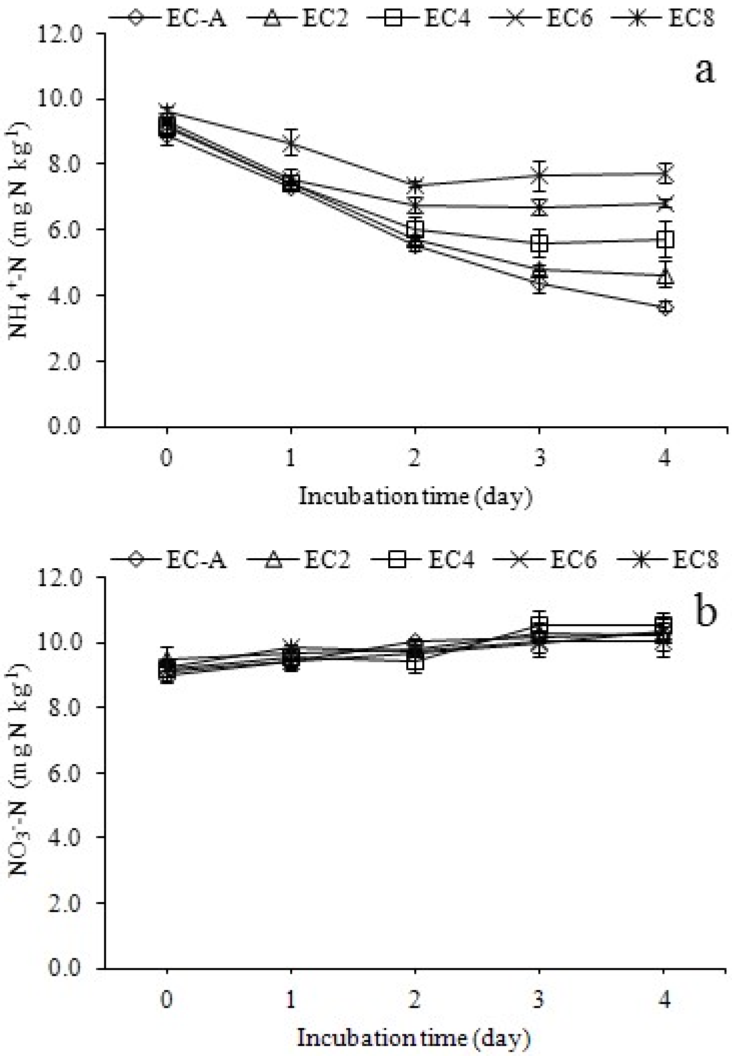

Figure 4-2 Changes in a) NH_4^+-N and b) NO_3^--N concentrations (mg N kg^{-1} soil) over a 4-day period incubated with NH_4NO_3 in treatments with different levels of salinity in peat-mineral soil mix. Vertical bars are standard errors of means (n = 6).

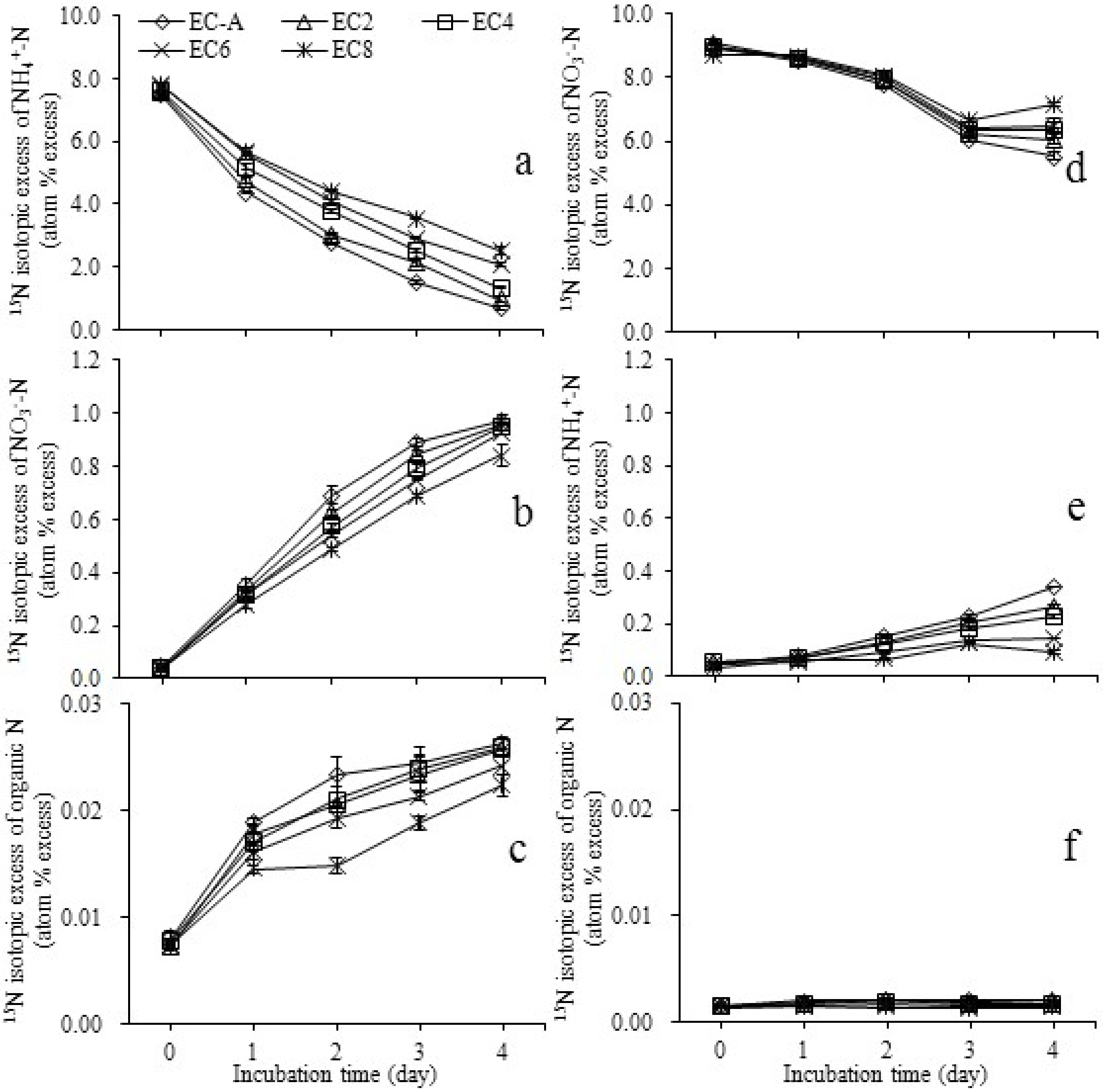

Figure 4-3 Changes in ^{15}N abundances (atom% excess) of a) NH$_4^+$-N, b) NO$_3^-$-N and c) organic N for samples incubated with ^{15}NH$_4$NO$_3$ and d) NO$_3^-$-N, e) NH$_4^+$-N and f) organic N for samples incubated with NH$_4{}^{15}$NO$_3$ over a 4-day period in treatments with different levels of salinity in peat-mineral soil mix. Vertical bars are standard errors of means (n = 3).

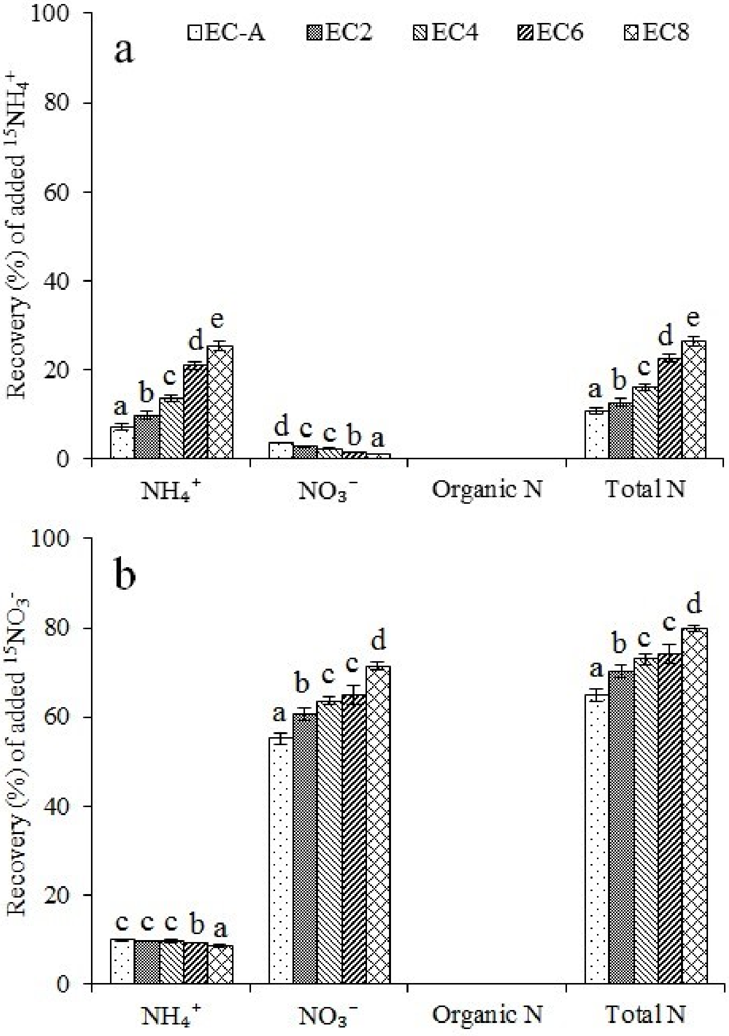

Figure 4-4 Recovery of added ^{15}N in the NH_4^+-N, NO_3^--N, organic N and total N pools after a 4-day incubation with a) $^{15}NH_4NO_3$ and b) $NH_4{}^{15}NO_3$ in treatments with different levels of salinity in peat-mineral soil mix. Different letters represent significant differences among different salinity levels for each N form at $p < 0.05$. Vertical bars are standard errors of means (n = 3).

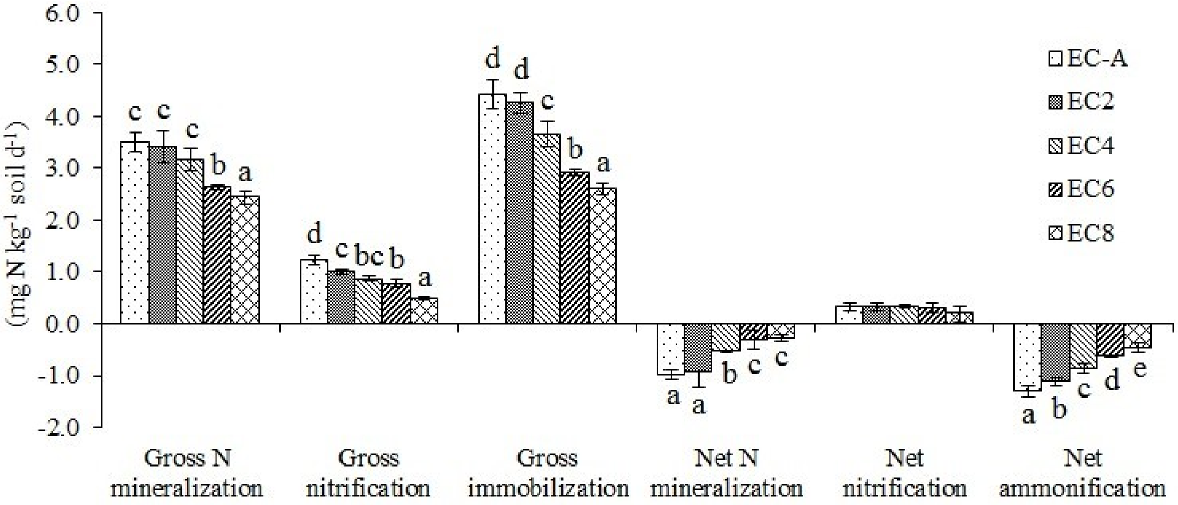

Figure 4-5 Gross and net N mineralization and nitrification, gross N immobilization and net ammonification rates after a 4-day incubation in treatments with different levels of salinity in peat-mineral soil mix. Different letters represent significant differences for each N transformation rate among different salinity levels at $p < 0.05$. Vertical bars are standard errors of means (n = 3).

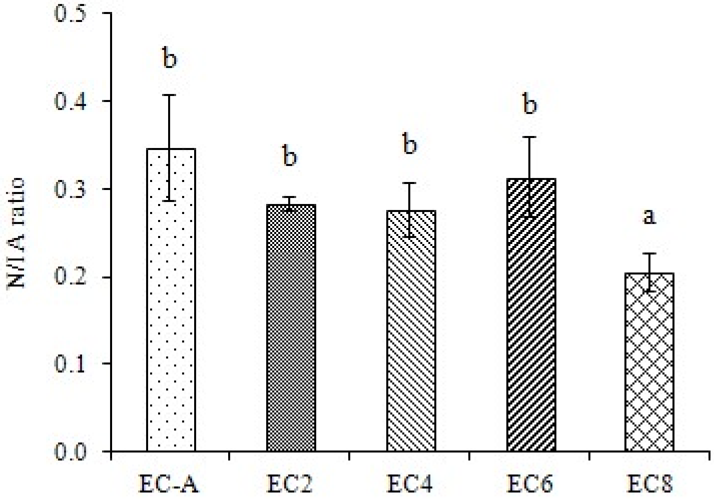

Figure 4-6 The ratio of gross nitrification rate to gross NH_4^+-N immobilization rate (N/IA ratio) after a 4-day incubation with NH_4NO_3 in treatments with different levels of salinity in peat-mineral soil mix. Different letters represent significant differences among different salinity levels at $p < 0.05$. Vertical bars are standard errors of means (n = 3).

References

Agarwal, A.S., Singh, B.R., Kanehiro, Y. 1971. Ionic effect of salts on mineral nitrogen release in an allophanic soil. Soil Sci. Soc. Am. Proc. 35:454-457.

Allen, J.A., Chambers, J.L., Stine, M. 1994. Prospects for increasing the salt tolerance of forest trees: A review. Tree Physiol. 14:843-853.

Andrews, J.A., Johnson, J.E., Torbert, J.L., Burger, J.A., Kelting, D.L. 1998. Mine soil and site properties associated with early height growth of eastern white pine. J. Environ. Qual. 27:192-199.

Badran, R.A.M. 1994. Cellulolytic activity of some cellulose decomposing fungi in salinized soils. Acta. Mycol. 29:245-321.

Barbour, S.L., Chanasyk, D., Hendry, J., Leskiw, L., Macyk, T., Mendoza, C., Naeth, A., Nichol, C., O'Kane, M., Purdy, B., Qualizza, C., Quideau, S., Welham, C. 2007. Soil Capping Research in the Athabasca Oil Sands Region. Volume 1: Technology Synthesis. Syncrude Canada Ltd., FortMcMurray. p. 49-88

Baumann, K., Marschner, P. 2013. Effects of salinity on microbial tolerance to drying and rewetting. Biogeochemistry112:71-80.

Beales, N. 2004. Adaptation of microorganisms to cold temperatures, weak acid preservatives, low pH, and osmotic stress: A review. Compr. Rev. Food Sci. Food Saf. 3:1-20.

Binkley, D., Hart, S.C. 1989. The components of nitrogen availability assessments in forest soils.

Adv. Soil Sci. 10:57-112.

Booth, M.S., Stark, J.M., Rastetter, E. 2005. Controls on nitrogen cycling in terrestrial ecosystems: A synthetic analysis of literature data. Ecol. Monogr. 75:139-157.

Cheng, Y., Wang, J., Mary, B., Zhang, J.B., Cai, Z.C., Chang, S.X. 2013. Soil pH has contrasting effects on gross and net nitrogen mineralizations in adjacent forest and grassland soils in central Alberta, Canada. Soil Biol. Biochem. 57:848-857.

Christenson, L.M., Lovett, G.M., Weathers, K.C., Arthur, M.A. 2009. The influence of tree species, nitrogen fertilization, and soil C to N ratio on gross soil nitrogen transformations. Soil Sci. Soc. Am. J. 73:638-646.

Chowdhury, N., Marschner, P., Burns, R.G. 2011. Response of microbial activity and community structure to decreasing soil osmotic and matric potential. Plant Soil 344:241-254.

Compton, J.E., Church, M.R. 2011. Salt additions alter short-term nitrogen and carbon mobilization in a coastal Oregon Andisol. J. Environ. Qual. 40:1601-1606.

Darrah, P.R., Nye, P.H., White, R.E. 1987. The effect of high solute concentrations on nitrification rates in soil. Plant Soil 97:37-45.

Davidson, E.A., Hart, S.C., Shanks, C.A., Firestone, M.K. 1991. Measuring gross nitrogen mineralization, immobilization, and nitrification by ^{15}N isotopic pool dilution in intact soil cores. Eur. J. Soil Sci. 42:335-349.

Elmajdoub, B., Barnett, S., Marschner, P. 2014. Response of microbial activity and biomass in rhizosphere and bulk soils to increasing salinity. Plant Soil 381:297-306.

Environment Canada. 2010. Canadian Climate Normals. Government of Canada Web. http://climate.weather.gc.ca/climate_normals/index_e.html. (Accessed 23 December 2013).

Fung, M.Y.P., Macyk, T.M. 2000. Reclamation of oil sands mining areas. In: Barmhisel, R.I., Darmody, R.G., Daniels, W.L. (Eds.), Reclamation of Drastically Disturbed Lands. Agronomy Monograph No. 41. American Society of Agronomy, Madison. p. 755-774.

Goodale, C.L., Aber, J.D. 2001. The long-term effects of land-use history on nitrogen cycling in northern hardwood forests. Ecol. Appl. 11:253-267.

Green, S.M., Cresser, M.S. 2008. Nitrogen cycle disruption through the application of deicing salts on upland highways. Water Air Soil Pollut. 188:139-153.

Harper, S.H.T., Lynch, J.M. 1985. Colonization and decomposition of straw by fungi. Trans. Br. Mycol. Soc. 85:655-661.

Hart, S.C., Nason, G.E., Myrold, D.D., Perry, D.A. 1994. Dynamics of gross nitrogen transformations in an old-growth forest: The carbon connection. Ecology 75:880-891.

Heilman, P. 1975. Effect of added salts on nitrogen release and nitrate levels in forest soils of the Washington coastal area. Soil Sci. Soc. Am. Proc. 39:778-782.

Hemstock, S.S., Quideau, S.A., Chanasyk, D.S. 2010. Nitrogen availability from peat amendments used in boreal oil sands reclamation. Can J. Soil. Sci. 90:165-175.

Hoyle, F.C., Murphy, D.V., Fillery, I.R.P. 2006. Temperature and stubble management influence microbial CO_2-C evolution and gross N transformation rates. Soil Biol. Biochem. 38:71-80.

Inubushi, K., Barahona, M.A., Yamakawa, K. 1999. Effects of salts and moisture content on N_2O

emission and nitrogen dynamics in Yellow soil and Andosol in model experiments. Biol. Fertil. Soils 29:401-407.

Irshad, M., Honna, T., Yamamoto, S., Eneji, A.E., Yamasaki, N. 2005. Nitrogen mineralization under saline conditions. Commun. Soil Sci. Plant Anal. 36:1681-1689.

Kayama, M., Quoreshi, A.M., Kitaoka, S., Kitahashi, Y., Sakamoto, Y., Maruyama, Y., Kitao, M., Koike, T. 2003. Effects of deicing salt on the vitality and health of two spruce species, *Picea abies* Karst., and *Picea glehnii* Masters planted along roadsides in northern Japan. Environ. Pollut. 124:127-137.

Kessler, S., Barbour, S.L., van Rees, K.C.J., Dobchuk, B.S. 2010. Salinization of soil over saline-sodic overburden from the oil sands in Alberta. Can. J. Soil Sci. 90:637-647.

Khan, K.S., Gattinger, A., Buegger, F., Schloter, M., Joergensen, R.G. 2008. Microbial use of organic amendments in saline soils monitored by changes in the 13C/12C ratio. Soil Biol. Biochem. 40:1217-1224.

Killham, K., Firestone, M.K. 1984. Salt stress control of intracellular solutes in Streptomycetes indigenous to saline soils. Appl. Environ. Microbiol. 47:301-306.

Kirkham, D., Bartholomew, W. 1954. Equations for following nutrient transformations in soil, utilizing tracer data. Soil Sci. Soc. Am. Proc. 18:33-34.

Laura, R.D. 1974. Effects of neutral salts on carbon and nitrogen mineralization of organic-matter in soil. Plant Soil 41:113-127.

Low, A.P., Stark, J.M., Dudley, L.M. 1997. Effects of soil osmotic potential on nitrification, ammonification, N-assimilation, and nitrous oxide production. J. Soil Sci. 162:16-27.

Mandeel, Q.A. 2006. Biodiversity of the genus Fusarium in saline soil habitats. J. Basic Microbiol. 46:480-494.

Martikainen, P.J. 1985. Nitrification in forest soil of different pH as affected by urea, ammonium sulfate and potassium sulfate. Soil Biol. Biochem. 17:363-367.

McClung, G., Frankenberger, W.T. 1987. Nitrogen mineralization rates in saline vs salt-amended soils. Plant Soil 104:13-21.

McCormick, R.W., Wolf, D.C. 1980. Effect of sodium chloride on CO_2 evolution, ammonification, and nitrification in a Sassafras sandy loam. Soil Biol. Biochem. 12:153-157.

McFee, W.W., Byrnes, W.R., Stockton, J.G. 1981. Characteristics of coal mine overburden important to plant growth. J. Environ. Qual. 10:300-308.

McMillan, R., Quideau, S.A., MacKenzie, M.D., Biryukova, O. 2007. Nitrogen mineralization and microbial activity in oil sands reclaimed boreal forest soils. J. Environ. Qual. 36:1470-1478.

Murphy, D.V., Recous, S., Stockdale, E.A., Fillery, I.R.P., Jensen, L.S., Hatch, D.J., Goulding, K.W.T. 2003. Gross nitrogen fluxes in soil: Theory, measurement and application of ^{15}N pool dilution techniques. Adv. Agron. 79:69-118.

Omar, S.A., Abdel-Sater, M.A., Khallil, A.M., Abd-Alla, M.H. 1994. Growth and enzyme activities of fungi and bacteria in soil salined with sodium chloride. Folia. Microbiol.

39:23-28.

Oren, A. 1999. Bioenergetic aspects of halophilism. Microbiol. Mol. Biol. Rev. 63:334-348.

Pathak, H., Rao, D.L.N. 1998. Carbon and nitrogen mineralization from added organic matter in saline and alkali soils. Soil Biol. Biochem. 30:695-702.

Reich, P.B., Grigal, D.F., Aber, J.D., Gower, S.T. 1997. Nitrogen mineralization and productivity in 50 hardwood and conifer stands on diverse soils. Ecology 78:335-347.

Renault, S., Paton, E., Nilsson, G., Zwiazek, J.J., MacKinnon, M.D. 1999. Responses of boreal plants to high salinity oil sands tailings water. J. Environ. Qual. 28:1957-1962.

Rodrigue, J.A., Burger, J.A. 2004. Forest soil productivity of mined land in the mid-western and eastern coalfield regions. Soil Sci. Soc. Am. J. 68:833-844.

Setia, R., Marschner, P., Baldock, J., Chittleborough, D., Smith, P., Smith, J. 2011. Salinity effects on carbon mineralization in soils of varying texture. Soil Biol. Biochem. 43:1908-1916.

Schimel, J.P., Scott, W.J., Killham, K. 1989. Changes in cytoplasmic carbon and nitrogen pools in a soil bacterium and a fungus in response to salt stress. Appl. Environ. Microbiol. 55:1635-1637.

Shrestha, R.K., Lal, R. 2011. Changes in physical and chemical properties of soil after surface mining and reclamation. Geoderma 161:168-176.

Ussiri, D.A.N., Lal, R. 2005. Carbon sequestration in reclaimed mine soils. Crit. Rev. Plant Sci. 24:151-165.

Verchot, L.V., Holmes, Z., Mulon, L., Groffman, P.M., Lovett, G.M. 2001. Gross vs net rates of N mineralization and nitrification as indicators of functional differences between forest types. Soil Biol. Biochem. 33:1889-1901.

Westerma, R.l., Tucker, T.C. 1974. Effect of salts and salts plus nitrogen-15-labeled ammonium chloride on mineralization of soil nitrogen, nitrification, and immobilization. Soil Sci. Soc. Am. Proc. 38:602-605.

Yan, N., Marschner, P. 2013. Response of soil respiration and microbial biomass to changing EC in saline soils. Soil Biol. Biochem. 65:322-328.

Yuan, B.C., Li, Z.Z., Liu, H., Gao, M., Zhang, Y.Y. 2007. Microbial biomass and activity in salt affected soils under arid conditions. Appl. Soil Ecol. 35:319-328.

Chapter 5 Nitrogen fertilization increased lodgepole pine and white spruce seedling growth in a reclaimed saline boreal forest soil

1 Introduction

The reconstruction of landscape after oil sands surface mining may increase soil salinity in reclaimed boreal forest soils in the Athabasca oil sands region (AOSR) of Alberta, Canada (Barbour et al., 2007). Salts, mainly sodium chloride (NaCl) and sodium sulfate (Na_2SO_4), may migrate from saline processed tailings sand (TS) or marine shale overburden (OB), that are produced from oil sands mining and are generally buried below the cover soil (Barbour et al., 2007). Sometimes salts are introduced into the cover soil when the saline OB material is mixed with the cover soil automatically. In addition, the upward movement of salts from the OB layers into the cover soil could raise the electrical conductivity (EC) level up to 6.0 dS m^{-1} in the lower part of peat-mineral soil mix (PMM) used as a cover soil (Kessler et al., 2010). According to the guidelines for reclamation to forest vegetation in the AOSR, if soil EC is greater than 4 dS m^{-1}, they are considered unfavorable for tree survival, plant growth and establishment of the forest ecosystem (Alberta Environment, 2010). For example, high soil salinity decreased osmotic potential, induced nutrient imbalance, and led to ion toxicity and influenced water and nitrogen (N) uptake, thus limiting plant growth (Aslam et al., 1984; Munns, 1993; Jacoby, 1994; Botella et al., 1997; Cornillon and Palloix, 1997; Dluzniewska et al., 2007; Munns and Tester, 2008).

Lodgepole pine (Pl, *Pinus contorta*) and white spruce (Sw, *Picea glauca*) are often planted for oil sands reclamation in the boreal forest region. However, boreal tree species are usually thought to have very limited tolerance to salinity (Allen et al., 1994). Although previous studies report that vegetation could survive on some natural sites with high salinity in boreal mixedwood forests due to relatively high soil moisture and nutrient availabilities in surface soils (Purdy et al., 2005; Lilles et al., 2010; Lilles et al., 2012), soil salinity is a challenge for the reestablishment of plant communities in reclaimed soils.

Nutrient availability, especially N availability, is another challenge for the reestablishment of plant communities in reconstructed soils, because mining and related activities may lead to severe loss of soil organic C and N due to the loss of topsoil and mechanical mixing of the A with the B and C horizon soils (Ussiri and Lal, 2005; Shrestha and Lal, 2011). Growth of planted trees could be affected by limited available N in reconstructed soils. To overcome low N availability, planted seedlings are usually fertilized in the initial 5 years after planting, and no fertilization is applied beyond that time (Fung and Macyk, 2000).

The detrimental effects of salinity on plant survival, growth, development and site productivity have been extensively reported (Boyer, 1982; Andrews et al., 1998; Renault et al., 1999; Rodrigue and Burger, 2004). Fertilization effects on seedling establishment and growth in oil sands reclamation have also been widely recognized (Khasa et al., 2005; Pinno et al., 2012; Sloan and Jacobs, 2013). Although beneficial effects of N fertilization on alleviation of salt stress have been reported for some annual herbaceous plant species (Villa-Castorena et al., 2003; Chen et al., 2010), no studies have been conducted to determine whether N fertilization plays a role in the amelioration of salt stress in boreal tree species. The understanding of the interactive effects of salt stress and N availability will be useful to develop management strategies to deal with growth limitation in saline soils with low N availability.

Therefore, the objective of this study was to evaluate the physiological and growth responses of Pl and Sw to N fertilization and salinity in a boreal forest soil in the AOSR in Alberta. I hypothesized that 1) seedling growth would be improved when N availability increases in saline boreal forest soils; and 2) the beneficial effect of N fertilization on seedling growth would be limited if soil salinity becomes the dominant factor restricting plant growth.

2 Materials and methods

2.1 Soil preparation, plant materials and growth conditions

The top 50 cm of mineral soil was collected from a boreal forest stand (56°09′N, 111°52′W) near Fort McMurray, Alberta after removing the forest floor layer. The mineral soil was passed through a 2 mm sieve and stored at 4 ℃ for later use. The PMM was formed by mixing the mineral soil with commercial peat moss and perlite with the ratio of 5:4:1 on a volumetric basis.

One-year-old container-grown (415D styroblocks^{TM}, Beaver Plastics Ltd., Acheson, Canada) Pl and Sw seedlings were obtained from the Smoky Lake Forest Nursery of Coast to Coast

Reforestation Inc. in Edmonton, Alberta, Canada. Seedlings that had gone through a chilling process were placed in a freezer at -3 ℃ for a six-week cold storage period prior to the greenhouse experiment. Seedlings were planted in pots with 15-cm diameter and 17-cm height in early January, 2014. The bottom of the pots was lined with a nylon mesh to prevent soil from falling through the holes. An aluminum pan was placed under each pot to collect leachates during irrigation events, and the leachates were reapplied back to the pots to prevent nutrient loss. Seedlings were placed in a controlled-environment greenhouse. Environmental conditions in the greenhouse were maintained with a day-time temperature of 24 ℃ and night-time temperature of 18 ℃, and 65% relative humidity. The photoperiod in the greenhouse was 18 h from 6 am to 12 pm, and photosynthetic photo flux density was 300-350 μmol m^{-2} s^{-1}. Pots were periodically rotated to minimize edge effects during the experiment.

2.2 Experimental design

The experiment used a 2 × 4 × 2 factorial design with two N fertilization rates, four salinity levels and two tree species. Each treatment was replicated five times. Seedlings were grown in the greenhouse for two weeks to allow them to recover from cold storage and to break dormancy before the treatments of N fertilizer and salinity. Half of the seedlings for each tree species received N fertilizer (applied as urea, 300 mg N $seedling^{-1}$, equivalent to 200 kg N ha^{-1}, N300), and the other half were not fertilized (N0) as the controls. Fertilizer was applied in solution; and the same amount of deionized water was added to the control. Seedlings were then subjected to 300 mL deionized water (S0), 50 (S50), 100 (S100) and 150 (S150) mmol L^{-1} NaCl. During the experiment, N fertilizer was split-applied for three times in a 20-day interval in a total of 300 mg N $seedling^{-1}$. Salinity treatments were also applied for three times in a 20-day interval one week after N fertilizer addition, and 300 mL of either deionized water or NaCl solution was added to the seedlings each time. Seedlings were irrigated with 200 mL deionized water every two days to maintain the soil moisture at 70% of water holding capacity.

2.3 Seedling physiological measurements

Seedlings were grown in the greenhouse for three months. Net photosynthetic rate (P_n), stomatal conductance (g_s) and transpiration rate (T) were measured in needles in the upper part of the crown before harvesting using a LI-6400XT portable photosynthesis system (LI-COR Inc., Lincoln, NE). After those measurements, needles were detached from the branches and scanned, and the SigmaScan Pro 5.0 software (Systat Software Inc., San Jose, CA) was used to calculate total leaf areas. Shoot water potential (Ψ_w) was measured in the distal 10-cm shoot using a Scholander-type pressure chamber (PMS Instrument Company, Albany, OR) before harvesting as described in Wan et al. (1999). Root hydraulic conductance was determined with a high pressure flow meter (HPFM) (Dynamax Inc., Houston, TX) after Ψ_w measurement as described in Tyree et al. (1995). Briefly, the stem of seedlings was excised 2 cm above the root collar, and the whole intact root system was connected to the HPFM system through the cut stem. Root systems were gradually pressurized by the pressurized water tank from 0 to 0.5 MPa to obtain a pressure-flow relationship. Root hydraulic conductivity (L_p) was calculated by dividing the root hydraulic conductance value by root dry weight.

2.4 Seedling growth measurements

The height and root collar diameter (RCD) of seedlings were measured with a meter stick and a digital vernier caliper (Traceable™, Thermo Fisher Scientific Inc., Waltham, MA), respectively, two weeks after planting and before harvest twelve weeks later. Height and RCD increments were calculated as the differences of height and RCD between the two measurements. The seedlings were harvested, washed with deionized water three times and separated into current-year and 1-year-old needles, stems and roots. After being oven-dried at 65 ℃ for 48 h, the dry weight (DW) of each component was determined.

2.5 Total C and N, and ^{13}C and ^{15}N abundance in seedlings

The oven-dried current-year and 1-year-old needles and roots were ground with a ball mill to pass through a 0.15 mm sieve for analyses of total C and N concentrations. Total C and N concentrations of roots were determined with an elemental analyzer (CE440, Exeter Analytical

Inc., North Chelmsford, MA).Total C and N concentrations, ^{13}C and ^{15}N abundance of current-year and 1-year-old needles were determined with an elemental analyzer (EuroEA3028-HT, EuroVector SpA., Tortona, Italy) coupled to a continuous-flow isotope ratio mass spectrometer (IRMS) (IsoPrime, GV Instruments Ltd., Manchester, UK). The ^{13}C and ^{15}N abundances were expressed as a deviation from international standards of atmospheric N_2 for ^{15}N and Pee Dee Belemite (PDB) for ^{13}C.

2.6 Analyses of soil properties

Soil properties of mineral soil, commercial peat moss and PMM including soil water content, pH, EC and concentrations of total C and N and inorganic N were measured before planting seedlings (Table 5-1). Inorganic N concentration in PMM was measured again after harvesting. The water content of each soil sample was measured by weighing a portion of the sample before and after oven-drying at 105 ℃ for 24 h. Soil pH was measured in deionized water using a digital-type DMP-2 mV/pH meter (Thermo Fisher Scientific Inc., Waltham, MA) with a soil to water ratio of 1:2 (w:v). Soil EC was measured by an AP75 portable waterproof conductivity/TDS meter (Thermo Fisher Scientific Inc., Waltham, MA) using 30 g of air-dried soil in 60 mL deionized water after shaking for 1 h at 25 ℃ and filtration through Whatman No. 42 filter papers. Concentrations of NH_4^+-N and NO_3^--N were determined using colorimetric methods as described in Keeney and Nelson (1982) and Miranda et al. (2001), respectively, using a UV-Vis spectrophotometer (Genesys^{TM} 10S, Thermo Fisher Scientific Inc., Waltham, MA). Fresh soil samples were extracted with 0.5 mol L^{-1} KCl at a 1:3 soil to solution ratio (w:v). After being shaken at 250 rpm on a mechanical shaker for 1 h, the samples were filtered through Whatman No. 42 filter papers. The KCl extracts were treated with reaction agents, and available NH_4^+-N and NO_3^--N concentrations determined as described earlier.

2.7 Statistical analyses

Since differences between tree species were not the focus of this study, a two-way analysis of variance (ANOVA) was applied to examine the statistical significance of seedling growth, biomass, physiological parameters, foliar and soil N concentrations and foliar $\delta^{13}C$ and $\delta^{15}N$ in response to salinity and N fertilization treatments for each tree species. If the interactions between salinity and N fertilization treatments were not significant, a one-way ANOVA was used to determine significant differences among different salinity levels within non-fertilized or fertilized seedlings, and a T-test was used to evaluate significant differences between non-fertilized and fertilized seedlings for the same salinity level. If the interactions were significant, the 'slice' function was used to detect significant differences by salinity or N fertilizer treatments within a specific level of the other treatment. Tukey's multiple comparison was used as the post-hoc test to detect significant differences at an α value of 0.05. The data of Ψ_w and L_p for Pl were logarithmically transformed to meet assumptions of normal distribution and homogeneous variance for ANOVA, and to meet the assumption of normal distribution for T-test. The non-transformed data were presented in this paper. All statistical analyses were conducted with the SAS software (SAS 9.2, SAS Institute Inc., Cary, NC).

3 Results

3.1 Seedling growth

Salinity and N fertilization affected Pl and Sw height and RCD increments (Table 5-2). Significant interactions were found between salinity and N fertilization in RCD increments for Pl, and in height increments for Sw. As salinity increased, height and RCD increments decreased in both non-fertilized and fertilized Pl and Sw seedlings (Table 5-3). The RCD increment in the non-saline treatment was two times greater than those in treatments with the highest salinity level. Salinity and N fertilization affected DW of different components in Pl and Sw seedlings except for the 1-year-old needles (Table 5-2), in which no differences were found among different salinity levels within non-fertilized or fertilized Pl and Sw seedlings (Table 5-3). The DW of current-year needles, stems and roots in Pl and Sw seedlings decreased as the salinity levels increased in both non-fertilized and fertilized seedlings. For the same salinity level, fertilized seedlings had higher total biomass than non-fertilized Pl and Sw seedlings.

3.2 Seedling physiological performance

Salinity and N fertilization significantly affected P_n, g_s T, Ψ_w and L_p in both Pl and Sw seedlings (Figure 5-1 and 5-2). For the same salinity levels, fertilized seedlings had higher P_n relative to non-fertilized Pl (Figure 5-1 A) and Sw seedlings (Figure 5-1 B) with the exception of the S150 treatment for both Pl and Sw seedlings, where fertilization did not affect P_n. Seedlings in the non-saline treatment had the highest P_n, and as salinity increased, P_n significantly decreased in both Pl and Sw seedlings in the N0 and N300 treatments.

Fertilization did not affect g_s in Pl (Figure 5-1 C) and Sw seedlings (Figure 5-1 D) within the same salinity level except for Pl seedlings in the S0 treatment, in which fertilization increased g_s. For Pl seedlings, g_s decreased with salinity regardless of fertilization. The g_s was higher in the S0 treatment than in the salinity treatments for Sw seedlings in N0 and N300, but there were no differences among the three salinity treatments.

Nitrogen fertilization did not affect T of Pl (Figure 5-1 E) and Sw seedlings (Figure 5-1 F) except for the S50 treatment in Sw seedlings, in which fertilization increased T. Transpiration rates also increased with increasing salinity in both Pl and Sw seedlings.

Shoot water potential decreased with increasing salinity regardless of the N treatment for both Pl (Figure 5-2 A) and Sw seedlings (Figure 5-2 B). Nitrogen fertilization decreased Ψ_w in the S50 and S100 treatments for Pl seedlings but did not affect Ψ_w in Sw seedlings, except that N fertilization decreased Ψ_w in the S150 treatment.

Nitrogen fertilization did not affect L_p in Pl (Figure 5-2 C) and Sw seedlings (Figure 5-2 D). For Pl seedlings, L_p decreased and then increased with salinity for both N0 and N300 treatments. Fertilized Pl seedlings in the S150 treatment had the highest L_p among all the treatments. However, for Sw seedlings, the L_p values were greater in the treatments with the highest salinity than in the other three salinity treatments for both N0 and N300, but there were no differences among the S0, S50 and S100 treatments.

3.3 Nitrogen concentrations in biomass and soil

Salinity and N fertilization affected N concentrations of current-year and one-year-old needles and roots in Pl and Sw seedlings, without N x salinity interactions (Table 5-4). Nitrogen concentrations in current-year and one-year-old needles and roots in fertilized Pl and Sw seedlings were about two times higher than that in the non-fertilized seedlings in each salinity level (Table 5-5). Nitrogen concentrations decreased with increasing salinity in Pl and Sw seedlings except for those in fertilized Sw seedlings, where no differences in N concentrations were found among different salinity levels.

Salinity and N fertilization affected concentrations of NH_4^+-N, NO_3^--N and inorganic N in soils three months after the treatments were applied (Table 5-4). Concentrations of NH_4^+-N, NO_3^--N and inorganic N in soils increased with the salinity in each N level for both Pl and Sw with the exception of concentrations of NO_3^--N and inorganic N in the N300 treatment for Pl, in which no differences were found among different salinity levels (Table 5-5). Nitrogen fertilization significantly increased concentrations of soil NH_4^+-N, NO_3^--N and inorganic N for both Pl and Sw regardless of the salinity levels. Concentrations of NO_3^--N and inorganic N were far greater in fertilized soils than those in non-fertilized soils in each salinity level.

3.4 Foliar $\delta^{15}N$ and $\delta^{13}C$ in seedlings

In the same salinity level, N fertilization increased foliar $\delta^{13}C$ and $\delta^{15}N$ in Pl and Sw seedlings (Figure 5-3). The salinity effects on foliar $\delta^{13}C$ and $\delta^{15}N$ were species specific (Figure 5-3). For non-fertilized Pl seedlings, salinity did not affect foliar $\delta^{13}C$ and $\delta^{15}N$ except for foliar $\delta^{13}C$ in the N0 treatment, in which it was higher than the other three salinity levels (Figure 5-3 A and C). However, foliar $\delta^{13}C$ increased and foliar $\delta^{15}N$ decreased with increasing salinity in fertilized Pl seedlings. On the other hand, foliar $\delta^{13}C$ increased and foliar $\delta^{15}N$ decreased with increasing salinity for non-fertilized Sw seedlings (Figure 5-3 B and D). For fertilized Sw seedlings, no difference in foliar $\delta^{13}C$ among different salinity levels were found, and foliar $\delta^{15}N$ in the S0 and S50 treatments were higher than those in the S100 and S150 treatments.

4 Discussion

Detrimental effects of soil salinity on plant growth have been shown in many studies. In this experiment, restricted plant growth and biomass in Pl and Sw seedlings were probably due to decreased net photosynthetic rates induced by salinity. This was consistent with the findings reported by Myers et al. (1990) in a halophytic grass, and by Tiwari et al. (1997) in rice. Ehlting et al. (2007) also found that salt stress reduced P_n and T of grey poplar (*Populus tremula* x *alba*) exposed to 75 mmol L^{-1} NaCl. Previous studies attributed the reduced photosynthesis to decreased stomatal conductance, restricted carboxylase activity and accumulation of Na^+ and Cl^- in leaf (Ungar, 1991; Bethke and Drew, 1992; Delfine et al., 1999).

Since N uptake is strongly dependent on water availability, and high salinity decreases osmotic water potential and water uptake, salt stress can significantly reduce uptake of inorganic N in several plant species (Hawkins and Lewis, 1993; Parida and Das, 2004; Rubinigg et al., 2005; Ehlting et al., 2007). In this experiment, N concentrations of needles and roots significantly decreased in the high salinity levels for both Pl and Sw seedlings, because salt-stressed plants could not uptake and utilize N as efficiently as plants under non-saline conditions (Pessarakli and Tucker, 1988; Bowman et al., 2006). Chen et al. (2010) reported increased Cl^- concentration and decreased cotton growth, and attributed the reduced N uptake of roots to the competitive inhibition of NO_3^--N uptake from Cl^- in saline soils (Xu et al., 2000; Abdelgadir et al., 2005). However, Ehlting et al. (2007) found that grey poplar exposed to 75 mmol L^{-1} NaCl had higher Na^+ and lower N concentration in all tissues, and they attributed the reduced uptake of ammonium and nitrate to competitive inhibition from Na^+ and decreased N demand for plant growth in a saline medium, respectively (Bradley and Morris, 1991; Rubinigg et al., 2005).

In contrast to the finding in González-Moreno et al. (1997) that salt stress enhanced respiration rates, high salinity levels significantly decreased respiration rates in both Pl and Sw seedlings. This was accompanied by reduced shoot water potential in this experiment, suggesting limited water uptake resulted from high salinity. On the other hand, differential responses of root hydraulic conductivity to salinity highlighted the different tolerance of Pl and Sw. Previous study showed that the application of salt resulted in the reduction of root hydraulic conductivity of *Phaseolus vulgaris* exposed to 30 mmol L^{-1} NaCl because of the decrease of aquaporin activity and osmotic potential and the toxicity of Na^+ and Cl^- (Calvo-Polanco et al., 2014). However, when exposed to 100 or 150 mmol L^{-1} NaCl, the root hydraulic conductivity increased in both Pl and Sw seedlings. The mechanism of the root hydraulic conductivity response to high salinity was not clear. It is probably because the high salt concentrations impaired the integrity of roots, and when pressure was applied during measurements, the roots were easily broken, and the root would lose regulation of water uptake and transport. Few previous studies observed recovery of decreased root hydraulic conductivity after salt addition (Wan, 2010; Aroca et al., 2011), and those that have attributed it to accumulated solutes in roots after long-term exposure to salts, which resulted in partial recovering of root hydraulic conductivity.

Foliar $\delta^{13}C$ has been shown to be a good indicator of water availability, as foliar $\delta^{13}C$ integrates soil water availability over time and is closely related to plant water use efficiency (Sun et al., 1996). The closure of stomata under water limitation decreases $^{13}CO_2$ diffusion rates, increases the fixation of $^{13}CO_2$, and reduces the discrimination against ^{13}C during photosynthesis, resulting in less negative $\delta^{13}C$ in plant tissues (Matsushima and Chang, 2007). Previous studies reported that N fertilization significantly increased foliar $\delta^{13}C$ in plants and they attributed the increased foliar $\delta^{13}C$ to enhanced carboxylation efficiencies by improved N nutrition (Livingston et al., 1999; Choi et al., 2005). In this study the increased foliar $\delta^{13}C$ in both Pl and Sw seedlings in the salinity treatments reflected the improved N nutrition after fertilization as well as the restricted water uptakes induced by salinity (Villa-Castorena et al., 2003). Fertilization significantly increased foliar $\delta^{15}N$ in both Pl and Sw seedlings, which was in agreement with Högberg (1991) and Högberg et al. (1992) that trees with high N availability showed a greater foliar δ^{15}, while trees with low N availability developed a lower foliar δ^{15}. The stronger retention of ^{15}N than ^{14}N in soil resulted in ^{15}N enrichment in needles (Högberg, 1991).

Nitrogen fertilization significantly increased P_n, N concentrations in needles and roots and foliar $\delta^{15}N$, indicating the alleviation of growth limitations that resulted in improved seedling growth and total biomass for both Pl and Sw. Previous studies showed that the alleviation from salinity following N fertilization was dependent on plant species, salinity level, N source and application rate, and other environmental conditions (Soliman et al., 1994; Gómez et al., 1996; Grattan and Grieve, 1999; Albassam, 2001; Tuna et al., 2007). Fertilization at 135 and 270 kg N ha^{-1}could increase N uptake and alleviate the inhibited growth of cotton under low to moderate salt conditions, whereas application rates as high as 405 kg N ha^{-1} showed no beneficial effects (Chen et al., 2010). Ammonium-fed herbaceous species were more sensitive to salt stress compared with nitrate-fed plants (Bourgeais-Chaillou et al., 1992; Hawkins and Lewis, 1993; Ashraf and Sultana, 2000). However, Flores et al. (2001) showed that NH_4^+-N addition could minimize the deleterious effects on biomass production in tomato plants exposed to 60 mmol L^{-1} NaCl, and attributed to increased concentrations of iron (Fe) and chlorophyll in salt-stressed plants, which would benefit for photosynthetic rates and N assimilation. On the other hand, Ehlting et al. (2007) concluded that nitrate had better performance than ammonium as N sources regarding the growth of grey poplar grown in saline soils. Gómez et al. (1996) also reported increased growth and yield of sweet pepper under saline conditions when nitrate was supplied. Accumulation of amino acids in plant tissues after N fertilization was reported to be responsible for the alleviation of the adverse effects from salinity. Ehlting et al. (2007) reported that concentrations of amino acids in roots and leaves of fertilized grey poplar seedlings significantly increased when exposed to 75 mmol L^{-1} NaCl. The amino acids as osmoprotectants were likely to counterbalance increased osmotic potential from Na^+ and Cl^- and protect membranes and metabolites by scavenging reactive oxygen species (ROS), thus protecting cells from further damage (Kishor et al., 1995; Mansour, 2000; Ehlting et al., 2007). However, no alleviation of growth inhibition from salinity by N fertilization was observed at high salinity levels in this study, which suggested that the deleterious effects of salinity outweighed the beneficial effects of fertilization on Pl and Sw seedling growth, and seedling growth was mainly influenced by salt stress.

5 Conclusions

Nitrogen fertilization alleviated salt stress for both Pl and Sw seedlings under low to medium salinity, and increased height and RCD growth and total seedling biomass, suggesting N fertilization is effective in enhancing seedling growth in saline boreal forest soils. Improved seedling growth was attributed to the increased N uptake by roots and subsequent higher N concentrations in needles, and increased net photosynthetic rates. However, the detrimental effects of high salinity on the seedlings could not be offset by N fertilization because of reduced net photosynthetic rates, stomatal conductance, transpiration rates and N and water uptake by roots, thus limiting seedling growth. In any case, salinity and N availability should be equally considered when planning for oil sands reclamation. In reclaimed saline boreal forest soils with low N availability, N fertilization would be effective to improve plant growth. However, the effectiveness of fertilization can be substantially reduced by high salinity in reclaimed soils.

Table 5-1 Properties of commercial peat moss (P), mineral soil (M) and peat-mineral soil mix (PMM) used in the greenhouse study.

Soil type	Soil pH	Soil EC[a] (dS m^{-1})	Total C (g kg^{-1})	Total N (g kg^{-1})	NH_4^+-N (mg kg^{-1})	NO_3^--N (mg kg^{-1})
P	4.37 (0.03)[b]	1.32 (0.054)	466.5 (5.5)	17.1 (0.07)	156.6 (4.2)	148.0 (3.36)
M	5.15 (0.03)	0.08 (0.001)	8.0 (0.6)	0.5 (0.03)	9.4 (0.3)	0.5 (0.04)
PMM	4.39 (0.01)	0.92 (0.011)	62.8 (0.6)	2.1 (0.23)	36.8 (1.0)	19.3 (1.65)

[a] EC stands for electrical conductivity.

[b] numbers in parentheses are standard errors of means (n = 3).

Table 5-2 ANOVA *p* values for the main effects of salinity and N application rate and their interactions on seedling growth and biomass of lodgepole pine (Pl) and white spruce (Sw). Four levels of salinity (S, 0, 50, 100 and 150 mmol L^{-1} NaCl) and two levels of N fertilization (N, 0 and 300 mg N $seedling^{-1}$) were studied.

Tree species	Treatment	Height increment	RCD[a] increment	Seedling biomass				
				Current-year needle	1-year-old needle	Stem	Root	Total
Pl	S	0.034	<0.001	<0.001	0.307	<0.001	0.004	<0.001
	N	<0.001	<0.001	<0.001	0.079	<0.001	0.006	<0.001
	S×N	0.965	0.019	<0.001	0.940	0.030	0.537	<0.001
Sw	S	<0.001	0.010	0.014	0.930	0.041	0.023	0.038
	N	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	S×N	0.023	0.777	0.571	0.787	0.818	0.037	0.296

[a] RCD stands for root collar diameter.

Table 5-3 Seedling growth and biomass of lodgepole pine (Pl) and white spruce (Sw) in response to salinity (S, 0, 50, 100 and 150 mmol L^{-1} NaCl) and N fertilization (N, 0 and 300 mg N $seedling^{-1}$) treatments three months after planting.

Tree species	Treatment	Seedling growth		Seedling biomass (g $seedling^{-1}$)				
		Height incre.[a] (cm)	RCD[b] incre.	Current-year needle	1-year-old needle	Stem	Root	Total
Pl	S0N0	7.1 a[*]	3.2 a	4.55 a	1.57	2.35 a	2.67 a	11.14 a
	S50N0	6.4 ab	2.5 b	4.34 a	1.68	1.80 ab	2.18 ab	10.00 ab
	S100N0	5.8 ab	1.7 c	3.80 ab	1.44	1.57 b	2.22 ab	9.03 b
	S150N0	4.6 b	1.4 c	3.17 b	1.26	1.37 b	1.47 b	7.27 c
	S0N300	8.0 A	3.9 A	8.29 A	1.63	3.07 A	2.28 A	15.27 A
	S50N300	6.9 A	3.4 A	8.41 A	1.77	2.96 A	2.50 A	15.64 A
	S100N300	6.7 AB	3.4 A	8.26 A	1.49	2.59 A	2.30 A	14.64 A
	S150N300	5.2 B	1.8 B	4.78 B	1.47	1.50 B	1.66 B	9.41 B
Sw	S0N0	12.4 a	2.7 a	3.21 a	1.92	4.07 a	2.10 a	11.30 a
	S50N0	10.6 b	2.4 a	2.70 ab	1.50	3.55 ab	2.08 a	9.83 ab
	S100N0	9.5 bc	2.1 a	2.89 ab	1.26	3.47 ab	1.48 ab	9.10 bc
	S150N0	8.3 c	1.2 b	2.30 b	1.33	2.63 b	0.92 b	7.18 c
	S0N300	15.0 A	3.3 A	3.55 A	2.04 A	4.30 A	3.42 A	13.31 A
	S50N300	11.1 B	2.6 A	2.61 B	1.34 B	4.14 A	2.38	10.47 B
	S100N300	10.0 BC	2.5 A	2.68 B	1.31 B	3.92 A	1.45	9.36
	S150N300	9.5 C	1.6 B	2.40 B	1.28 B	2.76 B	0.99 C	7.43 C

[a] incre. stands for increment

[b] RCD stands for root collar diameter.

[*] Different lower- and uppercase letters indicate significant differences ($p < 0.05$) within non-fertilized and fertilized seedlings, respectively (n = 5).

Table 5-4 ANOVA *p* values for the main effects of salinity and N application rate and their interactions on N concentrations of current-year and one-year-old needles and roots in lodgepole pine (Pl) and white spruce (Sw) seedlings and in soils. Four levels of salinity (S, 0, 50, 100 and 150 mmol L^{-1} NaCl) and two levels of N fertilization (N, 0 and 300 mg N $seedling^{-1}$) were studied.

Tree species	Treatment	Seedling			Soil		
		Current-year needle	1-year-old needle	Root	NH_4^+-N	NO_3^--N	Inorganic N
Pl	S	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	N	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	S×N	0.867	0.820	0.072	0.071	0.045	0.040
Sw	S	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	N	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	S×N	0.643	0.175	0.197	<0.001	0.001	<0.001

Table 5-5 Nitrogen concentrations of current-year and one-year-old needles and roots in lodgepole pine (Pl) and white spruce (Sw) seedlings and in soils in response to salinity (S, 0, 50, 100 and 150 mmol L^{-1} NaCl) and N fertilization (N, 0 and 300 mg N $seedling^{-1}$) treatments three months after planting.

Tree species	Treatment	Seedling (mg g^{-1})			Soil (mg kg^{-1})		
		Current-year needle	1-year-old needle	Root	NH_4^+-N	NO_3^--N	Inorganic N
Pl	S0N0	9.4 a[*]	8.1 a	12.1 a	4.9 c	0.3 c	5.2 d
	S50N0	7.8 b	7.6 a	9.9 b	6.6 b	0.4 c	7.0 c
	S100N0	6.3 c	6.4 ab	8.7 bc	7.6 ab	1.1 b	8.7 b
	S150N0	5.7 c	5.4 b	7.3 c	8.0 a	3.1 a	11.1 a
	S0N300	19.6 A	16.4 A	16.7 A	6.6 C	104.8	111.4
	S50N300	18.6 AB	16.3 A	16.5 A	7.0 BC	92.3	99.3
	S100N300	17.4 AB	14.2 B	14.9 AB	8.0 B	92.1	100.1
	S150N300	16.4 B	14.0 B	13.7 B	9.2 A	85.8	95.0
Sw	S0N0	10.8 a	12.6 a	14.5 a	4.8 b	1.6 c	6.4 c
	S50N0	10.7 a	11.5 a	12.4 b	5.0 b	3.4 b	8.4 bc
	S100N0	9.0 b	10.4 a	11.6 b	6.3 b	4.1 ab	10.4 b
	S150N0	6.2 c	7.1 b	11.7 b	8.7 a	5.2 a	13.9 a
	S0N300	22.5	19.7	20.9	11.5 B	108.4 B	119.9 B
	S50N300	21.4	20.0	20.4	13.6 B	104.2 B	117.8 B
	S100N300	20.2	18.3	19.7	13.5 B	136.8 A	150.3 A
	S150N300	18.8	17.4	20.1	26.9 A	139.2 A	166.1 A

[*] Different lower- and uppercase letters indicate significant differences ($p < 0.05$) within non-fertilized and fertilized seedlings, respectively (n = 5).

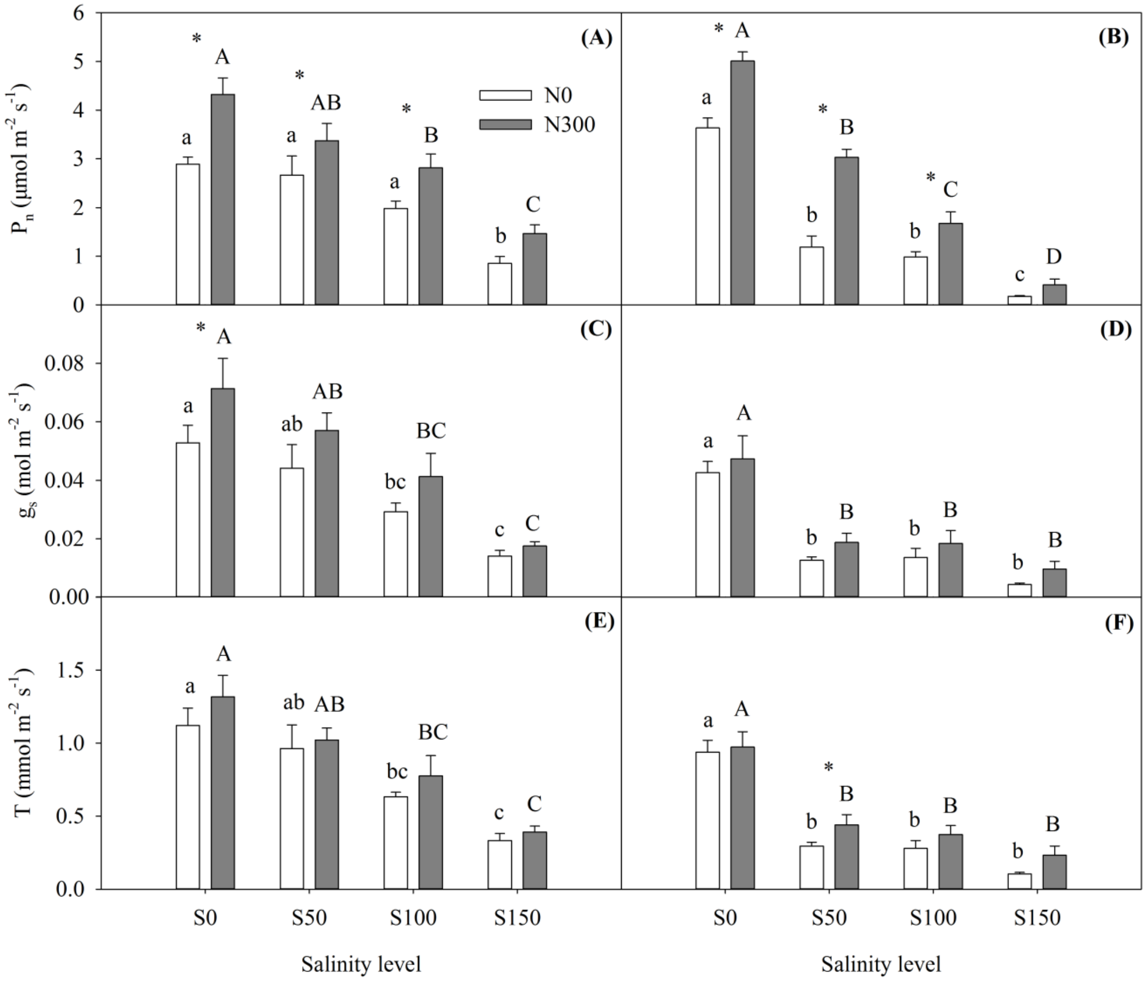

Figure 5-1 Net photosynthetic rate (P_n, A), stomatal conductance (g_s, C) and transpiration rate (T, E) in lodgepole pine, and P_n (B), g_s (D) and T (F) in white spruce in response to salinity (S, 0, 50, 100 and 150 mmol L^{-1} NaCl) and N fertilization (N, 0 and 300 mg N seedling^{-1}) treatments three months after planting. Different lower- and uppercase letters indicate significant differences ($p < 0.05$) within non-fertilized and fertilized seedlings, respectively. Asterisks indicate significant differences ($p < 0.05$) between non-fertilized and fertilized seedlings for the same salinity level. Vertical bars are standard errors of means (n = 5).

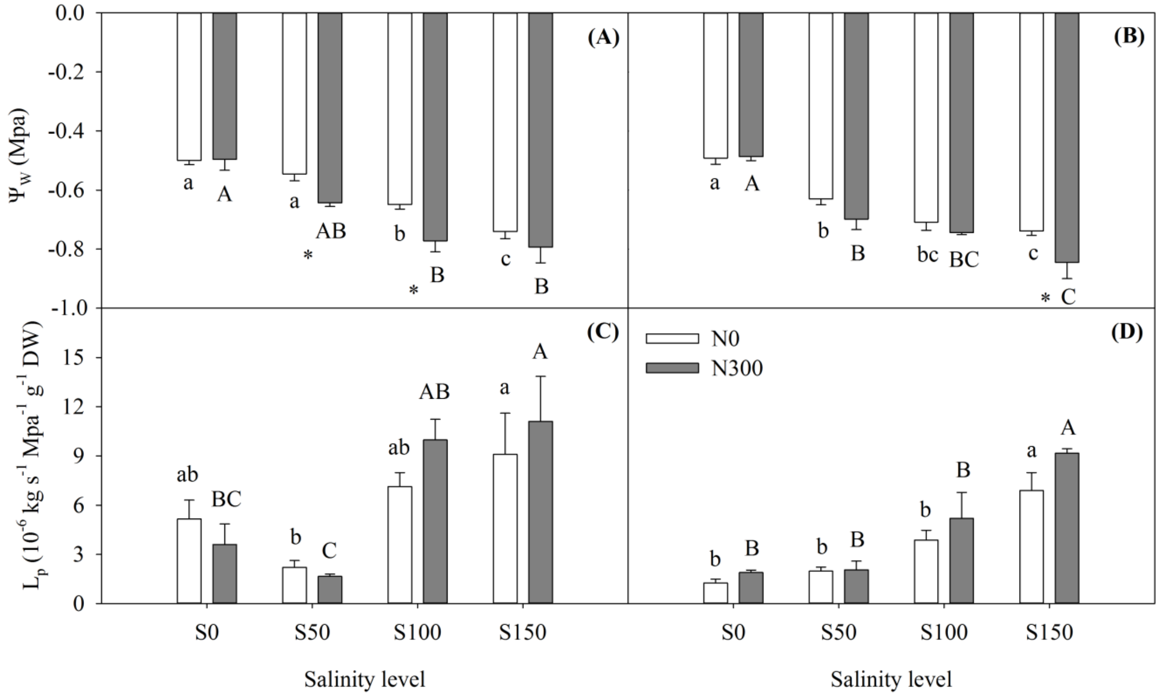

Figure 5-2 Shoot water potential (Ψ_w, A) and root hydraulic conductivity (L_p, C) in lodgepole pine, and Ψ_w (B) and L_p (D) in white spruce in response to salinity (S, 0, 50, 100 and 150 mmol L^{-1} NaCl) and N fertilization (N, 0 and 300 mg N seedling^{-1}) treatments three months after planting. Different lower- and uppercase letters indicate significant differences ($p < 0.05$) within non-fertilized and fertilized seedlings, respectively. Asterisks indicate significant differences ($p < 0.05$) between non-fertilized and fertilized seedlings for the same salinity level. Vertical bars are standard errors of means (n = 5).

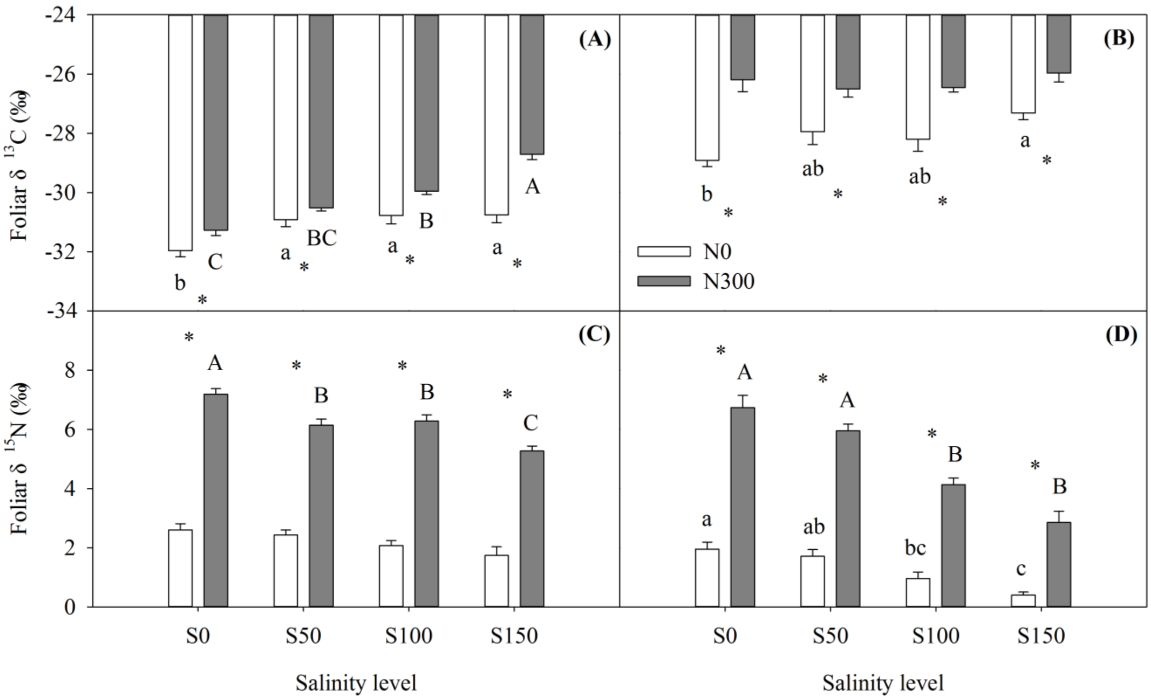

Figure 5-3 Foliar δ^{13}C (A) and δ^{15}N (C) in lodgepole pine current-year needles, and δ^{13}C (B) and δ^{15}N (D) in white spruce current-year needles in response to salinity (S, 0, 50, 100 and 150 mmol L^{-1} NaCl) and N fertilization (N, 0 and 300 mg N seedling^{-1}) treatments three months after planting. Different lower- and uppercase letters indicate significant differences ($p < 0.05$) within non-fertilized and fertilized seedlings, respectively. Asterisks indicate significant differences ($p < 0.05$) between non-fertilized and fertilized seedlings for the same salinity level. Vertical bars are standard errors of means (n = 5).

References

Abdelgadir, E.M., Oka, M., Fujiyama, H. 2005. Characteristics of nitrate uptake by plants under salinity. J. Plant Nutr. 28:33-46.

Albassam, B.A. 2001. Effect of nitrate nutrition on growth and nitrogen assimilation of pearl millet exposed to sodium chloride stress. J. Plant Nutr. 24:1325-1335.

Alberta Environment. 2010. Guidelines for Reclamation to Forest Vegetation in the Athabasca Oil Sands Region, 2nd Edition. Prepared by the Terrestrial Subgroup of the Reclamation Working Group of the Cumulative Environmental Management Association, Fort McMurray, AB. December 2009.

Allen, J.A., Chambers, J.L., Stine, M. 1994. Prospects for increasing the salt tolerance of forest trees: A review. Tree Physiol. 14:843-853.

Andrews, J.A., Johnson, J.E., Torbert, J.L., Burger, J.A., Kelting, D.L. 1998. Mine soil and site properties associated with early height growth of eastern white pine. J. Environ. Qual. 27:192-199.

Aroca, R., Porcel, R., Ruiz-Lozano, J.M. 2011. Regulation of root water uptake under abiotic stress conditions. J. Exp. Bot. 63:43-57.

Ashraf, M., Sultana, R. 2000. Combination effect of NaCl salinity and nitrogen form on mineral composition of sunflower plants. Biol. Plantarum. 43:615-619.

Aslam, M., Huffaker, R.C., Rains, D.W. 1984. Early effects of salinity on nitrate assimilation in barley seedlings. Plant Physiol. 76:321-325.

Barbour, S.L., Chanasyk, D., Hendry, J., Leskiw, L., Macyk, T., Mendoza, C., Naeth, A., Nichol, C., O'Kane, M., Purdy, B., Qualizza, C., Quideau, S., Welham, C. 2007. Soil Capping Research in the Athabasca Oil Sands Region. Volume 1: Technology Synthesis. Syncrude Canada Ltd., Fort McMurray, AB.

Bethke, P.C., Drew, M.C. 1992. Stomatal and non-stomatal components to inhibition of photosynthesis in leaves of *Capsicum annuum* during progressive exposure to NaCl salinity. Plant Physiol. 99:219-226.

Botella, M.A., Martínez, V., Nieves, M., Cerdá, A. 1997. Effect of salinity on the growth and nitrogen uptake by wheat seedlings. J. Plant Nutr. 20:793-804.

Bourgeais-Chaillou, P., Perez-Alfocea, F., Guerrier, G. 1992. Comparative effects of N-sources on growth and physiological responses of soyabean exposed to NaCl-stress. J. Exp. Bot. 43:1225-1233.

Bowman, D.C., Devitt, D.A., Miller, W.W. 2006. The effect of moderate salinity on nitrate leaching from bermudagrass turf: A lysimeter study. Water Air Soil Pollut. 175:49-60.

Boyer, J.S. 1982. Plant productivity and environment. Science 218:443-448.

Bradley, P.M., Morris, J.T. 1991. The Influence of salinity on the kinetics of NH_4^+ uptake in *Spartina alterniflora*. Oecologia 85:375-380.

Calvo-Polanco, M., Sa´nchez-Romera, B., Aroca, R. 2014. Mild salt stress conditions induce different responses in root hydraulic conductivity of *Phaseolus vulgaris* over-time. PLoS

ONE. 9(3), e90631. doi:10.1371/journal.pone.0090631.

Chen, W., Hou, Z., Wu, L., Liang, Y., Wei, C. 2010. Effects of salinity and nitrogen on cotton growth in arid environment. Plant Soil 326:61-73.

Choi, W.J., Chang, S.X., Hao, X. 2005. White spruce response to co-composted hydrocarbon-contaminated drilling waste: Effects of compost age and nitrogen fertilization. J. Environ. Qual. 34:1319-1327.

Cornillon, P., Palloix, A. 1997. Influence of sodium chloride on the growth and mineral nutrition of pepper cultivars. J. Plant Nutr. 20:1085-1094.

Delfine, S., Alvino, A., Villani, M.C., Loreto, F. 1999. Restrictions to carbon dioxide conductance and photosynthesis in spinach recovering from salt stress. Plant Physiol. 119:1101-1106.

Dluzniewska, P., Gessler, A., Dietrich, H., Schnitzler, J.P., Teuber, M., Rennenberg, H. 2007. Nitrogen uptake and metabolism in *Populus* x *canescens* as affected by salinity. New Phytol. 173:279-293.

Ehlting, B., Dluzniewska, P., Dietrich, H., Selle, A., Teuber, M., Hänsch, R., Nehls, U., Polle, A., Schnitzler, J.P., Rennenberg, H., Gessler, A. 2007. Interaction of nitrogen nutrition and salinity in grey poplar (*Populus tremula* x *alba*). Plant Cell Environ. 30:796-811.

Flores, P., Carvajal, M., Cerda, A., Martinez, V. 2001. Salinity and ammonium/nitrate interactions on tomato plant development, nutrition, and metabolites. J. Plant Nutr. 24:1561-1573.

Fung, M.Y.P., Macyk, T.M. 2000. Reclamation of oil sands mining areas. In: Barnhisel, R.I., Darmody, R.G., Daniels, W.L. (Eds.), Reclamation of Drastically Disturbed Lands. ASA, CSSA and SSSA, Madison, Wisconsin. p. 755-774.

Gómez, I., Pedreño, J.N., Moral, R., Iborra, M.R., Palacios, G., Mataix, J. 1996. Salinity and nitrogen fertilization affecting the macronutrient content and yield of sweet pepper plants. J. Plant Nutr. 19:353-359.

González-Moreno, S., Gómez-Barrera1, J., Perales, H., Moreno-Sánchez, R. 1997. Multiple effects of salinity on photosynthesis of the protist Euglena gracilis. Physiol. Plant 101:777-786.

Grattan, S.R., Grieve, C.M. 1999. Salinity-mineral nutrient relations in horticultural crops. Sci. Hortic. 78:127-157.

Hawkins, H.J., Lewis, O.A.M. 1993. Effect of NaC1 salinity, nitrogen form, calcium and potassium concentration on nitrogen uptake and kinetics in *Triticum aestivum* L. cv. Gamtoos. New Phytol. 124:171-177.

Högberg, P. 1991. Development of ^{15}N enrichment in a nitrogen-fertilized forest soil-plant system. Soil Biol. Biochem. 23:335-338.

Högberg, P., Tamm, C.O., Högberg, M. 1992. Variations in ^{15}N abundance in a forest fertilization trial: Critical loads of N, N saturation, contamination and effects of revitalization fertilization. Plant Soil 142:211-219.

Jacoby, B. 1994. Mechanisms involved in salt tolerance by plants. In: Pessarakli, M. (Ed.), Handbook of Plant and Crop Stress. Marcel Dekker Inc., New York, NY. p. 97-145.

Keeney, D.R., Nelson, D.W. 1982. Nitrogen-inorganic forms. In: Page, A. L. (Ed.), Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties, 2nd edition. ASA and SSSA, Madison, Wisconsin. p. 643-698.

Kessler, S., Barbour, S.L., van Rees, K.C.J., Dobchuk, B.S. 2010. Salinization of soil over saline-sodic overburden from the oil sands in Alberta. Can. J. Soil Sci. 90:637-647.

Khasa, D.P., Fung, M., Logan, B. 2005. Early growth response of container-grown selected woody boreal seedlings in amended composite tailings and tailings sand. Bioresour. Technol. 96:857-864.

Kishor, P.B.K., Hong, Z., Miao, G.H., Hu, C.A.A., Verma, D.P.S. 1995. Overexpression of D-pyrroline-5-carboxylate synthetase increases proline production and confers osmotolerance in transgenic plants. Plant Physiol. 108:1387-1394.

Lilles, E.B., Purdy, B.G., Chang, S.X., Macdonald, S.E. 2010. Soil and groundwater characteristics of saline sites supporting boreal mixedwood forests in northern Alberta. Can. J. Soil Sci. 90:1-14.

Lilles, E.B., Purdy, B.G., Macdonald, S.E., Chang, S.X. 2012. Growth of aspen and white spruce on naturally saline sites in northern Alberta: Implications for development of boreal forest vegetation on reclaimed saline soils. Can. J. Soil Sci. 92:213-227.

Livingston, N.J., Guy, R.D., Ethier, G.J. 1999. The effects of nitrogen stress on the stable carbon isotope composition, productivity and water use efficiency of white spruce (*Picea glauca*

(Moench) Voss) seedling. Plant Cell Environ. 22:281-289.

Mansour, M.M.F. 2000. Nitrogen containing compounds and adaptation of plants to salinity stress. Biol. Plant. 43:491-500.

Matsushima, M., Chang, S.X. 2007. Nitrogen and water availabilities and competitiveness of bluejoint: Spruce growth and foliar carbon-13 and nitrogen-15 abundance. Soil Sci. Soc. Am. J. 71:1547-1554.

Miranda, K.M., Espey, M.G., Wink, D.A. 2001. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide 5:62-71.

Munns, R. 1993. Physiological processes limiting plant growth in saline soils: Some dogmas and hypotheses. Plant Cell Environ. 16:15-24.

Munns, R., Tester. M. 2008. Mechanisms of salinity tolerance. Annu. Rev. Plant Biol. 59:651-681.

Myers, B.A., Neales, T.F., Jones, M.B. 1990. The influence of salinity on growth, water relations and photosynthesis in *Diplachne fuca* (L.). P. Beauv. ex. Roemer & Schultes. Aust. J. Plant Physiol. 17:675-691.

Parida, A.K., Das, A.B. 2004. Effects of NaCl stress on nitrogen and phosphorous metabolism in a true mangrove *Bruguiera parviflora* grown under hydroponic culture. J Plant Physiol. 161:921-928.

Pessarakli, M., Tucker, T.C. 1988. Dry matter yield and nitrogen-15 uptake by tomatoes under sodium chloride stress. Soil Sci. Soc. Am. J. 52:698-700.

Pinno, B.D., Landhäusser, S.M., MacKenzie, M.D., Quideau, S.A., Chow, P.S. 2012. Trembling aspen seedling establishment, growth and response to fertilization on contrasting soils used in soil sands reclamation. Can. J. Soil Sci. 92:143-151.

Purdy, B.G., Macdonald, S.E., Lieffers, V.J. 2005. Naturally saline boreal communities as models for reclamation of saline oil sand tailings. Restor. Ecol. 13:667-677.

Renault, S., Paton, E., Nilsson, G., Zwiazek, J.J., MacKinnon, M.D. 1999. Responses of boreal plants to high salinity oil sands tailings water. J. Environ. Qual. 28:1957-1962.

Rodrigue, J. A., Burger, J. A. 2004. Forest soil productivity of mined land in the mid-western and eastern coalfield regions. Soil Sci. Soc. Am. J. 68:833-844.

Rubinigg, M., Posthumus, F.S., Elzenga, J.T.M. 2005. Effect of NaCl salinity on nitrate uptake in *Plantago maritima* L. Phyton-Ann. REI Bot. 45:295-302.

Shrestha, R.K., Lal, R. 2011. Changes in physical and chemical properties of soil after surface mining and reclamation. Geoderma 161:168-176.

Sloan. J.L., Jacobs, D.F. 2013. Fertilization at planting influences seedling growth and vegetative competition on a post-mining boreal reclamation site. New For. 44:687-701.

Soliman, M.S., Shalabi, H.G., Campbell, W.F. 1994. Interaction of salinity, nitrogen, and phosphorus fertilization on wheat. J. Plant Nutr. 17:1163-1173.

Sun, Z.J., Livingston, N.J., Guy, R.D., Ethier, G.J. 1996. Stable carbon isotopes as indicators of increased water use efficiency and productivity in white spruce (*Picea glauca* (Moench) Voss) seedlings. Plant Cell Environ. 19:887-894.

Tiwari, B.S., Bose, A. Ghosh, B. 1997. Photosynthesis in rice under salt stress. Photosynth. 34:303-306.

Tuna, A.L., Kayab, C., Ashraf, M., Altunlu, H., Yokas, I., Yagmur, B. 2007. The effects of calcium sulphate on growth, membrane stability and nutrient uptake of tomato plants grown under salt stress. Environ. Exp. Bot. 59:173-178.

Tyree, M.T., Patiño, S., Bennink, J., Alexander, J. 1995. Dynamic measurements of root hydraulic conductance using a high-pressure flow meter in the laboratory and field. J. Exp. Bot. 46:83-94.

Ungar, I.A. 1991. Photosynthesis. In: Ungar, I.A. (Ed.), Ecophysiology of Vascular Halophytes. CRC Press Inc., Boca Raton, FL. p. 73-86.

Ussiri, D.A.N., Lal, R. 2005. Carbon sequestration in reclaimed mine soils. Crit. Rev. Plant Sci. 24:151-165.

Wan, X.C. 2010. Osmotic effects of NaCl on cell hydraulic conductivity of corn roots. Acta Biochim. Biophys. Sin. 42:351-357.

Wan, X., Landhäusser, S.M., Zwiazek, J.J., Lieffers, V.J. 1999. Root water flow and growth of aspen (*Populus tremuloides*) at low root temperatures. Tree Physiol. 19:879-884.

Xu, G., Magen, H., Tarchitzky, J., Kafkafi, U. 2000. Advances in chloride nutrition of plants. Adv. Agron. 68:97-150.

Villa-Castorena, M., Ulery, A.L., Catalan-Valencia, E.A., Remmenga, M.D. 2003. Salinity and nitrogen rate effects on the growth and yield of chile pepper plants. Soil Sci. Soc. Am. J.

67:1781-1789.

Chapter 6 Synthesis, conclusions and future research

1 Overview of study objectives

Reclaimed soils in the Athabasca oil sands region (AOSR) in Alberta often possess some properties that negatively influence tree growth. Those properties include low nutrient availability (McMillan et al., 2007; Hemstock et al., 2010), limited water availability (Naeth et al., 2011; Jung et al., 2014), high soil salinity (Renault et al., 1999), high pH (Howat, 2000), extensive soil compaction, and high concentrations of residual bitumen (Fung and Macyk, 2000). Under such conditions, plants may experience growth limitations and develop stunted growth (Fisher and Binkley, 2000). One such example is the foliar discoloration of planted trees observed in some reclaimed areas in the Suncor Energy Inc. Lease 86/17 in the AOSR. Poor growth of trees will affect the success of oil sands reclamation.

Initial objective of this research was to identify the limiting factors for growth of two tree species planted for oil sands reclamation, including lodgepole pine (*Pinus contorta*, Pl) planted in peat-mineral soil mix (PMM) over tailing sands (TS), and white spruce (*Picea glauca*, Sw) planted in PMM over overburden (OB) in reconstructed sites in the AOSR. Through analyses of foliar nutrient concentrations and soil chemical and physical properties, as well as comparisons of tree growth on sites with different site productivity, the main limiting factors for Sw and Pl growth were identified (Chapter 2). Low nutrient and water availabilities and high soil salinity have been reported in previous studies, and water availability was the concern of another parallel study by House (2014), therefore a single tree fertilization experiment was conducted to test the growth responses of Pl and Sw with poor tree growth to fertilization in reclaimed sites (Chapter 3). High salinity was reported to influence N cycling processes (Inubushi et al., 1999; Irshad et al., 2005; Cheng et al., 2013), therefore a laboratory incubation experiment was carried out to evaluate short-term effects of salinity (NaCl) on gross N transformations in PMM material collected from a reclaimed site using the ^{15}N tracing technique (Chapter 4). Chapter 5 investigated whether N fertilization would improve growth of salt-stressed Pl and Sw seedlings in a greenhouse experiment. Based on the research completed, understanding the interaction between soil salinity and N cycling, interactive effects of salinity and N fertilization on seedling growth would be obtained, and recommendations of proper management practices would be developed to correct growth limiting factors in some reclaimed sites in the AOSR. The key findings of this thesis research were listed in this chapter (Figure 6-1).

2 Summary of the research results

2.1 Nitrogen availability and tree growth

Limiting factors for Pl and Sw tree growth in the area studied were species and site specific. Significant correlation of mean annual growth of aboveground biomass (ABG), DBH (DBHG) or height (HG) with volumetric water content (VWC) in PMM and with foliar $\delta^{13}C$ in current-year needles both suggested that Pl planted on PMM over TS sites was restricted by low water availability. Low water holding capacity of TS substrate, abrupt changes of soil texture induced from reconstruction of multi-layered soils, relatively thin PMM thickness and high total plant community cover all could contribute to the low water availability in reclaimed sites.

On the other hand, positive relationships between tree growth (ABG, DBHG and HG) and soil inorganic N concentrations demonstrated that Sw planted on PMM over OB sites was limited by low soil N availability. Foliar N concentrations in current-year needles in all sites were less than the critical value of 13 mg g^{-1} that indicates N deficiency for Sw (Ballard and Carter, 1986) except for the high productivity sites. Soil inorganic N concentration and bulk density together explained 67% of the variation in the current annual increment of height (HI). Low N availability in reclaimed soils in the AOSR has been reported in previous studies (McMillan et al., 2007; Hemstock et al., 2010). Mining and related activities dramatically disturbed landscapes, and reconstruction using different reclamation materials formed varied soil physical and chemical properties. Loss of topsoil and mechanical mixing of the A with B and C horizon soils during surface mining resulted in severe loss of soil organic carbon and N (Ussiri and Lal, 2005; Shrestha and Lal, 2011). The mixture of peat and surface mineral soil used as cover soils was expected to increase organic matter content and improve nutrient availability, but low peat decomposition rates and net N mineralization and nitrification rates in cover soils due to small microbial population sizes, reduced enzymatic activity and low microbial diversity may affect N availability, thus limiting plant growth in the oil sand region (Reeder and Sabey, 1987; McMillan et al., 2007; Hemstock et al., 2010; Jung and Chang, 2012; Yan et al., 2012). McMillan et al. (2007) and Hemstock et al. (2010) compared N transformation rates in reclaimed and natural boreal forest soils and found that net N mineralization rate in peat-amended soils was significantly lower than that in natural soils. The differences could be attributed to inhibited nitrification by lower soil pH (Myrold, 2005), slow release of organic N by reduced microbial activity (Horwath and Paul, 1994) and lower N reserve and labile N content in reclaimed soils.

The two-year single tree fertilization experiment confirmed low N availability in Sw sites limiting Sw growth. Height, DBH and aboveground biomass growth of Sw significantly increased two growing seasons after application of N or complete fertilizers (N, P, K and micronutrients). In contrast, Pl growth was not improved by fertilization, which indicated that N availability was not the limiting factor for Pl growth. In the fertilized Sw trees, foliar N concentration and content in current-year needles increased in the first growing season, while fertilization effects were not detected in the following growing season. However, soil NH_4^+-N, NO_3^--N and dissolved organic N were not influenced by fertilization.

Boreal forests are generally regarded as being N limited because of higher N immobilization than N mineralization (Reich et al., 1997; Carmosini et al., 2003; Jerabkova et al., 2006). Immobilization can make nutrients unavailable for vegetation and suppress plant growth (Zimmerman et al., 1995). Emissions of nitrogen oxides increased in the last 40 years in the AOSR because of increasingly developed oil sands extraction (Hazewinkel et al., 2008), and chronic N emission and subsequent N deposition are expected to improve N availability in boreal forest soils; however, N deposition into the ecosystems remained relatively low (Laxton et al., 2010; Jung and Chang, 2012). Therefore, N deposition may not offer substantial relief for the demand for nutrients by the reestablishing vegetation in oil sands reclamation. In addition, reclaimed sites in the AOSR are usually fertilized during the first five years after planting, but research has not addressed what happens to plants when fertilization stops after five years, and whether reclaimed sites should be fertilized for longer periods.

2.2 Soil salinity and tree growth

Reclaimed soils in the AOSR are often affected by high salinity (Renault et al., 1999). Soil salinity and bulk density together explained 79% of the variation in the current annual increment of aboveground biomass (ABI) in the field study, which suggested that salinity and soil compaction were probably limiting Sw growth in reclaimed soils. Tree species in boreal forests were thought to be less tolerant to high levels of soil salinity (Allen et al., 1994). When soil electrical conductivity (EC) is greater than 4 dS m^{-1}, soils are unfavorable for tree survival, plant growth and establishment of the forest ecosystem (Alberta Environment, 2010). Although the average ECs in all Sw sites were 2.12 and 3.05 dS m^{-1} in the PMM and OB layer, respectively, which were both less than the salinity threshold of 4 dS m^{-1}, soil ECs in PMM or in OB layers were more than two times higher in low than in medium and high productivity sites (Studied sites were classified into low, medium and high productivity sites based on height and DBH). Soil EC in OB in low productivity sites even reached 4.71 dS m^{-1}. The diffusion of salts from saline OB to PMM layers probably resulted in increased soil salinity in cover soils (Merrill et al., 1980, 1983). High salinity can affect water availability and nutrient uptake (Kayama et al., 2003), and thus may have detrimental effects on plant survival and growth (McFee et al., 1981).

The greenhouse study evaluated the growth response of salt-stressed Pl and Sw seedlings (subjected to 0, 50, 100 and 150 mmol L^{-1} NaCl solutions) to N fertilization (0 and 300 mg seedling^{-1}). Salinity significantly decreased net photosynthetic rates (P_n), stomatal conductance, transpiration rates and N uptake, leading to reduced seedling growth and biomass for both Pl and Sw seedlings. The soil treated with 150 mmol L^{-1} NaCl had the lowest seedling growth and biomass among all the salinity treatments, demonstrating the detrimental effects of salinity on seedling growth. High soil salinity can decrease osmotic potential, induce nutrient imbalance and lead to ion toxicity (Munns, 1993; Jacoby, 1994; Cornillon and Palloix, 1997; Munns and Tester, 2008). In addition, high salinity could influence water uptake and N metabolisms such as N uptake and assimilation and amino acid and protein synthesis, thus reduce plant growth (Aslam et al., 1984; Botella et al., 1997; Dluzniewska et al., 2007). However, when the Pl and Sw seedlings were subjected to even 150 mmol L^{-1} NaCl, no seedlings died during the three-month experiment, which indicated high salt tolerance of the two tree species.

On the other hand, N fertilization significantly increased seedling growth in soils with low to medium salinity (50 and 100 mmol L^{-1} NaCl solutions). When the seedlings were subjected to the same level of salinity, the seedlings fertilized with 300 mg N seedling^{-1} showed higher P_n, N concentrations in needles and roots and greater seedling height and root collar diameter growth and total seedling biomass as compared to non-fertilized seedlings for both Pl and Sw. When the seedlings were subjected to 150 mmol L^{-1} NaCl, N fertilization could not improve seedling physiological properties and growth; on the contrary, it could increase salt stress and have negative influence on seedling growth, this may be because salt stress rather than N availability became the dominant factor limiting seedling growth.

2.3 Soil salinity and N transformation and availability

Microbes play important roles in N cycling and availability. High soil salinity could influence microbial activity and community structure (Yuan et al., 2007; Elmajdoub et al., 2014), thus significantly affecting N transformations. In the laboratory incubation experiment, gross N mineralization, nitrification, and N immobilization rates were all suppressed by high soil salinity, but they were not completely inhibited even when soil EC reached 8 dS m^{-1}. Gross N immobilization rates were greater than gross N mineralization rates regardless of salinity levels, resulting in negative net N mineralization rates and reduced N availability. This is in agreement with previous reports for boreal forests (Reich et al., 1997; Carmosini et al., 2003; Jerabkova et al., 2006). They attributed it to rich C source and limited N source in boreal forest soils. The NH_4^+-N immobilization was the dominant NH_4^+-N consumption process over nitrification, regardless of salinity levels. In contrast to gross nitrification rates, net nitrification rates were not significantly influenced by soil salinity in the soil studied. Our results suggested that inhibited N mineralization and nitrification rates in high soil salinity might be one of reasons resulting in low N availability in some saline reclaimed soils in the AOSR.

3 Conclusions

Tree growth in the studied reclaimed soils in the AOSR was limited by soil water and nutrient availabilities, soil salinity and compaction. Growth limitation varied for different tree species planted in different types of reconstructed soils. Lodgepole pine on PMM over TS was mainly affected by water availability, while N availability, soil salinity and compaction combined limited Sw growth on PMM over OB. The enhanced Sw growth after fertilization suggested the potential possibility of applying fertilizers to improve tree growth in reclaimed boreal forest soils in the AOSR. However, complete fertilizer did not show any advantage than N fertilizer applied alone indicated that N rather than other nutrients dominantly affected Sw growth. Lodgepole pine did not positively respond to fertilization indicating that, to some extent, other factors instead of N availability limited Pl growth. However, further research should be undertaken to confirm water limitation for Pl growth.

Nitrogen cycling and availability were negatively influenced by soil salinity. Increased salinity in reclaimed soils reduced gross N mineralization and nitrification rates, significantly decreased available N for plant growth. Moreover, in the N-limited boreal forests with high C source, microbes could strongly compete with trees for the limited amount of available N in the reclaimed soils, which was reflected by greater N immobilization than N mineralization rates when N was supplied during the laboratory incubation. Therefore, it is necessary to consider both salinity effects and microbial processes when applying N fertilizers to improve N availability in some saline reclaimed soils because a proportion of added N would be immobilized by microbes to make them unavailable to plants in a short term.

The greenhouse study demonstrated the effectiveness of N fertilization in enhancing seedling growth in soils with low to medium salinity. When seedlings were grown under low salinity, N fertilization could increase height and diameter growth and total seedling biomass by by increasing N concentrations in needles and net photosynthetic rates. When seedlings were subjected to medium to high salinity, reduced net photosynthetic rates, stomatal conductance, transpiration rates and N uptakes resulted in limited seedling growth, even when the seedlings were fertilized. This has implications of planning fertilization management to improve plant growth in oil sands reclamation.

4 Suggestions for future research

4.1 Explanations of low N availability in reclaimed soils

Limited N availability has been extensively reported in boreal forests (Jung and Chang, 2012; Yan et al., 2012). As pointed out by Reich et al. (1997) and Hemstock et al. (2010), low N availability could be due to slow organic matter decomposition rates, the high lignin content of the litter, and low microbial activities in soils. As suggested in this study the low N availability is a big problem in revegetation of reclaimed sites. Besides the reasons mentioned above, there are other explanations for the low N available in reclaimed soils. Disturbance during soil reconstruction significantly changed soil physical and chemical properties, leading to loss of organic matter and associated microorganisms. Moreover, high soil salinity would contribute to low N mineralization and nitrification rates as indicated in this study. Therefore, investigation on low N availability in reclaimed soils would be valuable for nutrient management of planted trees and beneficial for speeding up reclamation process.

4.2 *In situ* N transformation in saline reclaimed soils

The short-term laboratory study on the effect of salinity on N transformations provided a mechanistic understanding of the dynamics of soil gross N transformations as a response to changes in soil salinity. However, it is difficult to extrapolate the findings to field conditions because soil salinity changes gradually (over months or years) and microbial populations would have time to adjust to salt stress. In addition, soil processes are not affected by a single form of salt as was used in the laboratory incubation experiment; instead they are influenced by a combination of several different salts under field conditions. Moreover, laboratory studies could not reflect the real field conditions because soil storage, sieving, ^{15}N addition and salt addition all could change N transformation rates *in situ* (Booth et al., 2005). Therefore, more detailed studies on *in situ* N transformations in the field over the long-term would help elucidate the temporal changes of N flux in saline reclaimed soils. This may further explain whether microbial competition through N immobilization is an important factor leading to nutrient deficiency problems in oil sands reclaimed sites. Detailed N cycling processes would also help in evaluating the possible supply form of available N to tree growth and the risk of N loss through N leaching or denitrification.

4.3 Possibility of fertilization to improve tree growth in saline reclaimed soils

As is shown in previous studies that trees could survive on some natural sites with high salinity with the electrical conductivity as high as 23 dS m^{-1} in sub-soils in boreal mixedwood forests because of relatively high soil moisture and nutrient availabilities of surface soils (Lilles et al., 2010). This provided a strategy to deal with salinity problem in some saline reclaimed soils. However, the question whether tree seedlings can survive in dry conditions with high nutrient availability remains unresolved, and it is important to elucidate how seedlings respond to N and salinity under field conditions. It showed that appropriate depth of capping material over substrates could reduce the upward movement of salts to the rooting zone and at the same time increase water storage for tree growth, but how thick the cover soil should be above different substrates needs to be addressed in future research.

4.4 Water limitation for tree growth in saline reclaimed soils

The relationship between water availability and Pl growth in reclaimed sites needs to be further tested through field irrigation experiments. As shown in the study by Jung et al. (2014) conducted in the same research sites that water availability varied in reclaimed sites reconstructed using different substrates. Therefore, a better understanding of the reasons leading to limited water availability in sites using TS as a substrate could provide useful insights into the choice and layer configuration for soil reconstruction.

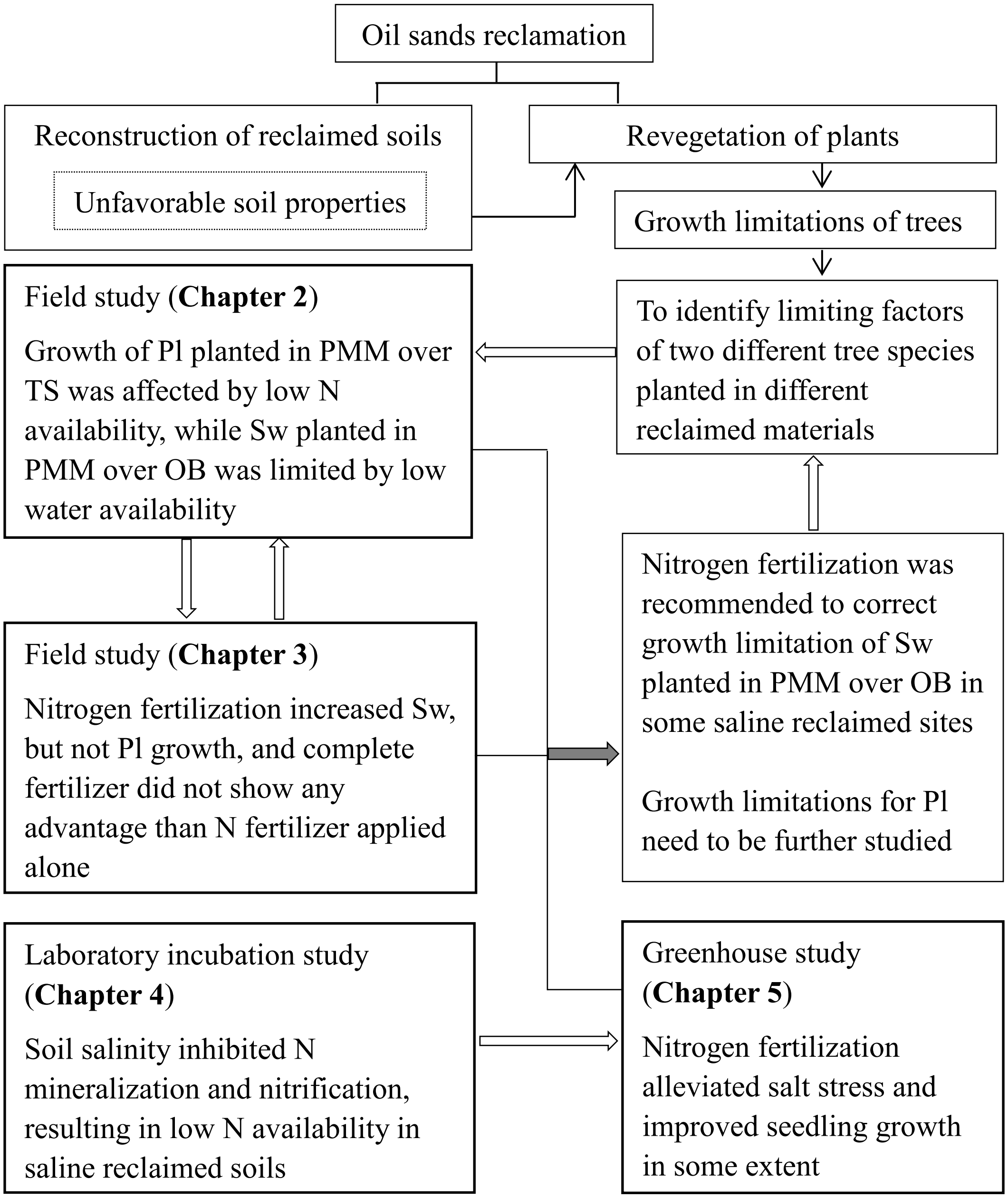

Figure 6-1 Flow chart of the key findings in the thesis research.

References

Alberta Environment. 2010. Guidelines for Reclamation to Forest Vegetation in the Athabasca Oil Sands Region. 2nd edition. Prepared by the Terrestrial Subgroup of the Reclamation Working Group of the Cumulative Environmental Management Association, Fort McMurray, Alberta. p. 332.

Allen, J.A., Chambers, J.L., Stine, M. 1994. Prospects for increasing the salt tolerance of forest trees: A review. Tree Physiol. 14:843-853.

Aslam, M., Huffaker, R.C., Rains, D.W. 1984. Early effects of salinity on nitrate assimilation in barley seedlings. Plant Physiol. 76:321-325.

Ballard, T.M., Carter, R.E. 1986. Evaluating Forest Stand Nutrient Status. Ministry of Forests, Victoria, British Columbia. p. 33.

Booth, M.S., Stark, J.M., Rastetter, E. 2005. Controls on nitrogen cycling in terrestrial ecosystems: A synthetic analysis of literature data. Ecol. Monogr. 75:139-157.

Botella, M.A., Martínez, V., Nieves, M., Cerdá, A. 1997. Effect of salinity on the growth and nitrogen uptake by wheat seedlings. J. Plant Nutr. 20:793-804.

Carmosini, N., Devito, K.J., Prepas, E.E. 2003. Net nitrogen mineralization and nitrification in trembling aspen forest soils on the Boreal Plain. Can. J. For. Res. 33:2262-2268.

Cheng, Y., Wang, J., Mary, B., Zhang, J.B., Cai, Z.C., Chang, S.X. 2013. Soil pH has contrasting effects on gross and net nitrogen mineralizations in adjacent forest and grassland soils in central Alberta, Canada. Soil Biol. Biochem. 57:848-857.

Cornillon, P., Palloix, A. 1997. Influence of sodium chloride on the growth and mineral nutrition of pepper cultivars. J. Plant Nutr. 20:1085-1094.

Dluzniewska, P., Gessler, A., Dietrich, H., Schnitzler, J.P., Teuber, M., Rennenberg, H. 2007. Nitrogen uptake and metabolism in *Populus* x *canescens* as affected by salinity. New Phytol. 173:279-293.

Elmajdoub, B., Barnett, S., Marschner, P. 2014. Response of microbial activity and biomass in rhizosphere and bulk soils to increasing salinity. Plant Soil 381:297-306.

Fisher, R.F., Binkley, D. 2000. Ecology and Management of Forest Soils. 3rd edition. John Wiley and Sons Inc., New York City, New York. p. 489.

Fung, M.Y.P., Macyk, T.M. 2000. Reclamation of oil sands mining areas. In: Barnhisel, R.I., Darmody, R.G., Daniels, W.L. (Eds.), Reclamation of Drastically Disturbed Lands. ASA, CSSA and SSSA, Madison, Wisconsin. p. 1082.

Hazewinkel, R.R.O., Wolfe, A.P., Pla, S., Curtis, C., Hadley, K. 2008. Have atmospheric emissions from the Athabasca oil sands impacted lakes in northeastern Alberta, Canada? Can. J. Fish. Aquatic Sci. 65:1554-1567.

Hemstock, S.S., Quideau, S.A., Chanasyk, D.S. 2010. Nitrogen availability from peat amendments used in boreal oil sands reclamation. Can. J. Soil. Sci. 90:165-175.

Horwath, W.R., Paul, E.A. 1994. Microbial biomass. In: Weaver, R.W., Angle, S., Bottomley, P., Bezdicek, D., Smith, S., Tabatabai, A., Wollum, A. (Eds.), Methods of Soil Analysis: Part

2-Microbiological and Biochemical Properties. SSSA, Madison, Wisconsin. p. 1121.

Howat, D.R. 2000. Acceptable Salinity, Sodicity and pH Values for Boreal Forest Reclamation. Alberta Environment, Edmonton, Alberta. p. 191.

Inubushi, K., Barahona, M.A., Yamakawa, K. 1999. Effects of salts and moisture content on N_2O emission and nitrogen dynamics in Yellow soil and Andosol in model experiments. Biol. Fertil. Soils 29:401-407.

Irshad, M., Honna, T., Yamamoto, S., Eneji, A.E., Yamasaki, N. 2005. Nitrogen mineralization under saline conditions. Commun. Soil Sci. Plant Anal. 36:1681-1689.

Jacoby, B. 1994. Mechanisms involved in salt tolerance by plants. In: Pessarakli, M. (Ed.), Handbook of Plant and Crop Stress. Marcel Dekker Inc., New York City, New York. p. 697.

Jerabkova, L., Prescott, C. E., Kishchuk, B. E. 2006. Nitrogen availability in soil and forest floor of contrasting types of boreal mixedwood forests. Can. J. For. Res. 36:112-122.

Jung, K., Chang, S.X. 2012. Four years of simulated N and S depositions did not cause N saturation in a mixedwood boreal forest ecosystem in the oil sands region in northern Alberta, Canada. For. Ecol. Manage. 280:62-70.

Jung, K., Duan, M., House, J., Chang, S.X. 2014. Textural interfaces affected the distribution of roots, water, and nutrients in some reconstructed forest soils in the Athabasca oil sands region. Ecol. Eng. 64:240-249.

Kayama, M., Quoreshi, A.M., Kitaoka, S., Kitahashi, Y., Sakamoto, Y., Maruyama, Y., Kitao, M., Koike, T. 2003. Effects of deicing salt on the vitality and health of two spruce species, *Picea* *abies* Karst., and *Picea glehnii* Masters planted along roadsides in northern Japan. Environ. Pollut. 124:127-137.

Laxton, D.L., Watmough, S.A., Aherne, J., Straker, J. 2010. An assessment of nitrogen saturation in *Pinus banksiana* plots in the Athabasca oil sands region, Alberta. J. Limnol. 69 (Suppl. 1):171-180.

Lilles, E.B., Purdy, B.G., Chang, S.X., Macdonald, S.E. 2010. Soil and groundwater characteristics of saline sites supporting boreal mixedwood forests in northern Alberta. Can. J. Soil Sci. 90:1-14.

McFee, W.W., Byrnes, W.R., Stockton, J.G. 1981. Characteristics of coal mine overburden important to plant growth. J. Environ. Qual. 10:300-308.

McMillan, R., Quideau, S.A., MacKenzie, M.D., Biryukova, O. 2007. Nitrogen mineralization and microbial activity in oil sands reclaimed boreal forest soils. J. Environ. Qual. 36:1470-1478.

Merrill, S.D., Doering, E.J., Power, J.F. 1980. Changes of sodicity and salinity in soils reconstructed on strip-mined lands. N. D. Farm Res. 37:13-16.

Merrill, S.D., Doering, E.J., Power, J.F., Sandoval, F.M. 1983. Sodium movement in soil-mine spoil profiles: Diffusion and convection. Soil Sci. 136:308-316.

Munns, R. 1993. Physiological processes limiting plant growth in saline soils: Some dogmas and hypotheses. Plant Cell Environ. 16:15-24.

Munns, R., Tester. M. 2008. Mechanisms of salinity tolerance. Annu. Rev. Plant Biol.

59:651-681.

Myrold, D.D. 2005. Transformation of nitrogen. In: David, M.S., Jeffry, J.F., Peter, G.H., David, A.Z. (Eds.), Principles and Applications of Soil Microbiology. 2nd edition. Prentice Hall, Upper Saddle River, New Jersey. p. 640.

Naeth, M.A., Chanasyk, D.S., Burgers, T.D. 2011. Vegetation and soil water interactions on a tailings sand storage facility in the Athabasca oil sands region of Alberta Canada. Phys. Chem. Earth. 36:19-30.

Reeder, J.D., Sabey, B. 1987. Nitrogen. In: Williams, R.D., Schuman, G.E. (Eds.), Reclaiming Mine Soils and Overburden in the Western United States: Analytic Parameters and Procedures. Soil Conservation Society of America, Ankeny, Iowa. p. 336.

Reich, P.B., Grigal, D.F., Aber, J.D., Gower, S.T. 1997. Nitrogen mineralization and productivity in 50 hardwood and conifer stands on diverse soils. Ecology 78:335-347.

Renault, S., Paton, E., Nilsson, G., Zwiazek, J.J., MacKinnon, M.D. 1999. Responses of boreal plants to high salinity oil sands tailings water. J. Environ. Qual. 28:1957-1962.

Shrestha, R.K., Lal, R. 2011. Changes in physical and chemical properties of soil after surface mining and reclamation. Geoderma 161:168-176.

Ussiri, D.A.N., Lal, R. 2005. Carbon sequestration in reclaimed mine soils. Crit. Rev. Plant Sci. 24:151-165.

Yan, E.R., Hu, Y.L., Salifu, F., Tan, X., Chen, Z.C., Chang, S.X. 2012. Effectiveness of soil N availability indices in predicting site productivity in the oil sands region of Alberta. Plant

Soil 359:215-231.

Yuan, B.C., Li, Z.Z., Liu, H., Gao, M., Zhang, Y.Y. 2007. Microbial biomass and activity in salt affected soils under arid conditions. Appl. Soil Ecol. 35:319-328.

Zimmerman, J.K., Pulliam, W.M., Lodge, D.J., Quinones-Orfila, V., Fetcher, N., Guzmán-Grajales, S., Parrotta, J.A., Asbury, C.E., Walker, L.R., Waide, R.B. 1995. Nitrogen immobilization by decomposing woody debris and the recovery of tropical wet forest from hurricane damage. Oikos 72:314-322.